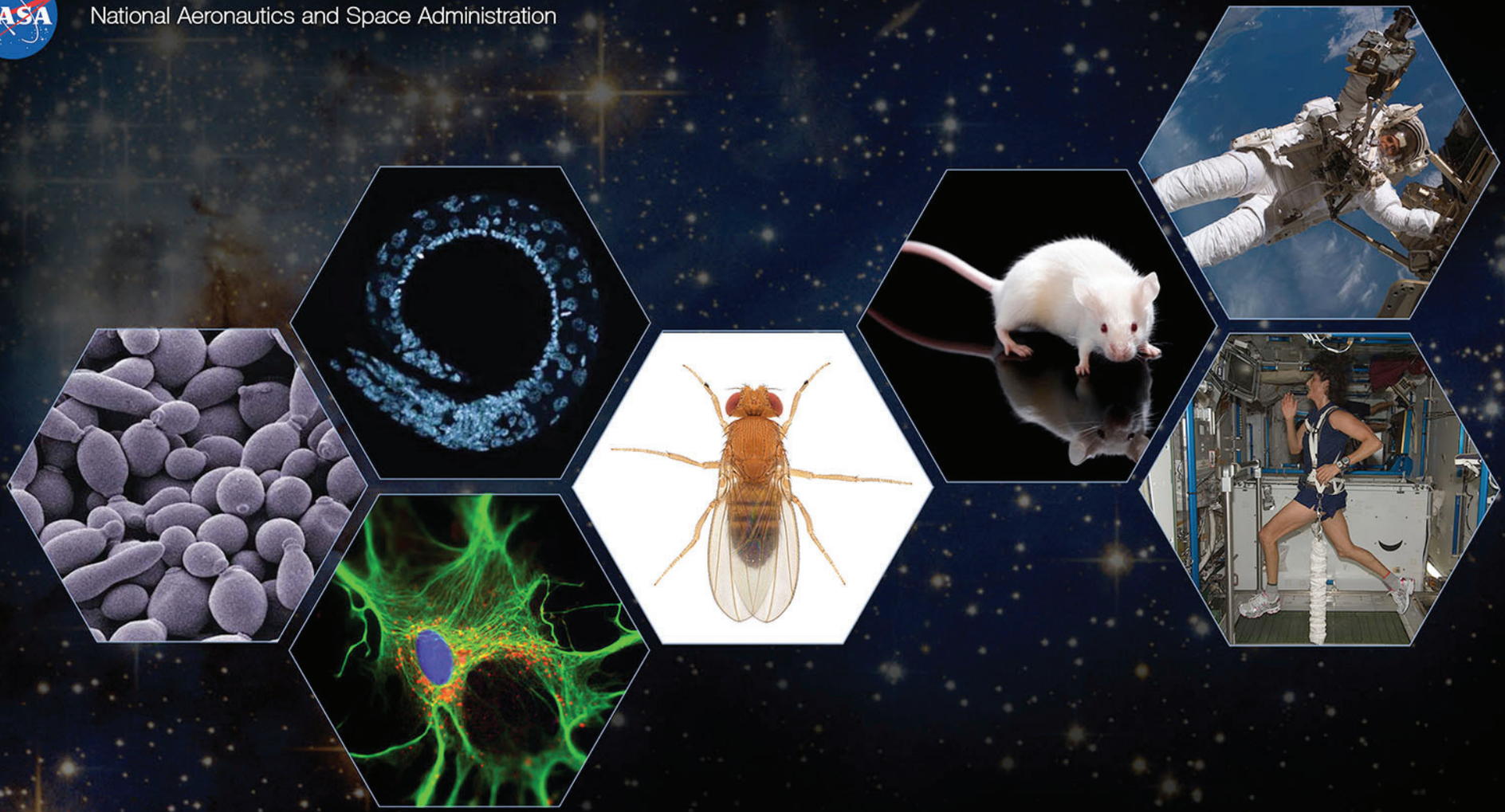
National Aeronautics and Space Administration



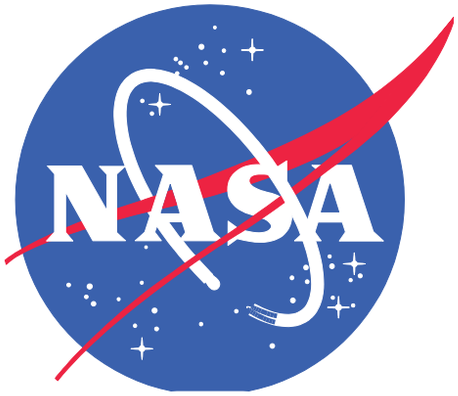
Translational Cell & Animal Research in Space

Ames Research Center

1965 - 2011

Edited by April E. Ronca, Kenneth A. Souza, Richard C. Mains

NASA/SP-2015-625



TRANSLATIONAL CELL and ANIMAL RESEARCH in SPACE 1965-2011

Edited by April E. Ronca, Kenneth A. Souza and Richard C. Mains
NASA Ames Research Center
Mountain View, CA

September 2015

NASA STI Program…in Profile

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This report is also available in electronic form at
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Contents

Preface

This publication includes one-page descriptions of experiments, grouped by science discipline area, for NASA ARC-managed animal and related cell spaceflight research from missions conducted between 1965 and 2011. Introductions are provided for each area, written by NASA-related research specialists, summarizing selected flight experiment results. The introductions reference the associated experiment descriptions (by mission I.D. and page no.), their related publications (by investigator(s) name and year) and, as warranted, link to useful research overview articles. They are meant to provide a window for users to find, review, and recognize the potential value of this space biology research for "translational" purposes to biomedical human space and Earth applications.

The experiment description format used herein was originally developed for a series of NASA publications titled "Life into Space." Two were initially produced as books (Souza, 1995; Souza, 2000) and are available through many university libraries. They were subsequently placed online (http://lis.arc.nasa.gov) with mission-related experiment content through 2003. NASA created a Life Sciences Data Archive (LSDA) in the 1990s (http://lsda.jsc.nasa.gov.), and the data from Life into Space was exported for use in that somewhat different format as the LSDA evolved. The NASA ARC LSDA staff used the same experiment description format to prepare content extracted from their Archive for the 2003–2011 period for the purposes of this publication.

Each experiment description page provides information on the investigator team, the research subjects, the ground-based controls (see explanation below), the flight hardware used, and selected publications, as available. The top-level summary information for each experiment includes objectives/hypothesis, approach or method, and available results. The content is written at a level intended to be generally understandable by a broad audience with interest in this subject.

Several ground-based control experiment designations are introduced in the Life into Space publication (Vol. II, 1991–1998, p. 22), and a short summary is also included here. Control experiments are designed to duplicate the flight environment except for microgravity and/or radiation, and often compare lab animal housing with spaceflight housing, or similar diet comparisons. Factors considered important to duplicate in flight and ground studies generally include: research subject type, gender, age, number; cage/habitat type; food and water; air temperature; light intensity and light/dark cycle; all subject-related operations; and experiment duration. Control experiment designations typically used in these experiment descriptions are listed below.

Vivarium Cage Control = animal subjects kept in standard lab conditions (housing, diet, lighting, temperature) or similarly for cells in standard lab culture conditions.

Basal Control = animal subjects (or similarly for cells) that are equivalent to flight subjects but sacrificed at launch to obtain baseline data.

Synchronous Control = experiments conducted on the ground at the same time as flight experiments with subjects in a flight-type cage/habitat and all other conditions held equal except unique aspects of the flight environment such as microgravity, radiation, and launch/reentry stresses.

Asynchronous (Delayed) Control = similar to Synchronous Control but using data from the spaceflight period to better duplicate test conditions such as temperature.

Gravity Simulation = subjects kept at simulated Earth-gravity conditions by living on an in-flight centrifuge or kept in a simulated

flight condition such as using the rodent tail suspension model for unloading (Morey-Holton, 2005).

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Introduction

Translational Cell and Animal Research (TCAR)

For nearly 50 years, the NASA Space Biology Program has funded, and Ames Research Center (ARC) has managed, a robust program of fundamental research including studies using a wide range of animal cells, tissues and organisms (http://www.nasa.gov/directorates/heo/slpsra/ and http://www.nasa.gov/ames/research/space-biosciences/). Much of this research was conducted on spacecraft in microgravity environments including diverse platforms such as: Gemini Spacecraft, US Biosatellites, Apollo Command Modules, Skylabs, Russian Biosatellites, NASA Space Shuttles, NASA/Mir, and most recently, the International Space Station (ISS). During the Space Shuttle Era (1981–2011), the science of space biology took an enormous step forward with 45 missions that afforded researchers with new opportunities to conduct systematic and complex experiments aimed at a deeper understanding of how life adapts to the space environment. Beginning in the 1990s, the products of these experiments, comprised of research summaries and rare, unused biospecimens, were collected and catalogued within the ARC Life Sciences Data Archiving Office, a branch of NASA's Life Sciences Data Archive (LSDA) managed from the NASA Johnson Spaceflight Center (http://lsda.jsc.nasa.gov)[1].

Report Rationale

The National Research Council Committee for the Decadal Survey on Biological and Physical Sciences in Space recently published their final results and recommendations [NRC, 2011]. Their "Integrative and Translational Research for Human Systems Panel" proposed ways that NASA can increasingly support "translational research in the space/life sciences." One key suggestion was to "improve central information networks to facilitate data sharing...to enhance the science results derived from flight opportunities." The efforts described herein are meant to support the Decadal Survey recommendations for improving this information and data sharing. This report compiles a subset of the cell and animal research in space supported by the NASA ARC Space Biosciences Division (formerly Life Sciences Division) from 1965 to 2011. These experiments, 382 in all, are deemed to be "translational," because they contain space biosciences' results relevant to human health in space and on Earth. Their primary rationale, of course, was to advance our knowledge of how space can influence basic processes in living systems. From both the mission and science viewpoints, future researchers can significantly benefit from the achievements and lessons learned from these efforts. Basic biologists, clinical scientists, flight medical personnel, and many others should find this report to be a valuable resource for guiding future space biomedical research. Laymen and students can also benefit from this comprehensive resource describing the rich history of space life sciences research.

In support of this effort, space biology research specialists in Bone Physiology, Cardiovascular/Cardiopulmonary Physiology, Developmental Biology, Immunology, Microbial Growth, Muscle Physiology, Neurosciences, and Regulatory Physiology were asked to review and prepare brief overviews of the progress made within their discipline. Each subject matter expert also considered translational impacts of the findings for human health in space and on Earth in their introduction section.

Human Health Risks in the Space Environment

Our experience with long-duration spaceflight shows that humans generally function well within the space environment. However, astronauts incur risks to their health due to the significant changes in physiology they experience while living and working in the

1 At the time of publication, many of the specimens from the portfolio of experiments described in this book remain available for scientific study. The LSDA continues to acquire unused specimens.

novel environment of space. The following examples illustrate the breadth of risks to astronaut health:

- Bone fracture
- Kidney stones
- Inter-vertebral disc injury
- Visual impairment
- Intra-cranial pressure
- Anemia
- Muscular atrophy
- Acute radiation syndrome and tissue degradation
- Radiation-induced carcinogenesis
- Altered immune system

Protecting humans in space requires a clear understanding of the physiologic changes that can occur and the degree of medical risk associated with that exposure. The NASA Human Research Program (HRP), located at Johnson Space Center (JSC), is concerned with crew health and performance in space. HRP has developed the Human Research Roadmap (HRR), a risk reduction strategy for human space exploration (http://humanresearchroadmap.nasa.gov/).) The HRR provides information, based on current data on known human health risks in the space environment, as well as potential countermeasures that include procedures, medications, devices, and other strategies that help keep astronauts healthy and productive during space travel and return to Earth.

Human research in space is essential for identifying and defining the human health risks, and developing countermeasures. However, conducting biomedical experiments on a flight crew can be difficult and involve many limitations, including:

- Operational constraints associated with conducting human research in space (such as test procedures that might impair crew function, limited crew time, and impracticality of isolating crewmembers and applying proper experimental controls such as diet, lighting, and temperature)
- Small non-homogeneous samples with large inter-subject variability that is difficult to analyze with good statistical certainty
- Use of countermeasures that confuse interpretation of data collected
- Limited knowledge base from Earth-bound control experiment cohorts (e.g., subjects with similar characteristics to crew members that live and work under analogous conditions to spaceflight)
- Significant privacy concerns regarding data and identification of human subjects.

For these reasons, research with animals (historically and currently) plays a vital role in advancing our understanding of biological responses and adaptation of humans to the space environment. Specific advantages of using animals in space biomedical research include:

- Invasive studies that can be carried out to determine mechanisms of adaptations to altered gravity, wound and fracture healing, and assess clinical and surgical procedures
- Feasibility of postmortem analyses
- Extensive baseline data and metadata are available from spaceflight and ground-based studies
- Availability of numerous genetically standardized and genetically modified subjects
- Continuous in-flight monitoring of various physiological parameters
- Statistical significance more readily achievable than with humans due to small size and larger number of subjects
- Drugs and diet can be tested and controlled
- Established models for different human systems/diseases can be utilized
- Higher risk space environment effects can be studied over life spans and generations within a relatively brief time pe-

riod. For example, the ISS increment of 180 days is roughly one-third of the rodent life span. Such studies have the potential to extrapolate important information to humans living in space well beyond 6 months.

Translational Research Insights

In 2002, the National Institutes of Health (NIH) began a process of charting a "roadmap" for biomedical research in the 21st century, identifying gaps and opportunities that crossed the boundaries of then-extant research institutions. Translational research is viewed as the process of applying ideas, insights, and discoveries, generated through basic scientific inquiry, to the treatment or prevention of human disease [Zerhouni, 2003]. A key initiative that came out of this review was a systematic effort to strengthen Translational Research defined by the NIH as the movement of discoveries in basic research (the Bench) to application at the clinical level (the Bedside). An additional key element of translational research is bi-directionality: the role of clinical research to inform and guide basic science questions. Broadly conceived, preclinical translation relies on a wide variety of basic and animal research activities, including (1) experiments conducted with cell and animal models; (2) sampling and analysis of human and/or animal tissues; and (3) computer-assisted modeling of drug, device, or diagnostic interactions with living systems.

The goals of translational research at NASA are to elucidate and understand the effects of spaceflight on astronaut health, to understand how gravity influences human health on Earth, and the development of countermeasures to those effects to benefit life in space and on Earth. Utilizing space platforms and ground-based facilities, NASA translational research forms the bridge between basic cell and animal research, space biomedicine, and their applications. Table 1 shows examples of translational relevance of space biology research to human health in space and on Earth.

Fortunately, significant synergism already exists at NASA between the Space Biology Program and the Human Health Countermeasures (HHC) element of the HRP. The common goals and approaches involve research on: (1) fundamental molecular and cellular mechanisms; (2) novel mechanisms and phenotypes; and (3) countermeasures and treatments for astronauts including drug testing, exercise protocols, and dietary requirements. There is a shared emphasis on a progression from preflight research to flight discovery and validation.

Improving Life in Space and on Earth

The biomedical concerns and risks of long-duration space exposures are not sufficiently known. The possibility exists that, unless effective countermeasures are developed and implemented, extended habitation on the ISS and eventual interplanetary travel may induce physiological and behavioral changes that degrade the health, safety, and productivity of crew members. Research in the post-Shuttle era is emphasizing long-duration studies of cells, tissues, and organisms, and generational changes in space, thus basic research on the ISS will begin to fill this gap.

Research in space can truly benefit life on Earth by uncovering knowledge of the basic biological mechanisms underpinning the organismal response to the spaceflight environment. Additionally, spaceflight missions provide a novel environment in which to develop and test countermeasures for aging, sedentary lifestyles, radiation exposure, and compromised immune system, among others. For example, preclinical tests of two pharmaceutical countermeasures in rodents have shown their effectiveness at preventing bone loss during spaceflight (see Bone Physiology Introduction) and have added novel data to the associated drug knowledge base for Food & Drug Administration (FDA) review.

Table 1. Translational Relevance of Space Biology Animal Research to Human Health in Space and on Earth.

Discipline (# Experiments)/ Model Organism or Cell Culture Types Studied	Effects on Cells and/or Animals	Implications for Astronaut Health	Potential Countermeasures in Space	Potential Earth Benefits
Bone Physiology (81) Chicken Gecko Quail Mouse Rat Rhesus Monkey	• Loss of bone density • Impaired bone healing • Cell and molecular mechanisms of bone loss emerging • Drug countermeasures to fight bone loss in mice appear effective	• Increased risk of fracture • Increased risk of kidney stone • Centrifugation in rats prevented negative effects of spaceflight on long-bone mechanical properties • Established and emerging drug therapies for bone loss in humans on Earth were tested in animals in space to confirm the efficacy in the weightless environment	• Weight-bearing activity essential to maintain bone density • Artificial gravity options under study • Drug treatment (Amgen) for lessening bone resorption and promoting formation received FDA approval in 2010 for treatment of osteoporosis. A second drug developed and FDA	• More comprehensive understanding of bone formation, growth, maintenance, and healing • Optimize treatment of osteoporosis and disuse arthritis in women and men
Cardiovascular/ Cardiopulmonary (25) Mouse Rat Rhesus Monkey	• Cardiac contraction tissue loss • Cardiac myocyte morphology changes • Impaired peripheral resistance responsiveness and cerebrovascular control • Increased intracranial pressure projected in rhesus monkey • Artificial gravity centrifuge with rats was protective of red blood cell loss	• Advances the integrated understanding of anatomy, physiology, and biochemistry underlying orthostatic hypotension and cardiovascular deconditioning • Analogous changes in cardiac muscle, vascular tissue and cerebrovascular control anticipated	• Need better understanding of potential intracranial pressure increase in micro-g on astronaut visual impairment • Anti-G suit essential for reentry and landing to minimize orthostatic blood shifts • Exercise essential to minimize cardiovascular deconditioning	• Improved understanding and treatment of cardiovascular disease in disuse, and post-bedrest deconditioning
Developmental Biology (54) Chicken Fruit Fly Frog Fish Quail Mouse Rat Rhesus Monkey Sea Urchin	• Impaired or delayed development of vestibular system elements • Some minor alterations in early development can be overcome by genomic factors • Pregnant rats flown in space delivered mostly normal pups postflight • No data yet on in-flight conception and birthing in mammals • Multi-generation studies successful in some non-vertebrates • Artificial gravity centrifuge with rats was protective of multiple micro-g decrements	• Sex differences in human response to spaceflight not yet widely reported • Human development studies not yet practical in spaceflight • Studies of in-flight conception and birthing in mammals needed • Multi-generation studies using all model systems needed, especially vertebrates and mammals	• Developmental biology studies in space have shed light on how sensorimotor systems are integrated and the role of gravity in modulation of sensorimotor function • Artificial gravity may counter several changes seen with spaceflight	• Understanding how gravity affects reproductive systems/fetal development could lead to improved conception rates and pregnancy outcomes • Provide new information on sensitivities of embryonic and postnatal development to the gravity vector • Advance understanding of early developmental programming • Identify cross-generational transmission of epigenetic changes • Understanding vestibular development likely to yield new insights into sensorimotor degradation associated with aging

Discipline (# Experiments)/ Model Organism or Cell Culture Types Studied	Effects on Cells and/or Animals	Implications for Astronaut Health	Potential Countermeasures in Space	Potential Earth Benefits
Immunology (23) Fruit Fly Human Cells Mouse Rat Rhesus Monkey	• Immune suppression (T-cells, transcription activation inhibited during spaceflight) • Increased susceptibility to infection • Multiple studies in human cells, fruit flies, rodents, and rhesus monkeys	• Animal/cell studies led to concept of tissue-specific immune responses, a central feature of terrestrial immune research • Spaceflight affects differentiation of cells from bone marrow • Phenotypic changes in immune cell populations may provide a biomarker for impaired host defense • Depressed T-Cell immune response	• Artificial gravity was protective of some immune suppression related changes (transcription) • Supplementation (diet, drugs)	• Important for study and treatment of immunodeficiency, infection, and tumor formation • Conceptual contributions (i.e., tissue-specific immune responses) • Aging population becomes bedridden and gravitationally "unloaded" as in space
Microbial Growth and Virulence (14) Bacteria Fungus Human Cells Yeast	• Increased virulence of microorganism-induced disease • Increased antibiotic resistance in microorganisms • Identification of a master molecular regulator, Hfq, that uniquely regulates spaceflight-induced characteristics of the disease-causing bacteria	• S. Typhimurium virulence cultured during spaceflight significantly elevated compared to Earth-grown cultures; could pose human hazards • Changes in virulence characteristics apply to a wide variety of micro-organisms and development of complex communities ("biofilms") • Increased virulence and antibiotic resistance plus decreased immune response is a concern	• Benefits of microorganisms in space include biological processing systems and probiotics to support crew health • Understanding potential hazards to the crew as allergens or agents of infectious disease • Countermeasures to increased virulence can emerge from understanding of basic mechanisms	• Identifying how microorganisms interact with their host and the environment could positively impact clinical environments and managing these issues • Understanding biofilms and preventing biofilm formation important for biotechnology/ synthetic biology applications
Muscle Physiology (58) Chicken Mouse Nematode Rat Rhesus Monkey	• Muscle atrophy • Muscle weakness and increased fatigability • Reduced muscle fiber attachment to tendon • Increased risk of muscle tearing with reduced healing capabilities	• Impaired skeletal muscle repair following trauma and reloading injury from intense exercise, reduced physical endurance • Nutrition deficits can contribute to muscle atrophy • Prevent spaceflight-induced conversion of slow- to fast-twitch muscle fibers to reduce muscle weakness and fatigue	• Optimize exercise to preserve slow-twitch muscle fibers, muscle mass, muscle length, and aerobic energy-deriving metabolism • Enhance nutrition (caloric intake) to prevent degradation of muscle for use as energy source • Commercial Biomedical Test Module-2, Myosin inhibitor (STS-188) under testing	• Develop exercise, nutrition, and drug therapies to preserve muscle strength and endurance • Translation of interventions to rehabilitation and sports medicine to treat debilitating conditions associated with musculoskeletal trauma, joint replacement, aging sarcopenia, spinal cord injury, stroke, and developmental defect disabilities found in cerebral palsies and muscular atrophies

Discipline (# Experiments)/ Model Organism or Cell Culture Types Studied	Effects on Cells and/or Animals	Implications for Astronaut Health	Potential Countermeasures in Space	Potential Earth Benefits
Neurophysiology (58) Cricket Fish Quail Mouse Newt Rat Rhesus Monkey Snail Toadfish	• Altered gravity sensing and circadian rhythms • Space motion sickness symptoms observed in squirrel monkeys paralleled crew's symptoms during flight • Significant cellular neuroplasticity to altered gravity in adult animals • Increased vestibular sensation in vertebrates and invertebrates • Effects on the angular vestibular-ocular reflex and gaze control • Single unit neural activity in the vestibular nuclei and cerebellar flocculus in rhesus monkey	• Altered circadian rhythms including sleep cycles • Altered distance perception, orientation, and visual tracking • Time course of neural adaptations similar in monkeys and humans • Loss of gravity vector impacts central blood pressure control mechanisms	• Structural changes in otoliths, modifications of neural interconnectivity including changes in synapse strength during long-duration flight • Artificial gravity needs study • Medication used routinely to ameliorate space motion sickness	• Important to the study and treatment of vestibular dysfunction (e.g., canalithiasis; Meniere's Disease; aging-related vestibular impairment)
Regulatory Physiology (69) Mouse Rat Rhesus Monkey	• Endocrine changes • Hematologic alterations • Altered metabolism, including metabolic dysfunction	• Identification of "integrators," particularly the endocrine system in concert with the nervous system that coordinate behavior, regulation of metabolism, nutrition and homeostatic processes, as well as cellular responses to environmental changes • Altered stress and sex hormones	• Similar spaceflight-induced changes in endocrine profiles in animals and humans provide opportunity for translational research • Relationship between nutritional adequacy and homeostasis • Evaluation of nutritional countermeasures, including flaxseed • Artificial gravity	• Relevant to the study and treatment of immunodeficiency, infection and tumor formation

Future Translational Research in Space

Translational research will continue to rely on model organisms. Model organisms are used in terrestrial and space biomedicine because they have well-known characteristics that allow them to be easily maintained, reproduced, and studied in a research laboratory and their genomes are well defined. Conducting basic research on model organisms enables scientists to better understand the cellular and molecular workings of the human body, and how disease propagates, because the origins of all living species evolved from the same life process that is shared by all living things. Model organisms can be plants, microbes (e.g., yeast), or animals (e.g., fruit flies, fish, nematode worms, amphibia, and rodents).

Just as the ISS has led to a new era of long-duration space habitation by the flight crew, the duration of study for basic cellular and animal experiments has been extended as well. Some new efforts and research directions are summarized below.

Rodent Research. Mammalian models are commonly used in biomedical research on Earth because of the many commonalities with humans in physiological and cellular responses, and genome elements. Genomes are well sequenced and understood; it is easy to create genetically altered strains to mimic human disease processes involving the cardiovascular, musculoskeletal, immune, nervous, and other physiological systems. Their short reproductive cycles and life span make them well suited to aging and multigenerational research. The Mouse Drawer System (MDS) flown by the Italian Space Agency in 2009 was a first attempt at a rodent habitat validation on the ISS, with mixed results. Since that time, NASA has modified the Animal Enclosure Module (AEM), which flew many experiments on the Space Shuttle, for use on the ISS. The validation flight for this hardware was launched on September 20, 2014.

Bioculture Research. A prior cell culture system flew successfully on 21 STS missions, but a new Bioculture System set to launch soon on the ISS provides many additional capabilities. It will contain 10 separate cassettes that operate in parallel and can contain and grow cells, microorganisms, and tissues over many months. New experiments will be accommodated that assess the space environment effects on biochemistry physiology, genetics, and gene expression of these living systems. “Omics” studies with cells and tissues grown in space can be used for drug discovery, countermeasure analyses, and infectious disease processes. Related studies can be conducted on tissue engineering, regeneration, and wound healing. The Bioculture System is designed so it can be used in conjunction with animal research on the ISS in a new way. Biosamples from flown animals can be transferred onboard to the Bioculture System for maintenance and analysis, thus avoiding complications from gravity effects associated with animal reentry and landing.

Fruit Fly Research. A new research habitat for fruit flies (Drosophila melanogaster) will be launched to the ISS. The fruit fly has common genes with humans, with more than half of human genes that map to diseases shared with the fruit fly genome. Genes that are conserved (shared) by fruit flies and humans are, for example, being studied on the precursor Heart Effect Analysis Research Team conducting Fly Investigations and Experiments in Space (HEART FLIES) payload on the ISS. The effects of spaceflight on function, morphology, and gene expression of fruit fly hearts will be studied to better understand the cardiovascular effects of spaceflight on humans, and to potentially identify countermeasures and treatment for astronauts. This precursor study, as warranted, will be repeated with many more measurement options available on the Fruit Fly Lab when it is ready for use.

GeneLab Research. The geneLab effort is focused on defining the "physiome" thorough omics systems biology approaches using cutting-edge omics technologies. These technologies allow the simultaneous examination of multiple, complex changes in deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNA), proteins, metabolites, and methylation states. Omics approaches generate vast datasets on the protein, transcript, lipid, and metabolite status of cells, paving the way for new areas of inquiry in genomics, transcriptomics, proteomics, metabolomics, and epigenomics. These exciting new approaches for describing changes in the animal and human physiome are rapidly advancing, thereby significantly improving our perspectives and understanding of biosystems and their functional integration. Next-generation deep sequencing machines promise to yield whole genome and organ-specific expression profiles at unprecedented speeds, enabling impressive scientific achievements and novel biological applications. The geneLab initiative is developing an "Open Access" bio-informatics data base system, which will allow the science community to input and freely access omics data generated from spaceflight experiments. The goal is to minimize cost and maximize value by extensive data sharing and research collaboration.

Conclusion

Exciting new opportunities for long-duration research on the ISS are emerging. Basic research studies of model organisms in the space environment form the first step in the translational pipeline to advance our understanding and treatment of human health in space and on Earth. This report brings forth the rich historical accomplishments of past spaceflight research funded by NASA Ames Research Center, and lends new perspective and guidance to new future space research opportunities. This new path is truly onward and upward.

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Experiment List

by Discipline and Flight Date

EXPERIMENT LIST BY DISCIPLINE

Sorted by Launch Date within Discipline

SCIENCE DISCIPLINE	MISSION/PAYLOAD	INVESTIGATOR	RESEARCH SUBJECT	LAUNCH DATE	PAGE
BONE PHYSIOLOGY					
Bone and Calcium Physiology	Biosatellite III	P.B. Mack	Macaca nemestrina (Pig-Tailed Monkey)	6/28/1969	28
Bone and Calcium Physiology	Cosmos 782 / Bion 3	C.W. Asling	*Rattus norvegicus* (Sprague-Dawley rat)	11/25/1975	29
Bone and Calcium Physiology	Cosmos 782 / Bion 3	C.W. Asling	*Rattus norvegicus* (Sprague-Dawley rat)	11/25/1975	30
Bone and Calcium Physiology	Cosmos 782 / Bion 3	I. Savostin-Asling	*Rattus norvegicus* (Sprague-Dawley rat)	11/25/1975	31
Bone and Calcium Physiology	Cosmos 782 / Bion 3	E.R. Morey-Holton	*Rattus norvegicus* (Sprague-Dawley rat)	11/25/1975	32
Bone and Calcium Physiology	Cosmos 936 / Bion 4	E.R. Morey-Holton	*Rattus norvegicus* (Sprague-Dawley rat)	8/13/1977	33
Bone and Calcium Physiology	Cosmos 936 / Bion 4	D.J. Baylink	*Rattus norvegicus* (Sprague-Dawley rat)	8/13/1977	34
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	E. Sabelman	*Rattus norvegicus* (Wistar rat)	9/25/1979	35
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	T.J. Wronski	*Rattus norvegicus* (Wistar rat)	9/25/1979	36
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	W.E. Roberts	*Rattus norvegicus* (Wistar rat)	9/25/1979	37
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	W.S.S. Jee	*Rattus norvegicus* (Sprague-Dawley rat)	9/25/1979	38
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	M. Judy	*Rattus norvegicus* (Wistar rat)	9/25/1979	39
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	J. Matthews	*Rattus norvegicus* (Wistar rat)	9/25/1979	40
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	L.E. Kazarian	*Rattus norvegicus* (Wistar rat)	9/25/1979	41
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	D. Simmons	*Rattus norvegicus* (Wistar rat)	9/25/1979	42
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	P. Tran Van	*Rattus norvegicus* (Wistar rat)	9/25/1979	43
Bone and Calcium Physiology	Cosmos 1129 / Bion 5	C.E. Cann	*Rattus norvegicus* (Sprague-Dawley rat)	9/25/1979	44
Bone and Calcium Physiology	Cosmos 1514 / Bion 6	C.E. Cann	*Macaca mulatta* (Rhesus monkey)	12/14/1983	45
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	C.E. Cann	*Macaca mulatta* (Rhesus monkey)	9/29/1987	46
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	C.E. Cann	*Rattus norvegicus* (Wistar rat)	9/29/1987	47
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	S.B. Arnaud	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	48
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	A.C. Vailas	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	49
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	E.R. Morey-Holton	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	50
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	S.B. Doty	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	51
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	L.P. Garetto	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	52
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	A.R. Hargens	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	53
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	D. Simmons	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	54
Bone and Calcium Physiology	Cosmos 1887 / Bion 8	P.J. Duke	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	55
Bone and Calcium Physiology	STS-29 / SSIP2	I.A. Fras	*Rattus norvegicus* (Long Evans rat)	3/13/1989	56
Bone and Calcium Physiology	Cosmos 2044 / Bion 9	P.J. Duke	*Rattus norvegicus (*Sprague-Dawley rat)	9/15/1989	57
Bone and Calcium Physiology	Cosmos 2044 / Bion 9	S.B. Arnaud	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	58
Bone and Calcium Physiology	Cosmos 2044 / Bion 9	E.R. Morey-Holton	*Rattus norvegicus (*Sprague-Dawley rat)	9/15/1989	59
Bone and Calcium Physiology	Cosmos 2044 / Bion 9	S.B. Doty	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	60
Bone and Calcium Physiology	Cosmos 2044 / Bion 9	L.P. Garetto	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	61
Bone and Calcium Physiology	Cosmos 2044 / Bion 9	C.E. Cann	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	62
Bone and Calcium Physiology	STS-40 / SLS-1	E.R. Morey-Holton	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	63

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Bone Physiology	STS-40 / SLS-1	A.V. Bakulin	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	64
Bone Physiology	STS-40 / SLS-1	A.S. Kaplansky	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	65
Bone Physiology	STS-40 / SLS-1	V.S. Oganov	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	66
Bone Physiology	STS-42 / IML-1	P.J. Duke	*Mus musculus* (Mouse) embryo cells	1/22/1992	67
Bone Physiology	STS-52 / PSE.02	W.W. Wilfinger	*Rattus norvegicus* (Sprague-Dawley rat)	10/22/1992	68
Bone and Calcium Physiology	Cosmos 2229 / Bion 10	C.E. Cann	*Macaca mulatta* (Rhesus monkey)	12/29/1992	69
Bone Physiology	Cosmos 2229 / Bion 10	S.B. Arnaud	*Macaca mulatta* (Rhesus monkey)	12/29/1992	70
Bone Physiology	Cosmos 2229 / Bion 10	A. LeBlanc	*Macaca mulatta* (Rhesus monkey)	12/29/1992	71
Bone Physiology	STS-54 / PARE.02	D.D. Bikle	*Rattus norvegicus* (Sprague-Dawley rat)	1/13/1993	72
Bone Physiology	STS-56 / PARE.03	W.E. Roberts	*Rattus norvegicus* (Sprague-Dawley rat)	4/8/1993	73
Bone Physiology	STS-56 / PARE.03	E.R. Morey-Holton	*Rattus norvegicus* (Sprague-Dawley rat)	4/8/1993	74
Bone Physiology	STS-57 / PSE.03	W.W. Wilfinger	*Rattus norvegicus* (Sprague-Dawley rat)	6/21/1993	75
Bone Physiology	STS-58 / SLS-2	E.R. Morey-Holton	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	76
Bone Physiology	STS-58 / SLS-2	G. Durnova	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	77
Bone Physiology	STS-58 / SLS-2	E. Zerath	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	78
Bone Physiology	STS-58 / SLS-2	C. Alexandre	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	79
Bone Physiology	STS-62 / PSE.04	W.W. Wilfinger	*Rattus norvegicus* (Sprague-Dawley rat)	3/4/1994	80
Bone Physiology	STS-59 / NIH.C1	R.K. Globus	*Rattus norvegicus* (Sprague-Dawley rat) cultured cells	4/9/1994	81
Bone Physiology	STS-59 / NIH.C1	D.A. Kulesh	*Rattus norvegicus* (Sprague-Dawley rat) L8 cell line	4/9/1994	82
Bone Physiology	STS-59 / NIH.C1	W.J. Landis	*Gallus gallus* (White leghorn chicken) cultured cells	4/9/1994	83
Bone Physiology	STS-66 / NIH.R1	R.B. Johnson	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	84
Bone Physiology	STS-66 / NIH.C2	A.L. Boskey	*Gallus gallus* (White leghorn chicken) embryos	11/3/1994	85
Bone Physiology	STS-63 / NIH.C3	R.K. Globus	*Rattus norvegicus* (Sprague-Dawley rat)	2/3/1995	86
Bone Physiology	STS-63 / NIH.C3	D.A. Kulesh	*Rattus norvegicus* (Sprague-Dawley rat) L8 cell line	2/3/1995	87
Bone Physiology	STS-63 / NIH.C3	W.J. Landis	*Gallus gallus* (White leghorn chicken) cultured cells	2/3/1995	88
Bone and Calcium Physiology	STS-78 / LMS	T.J. Wronski	*Rattus norvegicus* (Sprague-Dawley rat)	6/20/1996	89
Bone and Calcium Physiology	STS-80 / NIH.C6	R.J. Majeska	*Rattus norvegicus* (Sprague-Dawley rat)	11/19/1996	90
Bone and Calcium Physiology	Bion 11	L.C. Shackelford	*Macaca mulatta* (Rhesus monkey)	12/24/1996	91
Bone and Calcium Physiology	Bion 11	V.S. Oganov	*Macaca mulatta* (Rhesus monkey)	12/24/1996	92
Bone and Calcium Physiology	Bion 11	E. Zerath	*Macaca mulatta* (Rhesus monkey)	12/24/1996	93
Bone and Calcium Physiology	STS-95 / NIH.C8	S.B. Doty	*Gallus gallus* (White leghorn chicken) cartilage cells	10/29/1998	94
Bone and Calcium Physiology	STS-108 / UF-1	S.B. Doty	*Coturnix coturnix japonica* (Japanese quail egg)	12/5/2001	95
Bone and Calcium Physiology	STS-108 / CBTM	T. Bateman	*Mus musculus* (Mouse)	12/5/2001	96
Bone Physiology	Foton / M3	E. Almeida	*Pachydactylus turneri* (Gecko)	9/14/2007	97
Bone Physiology	STS-131 / BSP	A.R. Hargens	*Mus musculus* (B6.SJL-Ptprca Pepcb/BoyJ mouse)	4/5/2010	98
Cell and Molecular Biology	STS-131 / BSP	E. Almeida	*Mus musculus* (B6.SJL-Ptprca Pepcb/BoyJ mouse)	4/5/2010	99
Cell and Molecular Biology	STS-131 / BSP	S. Thomopoulos	*Mus musculus* (B6.SJL-Ptprca Pepcb/BoyJ mouse)	4/5/2010	100
Cell and Molecular Biology	STS-131 / BSP	D. Fitzgerald	*Mus musculus* (B6.SJL-Ptprca Pepcb/BoyJ mouse)	4/5/2010	101

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Developmental Biology	Cosmos 936 / Bion 4	J. Miquel	*Drosophila melanogaster* (Fruit fly)	8/13/1977	147
Developmental Biology	Cosmos 1129 / Bion 5	J.R. Keefe	*Rattus norvegicus* (Wistar rat)	9/25/1979	148
Developmental Biology	Cosmos 1514 / Bion 6	J.R. Alberts	*Rattus norvegicus* (Wistar rat)	12/14/1983	149
Developmental Biology	Cosmos 1514 / Bion 6	J.R. Keefe	*Rattus norvegicus* (Wistar rat)	12/14/1983	150
Developmental Biology	STS-40 / SLS-1	D.B. Spangenberg	*Aurelia* (Jellyfish)	6/5/1991	151
Developmental Biology	STS-47 / SL-J	K.A. Souza	*Xenopus laevis* (Frog)	9/12/1992	152
Developmental Biology	STS-65 / IML-2	D.B. Spangenberg	*Aurelia* (Jellyfish)	7/8/1994	153
Developmental Biology	STS-66 / NIH.R1	J.R. Alberts	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	154
Developmental Biology	STS-66 / NIH.R1	S.B. Hoath	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	155
Developmental Biology	STS-66 / NIH.R1	R.H. Renegar	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	156
Bone Physiology	STS-66 / NIH.R1	L.V. Serova	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	157
Developmental Biology	STS-66 / NIH.R1	L.V. Serova	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	158
Developmental Biology	Mir 19 / Incubator 2	S.B. Doty	*Coturnix coturnix japonica* (Japanese quail egg)	6/27/1995	159
Developmental Biology	Mir 19 / Incubator 2	C. Fermin	*Coturnix coturnix* (Japonica quail)	6/27/1995	160
Developmental Biology	Mir 19 / Incubator 2	B. Fritzsch	*Coturnix coturnix* (Japonica quail)	6/27/1995	161
Developmental Biology	Mir 19 / Incubator 2	G. Conrad	*Coturnix coturnix* (Japonica quail)	6/27/1995	162
Developmental Biology	Mir 19 / Incubator 2	P. Hester	*Coturnix coturnix* (Japonica quail)	6/27/1995	163
Developmental Biology	Mir 19 / Incubator 2	P. Anderson	*Coturnix coturnix* (Japonica quail)	6/27/1995	164
Developmental Biology	Mir 19 / Incubator 2	P. Lelkes	*Coturnix coturnix* (Japonica quail)	6/27/1995	165
Developmental Biology	Mir 19 / Incubator 2	B. Wentworth	*Coturnix coturnix (*Japonica quail)	6/27/1995	166
Developmental Biology	Mir 19 / Incubator 2	T. Shimizu	*Coturnix coturnix (*Japonica quail)	6/27/1995	167
Developmental Biology	STS-70 / NIH.R2	J.R. Alberts	*Rattus norvegicus* (Sprague-Dawley rat)	7/13/1995	168
Bone Physiology	STS-70 / NIH.R2	N.C. Partridge	*Rattus norvegicus* (Sprague-Dawley rat)	7/13/1995	169
Muscle Physiology	STS-70 / NIH.R2	S.C. Bodine-Fowler	*Rattus norvegicus* (Sprague-Dawley rat)	7/13/1995	170
Neurophysiology	STS-70 / NIH.R2	C.A. Fuller	*Rattus norvegicus* (Sprague-Dawley rat)	7/13/1995	171
Bone Physiology	STS-69 / NIH.C4	R.J. Majeska	*Rattus norvegicus* (Sprague-Dawley rat)	9/7/1995	172
Bone and Calcium Physiology	STS-72 / NIH.C5	A.L. Boskey	*Gallus gallus* (White leghorn chicken) cells	1/11/1996	173
Developmental biology	STS-72 / NIH.R3	K. Walton	*Rattus norvegicus* (Sprague-Dawley rat)	1/11/1996	174
Bone and Calcium Physiology	STS-76 / Biorack 1	M. Hughes-Fulford	*Mus musculus* (Mouse cells) osteoblasts	3/22/1996	175
Developmental Biology	NASA 2 / Incubator 3	P. Anderson	*Coturnix coturnix japonica* (Japanese quail egg)	3/22/1996	176
Developmental Biology	NASA 2 / Incubator 3	S.B. Doty	*Coturnix coturnix japonica* (Japanese quail egg)	3/22/1996	177
Developmental Biology	NASA 2 / Incubator 3	B. Wentworth	*Coturnix coturnix japonica* (Japanese quail egg)	3/22/1996	178
Developmental Biology	NASA 2 / Incubator 3	B. Fritzsch	*Coturnix coturnix japonica* (Japanese quail egg)	3/22/1996	179
Developmental Biology	NASA 2 / Incubator 3	G. Conrad	*Coturnix coturnix japonica* (Japanese quail egg)	3/22/1996	180
Developmental Biology	NASA 2 / Incubator 3	P. Hester	*Coturnix coturnix japonica* (Japanese quail egg)	3/22/1996	181
Developmental Biology	NASA 2 / Incubator 3	T. Shimizu	*Coturnix coturnix japonica* (Japanese quail egg)	3/22/1996	182
Developmental Biology	NASA 2 / Incubator 3	P. Lelkes	*Coturnix coturnix japonica* (Japanese quail egg)	3/22/1996	183
Developmental Biology	NASA 2 / Incubator 3	C. Fermin	*Coturnix coturnix* (Japonica quail)	3/22/1996	184

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Developmental Biology	STS-77 / ARF-01	H. Schatten	Sea urchin, painter (*Lytechinus pictus* and Strongylocentrotus	5/19/1996	186
Neurophysiology	STS-78 / LMS	D. Wolgemuth	*Oryzias latipes* (Medaka fish)	6/20/1996	187
Bone and Calcium Physiology	STS-81 / Biorack 2	M. Hughes-Fulford	*Mus musculus (*MC3T3 mouse) osteoblasts	1/12/1997	188
Developmental Biology	STS-81 / Biorack 2	J. Tash	*Strongelocentrotus pupuratus* (Sea urchin) sperm	1/12/1997	189
Endocrinology	STS-84 / Biorack 3	J. Tash	*Lytechinus pictus (*Sea urchin) sperm	5/5/1997	190
Bone and Calcium Physiology	STS-84 / Biorack 3	M. Hughes-Fulford	*Mus musculus* (MC3T3 mouse) osteoblasts	5/5/1997	191
Developmental Biology	STS-93 / NIH.B1a	H. Keshishian	*Drosophila melanogaster* (Fruit fly)	7/23/1999	192
Developmental Biology	STS-106 / NIH.B1b	H. Keshishian	*Drosophila melanogaster* (Fruit fly)	9/8/2000	193
Cell and Molecular Biology	STS-131 / STL-SCR	E. Almeida	*Mus musculus* (Mouse) embryonic stem cells	4/5/2010	194
Cell and Molecular Biology	STS-131 / BSP	M.I. Mednieks	*Mus musculus* (B6.SJL-Ptprca Pepcb/BoyJ mouse)	4/5/2010	195
Cell and Molecular Biology	STS-133 / BSP	M.I. Mednieks	*Mus musculus* (Mouse)	2/24/2011	196
Cell and Molecular Biology	STS-135 / STL2	E. Almeida	*Mus musculus* (Mouse) embryonic stem cells	7/8/2011	197
Cell and Molecular Biology	STS-135 / BSP	M.I. Mednieks	*Mus musculus* (Mouse)	7/8/2011	198
IMMUNOLOGY					
Immunology	Cosmos 782 / Bion 3	A.D. Mandel	*Rattus norvegicus* (Sprague-Dawley rat)	11/25/1975	205
Immunology	Cosmos 782 / Bion 3	L. Kraft	*Rattus norvegicus* (Sprague-Dawley rat)	11/25/1975	206
Immunology	Cosmos 1887 / Bion 8	A.D. Mandel	*Rattus norvegicus* (Wistar rat)	9/29/1987	207
Immunology	Cosmos 2044 / Bion 9	G. Sonnenfeld	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	208
Immunology	Cosmos 2044 / Bion 9	A.M. Mastro	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	209
Immunology	STS-40 / SLS-1	I.A. Popova	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	210
Immunology	STS-40 / SLS-1	I.V. Konstantinova	*Rattus norvegicus (*Sprague-Dawley rat)	6/5/1991	211
Immunology	Cosmos 2229 / Bion 10	G. Sonnenfield	*Macaca mulatta* (Rhesus monkey)	12/29/1992	212
Muscle Physiology	STS-54 / PARE.02	E.S. Miller	*Rattus norvegicus* (Sprague-Dawley rat)	1/13/1993	213
Immunology	STS-58 / SLS-2	I.V. Konstantinova	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	214
Immunology	STS-60 / Immune.1	R. Zimmermann	*Rattus norvegicus (*Sprague-Dawley rat)	2/3/1994	215
Immunology	STS-66 / NIH.R1	G. Sonnenfeld	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	216
Immunology	STS-63 / Immune.2	R. Zimmerman	*Rattus norvegicus* (Sprague-Dawley rat)	2/3/1995	217
Immunology	STS-77 / Immune.3	R. Zimmerman	*Rattus norvegicus* (Sprague-Dawley rat)	5/19/1996	218
Immunology	STS-84 / Biorack 3	C. Sams	*Homo sapiens* (human) T-cell	5/5/1997	219
Immunology	STS-121 / FIT	S. Bhattacharya	*Drosophila melanogaster* (Fruit fly)	7/4/2006	220
Immunology	21P / Leukin	A. Cogoli	*Rattus norvegicus* (Sprague-Dawley rat)	9/18/2006	221
Immunology	STS-126 / BONEMAC	S.K. Chapes	*Mus musculus* (C57BL/6J mouse bone marrow cells)	11/14/2008	222
Immunology	STS-131 / Mouse	M. Hughes-Fulford	*Mus musculus* (B6.SJL-Ptprca Pepcb/BoyJ mouse)	4/5/2010	223
Immunology	STS-133 / Mouse	R. Garofalo	*Mus musculus* (Mouse) viral pathogens	2/24/2011	224
Cell and Molecular Biology	STS-135 / BSP	R. Garofalo	*Mus musculus* (Mouse)	7/8/2011	225
Immunology	STS-135 / BSP	M. Hughes-Fulford	*Mus musculus* (Mouse)	7/8/2011	226

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MICROBIAL GROWTH AND VIRULENCE					
Microbiology	STS-42 / IML-1	C.V. Bruschi	*Saccharomyces cerevisiae* (Yeast)	1/22/1992	232
Microbiology	STS-81 / Biorack 2	B. Pyle	*Burkholderia cepacia* (Bacterium)	1/12/1997	233
Microbiology	Progress 13P /	C. Nickerson	*Saccharomyces cerevisiae* (Yeast)	1/29/2004	234
Microbiology	STS-115 / 12A	C. Nickerson	*Salmonella typhimurium, Pseudomonas aeruginosa* (Bacterium)	9/9/2006	235
Microbiology	STS-118 / SPEGIS	D. Niesel	*Streptococcus pneumoniae* (Bacteria)	8/8/2007	236
Microbiology	STS-123 / MDRV	C. Nickerson	*Salmonella typhimurium, Pseudomonas aeruginosa* (Bacterium)	3/11/2008	237
Microbiology	STS-123 / MDRV	D. Niesel	*Streptococcus pneumoniae* (Bacteria)	3/11/2008	238
Microbiology	STS-123 / MDRV	B. Pyle	*Pseudomonas aeruginosa* (Bacterium)	3/11/2008	239
Microbiology	STS-123 / MDRV	M. McGinnis	*Saccharomyces cerevisiae* (Yeast)	3/11/2008	240
Microbiology	STS-129 / SPEGIS-2	D. Niesel	*Streptococcus pneumoniae* (Bacteria)	11/16/2009	241
Microbiology	STS-131 STL Immune	C. Nickerson	HT-29 Human Intestinal Epithelial Cell Line, A549 Human Lung	4/5/2010	242
Cell and Molecular Biology	STS-132 / Micro-2	C. Collins	*Pseudomonas aeruginosa, Staphylococcus aureus* (Bacterium)	5/14/2010	243
Microbiology	STS-135 / Micro-4	T.G. Hammond	Yeast deletion series	7/8/2011	244
Cell and Molecular Biology	STS-135 / Micro-2A	C. Collins	*Pseudomonas aeruginosa, Staphylococcus aureus* (Bacterium)	7/8/2011	245
MUSCLE PHYSIOLOGY					
Muscle Physiology	Cosmos 936 / Bion 4	K. Castleman	*Rattus norvegicus* (Sprague-Dawley rat)	8/13/1977	250
Muscle Physiology	Cosmos 1129 / Bion 5	K. Castleman	*Rattus norvegicus* (Wistar rat)	9/25/1979	251
Muscle Physiology	Cosmos 1887 / Bion 8	D.A. Riley	*Rattus norvegicus* (Wistar rat)	9/29/1987	252
Muscle Physiology	Cosmos 1887 / Bion 8	S. Ellis	*Rattus norvegicus* (Wistar rat)	9/29/1987	253
Muscle Physiology	Cosmos 1887 / Bion 8	K.M. Baldwin	*Rattus norvegicus* (Wistar rat)	9/29/1987	254
Muscle physiology	Cosmos 1887 / Bion 8	F.W. Booth	Rattus norvegicus (Wistar rat)	9/29/1987	255
Muscle physiology	Cosmos 1887 / Bion 8	O.H. Lowry	Rattus norvegicus (Wistar rat)	9/29/1987	256
Muscle Physiology	Cosmos 1887 / Bion 8	V.R. Edgerton	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	257
Muscle Physiology	Cosmos 1887 / Bion 8	X. Musacchia	*Rattus norvegicus (*Sprague-Dawley rat)	9/29/1987	258
Muscle Physiology	Cosmos 2044 / Bion 9	X. Musacchia	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	259
Muscle Physiology	Cosmos 2044 / Bion 9	K.M. Baldwin	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	260
Muscle Physiology	Cosmos 2044 / Bion 9	F.W. Booth	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	261
Muscle Physiology	Cosmos 2044 / Bion 9	N. Weisbrodt	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	262
Muscle Physiology	Cosmos 2044 / Bion 9	N.G. Daunton	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	263
Muscle Physiology	Cosmos 2044 / Bion 9	A.C. Vailas	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	264
Muscle Physiology	Cosmos 2044 / Bion 9	B. Festoff	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	265
Muscle Physiology	Cosmos 2044 / Bion 9	W. Stauber	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	266
Muscle Physiology	Cosmos 2044 / Bion 9	V.R. Edgerton	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	267
Muscle Physiology	Cosmos 2044 / Bion 9	D.A. Riley	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	268

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Muscle Physiology	Cosmos 2044 / Bion 9	S. Ellis	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	269
Muscle Physiology	Cosmos 2044 / Bion 9	V.R. Edgerton	*Macaca mulatta (*Rhesus monkey)	9/15/1989	270
Muscle Physiology	Cosmos 2044 / Bion 9	A.C. Vailas	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	271
Muscle Physiology	Cosmos 2044 / Bion 9	A. Pedrini-Mille	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	272
Muscle Physiology	Cosmos 2044 / Bion 9	O.H. Lowry	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	273
Muscle Physiology	STS-41 / PSE.01	M. Cronin	*Rattus norvegicus* (Sprague-Dawley rat)	10/6/1990	274
Muscle Physiology	STS-40 / SLS-1	J.F.Y. Hoh	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	275
Muscle Physiology	STS-40 / SLS-1	D.A. Riley	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	276
Muscle Physiology	STS-40 / SLS-1	V.S. Oganov	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	277
Muscle Physiology	STS-40 / SLS-1	K.M. Baldwin	*Rattus norvegicus (*Sprague-Dawley rat)	6/5/1991	278
Muscle Physiology	STS-48 / PARE.01	M.E. Tischler	*Rattus norvegicus (*Sprague-Dawley rat)	9/12/1991	279
Muscle Physiology	Cosmos 2229 / Bion 10	S.C. Bodine-Fowler	*Macaca mulatta* (Rhesus monkey)	12/29/1992	280
Muscle Physiology	STS-54 / PARE.02	K.M. Baldwin	*Rattus norvegicus* (Sprague-Dawley rat)	1/13/1993	281
Muscle Physiology	STS-54 / PARE.02	B. Girten	*Rattus norvegicus* (Sprague-Dawley rat)	1/13/1993	282
Muscle Physiology	STS-58 / SLS-2	K.M. Baldwin	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	283
Muscle Physiology	STS-58 / SLS-2	D.A. Riley	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	284
Muscle Physiology	STS-58 / SLS-2	Y. Mounier	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	285
Muscle Physiology	STS-58 / SLS-2	J.F. Marini	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	286
Muscle Physiology	STS-58 / SLS-2	Y. Ohira	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	287
Muscle Physiology	STS-58 / SLS-2	T. Yoshioka	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	288
Muscle Physiology	STS-66 / NIH.R1	K.I. Clark	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	289
Muscle Physiology	STS-66 / NIH.C2	H.H. Vandenburgh	*Gallus gallus* (White leghorn chicken) skeletal muscle organoids	11/3/1994	290
Muscle Physiology	STS-70 / NIH.R2	R. Wassersug	*Rattus norvegicus* (Sprague-Dawley rat)	7/13/1995	291
Muscle Physiology	STS-77 / NIH.C7	H.H. Vandenburgh	*Gallus gallus* (White leghorn chicken) skeletal muscle organoids	5/19/1996	292
Muscle Physiology	Bion 11	B.S. Shenkman	*Macaca mulatta* (Rhesus monkey)	12/24/1996	293
Muscle Physiology	Bion 11	J.F. Marini	*Macaca mulatta* (Rhesus monkey)	12/24/1996	294
Muscle physiology	Bion 11	Y. Mounier	*Macaca mulatta* (Rhesus monkey)	12/24/1996	295
Muscle Physiology	Bion 11	D. Desplanches	*Macaca mulatta* (Rhesus monkey)	12/24/1996	296
Muscle Physiology	STS-90 / Neurolab	K.M. Baldwin	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	297
Muscle Physiology,	STS-90 / Neurolab	D.A. Riley	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	298
Muscle Physiology,	STS-90 / Neurolab	K. Walton	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	299
Muscle Physiology	STS-90 / Neurolab	N. Gonzales-Cadavid	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	300
Muscle Physiology	STS-90 / Neurolab	R. Wassersug	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	301
Muscle Physiology	STS-90 / Neurolab	F.W. Booth	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	302
Muscle Physiology	ICE-FIRST / Expedition 8	C. Conley	*Caenorhabditis elegans* (Nematode)	4/19/2004	303
Muscle Physiology	STS-118 / CBTM-2	H. Q. Han	*Mus musculus* (C57/B6 mouse)	8/8/2007	304
Muscle Physiology	STS-133 / BSP	J. Fitzgerald	*Mus musculus* (Mouse)	2/24/2011	305
Muscle Physiology	STS-135 / CBTM-3	R.J. Midura	*Mus musculus* (C57BL/6J mouse)	7/8/2011	306

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NEUROPHYSIOLOGY					
Neurophysiology	Biosatellite III	W.R. Adey	Macaca nemestrina (Pig-Tailed Monkey)	6/28/1969	312
Neurophysiology	Biosatellite III	W.R. Adey	Macaca nemestrina (Pig-Tailed Monkey)	6/28/1969	313
Neurophysiology	Biosatellite III	W.R. Adey	Macaca nemestrina (Pig-Tailed Monkey)	6/28/1969	314
Neurophysiology	Cosmos 1887 / Bion 8	N.G. Daunton	*Rattus norvegicus* (Wistar rat)	9/29/1987	315
Neurophysiology	Cosmos 1887 / Bion 8	O.H. Lowry	Rattus norvegicus (Wistar rat)	9/29/1987	316
Neurophysiology	Cosmos 2044 / Bion 9	B. Cohen	*Macaca mulatta* (Rhesus monkey)	9/15/1989	317
Neurophysiology	Cosmos 2044 / Bion 9	B. Jiang	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	318
Neurophysiology	Cosmos 2044 / Bion 9	M.J. Correia	*Macaca mulatta* (Rhesus monkey)	9/15/1989	319
Neurophysiology	Cosmos 2044 / Bion 9	O.H. Lowry	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	320
Neurophysiology	STS-40 / SLS-1	M.D. Ross	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	321
Neurophysiology	STS-40 / SLS-1	I.B. Krasnov	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	322
Neurophysiology	STS-40 / SLS-1	T.A. Leontovich	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	323
Neurophysiology	STS-40 / SLS-1	L.N. Dyachkova	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	324
Neurophysiology	STS-40 / SLS-1	L.M. Gershtein	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	325
Neurophysiology	STS-40 / SLS-1	I.B. Krasnov	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	326
Neurophysiology	STS-40 / SLS-1	I.B. Krasnov	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	327
Neurophysiology	STS-40 / SLS-1	T.A. Leontovich	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	328
Neurophysiology	STS-40 / SLS-1	C. Gharib	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	329
Neurophysiology	Cosmos 2229 / Bion 10	D.L. Tomko	*Macaca mulatta* (Rhesus monkey)	12/29/1992	330
Neurophysiology	Cosmos 2229 / Bion 10	B. Cohen	*Macaca mulatta* (Rhesus monkey)	12/29/1992	331
Muscle Physiology	Cosmos 2229 / Bion 10	V.R. Edgerton	*Macaca mulatta* (Rhesus monkey)	12/29/1992	332
Neurophysiology	Cosmos 2229 / Bion 10	M.J. Correia	*Macaca mulatta* (Rhesus monkey)	12/29/1992	333
Muscle Physiology	STS-54 / PARE.02	D.M. Desidero	*Rattus norvegicus* (Sprague-Dawley rat)	1/13/1993	334
Neurophysiology	STS-58 / SLS-2	M.D. Ross	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	335
Neurophysiology	STS-58 / SLS-2	I.B. Krasnov	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	336
Neurophysiology	STS-58 / SLS-2	I.B. Krasnov	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	337
Neurophysiology	STS-58 / SLS-2	J.B. Gabrion	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	338
Neurophysiology	STS-65 / IML-2	M.L. Wiederhold	*Cynops pyrrhogaster* (Newt)	7/8/1994	339
Neurophysiology	STS-66 / NIH.R1	M.E. DeSantis	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	340
Neurophysiology	STS-66 / NIH.R1	B. Fritzsch	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	341
Neurophysiology	STS-66 / NIH.R1	J.B. Gabrion	*Rattus norvegicus (*Sprague-Dawley rat)	11/3/1994	342
Neurophysiology	STS-66 / NIH.R1	J.L. Lambert	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	343
Neurophysiology	Bion 11	D.M. Rumbaugh	*Macaca mulatta* (Rhesus monkey)	12/24/1996	344
Neurophysiology	Bion 11	I.B. Kozlovskaya	*Macaca mulatta* (Rhesus monkey)	12/24/1996	345
Neurophysiology	Bion 11	A.M. Badakva	*Macaca mulatta* (Rhesus monkey)	12/24/1996	346

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Neurophysiology	STS-89 / CEBAS	M.L. Wiederhold	*Biomphalaria glabrata* (Snail)	1/22/1998	348
Neurophysiology	STS-89 / CEBAS	M. Schreibman	*Xiphophorus helleri* (Swordtail fish)	1/22/1998	349
Neurophysiology	STS-90 / Neurolab	S. Highstein	*Opsanus tau* (Oyster toadfish)	4/17/1998	350
Neurophysiology	STS-90 / Neurolab	G. Holstein	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	351
Neurophysiology	STS-90 / Neurolab	E.R. Horn	*Acheta domesticus* (cricket) eggs and larvae	4/17/1998	352
Neurophysiology	STS-90 / Neurolab	K. Kosik	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	353
Neurophysiology	STS-90 / Neurolab	B. McNaughton	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	354
Neurophysiology	STS-90 / Neurolab	R. Nowakowski	*Mus musculus* (Mouse)	4/17/1998	355
Neurophysiology	STS-90 / Neurolab	O. Pompeiano	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	356
Neurophysiology	STS-90 / Neurolab	J. Raymond	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	357
Neurophysiology	STS-90 / Neurolab	M.D. Ross	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	358
Neurophysiology	STS-90 / Neurolab	T. Shimizu	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	359
Neurophysiology	STS-90 / Neurolab	M.L. Wiederhold	*Biomphalaria glabrata* (Snail)	4/17/1998	360
Neurophysiology	STS-95 / VFEU	S. Highstein	*Opsanus tau* (Oyster toadfish)	10/29/1998	361
Neurophysiology	STS-108 / UF-1	J.D. Dickman	*Coturnix coturnix japonica* (Japanese quail egg)	12/5/2001	362
Neurophysiology	Foton / M2	R. Boyle	Helix aspersa, *Helix lucorum* (Snail)	6/20/2005	363
Neurophysiology	Foton / M3	R. Boyle	Helix aspersa, *Helix lucorum* (Snail)	9/14/2007	364
Neurophysiology	STS-131 / BSP	L. Hoffman	*Mus musculus* (B6.SJL-Ptprca Pepcb/BoyJ mouse)	4/5/2010	365
Neurophysiology	STS-133 / BSP	R. Boyle	*Mus musculus* (Mouse)	2/24/2011	366
Neurophysiology	STS-133 / BSP	S. Zanello	*Mus musculus* (Mouse)	2/24/2011	367
Neurophysiology	STS-135 / BSP	R. Boyle	*Mus musculus* (Mouse)	7/8/2011	368
Neurophysiology	STS-135 / BSP	S. Zanello	*Mus musculus* (Mouse)	7/8/2011	369
REGULATORY PHYSIOLOGY					
Regulatory Physiology	STS-40 / SLS-1	Y.V. Natochin	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	377
Regulatory Physiology	STS-58 / SLS-2	S.M. Ivanova	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	378
Regulatory physiology	STS-58 / SLS-2	L.V. Serova	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	379
Regulatory Physiology	STS-69 / BRIC-06	I. Block	*Physarum polycephalum* (acellular slime mold)	9/7/1995	380
Thermoregulation	Bion 11	C.A. Fuller	*Macaca mulatta* (Rhesus monkey)	12/24/1996	381
Regulatory Physiology	STS-90 / Neurolab	C. Wade	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	382
Regulatory Physiology	Foton / M2	E. Almeida	*Pleurodeles waltl* (Newt)	6/20/2005	383
Musculoskeletal	Foton / M2	E. Almeida	*Pachydactylus turneri* (Gecko)	6/20/2005	384
Hematology	Foton / M3	E. Almeida	*Pleurodeles waltl* (Newt)	9/14/2007	385
REGULATORY PHYSIOLOGY Chronobiology					
Chronobiology	Biosatellite III	W.R. Adey	Macaca nemestrina (Pig-Tailed Monkey)	6/28/1969	386
Chronobiology	Cosmos 1514 / Bion 6	F. Sulzman	*Macaca mulatta* (Rhesus monkey)	12/14/1983	387

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Chronobiology	Cosmos 2229 / Bion 10	C.A. Fuller	*Macaca mulatta* (Rhesus monkey)	12/29/1992	389
Chronobiology	Bion 11	A.M. Alpatov	*Macaca mulatta* (Rhesus monkey)	12/24/1996	390
Chronobiology	NASA-Mir / NASA 5	T.M. Hoban-Higgins	*Trigonoscelis gigas* (Black bodied beetle)	5/15/1997	391
Chronobiology	STS-90 / Neurolab	C.A. Fuller	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	392
REGULATORY PHYSIOLOGY Endocrinology					
Regulatory Physiology	Biosatellite III	N. Pace	*Macaca nemestrina* (Pig-Tailed Monkey)	6/28/1969	393
Endocrinology	Cosmos 782 / Bion 3	R.E. Grindeland	*Rattus norvegicus* (Sprague-Dawley rat)	11/25/1975	394
Endocrinology	Cosmos 1887 / Bion 8	L. Kraft	*Rattus norvegicus* (Wistar rat)	9/29/1987	395
Endocrinology	Cosmos 1887 / Bion 8	L. Kraft	*Rattus norvegicus* (Wistar rat)	9/29/1987	396
Endocrinology	Cosmos 1887 / Bion 8	D.E. Philpott	*Rattus norvegicus* (Wistar rat)	9/29/1987	397
Endocrinology	Cosmos 1887 / Bion 8	D. Holley	*Rattus norvegicus* (Wistar rat)	9/29/1987	398
Endocrinology	Cosmos 1887 / Bion 8	L.C. Keil	*Rattus norvegicus* (Wistar rat)	9/29/1987	399
Endocrinology	Cosmos 1887 / Bion 8	R.E. Grindeland	*Rattus norvegicus* (Wistar rat)	9/29/1987	400
Endocrinology	Cosmos 1887 / Bion 8	C.E. Cann	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	401
Endocrinology	Cosmos 1887 / Bion 8	R.E. Grindeland	*Rattus norvegicus* (Sprague-Dawley rat)	9/29/1987	402
Endocrinology	Cosmos 2044 / Bion 9	R. Amann	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	403
Endocrinology	Cosmos 2044 / Bion 9	D. Holley	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	404
Endocrinology	Cosmos 2044 / Bion 9	L.C. Keil	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	405
Endocrinology	Cosmos 2044 / Bion 9	R.E. Grindeland	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	406
Endocrinology	Cosmos 2044 / Bion 9	P.E. Sawchenko	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	407
Endocrinology	Cosmos 2044 / Bion 9	R.E. Grindeland	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	408
Endocrinology	STS-40 / SLS-1	K.V. Smirnov	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	409
Endocrinology	STS-40 / SLS-1	R. Gerzer	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	410
Endocrinology	STS-40 / SLS-1	R.E. Grindeland	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	411
Endocrinology	STS-46 / PHCF	W.C. Hymer	*Rattus norvegicus* (Sprague-Dawley rat)	7/31/1992	412
Endocrinology	Cosmos 2229 / Bion 10	R.E. Grindeland	*Macaca mulatta* (Rhesus monkey)	12/29/1992	413
Regulatory Physiology	STS-54 / PARE.02	A. Mortimer	*Rattus norvegicus* (Sprague-Dawley rat)	1/13/1993	414
Endocrinology	STS-58 / SLS-2	V.I. Loginov	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	415
Endocrinology	STS-58 / SLS-2	E.I. Alekseyev	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	416
Endocrinology	Bion 11	R.E. Grindeland	*Macaca mulatta* (Rhesus monkey)	12/24/1996	417
Endocrinology	STS-90 / Neurolab	P. Lelkes	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	418
Cell and Molecular Biology	STS-131 / BSP	J. Tash	*Mus musculus* (B6.SJL-Ptprca Pepcb/BoyJ mouse)	4/5/2010	419
Cell and Molecular Biology	STS-133 / BSP	J. Tash	*Mus musculus* (Mouse)	2/24/2011	420
Cell and Molecular Biology	STS-135 / BSP	J. Tash	*Mus musculus* (Mouse)	7/8/2011	421

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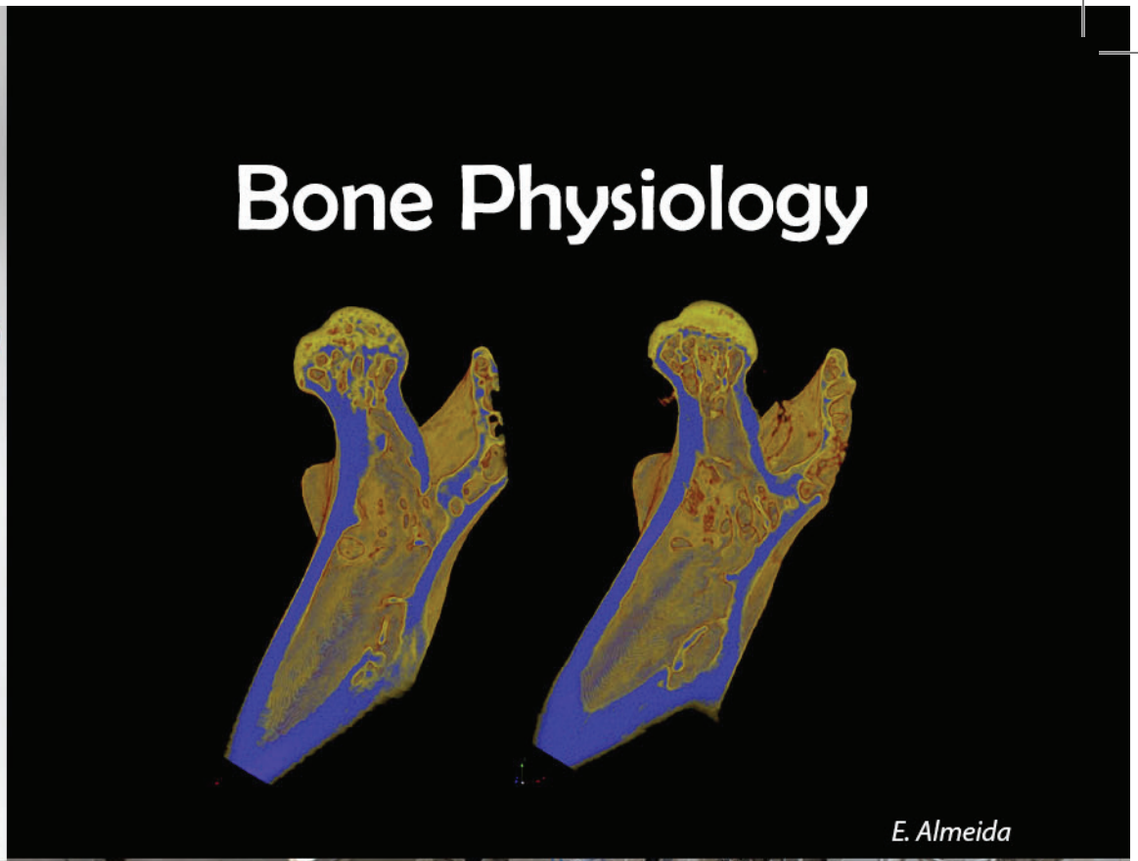
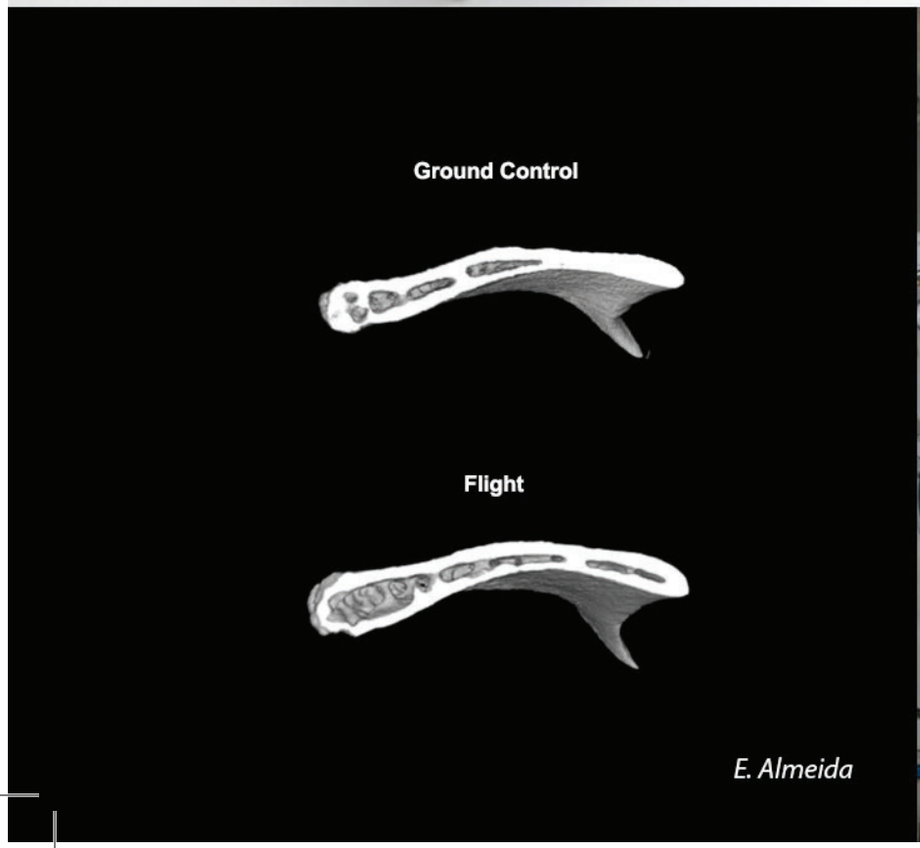
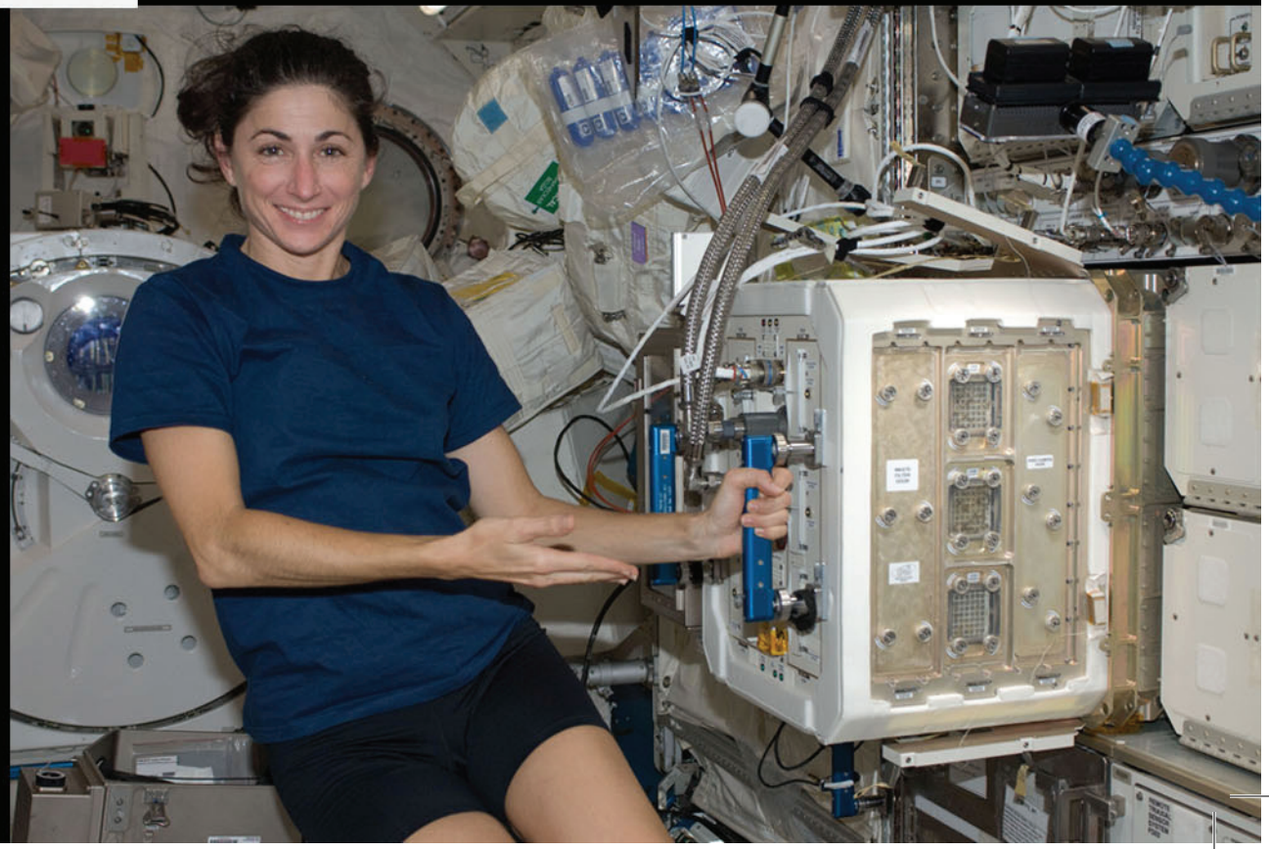
REGULATORY PHYSIOLOGY Hematology					
Hematology	Cosmos 782 / Bion 3	H. Leon	*Rattus norvegicus* (Sprague-Dawley rat)	11/25/1975	422
Hematology	STS-40 / SLS-1	R.D. Lange	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	423
Hematology	STS-40 / SLS-1	C.P. Alfrey	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	424
REGULATORY PHYSIOLOGY Metabolism/Nutrition					
Metabolism and Nutrition	Cosmos 782 / Bion 3	P. Brown	*Rattus norvegicus* (Sprague-Dawley rat)	11/25/1975	425
Metabolism and Nutrition	Cosmos 936 / Bion 4	S. Abraham	*Rattus norvegicus* (Sprague-Dawley rat)	8/13/1977	426
Metabolism and Nutrition	Cosmos 1129 / Bion 5	S. Abraham	*Rattus norvegicus* (Wistar rat)	9/25/1979	427
Metabolism and Nutrition	Cosmos 1129 / Bion 5	G. Pitts	*Rattus norvegicus* (Wistar rat)	9/25/1979	428
Metabolism and Nutrition	Cosmos 1887 / Bion 8	A.H. Merrill	*Rattus norvegicus* (Wistar rat)	9/29/1987	429
Metabolism and Nutrition	Cosmos 1887 / Bion 8	R. Phillips	*Rattus norvegicus* (Wistar rat)	9/29/1987	430
Metabolism and Nutrition	Cosmos 2044 / Bion 9	A.H. Merrill	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	431
Metabolism and Nutrition	Cosmos 2044 / Bion 9	S. Cormier	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	432
Metabolism and Nutrition	Cosmos 2044 / Bion 9	R. Phillips	*Rattus norvegicus* (Sprague-Dawley rat)	9/15/1989	433
Metabolism and Nutrition	Cosmos 2044 / Bion 9	C.A. Fuller	*Macaca mulatta* (Rhesus monkey)	9/15/1989	434
Metabolism and Nutrition	STS-40 / SLS-1	K.V. Smirnov	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	435
Metabolism and Nutrition	STS-40 / SLS-1	K.V. Smirnov	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	436
Metabolism and Nutrition	STS-40 / SLS-1	O. Szylit	*Rattus norvegicus* (Sprague-Dawley rat)	6/5/1991	437
Metabolism and Nutrition	Cosmos 2229 / Bion 10	C.A. Fuller	*Macaca mulatta* (Rhesus monkey)	12/29/1992	438
Metabolism and Nutrition	STS-58 / SLS-2	A.S. Kaplansky	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	439
Metabolism and Nutrition	STS-58 / SLS-2	O. Szylit	*Rattus norvegicus* (Sprague-Dawley rat)	10/18/1993	440
Metabolism and Nutrition	STS-66 / NIH.R1	L.V. Serova	*Rattus norvegicus* (Sprague-Dawley rat)	11/3/1994	441
Metabolism	Bion 11	C.A. Fuller	*Macaca mulatta* (Rhesus monkey)	12/24/1996	442
Metabolism and Nutrition	STS-90 / Neurolab	W. Rhoten	*Rattus norvegicus* (Sprague-Dawley rat)	4/17/1998	443
Cell and Molecular Biology	STS-135 / BSP	V.E. Wotring	*Mus musculus* (Mouse)	7/8/2011	444
Metabolism and Nutrition	STS-135 / BSP	S.M. Smith	*Mus musculus* (Mouse)	7/8/2011	445


Bone Physiology
E. Almeida


Ground Control
Flight
E. Almeida



Bone Physiology Introduction

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Bone is a dynamically remodeled tissue that evolved in the Earth's gravitational field and requires gravity, ground-reaction forces or muscle-mediated mechanical forces (i.e., loading) for maintenance of its mineral content, structure, and strength. In the microgravity environment of spaceflight, significant bone loss occurs in astronauts in the absence or reduction of these forces, and the application of various countermeasures (artificial gravity, resistance exercise, pharmaceuticals, special diet, etc.) are essential to optimize human health, structural function, and adaptability to gravity upon return to Earth or on the surface of extraterrestrial bodies. The bone loss experienced by astronauts during weightlessness is seen in both the highly porous spongy bone (cancellous) and the dense cortical weight-bearing bones. Translational studies using model organisms and cell cultures have been used, and a body of evidence regarding the basic cellular and molecular mechanisms underlying the organismal response to spaceflight has been created. This knowledge will aid the development and testing of countermeasures. Additionally, translational science can be used to answer questions relating to skeletal development and wound repair during spaceflight.

We now know that skeletal homeostasis depends on a balance of the activities and signaling of three kinds of bone cells along with their replenishment through proliferation and differentiation of stem cell progenitors or precursors. *Osteoblasts* (mesenchymal-lineage cells located on periosteal, endosteal, and cancellous bone surfaces) have an important function in bone formation. *Osteoclasts* (hematopoietic-lineage cells located on bone surfaces) execute bone resorption—the breakdown of bone mineral and matrix. *Osteocytes* (terminally differentiated osteoblasts that become embedded within the skeletal tissue in cave-like lacunae) are thought, in part, to regulate both bone formation and resorption. The osteocyte is also considered the putative cell for sensing gravity and mechanical loading due to its abundant number and location in bone tissue. Other organs communicate with the bone cells to affect skeletal changes. For example, endocrine-regulated feedback control exists between the skeleton, intestines, and kidneys to establish and regulate calcium homeostasis. Specifically, calcium levels in the blood are tightly controlled by two peptide hormones (parathyroid hormone and calcitonin) and a third hormone, vitamin D, by mobilizing calcium from bone and modifying calcium absorption in the intestine and excretion in the kidney [Cann, 1997]. Assessment of the effects of spaceflight and microgravity on bone should consider both structural quantification and biochemical factors that inform the state of turnover of the mineral reservoir.

Much of the translational work to date has used growing rats to study changes in skeletal structure and metabolism. However, adult (post-pubescent) mice have also been flown. To date, there has not been a direct comparison of the response of male versus female to spaceflight. In general, both sexes experience bone loss, presumably through similar mechanisms.

Bone

Spaceflight decreases bone formation in growing rats. Postflight analyses of growing rats after a 20-day spaceflight showed a striking decrease in bone formation resulting from a decrease in the number or activity of osteoblast cells in the humerus and tibia. There was a significant return of bone formation to preflight levels within 25 days of recovery [Morey-Holton and Baylink, 1978] [Cosmos 782 / Bion 3, p. 32].

Male rats were flown in space for 18–20 days in individual cages, and a large team of investigators carried out measurement of several bone parameters postflight [Wronski, 1980] [Cosmos 1129 / Bion 5, p. 36]; [Roberts et al., 1981] [Cosmos 1129 / Bion 5, p. 37]; [Kimmel and Jee, 1980] [Cosmos 1129 / Bion 5, p. 38]; [Judy, 1981] [Cosmos 1129 / Bion 5, p. 39]; [Matthews, 1981] [Cosmos 1129/ Bion 5, p. 40]; [Cann and Adachi, 1983] [Cosmos 1129 / Bion 5, p. 44]. The analyses indicated that changes in the long bones (tibia and humerus) during spaceflight included decreased bone formation; decreased bone trabecular volume, density, and strength; increased fat content of bone marrow; and alterations in osteoblasts and osteoclasts. Generally, measurements made on non-weightbearing bone (rib, calvaria, vertebra, maxilla) showed no significant changes in the growing animals [Wronski, 1980] [Cosmos 1129 / Bion 5, p. 36]; [Roberts et al., 1981] [Cosmos 1129 / Bion 5, p. 37]; [Kimmel and Jee, 1980] [Cosmos 1129 / Bion 5, p. 38]; [Judy, 1981] [Cosmos 1129 / Bion 5, p. 39]; [Matthews, 1981] [Cosmos 1129/ Bion 5, p. 40]; [Cann and Adachi, 1983] [Cosmos 1129 / Bion 5. p. 44], and [Morey-Holton et al., 1992] [STS-40 / SLS-1, p. 63]. Calcium tracer kinetic studies showed that the increased bone resorption, normalized by calcium turnover, does not decrease during flight and that bone loss on longer flights will likely continue [Cann, 1997; Cann and Adachi, 1983].

Housing needs to be considered in animal experiment design. A comprehensive spaceflight study comparing singly housed to group-housed rats was conducted to evaluate bone changes postflight. Group-housed rats had fewer bone changes and faster recovery than singly housed rats suggesting that on-orbit animal activity levels can have an effect [Morey-Holton et al., 1992] [STS-40 / SLS-1, p. 60].

Mice also show bone loss during spaceflight. In post-pubescent animals, weight-bearing bones were found to be an active site of bone loss during short-duration (<16 days) microgravity exposure [Bateman, 2004] [STS-108 / CBTM, p. 96]; [Blaber et al., 2013] [STS-131 / BSP, p. 99]; [Spatz et al., 2013] [STS-135 / CBTM-3, p. 104]. Thus, rat and mouse models exist for future translational studies examining the effects of spaceflight on skeletal structure.

Bone Cell and Molecular Responses

A wide range of cell and molecular evidence suggests that osteogenic activity associated with bone formation is slowed in spaceflight. This was seen in the rat periodontal ligament between tooth and jaw as decreased cell nuclear volumes [Roberts et al., 1981] [Cosmos 1129 / Bion 5, p. 37]. Another study using osteoblast cell cultures showed that following spaceflight the cells are less differentiated, and glucose utilization and lactate production is significantly lower. These data suggest that spaceflight may inhibit energy metabolism and protein synthesis in these cells [Globus et al., 1995] [STS-59 / NIH.C1, p. 81].

The pelvic and femoral regions of mice were found to be an active site of rapid bone loss during 15 days of microgravity. In addition to observations of osteoclastic-driven bone degradation, indices of cell cycle arrest were observed suggesting a degraded capability for tissue regeneration during spaceflight [Blaber et al., 2013] [STS-131 / BSP, p. 99]. Additionally, evidence for osteocytic osteolysis was generated, indicative of an additional mode of bone resorption.

Three 9- to 10-day STS missions were flown using an osteoblast cell line derived from embryonic mouse calvaria cultured within an ESA Biorack system under both microgravity and 1-g conditions. Gene expression changes were seen associated with reduced cell growth and abnormal cell morphology [Hughes-Fulford et al., 1998] [STS-76 / Biorack 1, p. 175]. Gene expression changes were also seen suggesting an inhibition of osteoblast cytoskeleton

growth associated with microfilament collapse or microtubule polymerization [Hughes-Fulford, 2003] [STS-81 / Biorack 2, p. 188] [STS-84 / Biorack 3, p. 191].

Altogether, rodent research has produced broad information on the mammalian response to spaceflight that can be used to better treat spaceflight-induced bone loss in astronauts.

Cartilage and Fibrous Tissue

In addition to bone and muscle, spaceflight has deleterious effects on cartilage and ligaments. Microgravity exposure of 15 days in mice produced a marked spinal disc height loss at the lower lumbar level [Bailey et al., 2012][STS-131 / BSP, p. 98] that may have relevance to the high incidence of lumbar disc herniation in astronauts.

Ongoing analyses indicate the articular cartilage of the knee [Fitzgerald and Moscibrocki, 2012] [STS-131 / BSP, p. 101] and tendons of the rotator cuff [Thomopoulos, 2011] [STS-131 / BSP, p. 100] show deleterious changes after 15 days of spaceflight. Altogether, these results suggest systemic changes occur in the organismal connective tissue during adaptation to the spaceflight environment.

Translational Areas

Preclinical Pharmaceutical Countermeasure Testing. In astronauts, FDA-approved drugs or drugs undergoing clinical trials to combat osteoporosis or disuse could play an important role in mitigating spaceflight-induced bone loss. For example, Amgen received FDA approval in 2010 for a human analog to OPG called Denosumab (also known as Prolia) for use in the treatment of female and male osteoporosis. The sclerostin-antibody drug (Romosozumab) for human use has been developed, and FDA review is anticipated to finish in 2017. As was demonstrated on STS-108 and STS-135, preclinical research using model organisms can validate the drug effectiveness during spaceflight and advance the drug's countermeasure readiness level.

The ARC Animal Enclosure Module was used to fly female mice in support of a broad partnership of investigators including two key commercial participants, Amgen and BioServe Space Technologies [STS-108 / CBTM] [STS-118 / CBTM-2] [STS-135 / CBTM-3]. On two 12- to 13-day missions, pharmaceutical bone loss countermeasures were tested. On STS-108 an experimental bone anti-resorptive agent, osteoprotegerin (OPG), was administered to one group of flight mice, and another flight group received a placebo. OPG is a naturally produced inhibitor of a key receptor on osteoclast precursor cells blocking their maturation to osteoclasts. Postflight analysis showed that bone mineral density of treated mice was greater than the in-flight controls through blockade of bone resorption [Bateman, 2004] [STS-108 / CBTM, p. 96]. On STS-135 an experimental sclerostin antibody was similarly administered to the mice. Sclerostin is naturally secreted by osteocytes that inhibit bone formation by osteoblasts. Treatment with the antibody to sclerostin reduces the inhibition of bone formation and, as a result, bone structure and strength are increased in space-flown mice or hindlimb-unloaded mice [Spatz et al., 2013] [STS-135 / CBTM-3, p. 104]. A slide summary of the general results is available online (http://www.slideshare.net/astrosociety/issrdc-2013-07170800stodieck).

Artificial Gravity Countermeasures. A single space-based study has been done on the efficacy of centrifugation to prevent spaceflight-induced bone loss. In brief, on-orbit, short-arm centrifugation at 1g prevented the decrease in mechanical stiffness of the femur resulting from spaceflight (spaceflight reduced stiffness by 30 percent compared to ground controls), and the postflight recovery of bone structure and strength was accelerated in animals centrifuged on-orbit [Spengler et al., 1983][Cosmos 936 / Bion 4, p. 33].

Ground-Based Models. The Hindlimb Unloading Rodent Model [Morey-Holton et al., 2005] was developed at NASA ARC in the early 1970s and has become a widely used spaceflight analog because it allows chronic unloading of the hindlimbs in semi-ambulatory rodents. About 25 percent of the studies published focus on bone or calcium metabolism, and often this ground-based model is used as a spaceflight-precursor experiment modality to study the effects of musculoskeletal disuse [Shirazi-Fard et al., 2014], or as a direct comparison to spaceflight.

Skeletal Development. Embryogenesis studies with quail eggs during spaceflight at both microgravity and on a 1-g centrifuge studied cellular differentiation and developing cartilage and bone, and the mineralization processes in limbs. No differences were seen at day 6, but at day 12 the group at 1g showed increased body weight and longer limbs. Because the embryonic limbs are largely cartilage, the lack of effect of the two gravity levels suggests a differential impact between cartilage and maturing bone [Doty et al., 2005] [STS-108 / UF-1, p. 95].

Wound Repair. A bone healing study was conducted comparing a 5-day spaceflight with synchronous weight-bearing and hindlimb suspension controls using 7-month-old rat subjects [Kirchen et al., 1995] [STS-29 / SSIP, p. 56]. The four rats in each group were given a surgical right hindlimb midshaft fibular osteotomy 5 days before launch. Histologic examinations of all rat groups immediately postflight showed a callus had formed after 10 days. Chondrogenesis (formation of cartilage) was more advanced in the weight-bearing rats than the other two groups supporting the view that healing was impaired.

These examples of translational research to clinical medicine will likely continue as longer duration rodent studies begin on the International Space Station (ISS) with the activation of the Rodent Research Facility (late 2014). Possible bone physiology studies may be conducted to quantify the dynamics of bone loss during flight (and a comparison to ground-based models) and to test countermeasures, including detailed assessments of the side effects of these pharmaceuticals. Related studies on bone healing are another obvious area for future research.

List of referenced flight experiments:

Cosmos 782 / Bion 3, E.R. Morey-Holton, Quantitative Analysis of Selected Bone Parameters

Cosmos 936 / Bion 4, E.R. Morey-Holton, Quantitative Analysis of Selected Bone Parameters

Cosmos 1129 / Bion 5, C. Cann, Bone Resorption in Rats During Spaceflight

Cosmos 1129 / Bion 5, J. Matthews, Quantitative Analysis of Selected Bone Parameters - Mineralization in the Long Bones

Cosmos 1129 / Bion 5, M. Judy, Quantitative Analysis of Selected Bone Parameters - Trabecular Spacing and Orientation in the Long Bones

Cosmos 1129 / Bion 5, T. Wronski, Quantitative Analysis of Selected Bone Parameters

Cosmos 1129 / Bion 5, W. Jee, Quantitative Analysis of Selected Bone Parameters - Bone Elongation Rate and Bone Mass in Metaphysics of Long Bones

Cosmos 1129 / Bion 5, W. Roberts, Quantitative Analysis of Selected Bone Parameters: Supplemental Report 1 - Effects of Weightlessness on Osteoblasts

STS-29 / SSIP, A. Fras, The Effects of Weightlessness in Spaceflight on the Healing of Bones

STS-40 / SLS-1, E.R. Morey-Holton, Bone, Calcium and Spaceflight

STS-59 / NIH.C1, R. Globus, The Effects of Hypogravity on Osteoblast Differentiation

STS-76 / Biorack 1, M. Hughes-Fulford, Microgravity Effects on Bone Cell Gene Expression

STS-81 / Biorack 2, STS-84 / Biorack 3, M. Hughes-Fulford, Microgravity Effects on Bone Cell Gene Expression

STS-84 / Biorack 3, M. Hughes-Fulford, Microgravity Effects on Bone Cell Gene Expression

STS-108 / CBTM, T. Bateman, Commercial Biomedical Testing Module (CBTM)

STS-108 / UF-1, S. Doty, Skeletal Development in Embryonic Quail

STS-118 / CBTM-2, H.Q. Han and D. Lacey, Commercial Biomedical Testing Module-2 (CBTM-2)

STS-131 / BSP, A. Hargens, Rodent Spine Deconditioning After 15 Days of Microgravity

STS-131 / BSP, D. J. Fitzgerald, The Response of Articular Cartilage to Microgravity

STS-131 / BSP, E. Almeida, The Role of the p21/p53 Pathway in Spaceflight-Induced Tissue Degeneration

STS-131 / BSP, S. Thomopoulos, The Effect of Weightlessness on the Tendon-to-Bone Insertion

STS-135 / CBTM-3, M.L. Bouxsein, Assessment of Anti-Sclerostin Antibody as a Novel Anabolic Therapy for Prevention of Spaceflight-Induced Skeletal Fragility in Mice

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Hughes-Fulford, M.; Tjandrawinata, R.; Fitzgerald, J.; Gasuad, K.; and Gilbertson, V.: Effects of Microgravity on Osteoblast Growth. Am. Soc. for Gravitational and Space Biology, vol. 11, no. 2, May 1998, pp. 51–60.

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Biosatellite III

Launch Date 6/28/1969

Landing Date 7/7/1696

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study

Investigation of Bone Density Changes in Various Sites of the Skeletal Anatomy of a Primate

Science Discipline

Bone and Calcium physiology

Investigator — ***Institute***

P.B. Mack — Texas Women's University

Co-Investigator(s) — ***Institute***

None

Research Subject(s)

Macaca nemestrina (Pig-Tailed Monkey)

Ground-Based Controls

Laboratory (Flight Backup Subjects)

Key Flight Hardware

Primate Life Support System

Selected Publications

Mack, P.B.: Bone Density Changes in a Macaca nemestrina Monkey During the Biosatellite III Project. BIOSPEX: Biological Space Experiments, NASA TM-58217, 1979, p. 122.

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Objectives/Hypothesis

Immobilization associated with flight has been found to definitely be associated with decreases in skeletal density in human subjects in prior studies. Using the x-ray radiographic method, this study was to find changes in bone density that might occur during weightlessness in the non-human primate.

Approach or Method

Several series of bone radiographs were taken preflight to ascertain initial skeletal density in seventeen anatomic sites. The values obtained from scanning sections of bones were equated in terms of mass of calcium hydroxyapatite, the major mineral component of bone. Additional radiographs were also taken postflight.

Results

Postflight density losses at the sites analyzed ranged from -1.71% to -17.52%, compared to 0.12% to -10.72% for ground controls. The bone density losses in the flight animal were considered to be due to immobilization coupled with the aggregate stresses of the flight environment.

Cosmos 782 / Bion 3

Launch Date 11/25/1975

Landing Date 12/15/1975

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Histological Studies on the Tibial Bone of Rats in the 1975 Cosmos 782 Flight: I. Endochondral Osteogenesis; Medullary Bone Turnover

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
C.W. Asling — University of California, San Francisco

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Asling, C.W.: Histological Studies on the Tibial Bone of Rats in the 1975 Cosmos 782 Flight: I. Endochondral Osteogenesis; Medullary Bone Turnover: Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 782. S.N. Rosenzweig and K.A. Souza, eds., NASA TM-78525, 1978, pp. 276–290.

Objectives/Hypothesis
This experiment concerns the histological evaluation of endochondral ontogenesis in rats subjected to weightlessness in Earth-orbit for 19 days. Balances between cartilage formation and resorption, and medullary bone formation and resorption, were analyzed in the proximal segment of the tibia.

Approach or Method
Tibial lengths, appearance of the proximal tibial epiphysis and metaphysis, and measurements of the bony spongiosa in the latter, were determined for flight rats and compared to ground controls. Fixated samples were stained with hematoxylin and eosin, and Mallory's triple stain (aniline blue, orange G, and acid fuchsin), and studied histologically. Measurements were made whenever possible to provide quantitative support for the qualitative histological evaluations.

Results
Growth retardation was not marked, falling between a negligible level and 25%. Bone formation was slightly impaired in synchronous controls, and to an appreciably greater extent in flight animals. Bone resorption was moderately accelerated in synchronous controls, markedly more so in the flight animals, to an extent under which virtually all preflight medullary bone was removed and, in addition, a substantial fraction (1/4 –1/2) of that formed during flight was also resorbed. Although the results suggest that disuse atrophy and other restrictions during the experiment account for part of the imbalance, the condition of weightlessness added considerable further imbalance.

Cosmos 782 / Bion 3

Launch Date 11/25/1975

Landing Date 12/15/1975

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Histological Studies on the Tibial Bone of Rats in the 1975 Cosmos 782 Flight: II. Micrographic Study of the Cortical Bone

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
C.W. Asling — University of California, San Francisco

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Asling, C.W.: Histological Studies on the Tibial Bone of Rats in the 1975 Cosmos 782 Flight: II. Microradiographic Study of Cortical Bone: Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 782. S.N. Rosenzweig and K.A. Souza, eds., NASA TM-78525, 1978, pp. 291–307.

Objectives/Hypothesis
Conceivably, a decrease in the mineral content of a bone (as in hypogravity) could result from a shift in the balance of internal remodeling, in which a smaller proportion of osteons would be of the older, high-mineral-density type, and a larger proportion of the recently formed and less mineralized type. Based on this hypothesis, a study of rat cortical bone was made by microradiography on sample tibia from rats subjected to prolonged weightlessness.

Approach or Method
Thin-ground transverse sections of the tibial cortex, embedded in polystyrene plastic, were microradiographed in contact with high-resolution spectrographic film. The resulting films were examined microscopically, and output from a photomultiplier tube was used to represent mineral densities, judged radiographically, from morphometric principles determined from mathematical bases. Resolving power of the system was such that while the lacunae of individual osteocytes were not recognized as “porosity,” all but the smallest channels in the bone specimen were thus recognized.

Results
Results indicate that ranges of mineral densities of vivarium controls in 3-month-old animals were in accord with findings on human juvenile bone. The synchronous controls showed increased porosity and a shift in mineral balance toward a larger proportion showing low mineral content, suggesting increased resorption. In the flight animals, lower levels of porosity, as compared to controls, and the tendency toward a uniform distribution of mineral values, suggest a sampling error. It is also possible that with the prolonged period between sacrifice and bone fixation, the products of marrow cytolysis may have redistributed mineral contents of adjacent bone so as to obscure any real difference that may have existed.

Cosmos 782 / Bion 3

Launch Date 11/25/1975 **Landing Date** 12/15/1975

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Mineralization in Teeth and Jaws, as Judged Radiographically, in the Rats of the Cosmos 782 Experiment

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
I. Savostin-Asling	University of California, San Francisco

Co-Investigator(s)	Institute
Asling, C.W.	University of California, San Francisco
Ellis, S.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Savostin-Asling, I.; Asling, C.W.; and Ellis, S.: Mineralization in Teeth and Jaws, as Judged Radiographically, in Rats of the Cosmos 782 Experiment: Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 782. S.N. Rosenzweig and K.A. Souza, eds., NASA TM-78525, 1978, pp. 308–320.

Objectives/Hypothesis
This study presents data on the mineralization of the teeth in rats subjected to prolonged spaceflight. Although the pitfalls inherent in quantitative radiologic studies were kept in mind, there was an advantage in knowing the resorption in the spongy bone and the ambiguous result on dense bone in the same animals. Special efforts were made to standardize the regions of tooth structure measured, in the hope that masses of tissue in low experimental reactivity might not obscure more highly reactive sites.

Approach or Method
Heads were divided sagittally and x-rayed. Optical densities were measured on the films with a densitometer calibrated in American Standard Diffuse Density units. Limitations were principally: 1) that readings would be taken at some reasonable distance from the apex (region of tooth formation) to reach the mineralized band of structure, and 2) that readings would not be over the tip of the tooth since the greatest part of the tooth was in fact closed in thin bone. While the density readings followed a logarithmic scale, the light transmission values were on an arithmetic percentage scale.

Results
Although enamel values in the flight animals, both at recovery and 25 days postflight, seemed lower than controls, the difference was not significant. Other findings suggest a small amount of mineral loss may have occurred during the experimental period. Mineral repletion was not observed during the recovery period. The thin bone of the mandibular body was slightly reduced in both synchronous and flight rats, but rose to the level of vivarium controls after the recovery period. Changes of similar direction were found in the heavy bone underlying the molar teeth, suggesting that the stimulus of chewing was reduced in these groups, with restoration of a firmer diet during the recovery period increasing masticatory activity and improving bone structure.

Cosmos 782 / Bion 3

Launch Date 11/25/1975

Landing Date 12/15/1975

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Quantitative Analysis of Selected Bone Parameters

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
E.R. Morey-Holton — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
Baylink, D.J. — VA Hospital, Seattle

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Morey, E.R. and Baylink, D.J.: Inhibition of Bone Formation During Spaceflight. Science, vol. 201, 1978, pp. 1138–1141.

Holton, E.M.: Effects of Weightlessness on Bone and Muscle of Rats. Space Gerontology, NASA CP-2248, 1982, pp. 59–66.

Objectives/Hypothesis
If bones are formed in relation to gravitational stresses, one would anticipate that prolonged recumbency and/or prolonged weightlessness would be associated with hypercalciuria, bone demineralization, and osteoporosis. To better understand the effect of spaceflight on bone, parameters including formation and mineralization, resorption, length, density and pore size distribution, and bone mechanical properties were studied in rats both immediately postflight and at 25 days postflight.

Approach or Method
Bone density and pore size distribution were measured by mercury porosimetry in the left humerus, while humerus mechanical properties were evaluated with a standard torsion test machine. Bone formation, mineralization, and resorption rates were determined by quantitative histological techniques using the left tibia, while osteoblastic and osteoclastic cell populations were determined from the right. Length measurements were made with calipers, and correlation, regression, and covariance analyses were made by means of computer programs based on standard statistical methods.

Results
Spaceflight had little effect on the bone porosity parameters measured, while the flight and synchronous animals (compared to vivarium controls) did show a significant decrease in bone density immediately postflight. The most striking effects were those on bone formation; all parameters investigated in the flight animals immediately after flight were significantly decreased from both vivarium and synchronous controls. An arrest line was found at both the endosteum and the periosteum of flight animals suggesting that a complete cessation of bone growth occurred during the flight. By 25 days postflight, flight animals showed a significant increase in formation, suggesting that a rebound in bone formation had occurred following flight.

Cosmos 936 / Bion 4

Launch Date 8/13/1977

Landing Date 8/22/1977

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Quantitative Analysis of Selected Bone Parameters

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
E.R. Morey-Holton	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Turner, R.T.	American Lake VA Medical Center
Baylink, D..J.	American Lake VA Medical Center

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Centrifuged Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications

Holton, E.M.: Effects of Weightlessness on Bone and Muscle of Rats. Space Gerontology, NASA CP-2248, 1982, pp. 59–66.

Turner, R.T.; Morey, E.R.; Liu, C.; and Baylink, D.J.: Altered Bone Turnover During Spaceflight. Physiologist, supl., vol. 22, 1979, pp. S73–S74.

Spengler, D.M.; Morey, E.R.; Carter, D.R.; Turner, R.T.; and Baylink, D.J.: Effects of Spaceflight on Bone Strength. Physiologist, supl., vol. 22, 1979, pp. S75–76.

Objectives/Hypothesis
Changes in calcium homeostasis present a potential problem during prolonged spaceflight. Because mechanical forces imposed by muscle utilization and gravity influence bone turnover, prolonged recumbency and/or prolonged weightlessness with continuous hypercalciuria and bone loss could ultimately result in osteoporosis. To better understand the effect of spaceflight and gravity on bone, the following parameters were studied in stationary and centrifuged space-flown rats both immediately following recovery and 25 days postflight: formation and mineralization, resorption, length, density and pore size distribution, and mechanical properties.

Approach or Method
Density and pore size distribution were measured in the left femur by mercury porosimetry; mechanical parameters were evaluated with a standard torsion test machine. The rate of bone formation and resorption was determined in the left tibia by quantitative histological techniques; in addition, osteoblast and osteoclast cell number was determined.

Results
The data obtained demonstrate that: no gross change in endosteal bone resorption occurs during flight or postflight; mean periosteal bone formation rate decreases about 45% and is not corrected by centrifugation; the decrease in formation rate may be due, in part, to a cessation of bone formation that occurs sometime after the 11th day of flight and continues until the 2nd postflight day; although centrifugation did not correct the defect in periosteal bone formation rate during flight, it appears to hasten the recovery following flight; femur stiffness decreases about 30%; and centrifugation did correct the defect in bone mechanical properties. All perturbations normalized by 25 days postflight. The reduction of mechanical stress is probably not sufficient to account for the decreased rate of bone formation because a comparable decrease occurred in the flight centrifuged rats. However, the mechanical strength of the femur was not reduced in these animals, and bone formation was apparently reinitiated immediately upon recovery in centrifuged rats, whereas it was delayed 2 to 3 days in flight rats.

Cosmos 936 / Bion 4

Launch Date 8/13/1977

Landing Date 8/22/1977

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Quantitative Analysis of Selected Bone Parameters: Formation of Ectopic Bone in Implanted Matrices

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
D.J. Baylink	American Lake VA Medical Center

Co-Investigator(s)	Institute
Shvets, V.N.	Institute of Biomedical Problems
Turner, R.T.	American Lake VA Medical Center
Morey-Holton, E.R.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Shvets, V.N.; Baylink, D.J.; Thompson, E.R.; and Holton, E.: Quantitative Analysis of Selected Bone Parameters: Formation of Ectopic Bone in Implanted Matrices. Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 936. S.N. Rosenzweig and K.A. Souza, eds., NASA TM-78526, 1978, pp. 179–183.

Objectives/Hypothesis
Undenatured bone matrix, demineralized and lyophilized, induces bone formation when implanted in muscle or fascia of host animals. This model was employed by Soviet scientists to evaluate the effects of spaceflight on bone formation in implanted matrices. Approximately half the implanted matrices recovered from basal, synchronous, vivarium control, and flight groups of rats were sent to the U.S. for independent evaluation. These were accompanied by samples of unimplanted bone matrix.

Approach or Method
Representative samples of ectopic bone recovered from each host rat were dried, weighed, and digested in 6N HCL. Calcium content was determined on an aliquot by atomic absorption spectrophotometry, and μg Ca/mg dry weight of sample was calculated. Specimens were prepared by a method that has been developed to preserve enzyme activity in mineralized bone, thus permitting the identification of osteoclasts as well as osteoblasts. Specimens were cut into 2 to 4 segments and embedded for sagittal sectioning, producing 57 tissue blocks, each containing from 1 to 3 segments of the specimen. Sections were stained by the Goldner technique, and a method intended to differentiate osteoblasts and osteoclasts by taking advantage of the high RNA content of the former and acid phosphatase activity of the latter, and examined microscopically.

Results
A subjective study of slides suggests that the only areas stained green by the Goldner technique are mineralized bone, in contrast to what has been found in rat and human bone, and in unimplanted bone matrix. In these, all “mature” or once mineralized matrix stains green even after being completely demineralized, and only newly formed osteoid stains red. If that were the case in the implanted matrices, all the old, implanted matrix except its normal borders of osteoid would be stained green, as well as the mineralized bone formed during the time the matrix was implanted in the host rat. One would then expect the red-stained area to be limited to the osteoid of the original matrix and the osteoid formed during the period of implantation that had not yet mineralized. Instead, it seems (but is not yet proven) that all the old matrix, whether once mineralized or not, stained red. This suggests that a change occurred in the implanted matrix that could not be demonstrated in the specimens of the unimplanted matrix, reflected in a change of its tinctorial properties, and deserves further study.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979 **Landing Date** 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Fetal and Neonatal Rat Bone and Joint Development Following In Utero Spaceflight

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
E. Sabelman	University of California, San Francisco

Co-Investigator(s)	Institute
Morey-Holton, E.R.	NASA Ames Research Center (ARC)
Arnaud, C.D.	University of California, San Francisco

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications
Sabelman, E.E.; Holton, E.M.; and Arnaud, C.D.: Fetal and Neonatal Rat Bone and Joint Development Following in Utero Spaceflight. Final Reports of U.S. Rat Experiments Flown on the Soviet Satellite Cosmos 1129. M.R. Heinrich and K.A. Souza, eds., NASA TM-81289, 1981, pp. 363–404.

Objectives/Hypothesis
The thrust of this study was altered by circumstances from the initial proposal to investigate the effects of prenatal exposure to spaceflight on rat limb development. Because no litters were born following in-flight impregnation, the only specimens were produced by vivarium and postflight pregnancies. An effort was made to measure differences between groups of specimens attributable to maternal housing, diet, or stress, which must be compensated for in future mammalian embryology experiments in space.

Approach or Method
After separation into single limbs, with skin removed to mid-tibia, specimens were superficially examined and macrophotographed at 3x. With input from a microscope and camera, radiographic density was measured using an image analysis system consisting of a camera, a video-digitizer terminal, and computer. Data were obtained on maturation of ossification centers; orientation of collagen fibers in the bone, tendon, and ligament; joint surface texture; and spatial relationships of bones and the hindlimb. A comparison was also conducted between vivarium (standard) and flight-type (Soviet paste) diets.

Results
No overt anatomical abnormalities were noted in domestic or Soviet specimens. Weights of offspring whose dams were fed Soviet diet did not differ significantly until after day 15 from standard diet controls. In pups less than 2 days old, tendon and ligament insertions tended to merge with the perichondrium rather than penetrate into underlying cartilage or bone. The texture of the minuscule fibrocartilage was distinct from adjacent ligament, with prominent cell lacunae that could be indicators of states of mechanical stress. Because of unknown degrees of sensitivity of bone and joint maturation of Czech Wistars to such factors as litter size, gestation age, uterine position, or birth order, this data should be gathered for all specimens, in addition to the age and weight at sacrifice. Certainly, environmental parameters to which flight and synchronous control groups were exposed, such as noise, vibration, and possible hypoxia, should be measured during future studies. Computer reconstructions of knee and hip show promise as a means of investigating the etiology of congenital hip dislocation.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Quantitative Analysis of Selected Bone Parameters

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
T.J. Wronski	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Morey-Holton, E.R.	NASA Ames Research Center (ARC)
Cann, C.E.	University of California, San Francisco
Baylink, D.J.	American Lake VA Medical Center

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications

Wronski, T.J.; Morey-Holton, E.; and Jee, W.S.: Cosmos 1129: Spaceflight and Bone Changes. Physiologist, supl., vol. 23, no. 6, 1980, pp. S79–S82.

Wronski, T.J. and Morey-Holton, E.R.: Effect of Spaceflight on Periosteal Bone Formation in Rats. American Journal of Physiology, vol. 244, 1983, pp. R305–R309.

Wronski, T.J. and Morey, E.R.: Alterations in Calcium Homeostasis and Bone During Actual and Simulated Spaceflight. Medicine and Science in Sports and Exercise, vol. 15, no. 5, 1983, pp. 410–414.

Objectives/Hypothesis
According to previous spaceflight research, differential effects are noted not only between weight-bearing and non-weight-bearing bones, but also are seen within different regions of the same bone. Therefore, one of the objectives of this study was to determine growth at the periosteum in different regions both in weight-bearing (tibia) and non-weight-bearing (rib) bones. Following both Cosmos 782 and 936, an arrest line was found in all flight rats and was both more distinct and more extensive than in controls. Another objective of this study was to obtain more precise measurements and further define this arrest line.

Approach or Method
The Merz grid was used to quantify the rate of periosteal bone formation in the tibial and humeral diaphysis, and to aid in quantification of the fractional area of trabecular bone and the fractional area of fat in the bone marrow of the proximal tibial metaphysis. Additional cross sections of the tibial diaphysis were used for chemical characterization of the arrest lines.

Results
The skeletal alterations induced by spaceflight were determined to be a reduced rate of periosteal bone formation in the tibial and humeral diaphyses, and a decreased trabecular bone volume and an increased fat content of bone marrow in the proximal tibial metaphysis. Inhibition of periosteal bone formation in the humerus was not as dramatic as in the tibia, probably due to the lower rate of periosteal bone formation in the humerus relative to the tibia. An increased incidence of arrest lines was seen in flight animals. The staining properties of these arrest lines and the demonstration that osteocyte canaliculi rarely pass through them, suggest that they represent a cessation of bone matrix formation followed by reinitiation of bone formation at a later time. The rate of periosteal bone formation in the rib was not significantly decreased during spaceflight, possibly due to its non-weight-bearing nature; periosteal bone formation rate in the rib may be too low to exhibit a significant change during a relatively short flight. Endosteal bone resorption was not affected markedly by spaceflight.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Quantitative Analysis of Selected Bone Parameters: Supplemental Report 1: Effects of Weightlessness on Osteoblast

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
W.E. Roberts	Indiana University

Co-Investigator(s)	Institute
Mozsary, P.G.	University of the Pacific
Morey-Holton, E.R.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications
Roberts, W.E. and Mozsary, P.G.: Quantitative Analysis of Selected Bone Parameters: Supplemental Report 1: Effects of Weightlessness on Osteoblast Differentation in Rat Molar Periodontium. Final Reports of U.S. Rat Experiments Flown on the Soviet Satellite Cosmos 1129. M.R. Heinrich and K.A. Souza, eds., NASA TM-81289, 1981, pp. 127–148.

Roberts, W.E.; Mozsary, P.G.; Morey, E.R.: Suppression of Osteoblast Differentiation During Weightlessness. Physiologist, supl., vol. 24, 1981, pp. S75-S76.

Objectives/Hypothesis
Marked depression or arrest of bone formation has been associated with spaceflight and simulated weightlessness. The mechanism of this suppression of osteogenesis is unclear, but probably involves altered induction. Data indicate that nuclear volume frequency distributions are an effective means of assaying preosteoblast differentiation in a population of connective tissue cells. Thus, the objective of this experiment was to determine whether spaceflight would alter cellular induction in the fibroblast-like cells in the rat periodontal ligament (PDL).

Approach or Method
PDL, the osteogenic interface between tooth and bone, was morphometrically analyzed. The region studied was a 300 μm length of midroot PDL on the medial aspect of the medial root of the maxillary first molars. Volumes for 100 nuclei from throughout the width of the PDL were determined, and frequency distributions of nuclear volume for each group were calculated. Studies were conducted on rats sacrificed at recovery and 6 and 29 days postflight.

Results
Immediately postflight, PDL width and total cell number were decreased. Frequency distributions of nuclear volume revealed that presumptive preosteoblasts (nuclei $\geq$ 130 μm-3) were particularly depressed. Compared to vivarium controls, frequency distributions of nuclear volume revealed a relative increase in smaller nuclei ($\leq$ 80 μm–3) at the expense of these larger nuclei. No significant differences in interzone mean nuclear volumes were observed for the groups sacrificed at 6 and 29 days postflight. This study suggests that depleted numbers of preosteoblasts may be an important factor in the arrest of bone formation during weightlessness. Data are consistent with either a defect in proliferation and/or differentiation. Additional cell kinetic studies utilizing 3H-thymidine are needed to define the mechanism of this important aerospace problem.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Quantitative Analysis of Selected Bone Parameters: Bone Elongation Rate and Bone Mass in Metaphysis of Long Bones

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
W.S.S. Jee	University of Utah

Co-Investigator(s)	Institute
Kimmel, D.B.	University of Utah
Smith, C.	University of Utah
Dell, R.B.	University of Utah

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications
Jee, W.S.; Wronski, T.J.; Morey, E.R.; and Kimmel, D.B.: Effects of Spaceflight on Trabecular Bone in Rats. American Journal of Physiology, vol. 244, no. 3, 1983, pp. R310–R314.

Kimmel, D.B. and Jee, W.S.S.: A Quantitative Histologic Analysis of the Growing Long Bone Metaphysis. Calcified Tissue International, vol. 32, 1980, pp. 113–122.

Objectives/Hypothesis
The purpose of this study was to determine whether bone elongation rate and bone cell number in the metaphysis of long bones were altered by Cosmos 1129 spaceflight. An attempt was made to measure the rate of bone elongation directly in the proximal tibial and humeral metaphysis by the use of tetracycline labeling.

Approach or Method
The proximal tibial and humeral metaphysis from groups of animals sacrificed at recovery (R-0) and 6 (R+6) and 29 days (R+29) postflight was measured using quantitative light microscopic techniques. One section of the proximal humerus of each animal was randomly selected. The following parameters were calculated for each band analyzed in the metaphysis: fractional bone and fractional calcified cartilage volumes; osteoblast, osteoprogenitor cell, and osteoclast nucleus numbers; ratios of osteoblast, osteoprogenitor cell, and osteoclast nuclei to surface area of bone; and fractional fatty marrow volume.

Results
The study demonstrated a reduction in bone and calcified cartilage volume in flight and synchronous animals, in a region of the metaphysis where a maximum of calcified tissues was seen in vivarium controls. This was associated with a decreased number of functional bone cells (osteoblasts and osteoclasts) in both flight and (probably) synchronous groups. It was also clear that the metaphysis had returned to normal by the end of the 29-day recovery period. The fatty marrow volume was increased only in flight groups R-0 and R+6, but was normal in R+29 animals. The decreased amount of bone and calcified cartilage is believed to be the result of a temporarily slowed or arrested production of calcified cartilage as a substrate for bone formation. This would have resulted from slowed bone elongation during flight and synchronous control conditions. Because the synchronous group seemed to show significant changes quite similar to the flight animals, these data indicate that the general stress, as well as the flight itself, had an effect on the rate of bone elongation.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Quantitative Analysis of Selected Bone Parameters: Trabecular Spacing and Orientation in the Long Bones

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
M. Judy	Baylor University Medical Center

Co-Investigator(s)	Institute
None	

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control

Key Flight Hardware
Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications
Judy, M.M.: Quantitative Analysis of Selected Bone Parameters: Supplement 3A: Trabecular Spacing and Orientation in the Long Bones. Final Reports of U.S. Rat Experiments Flown on the Soviet Satellite Cosmos 1129. M.R. Heinrich and K.A. Souza, eds., NASA TM-81289, 1981, pp. 177–198.

Objectives/Hypothesis
Past data suggest mineral loss is prevalent in weight-bearing bones following spaceflight. The primary goal of this research was to quantitatively determine, by optical diffraction techniques, changes in trabecular spacing (area density) and orientation under effects of weightlessness. One major goal of the research was to study changes throughout the area and height of both primary and secondary spongiosa in a trabecular region which is primarily weight bearing.

Approach or Method
Optical diffraction measurements were employed to determine the magnitude of changes in mean trabecular spacing and in mean trabecular orientation. The trabecular region immediately below the inferior-directed convexity of the cartilage growth plate, which is functionally related predominantly to sustaining mechanical forces of weight bearing and locomotion, was studied.

Results
Values of the ratio of mean trabecular spatial density, in a region 300 μm distal to the downward convexity in the cartilage growth plate, to the value adjacent to the plate, were significantly smaller ($p \leq 0.2$) for the flight animals than values for vivarium control animals. No significant differences were found in proximal regions, or detected in mean trabecular orientation. The increase in the ratio of trabecular spacing at 300 μm distal to that at the cartilage plate in the flight animals means that the linear trabecular density at this distance decreased under the reduced loading of weightlessness. Decreased values of trabecular spatial density, and of both osteoblastic activity and trabecular cross-sectional area noted in collateral research, suggest decreased modeling activity under weightlessness.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Quantitative Analysis of Selected Bone Parameters: Mineralization in the Long Bones

Science Discipline
Bone and Calcium Physiology

Investigator
J. Matthews

Institute
Baylor University Medical Center

Co-Investigator(s)
None

Institute

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications
Matthews, J.L.: Quantitative Analysis of Selected Bone Parameters: Supplemental Report 3B: Mineralization in the Long Bones. Final Reports of U.S. Rat Experiments Flown on the Soviet Satellite Cosmos 1129. M.R. Heinrich and K.A. Souza, eds., NASA TM-81289, 1981, pp. 199–228.

Objectives/Hypothesis
The major objectives of this experiment were: 1) a microscopic study of growth plates and metaphyseal trabeculae to assess the type and functional state of bone cells and to characterize the zone of calcification of the cartilaginous growth, and 2) an optical birefringent study of the trabeculae in order to assess trabecular number, size, shape, and orientation, as it is presumed that this metabolically active bone will reflect subtle changes that may result from a zero-gravity condition.

Approach or Method
Thin slices (< 1 μm) of tibial metaphysis and epiphyseal growth plate were examined with a transmission electron microscope. A checklist of cell organelles, inclusions, and ultrastructural features were used during examination of all tissue sections in an effort to give a complete descriptive profile of the bone cells and matrix of representative sections of all experimental bones.

Results
Differences noted in osteoblasts of flight animals include: a reduced nucleolus (78%); an increase in heterochromatin; dispersion of Golgi components; a reduction in number but increase in size of Golgi vesicles (a result of fusion?); reductions in rough surfaced endoplasmic reticulum, the number and size of rough endoplasmic reticulum cysternae, and evidence of pinocytotic activity; as well as an overall flattening of the cell to characterize a decrease in cell metabolic activity, particularly its protein synthesizing and secreting activity. Reduction of new mineral nodules and irregularity of mineral surface contour suggest that the newly secreted osteoid was immature. Large number of nuclei per cell, reduction in brush border, and cytoplasmic vacuoles in the flight group are indicative of a reduction of activity for each osteoclast. Some resorption activity was noted, however, and shallow matrix cavities in flight bone were observed. Whether each clast ultimately resorbs the same bone volume, simply requiring more time, will have to be established by double tetracycline studies.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Vertebral Body Strength of Rat Spinal Columns

Science Discipline
Bone and Calcium Physiology

Investigator
L.E. Kazarian

Institute
Air Force Aerospace Medical Research Laboratory

Co-Investigator(s)
None

Institute

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications
Eurell, J.A. and Kazarian, L.E.: Quantitative Histochemistry of Rat Lumbar Vertebrae Following Spaceflight. American Journal of Physiology, vol. 244, 1983, pp. R315–R318.

France, E.P.; Oloff, C.M.; and Kazarian, L.E.: Bone Mineral Analysis of Rat Vertebra Following Spaceflight: Cosmos 1129. Physiologist, supl., vol. 25, no. 6, 1982, pp. S147–S148.

Kazarian, L.E.; Collins, G.; Muhic, L.; and Becton, F.: Strength Characteristics of Rat Spinal Columns. Cosmos 1129 Advances in Physiological Sciences. Journal of Gravitational Physiology, vol. 19, 1981, pp. 129–138.

Objectives/Hypothesis
This investigation undertook a comparative analysis of biomechanical property data of rat vertebral bodies following spaceflight exposure. The purpose of this study was to shed light on the effects of environment, relative vertebral body position, and loading rate on groups of rats sacrificed immediately at spacecraft recovery (R-0), and 6 (R+6) and 29 days (R+29) postflight.

Approach or Method
Vertebral centra properties were determined by subjecting the specimens to simple compression loading; a screw gear test machine applied a load in compression by the motion of a movable crosshead. The values of stiffness, ultimate load, displacement to ultimate load, and energy to ultimate load were analyzed by analysis of variance (ANOVA) and Duncan's Multiple Range test.

Results
At R+0 in the flight animals, all of the properties showed that the vertebral body exhibits an increasing susceptibility to fracture. This reduction of bone strength was not homogeneous and dependent on vertebral level. The R+6 recovery data were inconclusive because they varied above and below the R+0 data. At R+29, ultimate load values showed a statistically significant increase in bone strength approaching that of the vivarium group. The results relating to the synchronous group were not consistent in that at the end of the 29-day recovery period, the ultimate load data, in some cases, was greater than the ultimate load data of the vivarium.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effect of Spaceflight on Osteogenesis and Dentinogenesis in the Mandibles of Rats

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
D. Simmons	Washington University School of Medicine, St. Louis

Co-Investigator(s)	Institute
Russell, J.E.	Washington University, St. Louis
Winter, R.	Washington University, St. Louis
Rosenberg, G.D.	Indiana/Purdue University

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications
Simmons, D.J. and Rosenberg, G.D.: The Effects of Spaceflight on the Mineralization of Rat Incisor Dentin. Proceedings of the Society for Experimental Biology and Medicine, vol. 175, 1984, pp. 429–437.

Rosenberg, G.D. and Simmons, D.J.: Time Varying Calcium, Phosphorus and Sulfur Concentrations in Rat Incisors: Three Effects of Spaceflight (Cosmos 1129 Biosatellite). The Chemistry and Biology of Mineralized Connective Tissue, A. Veis, ed., Elsevier North Holland, Inc., 1981, pp. 295–297.

Objectives/Hypothesis
Efforts to understand how prolonged spaceflight affects changes in calcium homeostasis and bone formation-resorption have been pursued on past Cosmos missions. This study examined the effect of spaceflight on the integrated growth and remodeling of a non-weight-bearing bone, the mandible and teeth, to determine how microgravity affects tissues in a skeletal element that is only supplied with a large antigravity muscle (masseter).

Approach or Method
Animals were injected with 1.0 mg/kg body weight of Declomycin to mark forming and mineralizing surfaces of bone and dentin, 3 days prior to loading. The left jaw was divided into three regions: premolar, molar, and postmolar. All sections were examined by UV microscopy to reveal the distribution of tetracycline time markers. Dentinogenesis was estimated in the portion of the mandibular incisor that lay within the diastema. Polished slabs of the lower incisors were scanned to measure local variations in calcium, phosphorus, and sulfur.

Results
The total calcium, inorganic phosphorus, and hydroxyproline levels in the jaws and incisors of the flight rats were normal. Gravity-density fractionation studies suggested, however, that spaceflight caused a delay in the normal maturation of bone and mineral matrix; these values were normalized at R+6 and were fully corrected by R+29. The teeth were spared. The circadian and ultradian patterns of calcified dentin were normal during spaceflight and recovery periods, but the enamel rhythms displayed a greater amplitude of sulfur concentrations and thus abnormal calcium/sulfur ratios during exposure to microgravity. The only derangements detected were in the quality of the matrix and mineral moieties. The highest density fractions of the flight rat bones had 30% less mineral and collagen than the corresponding fractions from the control rat bone. These changes suggest that there was a delay in the maturation of collagen (a lack of intramolecular cross-links?) and apatite mineral in the flight animals.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study

The Effect of Spaceflight on Osteogenesis and Dentinogenesis in the Mandible of Rats: Supplement 1: The Effects of Spaceflight on Alveolar Bone Remodeling in the Rat Mandible

Science Discipline

Bone and Calcium Physiology

Investigator	Institute
P. Tran Van	Yale University School of Medicine

Co-Investigator(s)	Institute
Vignery, A.	Yale University School of Medicine
Baron, R.	Yale University School of Medicine

Research Subject(s)

Rattus norvegicus (Wistar rat)

Ground-Based Controls

Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications

Simmons, D.J.; Russell, J.E.; Winter, F.; Baron, R.; Vignery, A.; Tran, V.T.; Rosenberg, G.D.; and Walker, W.: Bone Growth in the Rat Mandible During Space flight. Physiologist, supl., vol. 23, no. 9, 1980, pp. S87–S90.

Simmons, D.J.; Russell, J.E.; Winter, F.; Tran, V.P.; Vignery, A.; Baron, R.; Rosenberg, G.D.; and Walker, W.W.: Effect of Spaceflight on the Non-Weight-Bearing Bones of Rat Skeleton. American Journal of Physiology, vol. 244, no. 3, Mar. 1983, pp. R319–R326.

Objectives/Hypothesis

Under spaceflight conditions, and assuming animals are eating normally, alveolar bone should be subjected to only slightly different mechanical conditions, which should not induce marked changes in bone remodeling if all other metabolic conditions are unchanged. On the other hand, if the changes observed in the long bone are due totally or in part to systemic changes, alveolar bone remodeling should also be affected, and even more markedly considering its high normal turnover rate.

Approach or Method

The molar area was dissected from the right lower jaw and embedded without decalcification. Horizontal sections (4 μm) from the cervix to the apex of the root were prepared with a microtome. Sections from the middle part of the buccal root of the first molar were stained for examination; one section out of every five of these was prepared for fluorescent microscopic analysis. A technique of dynamic histomorphometry was used to determine the extent and duration of each phase in the bone remodeling sequence, the mean calcification rate, and the amount of bone mineralized per day.

Results

The results obtained showed the absence of effects of spaceflight upon the balance between bone formation and resorption. There was, however, a slight but constant decrease in the alveolar bone turnover rate. This decreased remodeling activity, although present in the synchronous animals, was significantly lower in the flight animals, even after a 3-week recovery period. In terms of bone remodeling activity, results indicated a decrease in the birthrate of new Basic Multicellular Units (BMU) at the tissue level rather than an abnormal activity at the BMU or the cellular levels. The most dramatic effect of spaceflight was observed along the periosteal surface, and especially in areas not contiguous with (covered with) masticatory muscles, where bone formation almost stopped completely during the flight period. As this bone was submitted to the same mechanical forces in the flight animals and controls, it was concluded that factors other than mechanical loading might be involved in the decreased bone formation during spaceflight.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Bone Resorption in Rats During Spaceflight

Science Discipline
Bone and Calcium Physiology
Renal, Fluid and Electrolyte Physiology

Investigator	Institute
C.E. Cann	University of California, San Francisco

Co-Investigator(s)	Institute
Adachi, R.R.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages
Cosmos 1129 Russian Hardware Suite

Selected Publications

Cann, C.E. and Adachi, R.R.: Bone Resorption and Mineral Excretion in Rats During Spaceflight. American Journal of Physiology, vol. 244, 1983, pp. R327–R331.

Cann, C.E.; Adachi, R.R.; and Holton, E.M.: Bone Resorption and Calcium Absorption in Rats During Spaceflight. Physiologist, supl., vol. 23, no. 6, 1980, pp. S83–S86.

Cann, C.E. and Adachi, R.R.: Bone Resorption in Rats During Spaceflight. Final Reports of U.S. Rat Experiments Flown Aboard the Soviet Biosatellite Cosmos 1129. M.R. Heinrich and K.A. Souza, eds., NASA TM-81289, 1981, pp. 427–438.

Objectives/Hypothesis
Calcium metabolism is altered in weightlessness. In humans, bone loss occurs and urinary calcium output is increased. Experiments on previous Cosmos flights have shown that bone formation in the tibia is depressed in young, growing rats, but no direct information has been obtained about bone resorption, or any of the other calcium metabolic parameters such as excretion, absorption, or net calcium balance. The basis of the present study was to determine the response of calcium homeostasis and bone to weightlessness.

Approach or Method
Calcium tracer kinetic methods were used in this study. In the normal situation, both bone and dietary calcium are made up of natural calcium and thus are labeled with stable isotopic tracers such as 48Ca. If one removes 48Ca from the diet, however, then dietary calcium is distinguished from bone calcium by lack of this tracer. This is done by replacing natural dietary calcium with isotopically-separated ~100% 40Ca. As calcium is excreted from the serum, it is replaced by calcium coming from both bone and diet. As the only source of 48Ca is the bone, the amount found in serum represents the fraction of calcium turnover coming directly from bone. Animals were started on the tracer diet at the time of loading into flight hardware. Specimens received from the Soviets following flight were the rib cage (left and right) from each animal and approximately 50% of each 2-day excreta collection from each animal. The muscle from each rib cage was used as an indicator of tracer activity in the serum.

Results
Bone resorption expressed as the fraction of exchangeable calcium pool coming from bone was 0.690 ± 0.089 in the flight animals versus 0.675 ± 0.085 in synchronous controls, measured at the end of the flight period. Bone resorption rate at the end of the flight period was 15.7 mg Ca/day in flight rats and 20.2 mg Ca/day in the controls. It is significant that resorption normalized by calcium turnover does not decrease during flight, so that the decrease seen in the bone resorption rate is probably secondary to a decrease in total calcium turnover. Of particular interest may be the fact that while bone resorption rate decreased during flight, it was still 75–80% of normal at the end of the flight. This may indicate that bone loss on even longer flights will continue. The continual imbalance of bone formation and breakdown, and the large excretion of other minerals (sodium, potassium) from the body indicate that mineral homeostasis does not adapt to weightlessness.

Cosmos 1514 / Bion 6

Launch Date 12/14/1983

Landing Date 12/19/1983

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Calcium Metabolism and Correlated Endocrine Measurements in Primates

Science Discipline
Bone and Calcium Physiology
Renal, Fluid and Electrolyte Physiology

Investigator	Institute
C.E. Cann	University of California, San Francisco

Co-Investigator(s)	Institute
Patterson-Allen, P.	NASA Ames Research Center (ARC)
Adachi, R.R.	NASA Ames Research Center (ARC)
Ushakov, A.S.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Laboratory Control

Key Flight Hardware
Cosmos Primate-BIOS
Cosmos 1514 Russian Hardware Suite

Selected Publications
Cann, C.E.; Patterson-Allen, P.; Adachi, R.R.; and Ushakov, A.: Calcium Metabolism and Correlated Endocrine Measurements in Primates. Final Reports of U.S. Monkey and Rat Experiments Flown on the Soviet Satellite Cosmos 1514. R.C. Mains and E.W. Gomersall, eds., NASA TM-88223, 1986, pp. 129–144.

Objectives/Hypothesis
The problem of calcium loss from the body during spaceflight had been long recognized; however, little is known about the initiating mechanism of this loss. Two hypotheses have been suggested in explanation: 1) a primary increase in bone resorption due to decreased mechanical stress on the bone, or 2) a primary renal calcium leak. This experiment was designed to measure the initial responses of the calcium homeostatic system in nonhuman primates subjected to the spaceflight environment.

Approach or Method
The basis for this study was the “tracer” technique, in which direct measurements of changes in bone resorption are possible through isotopically labeled calcium. Fifteen days prior to launch, flight and control subjects were started on a diet laced with the isotopically labeled calcium. Excretion samples were collected pre- and postflight, and amounts of labeled calcium were detected by means of irradiation. Single lateral x-rays were obtained for the right and left arms and legs of one flight, two synchronous, and one laboratory monkey to provide high contrast and good radiographic detail for bone. No serum samples were received for the flight experiment, so the planned endocrine studies were not done.

Results
Results indicate that after 5 days of flight, the fraction of circulating calcium that comes from the bone was increased. A major point to be noted is that, even with a wide fluctuation in dietary calcium intake, urinary calcium output remained fairly constant over the 2- to 3-week period. Similar results have been noted in immobilized monkeys. However, the very low variation when expressed as calcium/creatinine suggests a technical explanation having to do with collection. Gross skeletal changes were not observed, but there was a suggestion of subtle changes as determined from the high-quality radiographs. Qualitative assessment of the films for cortical porosity suggests that one of the flight monkeys may have experienced a state of slightly increased bone turnover because of the presence of some intracortical striations in the radial and ulnar cortex. Data indicate that the methods used to study calcium metabolism during spaceflight were sufficient to answer the questions asked.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Analysis of Radiographs and Biosamples From Primate Studies

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
C.E. Cann	University of California, San Francisco

Co-Investigator(s)	Institute
Rakhmanov, A.	Institute of Biomedical Problems
Korolkov, V.I.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Cosmos 1887 Russian Hardware Suite

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Cann, C.E.; Rakhmanov, A.; and Karolkov, V.: Analysis of Radiographs and Biosamples From Primate Studies. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 513–519.

Objectives/Hypothesis
This experiment was designed to serially study the growth and development of the juvenile primate peripheral skeleton, and to determine if a 2-week period of spaceflight affected this development. This design was chosen because in the juvenile primate (3–5 kg), the skeleton is still undergoing rapid development at this stage, with longitudinal growth at the unfused metaphyseal-epiphyseal junctions, and periosteal and endosteal architectural modeling. This provides the possibility to detect an effect of microgravity by a change in the normal rate of growth and modeling.

Approach or Method
Serial high-contrast radiographs were obtained of both arms and the right leg of two flight and four control monkeys. This investigation included a significant amount of preflight testing and development of optimal radiograph techniques in the U.S.S.R. Serial radiographs were taken of flight candidate, flight, and control monkeys from a period 60 days prior to launch to 2 weeks after the postflight control (synchronous) experiment.

Results
Longitudinal growth of the tibia, radius, and ulna was linear over this period in the control monkeys. In the flight monkey for whom the feeder malfunctioned, there were significant decreases in growth of long bones. There were also hypermineralized growth arrest lines produced in the distal radial and ulnar metaphyses following resumption of growth. In the other flight monkey, there was a suggestion of decreased long bone growth during flight and immediate postflight periods, but this recovered by the end of the postflight control experiment. There was also an increase in intracortical resorption, indicative of skeletal activation. No major changes in cortical thickness or other parameters were noted, but modifications of the techniques to obtain very high quality radiographs in further studies should allow subtle changes in these processes to be quantified.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Trace Element Balance in Rats During Spaceflight

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
C.E. Cann	University of California, San Francisco

Co-Investigator(s)	Institute
Patterson-Buckendahl, P.	University of California, San Francisco
Durnova, G.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Cann, C.E.; Patterson-Buckendahl, P.; Durnova, G.; and Kaplansky, A.: Trace Element Balance in Rats During Spaceflight. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 151–155.

Objectives/Hypothesis
Little is known about the effects of spaceflight in an older skeleton. Limited data suggest that bone resorption is increased after 5 days, but no data are available about other metabolic effects. The response of a more slowly growing skeleton may be different than that of a younger animal, similar to the different responses seen in adolescents and adult humans to immobilization. This experiment was designed to investigate changes occurring in skeletal and mineral homeostasis in older, space-flown rats.

Approach or Method
Vertebral specimens from flight and synchronous, vivarium, and basal control rats were obtained for analysis. Selected vertebrae (fourth lumbar), were weighed, separated into four parts, lyophilized to constant weight, then ground to a fine powder. Osteocalcin was measured in extracts of the powder with a rat-specific osteocalcin assay. Calcium was measured using atomic absorption spectrophotometry, and phosphorus was determined using a modified Fiske-Subarow method. In addition, a pooled excreta collection and samples of the flight paste diet were analyzed.

Results
The differences between flight and control animals were minimal. Mass of the whole vertebrae increased 6.2% in synchronous rats compared to less than 2% when compared to basal controls, suggesting a decreased rate of bone growth in flight. The increased osteocalcin concentration in the posterior spine of flight rats, as compared to all controls, suggests a higher state of maturation of this compact bone, possibly due to a slowed turnover with the removal of both dorsal-to-ventral loading as well as torsional muscle pulls in spaceflight. This is similar to differential effects of long-term microgravity exposure seen in the vertebral body and posterior elements in cosmonauts.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Distribution and Biochemistry of Mineral and Matrix in the Femurs of Rats

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
S.B. Arnaud	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Durnova, G.	Institute of Biomedical Problems
Mechanic, G.L.	University of North Carolina
Bromage, T.G.	The London Hospital Medical College

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Mechanic, G.L.; Arnaud, S.B.; Boyde, A.; Bromage, T.G.; Buckendahl, P.; Elliott, J.C.; Katz, E.P.; and Durnova, G.N.: Regional Distribution of Mineral and Matrix in the Femurs of Rats Flown on Cosmos 1887 Biosatellite. FASEB Journal, vol. 4, no. 1, 1990, pp. 34–40.

Objectives/Hypothesis
Previous analyses of the composition of mineral and matrix in the bone of young rats following spaceflight has revealed deficits in calcium, phosphorus, and osteocalcin, a noncollagenous protein, without an associated decrease in collagen. To characterize the location and nature of this mineralization defect in a weight-bearing long bone, the femur, this study attempted to relate the spatial distribution of mineral in situ in the proximal, central, and distal thirds of the femoral diaphysis to the biochemical composition of the bone from the same areas.

Approach or Method
Localizing mineralization activity in the diaphysis of the femur to proximal, central, and distal thirds was determined by chemical analysis of pooled bone samples. Because statistical analyses of a determination from a single pool could not be done, a value two standard deviations above or below the error of the method was used to denote the difference in two comparison groups (i.e., exceeding 6% for calcium, 12% for phosphorus, and 10% for hydroxyproline and osteocalcin, etc.). A new technique, x-ray microtomography, with a resolution of 26 microns, was used to obtain semi-quantitative data on mineral distribution in reconstructed sections of wet whole bone.

Results
Biochemical analyses revealed lower concentrations of calcium, phosphorus, and osteocalcin, but not collagen, in the distal half of the diaphysis of flight animals compared to synchronous controls. Collagen concentration was reduced only in the proximal half of the diaphysis. X-ray microtomography indicated a longitudinal gradient of decreasing mineralization toward the distal diaphysis similar to the biochemical analysis. Image analysis of cross sections by backscattered electrons in a scanning electron microscope revealed patterns of mineral distributions that varied with the site of the section in flight and synchronous controls. Circulating parameters of skeletal metabolism revealed differences in serum calcium, osteocalcin, and alkaline phosphatase in the flight groups suggestive of steroid hormone excess, a phenomenon supported by the finding of enlarged adrenal glands.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study

Biomedical, Biochemical, and Morphological Alterations of Muscle and Dense, Fibrous Connective Tissues During 14 Days of Spaceflight

Science Discipline

Bone and Calcium
Physiology Metabolism and
Nutrition Muscle Physiology

Investigator	Institute
A.C. Vailas	University of California, Los Angeles

Co-Investigator(s)	Institute
Kaplansky, A.S.	Institute of Biomedical Problems
Zernicke, R.	University of California, Los Angeles
Grindeland, R.E.	NASA Ames Research Center (ARC)

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Vailas, A.C.; Zernicke, R.F.; Grindeland, R.E.; Kaplansky, A.; Durnova, G.N.; Li, K.C.; and Martinez, D.A.: Effects of Spaceflight on Rat Humerus Geometry, Biomechanics, and Biochemistry. FASEB Journal, vol. 4 , no. 1, 1990, pp. 47–54.

Manske, S.L.; Boyd, S.K.; and Zernicke, R.F.: Muscle and Bone Follow Similar Temporal Patterns of Recovery From Muscle-Induced Disuse Due to Botulinum Toxin Injection. Bone. Jan. 2010, vol. 46, no. 1, pp. 24–31. Epub Oct. 21, 2009.

Objectives/Hypothesis

In consideration of the differences between the rodent experiments on U.S.S.R. and U.S. spaceflights, this experiment was designed to generate comparative data about the sensitivity of cortical bone (humerus) and trabecular bone (vertebral, T7) to caging environment, diet, and rat strain differences. Specifically this study provided data regarding the biochemical, biomechanical, and morphological characteristics of selected connective tissues (humerus, vertebral body, tendon, and skeletal muscle) for growing rats, maintained under different feeding and caging conditions (including weightlessness).

Approach or Method

Studies of biochemical, biomechanical, and morphological characteristics of selected connective tissues (humerus, vertebral body, tendon, and skeletal muscle) were performed on flight and control animals. Additionally, for 2 weeks, male Taconic-Sprague Dawley and Czechoslovakian-Wistar rats were maintained in flight simulation cages (one rat/cage = U.S.; ten rats/cage = U.S.S.R.) and fed U.S.S.R. or U.S. diets to compare different combinations of dietary and caging procedures (U.S. diet in U.S.S.R. cage, U.S.S.R. diet in U.S. cage, etc.).

Results

Using the basal group for comparison, during the 12.5-day period, the humeral lengths increased 4.2% for vivarium controls, 1.4% for synchronous controls, and 0.04% for flight rats. The average flexural rigidity (bending stiffness) of flight humeri were significantly less than the vivarium (40%) and synchronous (35%) controls, but the average flexural rigidity of the flight humerus was not different from the basal control group. The flight group had an average ventral body (L6) compressional stiffness that was 39% less than vivarium, 46% less than synchronous, and 16% less than basal controls. There seemed to be no significant effect upon the collagen concentration in various types of skeletal muscles. On average all rats increased (>60%) their body mass, and there were no differences among humeral lengths for different groups. The vertebra (T7) displayed no significant structural differences, but material properties were influenced by all three factors; generally, the combination of factors that produced significantly greater material properties were U.S.S.R. caging and diet, and the Wistar rat strain.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Gravity and Skeletal Growth: I. Gravity and Skeletal Growth

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
E.R. Morey-Holton	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Berretta, D.	University of Pennsylvania
Doty, S.B.	Colombia University
Roberts, W.E.	Indiana University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Morey-Holton, E.R.; Hargens, A.; Gonsalves, M.; Berretta, D.; Doty, S.; Roberts, W.; Garetto, L.; Kaplansky, A.; Durnova, G.; Gott, S.; and Rydevik, B.: Gravity and Skeletal Growth: I. Gravity and Skeletal Growth. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 113–122.

Objectives/Hypothesis
Past data suggest that matrix turnover is altered during spaceflight, which, in turn, decreases the amount of bone added; either this defect alters the size of bone crystals formed or the flight environment somehow impedes crystal growth independent of matrix production. This suppressed bone formation may be a major factor in the failure of bone to increase its strength. The objectives of the Cosmos 1887 study were to continue the investigation of the effects of spaceflight on bone in growing rats.

Approach or Method
Strength tests on humeri, analysis of bone composition, including maxillae w/teeth, calvaria, tibial shaft, and thoracic vertebra (general overview) were performed. Studies also included measuring bone area, bone electrophysiology, bone vascularity, osteoblast morphology, and osteoblast histogenesis. Electrophoresis was used as a direct method for determining the zeta potential for particles. The bone particle velocity was measured in a fluid with a given ion concentration while an electric field of known amplitude was applied. The ratio of a particle velocity to the electric field amplitude was defined as the electrophoretic mobility (EPM), which, in turn, was proportional to the zeta potential.

Results
The flight group lost an average of 13 g over a 20-day period compared to the basal group (–0.65 g/day). Visual observations of tibial cross sections under bright-field or polarized light did not show any obvious differences. The synchronous and vivarium control groups have very similar EPMs, while the basal group is slightly less electronegative and the flight group is more electronegative than the controls. The synchronous animals, which gained the most weight, had essentially normal zeta potential values, while the flight group had a more negative potential, indicating net bone formation, than all other groups. Interestingly, the potential in the flight rats is in the opposite direction of osteoporotic bone (that is, more negative rather than more positive EPM values). Whether the value reflects increased matrix synthesis during flight or postflight recovery is not known.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study

Gravity and Skeletal Growth: II. Morphology and Histochemistry of Bone Cells and Vasculature of the Tibia From Cosmos 1887

Science Discipline

Bone and Calcium Physiology

Investigator	Institute
S.B. Doty	Colombia University

Co-Investigator(s)	Institute
Durnova, G.	Institute of Biomedical Problems
Kaplansky, A.S.	Institute of Biomedical Problems
Morey-Holton, E.R.	NASA Ames Research Center (ARC)

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Doty, S.B.: Localization of Calcium Stimulated Adenosine Triphosphatase Activity in Blood Vessels of the Skeleton. Physiologist, supl., vol. 28, no. 6, 1985, pp. S125–S126.

Doty, S.B.; Morey-Holton, E.R.; Durnova, G.N.; and Kaplansky, A.S.: Cosmos 1887: Morphology, Histochemistry, and Vasculature of the Growing Rat Tibia. FASEB Journal, vol. 4, no. 1, 1990, pp. 16–23.

Objectives/Hypothesis

In a previous study of animals from Spacelab-3, the osteoblast appeared to be slightly smaller in size following 7 days of flight. These osteoblasts were found along the endosteal surface of the diaphyseal bone and tended to have a more uniform size as compared to osteoblasts along the trabeculae in the metaphyseal region of long bones. Therefore, this study concentrated on the cells found within the diaphyseal area of long bones. The objectives here were to investigate the relationships between vascular morphology, lipid accumulation, and osteocyte and osteoblast morphology in weight-bearing long bones.

Approach or Method

Electron microscopy, light microscopy, and enzyme histochemistry were used to study the effects of spaceflight on metaphyseal and cortical bone of the rat tibia. Following fixation and decalcification, 50-micrometers-thick sections were obtained with a vibratome, and sections incubated in the various media alkaline or acid phosphatase, or NADPase. Staining for lipids was carried out on frozen sections of fixed tissues and embedded vibratome sections. Morphometry was carried out on light or electron microscopy using an interactive data analysis system.

Results

Light microscopy of trabecular and cortical bone and the included osteoblast population showed no obvious morphological differences as a result of spaceflight. In the diaphyseal bone from flight animals, blood vessels near the periosteal surface often showed very dense intraluminal deposits. Also, in the periosteal region, many osteocytic lacunae were found devoid of osteocytes and sometimes filled an osmiophilic substance. Flight animals contained reactive saccules which averaged 11.3 ±6.1 saccules per cell, and the vivarium controls averaged 14.4 ±3.4 saccules per cell, which contained reaction product. The vasculature of the flight animals was definitely less reactive than the control groups. Results of other measurements indicate that more vascular space per area of bone existed in flight animals as compared to the simulated controls. This data suggest that a vascular change may occur during flight, which would then influence the bone forming ability of the osteoblasts in weight-bearing bones.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study

Gravity and Skeletal Growth: III. Nuclear Volume Analysis of Osteoblast Histogenesis in Periodontal Ligament Cells of Cosmos 1887 Rats

Science Discipline

Bone and Calcium Physiology

Investigator	Institute
L.P. Garetto	Indiana University

Co-Investigator(s)	Institute
Roberts, W.E.	Indiana University
Gonsalves, M.R.	NASA Ames Research Center (ARC)
Kaplansky, A.S.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Fielder, P.J.; Morey, E.R.; and Roberts, W.E.: Osteoblast Histogenesis in Periodontal Ligament and Tibial Metaphysis During Simulated Weightlessness. Aviation, Space, and Environmental Medicine, vol. 57, 1986, pp. 1125–1130.

Garetto, L.P.; Gonsalves, M.R.; Morey, E.R.; Durnova, G.; and Roberts, W.E.: Preosteoblast Production 55 Hours after a 12.5 Day Spaceflight on Cosmos 1887. FASEB Journal, vol. 4, no. 1, 1990, pp. 24–28.

Objectives/Hypothesis

Bone is a mechanically sensitive tissue that is particularly responsive to gravitational forces. An understanding of changes in bone mass is important because it functions not only as structural support, but also as a metabolic source of calcium. The periodontal ligament (PDL), a well-defined cell kinetic model for assessing the proliferation and differentiation of the cells associated with osteoblast histogenesis, was used to study the effect on osteoblast production.

Approach or Method

PDL, the osteogenic interface between tooth and bone, was morphometrically analyzed. Three-μm sections of methyl methacrylate embedded maxillary halves were stained with hematoxylin and eosin. Only PDL samples with a resorbing (scalloped with occasional osteoclasts) or resting (no morphological evidence of active resorption or formation) alveolar bone margin were selected for analysis. Nuclear volume analysis of cells in the PDL midroot area was performed and statistically considered.

Results

Compared to synchronous controls, this flight treatment resulted in a 40% decrease in less differentiated osteoblast progenitor cells, a 42% increase in preosteoblasts (immediate precursors to osteoblasts), and increased numbers of PDL fibroblast-like cells within 25 μm of the bone surface. These results are consistent with a postflight osteogenic response in PDL adjacent to previously resting or resorbing alveolar bone surfaces. This osteogenic response occurred despite physiological stress in the flight animals that resulted in a highly significant ($p \geq 0.001$) increase in adrenal weight. The data suggest that following spaceflight there is a strong and rapid recovery mechanism for osteoblast differentiation that is not suppressed by physiological stress.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Gravity and Skeletal Growth: IV. Intervertebral Disc Swelling Pressure Associated With Microgravity

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
A.R. Hargens	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Gott, S.A.	University of California, San Diego
Rydevik, B.	University of Goteborg, Sweden
Durnova, G.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications

Murthy, G. and Hargens, A.R.: Intervertebral Disc Swelling Pressures in Normal Gravity and Microgravity American Society for Gravitational and Space Biology Bulletin, vol. 6, no. 1, 1992, p. 88.

Hargens, A.R. and Mahmod, M.: Decreased Swelling Pressure of Rat Nucleus Pulposus Associated with Simulated Weightlessness. Physiologist, supl., vol. 32, no. 1, 1989, pp. S23–S24.

Glover, M.G.; Hargens, A.R.; Mahmood, M.M.; Gott, S.; Brown, M.D.; and Garfin, S.R.: A New Technique for the in vitro Measurement of Nucleus Pulposus Swelling Pressure. Journal of Orthopedic Research, vol. 9, 1991, pp. 61–67.

Objectives/Hypothesis
The back pain experienced by space travelers during exposure to microgravity may be caused by spinal lengthening due to swelling of their discs, a subsequent stretching of anterior and/or posterior spinal ligaments. Other work has documented that pooling of lumbar discs from the rat spine allows sufficient disc material for direct measurements of swelling pressure in this species. Therefore, studies of Cosmos 1887 rats allowed testing of the hypothesis that microgravity causes fluid uptake and decreased swelling pressure within the intervertebral disc of flight rats compared to ground-based, control rats.

Approach or Method
To examine fluid movement into discs, equilibrium swelling pressure of nucleus pulposus from flight rats was compared to controls. Measurements were made with a new compression-type osmometer that allowed direct measurement of swelling pressure.

Results
No significant differences were found in the swelling pressures between the flight and control groups of rat nucleus pulposus. Swelling pressure ranged between 622–690 mmHg. Because of the extended period between the time that the flight rats returned to Earth and the time of death (53–56 hours), it was concluded that the flight animals already were fully readapted to normal gravity in terms of fluid movement into and out of their intervertebral discs.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Maturation of Bone and Dentin Matrices in Rats Flown on Cosmos 1887

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
D. Simmons	University of Texas Medical Branch

Co-Investigator(s)	Institute
Grynpas, M.	University of Toronto
Rosenberg, G.D.	Indiana/Purdue University
Durnova, G.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Simmons, D.J.; Grynpas, M.D.; and Rosenberg, G.D.: Maturation of Bone and Dentin Matrices in Rats Flown on the Soviet Biosatellite Cosmos 1887. FASEB Journal, vol. 4, no. 1, 1990, pp. 29–33.

Objectives/Hypothesis
Maintaining musculoskeletal integrity in astronauts during prolonged spaceflight is important. This concern translates to questions about the role of gravity in calcium-mediated physiological mechanisms. The particular interest here in the skeletal status of rats flown in space focused upon the normality of the matrix and mineral moieties deposited in the bones and teeth. This report deals with the analyses of various weight and non-weight-bearing bones and incisor dentin from rats.

Approach or Method
The chemistry, hydroxyapatite crystal size, and maturation of bone and dentin (utilizing the mandible, skull cap or calvarium, and fifth lumbar vertebra) were characterized, including bone ash analysis, gradient density analysis, and x-ray diffraction. Analyses for calcium, phosphorus, magnesium, and zinc in bone and teeth were made on continuous traverses with a microprobe and appropriate crystal sensors.

Results
Flight calvarial and vertebral bone ash were subnormal, but contained a normal percent composition of Ca, P, and Mg. These tissues varied from the norm by having lower Ca/P and higher Ca/Mg ratios than any of their age-matched controls (Vivarium and Synchronous). Gradient density analyses (calvaria) indicated a strong shift to the lower specific gravity fractions, which was commensurate with impaired rates of matrix-mineral maturation. X-ray diffraction data were confirmatory. Bone hydroxyapatite crystal growth in flight rats was preferentially altered in a way to reduce the dimension of their C-axis. Flight rat dentin was normal with respect to age-matched control Ca, P, Mg, and Zn concentrations and Ca/P and Ca/Mg ratios. These observations affirm the concept that microgravity adversely affects the maturation of newly formed matrix and mineral moieties in bone.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Morphometric and EM Analysis of Tibial Epiphyseal Plates From Cosmos 1887 Rats

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
P.J. Duke	University of Texas Dental Branch

Co-Investigator(s)	Institute
Montufar-Solis, D.	University of Texas Dental Branch
Durnova, G.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications

Duke, P.J.; Durnova, G.; and Montufar-Solis, D.: Histomorphometric and Electron Microscopic Analyses of Tibial Epiphyseal Plates From Cosmos 1887 Rats. FASEB Journal, vol. 4, no. 1, 1990, pp. 41–46.

Montufar-Solis, D. and Duke, P.J.: Effects of Strain, Diet and Housing on Rat Growth Plates: a Cosmos ’87–Spacelab-3 Comparison. Physiologist, supl., vol. 34, no. 1, 1991, pp. S183–S184.

Montufar-Solis, D. and Duke, P.J.: Changes in the Rat Growth Plates Due to Strain, Diet and Housing. American Society for Gravitational and Space Biology Bulletin, vol. 3, no. 1, 1989, p. 114.

Objectives/Hypothesis
Studies have shown that bone formation ceases during spaceflight, and that the matrix formed during flight does not mature normally and consequently cannot mineralize normally. The cartilaginous epiphyses of the long bones of space rats have received less attention, even though some of the changes that are seen in bone (e.g., decreased length and decreased trabecular mass) must originate in the epiphyseal region. The objectives of the present study were to look for differences in plate parameters, and cell and matrix ultrastructure, in proximal tibial epiphyseal plates of space-flown rats.

Approach or Method
Light and electron microscopy studies were carried out on decalcified tibial epiphyseal plates. Height and cell number per zone and plate, shape of plates, and size of collagen fibrils were determined. A series of micrographs were taken in each zone, and measurements of collagen fibril length and width were made using a digitizing tablet. Means per section were averaged to obtain means per animal, and statistical analysis was performed.

Results
Flight animals had more cells than synchronous controls in the proliferative zone and less in the hypertrophic/calcification region. The total number of cells, however, was significantly higher in flight animals. No differences were found for perimeter or shape factor of growth plates, but area was significantly lower in flight animals in comparison to synchronous controls. Collagen fibrils in flight animals were shorter and wider than in synchronous controls. The time required for a cell to cycle through the growth plate is 2–3 days, so most of the cells and matrix present were formed after the animals had returned to 1 g and probably represent stages of recovery from microgravity exposure.

STS-29 / SSIP2

Launch Date 3/13/1989 **Landing Date** 3/18/1989

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effects of Weightlessness in Spaceflight on the Healing of Bones

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
I.A. Fras — Binghamton Central High School

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Long Evans rat)

Ground-Based Controls
Hindlimb Suspended Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Kirchen, M.E.; O'Connor, K.M.; Gruber, H.E.; Sweeney, J.R.; Fras, I.A.; Stover, S.J.; Sarmiento, A.; and Marshall, G.J.: Effects of Microgravity on Bone Healing in a Rat Fibular Osteotomy Model: Clinical Orthopaedics & Related Research, No. 318, Sept. 1995, pp. 231-242.

Objectives/Hypothesis
This experiment was designed to study the effects of space on changes in bone growth and repair. The experiment was originally flown on STS-51L, the Space Shuttle Challenger, which was tragically lost 73 seconds into flight as a result of a fuel leak. The re-manifested experiment extended previous objectives by comparing the effects of the gravity and microgravity environments on bone healing, with addition of the tail-suspension model to the ground controls. The tail-suspension model allowed the aspect of weight-bearing to be differentiated from other gravity effects by elevating the rear legs of the animal during the control experiment, and hence removing the animal's weight from the bones to be examined.

Approach or Method
Weight-bearing, suspended, and microgravity (flight) environments were used to study fracture repair. The five-day flight allowed the comparison of early stages of callus formation using histologic criteria. Seven-month-old rats underwent a right midshaft fibular osteotomies. A bone saw and 0.8 mm bur were used in performing the osteotomies. The time of suspension was equal to the time inflight, and postflight all groups were euthanized simultaneously. Histologic examination of the fracture was performed on the injured fibula from each rat.

Results
In each of the three groups, a callus had formed. Angiogenesis (AG), primitive mesenchymal cell (PMC) ingrowth, chondrogenesis and periosteal new bone characterized the calluses from weight-bearing bones. Calluses from the suspended group were not as well developed, in that AG, PMCs and cartilage cells were dominant; there was no new bone in the calluses or periostea. Fracture healing in the flight group was the least well developed: predominant features were AG and PMC ingrowth; chondrogenesis and periosteal activity were minimal; and there was no new bone formation. Comparing all three environments, it was concluded that weight-bearing has a positive effect on callus formation, while the suspended and weightless environments delay bone fracture healing. This delay indicates the need for investigation of the physiological parameters that contribute to the process of fracture healing in these environments.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study

Morphometric and EM Analyses of Tibial Epiphyseal Plates From Cosmos 2044 Rats

Science Discipline

Bone and Calcium Physiology

Investigator	Institute
P.J. Duke	University of Texas Dental Branch

Co-Investigator(s)	Institute
Montufar-Solis, D.	University of Texas Dental Branch
Durnova, G.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Duke, P.J.; Durnova, G.; Montufar-Solis, D.: Altered Cartilage Differentiation in Tibial Epiphyseal Plates of Cosmos Rats. Proceedings of the 4th European Symposium on Life Sciences Research in Space, V. David, ed., Trieste, Italy, May 28–June 1, 1990, pp. 269–273.

Montufar-Solis, D.; Duke, P.J.; and Durnova,G.: Spaceflight and Age Affect Tibial Epiphyseal Growth Plate Histomorphometry. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S19–S25.

Objectives/Hypothesis

Previous studies have shown that spaceflight has significant effects on cartilage differentiation within the growth plate, changes that could lead to the observed decreases in linear bone growth and trabecular bone mass. In this study, growth plates of rats flown aboard Cosmos 2044 were analyzed by the same histomorphometric and electron microscopic methods used for other flights and compared to appropriate controls.

Approach or Method

Epiphyses of right tibias were cut from the shaft just above the tibial crest and then split in half in the sagittal plane. One half was decalcified and split again in the sagittal plane; outer portions were embedded for electron microscopy, and middle portions were embedded for light microscopy. The undecalcified half was also split in the sagittal plane; the inner portion was embedded for differential staining of calcified cartilage and the outer portion was split for immunohistochemistry and microprobe studies. Area, perimeter, and shape factor were determined by computerized planimetry. Height and cell number per zone and plate were determined at the light microscopy level. Collagen fibril size and proteoglycan granules per area were determined from micrographs measured on a digitizing tablet.

Results

Flight rats had an increase in height of the proliferative zone (PZ) that was significantly greater than vivarium controls, and a decrease in the hypertrophic, calcification zone (HZC) that differed significantly from all controls. Cell number in the PZ was significantly greater than synchronous controls, and a decrease in cell number in the HZC was significantly different from vivarium animals. Computerized planimetry studies showed no differences between any of the groups with regard to plate area, perimeter, or shape factor. Electron microscopy studies found no difference in collagen fibril length and width, perhaps due to alteration of fibrils with age. Similarly, the lack of response in tail-suspended animals was also attributed to age.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study

Bone Biochemistry and Mineral Distribution in the Femurs of Rats after Two Weeks in Space: Circulating Parathyroid Hormone, Calcitonin, and Osteocalcin

Science Discipline

Bone and Calcium Physiology
Endocrinology

Investigator	Institute
S.B. Arnaud	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Fung, P.	NASA Ames Research Center (ARC)
Popova, I.A	Institute of Biomedical Problems
Morey-Holton, E.R.	NASA Ames Research Center (ARC)

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Arnaud, S.B.; Fung, P.; Popova, I.A.; Morey-Holton, E.R.; and Grindeland, R.E.: Circulating Parathyroid Hormone and Calcitonin in Rats After Spaceflight. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S169–S173.

Objectives/Hypothesis

The functional state of the parathyroid gland and the calcitonin cells of the thyroid is critical to the alterations in calcium and bone metabolism during spaceflight because of the major role each hormone has in their regulation. The purpose of this experiment was to document the early postflight levels of two calcium-regulating hormones. Postflight evidence for impaired calcium homeostasis from exposure to spaceflight, as well as the status of these hormones, was reported.

Approach or Method

Blood was collected through a heparinized funnel after decapitation and the plasma separated. Parathyroid hormone (PTH) and calcitonin (CT) were measured by radioimmunoassay kits, modified for samples of 50 μl, and compared to appropriate controls. The parathyroid hormone assay uses an antibody raised to an N-terminal fragment of human hormone and human standard. The calcitonin assay used an antibody raised to synthetic human hormone, human standards, and tracer. Analysis techniques included student's t-test, analysis of variance, and regression analysis computer programs.

Results

Measurable differences in opposite directions for apparently unrelated reasons were found in plasma concentrations of both PTH and CT. Slight increases in flight PTH, compared to synchronous animals, were indicative of mild hyperparathyroidism, most likely related to impaired kidney function, supported by increases in serum magnesium, phosphorous, creatinine. Plasma CT, on the other hand, in 14-week-old rats, failed to show the normal increase with age after 2 weeks of tail suspension, similar to the postflight result. Postflight circulating levels of PTH appear to reflect disturbances in calcium homeostasis from impaired renal function and of CT in growth connected with the flight. Bone biochemistry revealed mineral deficits and changes in osteocalcin and reducible cross-links in the distal femoral diaphysis. Of interest were similar changes in cross-links but not mineral composition in five controls.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Gravity and Skeletal Growth: I. Gravity and Skeletal Growth

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
E.R. Morey-Holton	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Durnova, G.	Institute of Biomedical Problems
Kaplansky, A.S.	Institute of Biomedical Problems
Doty, S.B.	Columbia University

Research Subject(s)
*Rattus norvegicus (*Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Morey-Holton, E.; Globus, R.K.; Kaplansky, A.; and Durnova G.: The Hindlimb Unloading Rat Model: Literature Overview, Technique Update and Comparison With Space Flight Data. Advances in Space Biology and Medicine, vol. 10, 2005, pp. 7–40.

Objectives/Hypothesis
Experiments flown on Cosmos 1887 were complicated by unexpected postflight processing delays. To differentiate between flight response with minimal recovery time and flight response with extended recovery superimposed, this experiment was repeated with the addition of a tail-suspended model as a control group. Data from this study are difficult to compare with that from Cosmos 1887 due to a 25-day age difference; the larger bones of these animals agree with the older age of the rats.

Approach or Method
Bone area and perimeter were measured at the tibiofibular junction of space-flown and ground-control rats. Bone vascularity and bone cells within the tibial diaphysis, and collagen fibrils in the tendons of the foot, were studied at the light and electron microscope level. The portion of the tibial shaft immediately proximal to the tibiofibular junction was sawed into 50-μm cross sections, mounted on slides, and exposed to incident and polarized light.

Results
Visual observations of tibial cross sections under brightfield or polarized light did not show any obvious differences. Likewise, area and periosteal perimeter measurements showed no significant differences. The lack of any increase in bone mass during the flight period indicates that animals were adults; thus bone mass was not accumulating rapidly during the flight period. Larger, adult rats may require a longer flight period to demonstrate bone changes, particularly in cortical bone since the skeleton is turning over more slowly.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study

Gravity and Skeletal Growth: II. Morphological Studies of Bone and Tendon

Science Discipline

Bone and Calcium Physiology

Investigator	Institute
S.B. Doty	Columbia University

Co-Investigator(s)	Institute
Durnova, G.	Institute of Biomedical Problems
Kaplansky, A.S.	Institute of Biomedical Problems
Morey-Holton, E.R.	NASA Ames Research Center (ARC)

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Doty, S.B.; Morey-Holton, E.R.; Durnova, G.N.; and Kaplansky, A.S.: Morphological Studies of Bone and Tendon in Post Spaceflight Rats. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. 10S–13S.

Objectives/Hypothesis

Previously described results from Cosmos 1887 noted that drastic changes occurred in the vascular system within the diaphyseal bone, evidently in response to the microgravity experience. In addition to the study of the vascular supply to the periosteal region and the determination of osteoblast activity, this study also investigated the structural integrity of the rat foot. This tissue is sensitive to changes in mechanical stress, and it was thought that the reduced activity imposed by spaceflight might also cause structural changes in these tendons.

Approach or Method

Electron and light microscopy, histochemistry, and morphometric techniques were combined in this study to determine the effects of microgravity on bone cells and the vasculature within the diaphysis of the rat tibia. Silver staining of 3- to 5-μm sections was carried out to visualize collagen fibers. Osteoblasts were stained for acid phosphate activity to outline the Golgi complex. Cytoplasmic RNA was stained with pyronine so the Golgi complex free of RNA could be visualized by negative contrast. Measurements were made at 320x so that the Golgi presence could be easily determined.

Results

Vasculature changes at the periosteal and sub-periosteal region were not apparent. Electron microscopy showed that vascular inclusions were present in flight rats; however, the blood vessels themselves appeared undamaged. Electron microscopy of the tendons of the foot showed some collagen fibril disorganization but this was not noticeable by light microscopy. Investigations of osteoblasts lining the endosteal surface indicated a reduction in activity, but morphometric measurements suggested that these alterations were not significantly different from controls. The general absence of vascular changes in this study is interesting because this flight was similar in many respects to Cosmos 1887, one notable difference being the almost 55-hour delay before recovery of 1887. This raises the possibility that vascular changes could have occurred when the animals became weight bearing following reentry. Following a non-weight-bearing period, the resumption of full weight bearing may be detrimental to the vascular system, especially at the periosteal surface of the bone.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Gravity and Skeletal Growth: III. Recovery of Osteogenic Potential

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
L.P. Garetto	Indiana University

Co-Investigator(s)	Institute
Morey-Holton, E.R.	NASA Ames Research Center (ARC)
Roberts, W.E.	Indiana University
Durnova, G.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Garetto, L.P.; Morey, E.R.; Durnova, G.N.; Kaplansky, A.S.; and Roberts, W.E.: Preosteoblast Production in Cosmos 2044 Rats: Short-Term of Osteogenic Potential. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S14–S18.

Nuclear Volume Analysis of Osteoblast Histogenesis in the Rat Tibial Metaphysis During Simulated Weightlessness. Fundamentals of Bone Growth, A.D. Dixon, B.G. Sarnat, and D.A.N. Hoyte, eds., 1991, pp. 397–405.

Objectives/Hypothesis
Data from previous spaceflight missions suggest an initial inhibition of osteoblast histogenesis followed by a postflight recovery response. This study of rat maxillary molar periodontal ligament (PDL) allowed further investigation of the osteoblast histogenesis recovery pattern.

Approach or Method
Samples were demineralized, embedded in plastic, sectioned (4 μm) in the mid-sagittal plane of the medial root of the first molar, and stained with hematoxylin and eosin. An ocular micrometer was used to measure the major and minor nuclear dimensions of 100 cells in each PDL under oil immersion at 1,000x. Nuclear volume for each cell sampled was calculated according to the formula for a prolate spheroid. The fractional distribution of each cell type was expressed as group mean ±SEM. PDL width was also measured at three levels within the mid-root region of the medial root of the first molar.

Results
A comparison of PDL cell populations from the control groups showed no significant differences. Similarly, no differences were seen in any of the nuclear volumes or observed in the width of the PDL. Compared to previous spaceflight experiments, the data are consistent with a postflight response to replenish preosteoblast and restore osteogenic potential. The lack of difference at the sampling time of this study coincides with a crossover point (a point where cell kinetic compartment size passes through the normal range on its way from a suppressed value to a supercompensated level) in the recovery response process.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Mineral Distribution and Balance in Rats During Spaceflight

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
C.E. Cann	University of California, San Francisco

Co-Investigator(s)	Institute
Serova, L.V.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal, Vivarium, Synchronous, Tail-Suspended; Pooled Control Samples; Cosmos 1887 Pooled Basal Control Samples

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Cann, C.; Patterson-Buckendahl, P.A.; Durnova, G.; and Kaplansky, A.: Mineral Distribution and Balance in Rats During Spaceflight. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 1, J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-108802, 1994, pp. 225–233.

Objectives/Hypothesis
Previous studies of mineral distribution and homeostasis have shown significant effects of spaceflight on the mineral composition of bones and the handling of major trace minerals by the intestine. Data from Cosmos 1129 and 1887 have shown a significant increase in fecal ash, predominantly due to progressive increases of excreted sodium and potassium, and to a lessor extent, calcium. Analyses of vertebrae from Cosmos 1887 further suggested that flight bones were more highly mineralized, consistent with other spaceflight studies. This experiment was primarily to add supplementary and supporting data to these findings, especially for the excreta analyses where measurements were made from pooled samples.

Approach or Method
Fifth lumbar vertebrae were split into two segments; separate segments were lyophilized and weighed, and then analyzed as separate samples. In contrast to samples from Cosmos 1887, only isolated vertebral bodies were received; therefore, regional analysis of the vertebral bodies vs. the posterior elements could not be done. Samples of pooled excreta from flight and control groups, each representing half of the total pool for that group, were cleaned, mixed well, and dried. Two random samples (~5 g each) were taken for analysis. Aliquots were weighed, dried, and reweighed to determine wet/dry weight ratio for the sample. Ashing occurred at 500°C for 48 hours, and the residue was weighed and dissolved in ultra pure nitric acid for element analysis. Calcium, magnesium, zinc, manganese, and copper were analyzed by atomic absorption spectrophotometry, and phosphorus by ammonium molybdate procedure. Analyses were done in duplicate and results were averaged. Samples from Cosmos 1887 basal group were included as control for comparisons between flights.

Results
Bones from flight animals were somewhat smaller than those of synchronous, and significantly smaller than those of vivarium animals, due to variance among synchronous rats. Some of this variance may have resulted from an inconsistent separation of posterior elements from the vertebral bodies, due to the fact that the whole vertebral body was not received. This variance also seemed to have prevented significant results in calcium and osteocalcin concentrations between rat bones. Among fecal samples, there were some inconsistent differences in trace element concentrations, probably attributable to dietary differences among groups. No major differences were found between flight and control groups, although calcium, phosphorus, and magnesium were lower in the basal group. Dietary differences complicated comparisons with Cosmos 1887 results. However, total excreted mass was higher in flight animals, suggesting that the intestine is much less efficient in metabolizing the organic components of the diet, even while maintaining reasonable efficiency for the mineral components.

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Bone, Calcium, and Spaceflight

Science Discipline
Bone Physiology

Investigator	Institute
E.R. Morey-Holton	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Cann, C.E.	University of California, San Francisco
Doty, S.B.	Hospital for Special Surgery
Roberts, W.E.	Indiana University
Vailas, A.C.	University of Wisconsin

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)
Animal Enclosure Module (AEM)

Selected Publications
Morey-Holton, E.; Cann, C.E.; and Doty, S.B.: Biomineralization and Spaceflight. American Gravitational and Space Biology, vol. 6, no. 1, 1992, p. 99.

Doty, S.B.: Space Flight and Bone Formation. Materwiss Werksttech, vol. 35, no. 12, Dec. 2004, pp. 951–961.

Objectives/Hypothesis
The purpose of this experiment was to delineate the early changes that occur in both weight-bearing and non-weight-bearing bone tissues in growing animals in different cage configurations during spaceflight, and to relate those changes to alterations in cell proliferation and mineralization, bone subcellular characteristics, and bone biomechanics. Other objectives were to delineate the early changes that occur in both weight-bearing and non-weight-bearing bone tissues in growing animals, in different cage configurations during spaceflight, and to relate those changes to alterations in calcium metabolism.

Approach or Method
Bone markers for measuring mineralization rates were injected intraperitoneally into all animals before and after launch. Bone mass and length were measured. Bone tissues were processed for bone progenitor populations and matrix synthesis using histomorphometric and autoradiographic techniques. Alkaline phosphatase and Golgi activity of the osteoblasts and perivascular cells were investigated in humoral heads. Femur, L1 vertebra, and calvaria were processed for mineralization. Nuclear volume of osteablast cells was investigated in the maxilla. Humerus, tibia, and L4 vertebra were processed for density, calcium, collagen parameters, and mechanical properties.

Results
Group-housed rats (AEM) had fewer bone changes and a faster recovery than singly housed animals. Bone mineralization rates showed significant suppression at the periosteal, but not corticoendosteal, surface during flight, and singly housed flight rats showed a greater suppression. Structural properties indicated that the flight had little effect on the humerus of either singly or group-housed rats. Also, not all regions of the bones, or all bones, were affected by flight; in the long bones, the periosteal surface showed suppression of formation while the endosteal surface showed little change, and no changes were noted in the ribs, calvaria, vertebra, or maxilla, suggesting that the response to spaceflight is not uniform throughout the skeleton.

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Bone Biomechanics

Science Discipline
Bone Physiology

Investigator	Institute
A.V. Bakulin	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Morey-Holton, E.R.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Bakulin, A.V. and Morey-Holton, E.R.: Bone Biomechanics. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, p. 31.

Objectives/Hypothesis
Study of mechanical properties of mammalian bones performed previously in Cosmos flights and ground-based simulation experiments has demonstrated variations in bone strength and deformability during and after exposure to microgravity. This experiment studied mineralization parameters and mechanical properties of bones in response to repeated cyclic loading in rats after spaceflight.

Approach or Method
Bone mechanical properties were examined using samples prepared from the head of femur. The stress-deformation curve was recorded simultaneously with sample compression in every cycle. Tests were discontinued when the sample was destroyed. Proximal and distal epiphysis of the femur were put into 100% ethanol to determine porosity. Samples were then exposed to dry ashing, and the ash residue was weighed for volume content of the mineral component in bone.

Results
No significant changes of the general indices of mineralization were found; the results obtained speak only of a trend towards a decreased mineralization compared to the control. Meanwhile, a significant deterioration of mechanical bone properties (bone as a material) was observed. The preliminary analysis of the cyclic compression results revealed significant differences in the bone tissue behavior after the 9-day period of the postflight adaptation; these differences might be attributed to the increase of the low mineralized young structures' content in bone tissue.

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Metabolic and Structural Changes in Bone and Systems Regulating Bone Growth and Metabolism

Science Discipline
Bone Physiology

Investigator	Institute
A.S. Kaplansky	Institute of Biomedical Problems (IMBP)

Co-Investigator(s)	Institute
Morey-Holton, E.R.	NASA Ames Research Center (ARC)
Durnova, G.	Institute of Biomedical Problems
Popova, I.A.	Institute of Biomedical Problems
Ilyina-Kakueva, E.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Kaplansky, A.S.; Popova, I.A.; and Morey-Holton, E.R.: Metabolic and Structural Changes in Bone and Systems Regulating Bone Growth and Metabolism. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, p. 31.

Objectives/Hypothesis
Studies of the effects of short-term exposure to weightlessness on rat bone yielded ambiguous results. For instance, Cosmos-1667 experiments revealed distinct signs of osteoporosis of the spongiosa of proximal metaphyses, while the SL-3 flight study of proximal metaphyses of rat humerus bones did not show clear indications of spongy bone osteoporosis. This study was conducted as a comprehensive morphological and biochemical investigation of changes in bones, and systems regulating bone metabolism, at an early stage of adaptation to microgravity. The experiment focused on bones, blood plasma, and endocrine systems that participate in bone metabolism regulation.

Approach or Method
Content of cyclic nucleotides and activity of acid and alkaline phosphates were determined. Limb bones and lumbar vertebrae were subjected to histomorphometric examinations. Elementary analysis of bones included trace elements and were conducted by the neutron-activation method. In the blood plasma, calcitonin, parathyroid hormone, corticosterone, calcium, sodium, potassium, phosphorus, and acid and alkaline phosphates were biochemically measured. The thyroid gland (C-cells) and adrenopituitary gland were also removed, and histologic and histomorphometric examinations were performed.

Results
Results demonstrate the appearance of initial minor signs of the developing osteoporosis in the spongiosa of proximal metaphyses of tibiae, represented as a decrease in volume density of secondary spongiosa and an increase of bone resorption surface. Such changes correlate with biochemical data demonstrating a tendency towards a decrease in alkaline phosphatase activity (an enzyme of bone formation) and an increase in activity of tartrate-resistant acid phosphatase (an enzyme of bone resorption). Neutron-activation analysis revealed a decreased bone content of such macroelements as calcium, phosphorus, sodium, and chloride, which, in accord with a depressed functional activity of thyroid C-cells producing calcitonin, is necessary for normal mineralization of bone matrix. In accord with previous studies, higher calcium and lower phosphorus blood content confirmed mineral metabolism disturbances. In the pituitary, a depression of somatotrophic activity occurred (a decrease of synthesis and secretion of growth hormone).

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Osteogenesis: Tissue Factors of Regulation

Science Discipline
Bone Physiology

Investigator	Institute
V.S. Oganov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Kabitskaya, O.E.	Institute of Biomedical Problems
Morey-Holton, E.R.	NASA Ames Research Center (ARC)
Sumarokov, D.D.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Oganov, V.S. and Morey-Holton, E.R.: Osteogenesis: Tissue Factors of Regulation. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, p. 32.

Objectives/Hypothesis
Bone physiological restructuring at a local level is regulated by short-distant factors that are synthesized by bone cells and released as a result of destruction of extracellular bone organic matrix. This experiment studied osteogenic potentials and the activity of osteo-induction inhibitor in the bone of space-flown rats, with special attention to bone morphogenetic protein (BMP).

Approach or Method
Osteo-inductive activity of bone matrix was determined, and the biological activity of osteo-induction inhibitor in the bone was measured. The concentration of BMP was evaluated with respect to the degree of ectopic osteogenesis induced by its demineralized matrix, determined in relation to the inhibition, or degree of inhibition, of ectopic osteogenesis produced by a standard matrix. A method of induction of ectopic osteogenesis by femur demineralized matrix of flight and control rats (donors) was used; the concentration of elements in the mineral component was determined after implantation.

Results
Results suggest that in space osteo-inductive activity of bone matrix increases but remains unaltered in qualitative terms. The amount of de novo generated bone was not large in recipient rats (less than in the controls) but the level of its mineralization was significant. At R+9, osteo-inductive potentials of the matrix decreased and inhibitory activity increased, meaning bone regenerative potentials declined, thus stimulating osteoporosis.

Launch Date 1/22/1992 **Landing Date** 1/30/1992

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Chondrogenesis in Micromass Culture of Mouse Limb-bud Mesenchyme Exposed to Microgravity (CELLS)

Science Discipline
Bone Physiology

Investigator / Institute
P.J. Duke — University of Texas Dental Branch

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse) embryo cells

Ground-Based Controls
Delayed 2-Hour Controls

Key Flight Hardware
Biorack, Passive Thermal Conditioning Unit (PTCU)

Selected Publications
Duke, J.P.: Teratogenic Effects of Gravity Change: the Concept of Allometric Growth. The Physiologist, supl., vol. 36, no. 1, 1993, pp. S34–S37.

Duke, P.J.; Montufar-Solis, D.; and Daane, E.: Teratogenic Effects of Gravitational Changes. Advances in Space Research, vol. 14, no. 8, 1994, pp. 281–287.

Duke, J.P. and Montufar-Solis, D.: Proteoglycans in Micromass Cultures of Embryonic Mouse Limb Mmesenchymal Cells: Preliminary Studies for the “CELLS” Spaceflight Experiment. The Physiologist, supl., vol. 35, no. 1, Feb. 1992, pp. S41–S42.

Objectives/Hypothesis
The effect of microgravity on cartilage development is important due to the critical role of chondrogenesis in skeletal development through endochondral ossification. In vivo studies have indicated altered cell kinetics in microgravity, but systemic effects are a contributing factor. The cells experiment was designed to determine whether cells sensitive to microgravity in vivo would retain their sensitivity in vitro. The hypothesis was that cell cultures in space would produce less cartilage, less Type II collagen, and less cartilage proteoglycan, and that the aggregative state of the collagen and proteoglycan cells produced would be different ultrastructurally from that of centrifuged cells.

Approach or Method
Cultures were obtained from the hind- and forelimbs of rat embryos. Cultures were exposed to four different experimental conditions: microgravity (in-flight), 1g simulated in flight, 1g ground, and 1.4 g ground centrifuge. Samples were taken every 24 hours and frozen for further biochemical analysis. Samples were examined postflight using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Other samples were stained with Alcian Blue and examined using light microscopy to determine cartilage growth and the size and number of nodules per unit area. Immunohistochemistry was performed to locate and study the relation between proteoglycans and collagens. Enzyme-linked-immunosorbent-assays (ELISA) were performed to determine collagen II production.

Results
Significant detachment of cell layers occurred in all groups. In areas where layers were intact, nodules formed of varying sizes but with no difference between flight and ground cultures. SEM of samples show definite differences between ground and flight cultures by day four. Flight cultures formed aggregates of cells with abnormally smooth surfaces. Cultures also exhibited unusual ruffling structures ranging in complexity from a single sheet to a rosette. TEMs show that within flight cell cultures, cells did undergo the shape change from flattened to rounded that is associated with chondrogenesis, but the associated proliferation of RER and production of matrix did not occur.

STS-52 / PSE.02

Launch Date 10/22/1992 **Landing Date** 11/1/1992

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Effect of Weightlessness on Bone Histology, Physiology, and Mechanics

Science Discipline
Bone Physiology

Investigator	Institute
W.W. Wilfinger	Pennsylvania State University

Co-Investigator(s)	Institute
Rodan, G.A.	Merck & Co., Inc.
Hymer, W.C.	Pennsylvania State University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Controls, Vivarium Controls

Key Flight Hardware
Ambient Temperature Recorder (ATR)

Selected Publications
Borkowski, G.L.; Wilfinger, W.W.; and Lane, P.K.: Laboratory Animals in Space Life Science Research. AWIC Newsletter, 1995, vol. 6, pp. 1–7.

Goodwin, T. and Powers, M.: Logistics of Maintaining Rats on a NASA Space Shuttle. 5th FELASA Symposium, Brighton, UK, June 8–11, 1993.

Objectives/Hypothesis
Degenerative changes observed in the musculoskeletal systems of astronauts and experimental animals during prolonged exposure to weightlessness parallel the slower changes in bone and muscle mass seen during the aging process on Earth. This experiment aimed to use this similarity to test the effectiveness of a Merck & Co. proprietary compound (MK-217) in preventing bone loss, for possible future use in treating disuse osteoporosis. The morphological and physiological effects of MK-217 on bone formation and resorption during a 9-day spaceflight were measured. This experiment also used the data collected to analyze the effectiveness of the bone unloading experienced during microgravity exposure as a model for disuse osteoporosis.

Approach or Method
Prior to flight, rats received injections of either MK-217 or a vehicle control. All rats received injections of the bone marker calcein. Postflight, all rats received injections of a second bone marker, oxytetracycline. Urine, blood, fecal, and oropharyngeal samples were taken. The animals were then euthanized and immediately shipped to the commercial laboratory for further analysis of the effects of microgravity and the proprietary compound on cortical bone formation and resorption.

Results
The Merck compound was reported to significantly reduce microgravity-induced endocortical bone resorption in comparison with saline-treated controls. Periosteal bone formation was significantly reduced in the flight animals and the delayed synchronous control group (housed in flight hardware) compared to the 22°C vivarium controls. Either the elevated temperature in the orbiter or the flight hardware appeared to contribute to decreased bone formation.

Cosmos 2229 / Bion 10

Launch Date 12/29/1992

Landing Date 1/10/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Calcium Metabolism and Correlated Endocrine Measurements in Primates During Cosmos

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
C.E. Cann	University of California, San Francisco

Co-Investigator(s)	Institute
Buckendahl, P.	University of California, Santa Cruz
Arnaud, C.D.	University of California, San Francisco

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
Cann, C.E.: Response of the Skeletal System to Spaceflight. Fundamentals of Space Life Sciences, vol. 1, 1997, pp. 83–103.

Patterson-Buckendahl, P.; Kvetnansky, R.; Fukuhara, K.; Cizza, G.; and Cann, C.: Regulation of Plasma Osteocalcin by Corticosterone and Norepinephrine During Restraint Stress. Bone, vol. 17, no. 5, Nov. 1995, pp. 467–472.

Objectives/Hypothesis
Previous Cosmos studies have shown an increase in bone resorption after 5 days of spaceflight. They have also shown a decrease in bone mineralization and growth. These effects may be due to microgravity or the stress of spaceflight. The purpose of this experiment was to examine changes in bone and bone regulation parameters to further understand the mechanisms that regulate bone growth and strength.

Approach or Method
Two male Rhesus monkeys were flown on the biosatellite Cosmos 2229. Measurements were taken on both flight monkeys and ground control monkeys. The length of the tibia, radius, and ulna were measured at pre- and postflight intervals to determine changes in bone length. Serum levels of calcium, parathyroid hormone, osteocalcin, and 25 hydroxy vitamin D were measured pre- and postflight to determine regulatory factors that may affect bone parameters.

Results
Cosmos 2229 endocrine studies suggest an increase in serum calcium immediately postflight, but it is not as convincing as the data from human studies obtained in flight. There is a clear decrease in 25 (OH) D during the postflight period, but beginning later, not immediately postflight. This may be a response to changes in dietary intake of vitamin D during the flight and postflight periods. There was no change in serum osteocalcin (an indicator of bone formation), but this parameter is not as sensitive as histomorphometry for quantification of changes in bone formation.

Cosmos 2229 / Bion 10

Launch Date 12/29/1992 **Landing Date** 1/10/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Bending Stiffness of the Tibia in Young Rhesus Monkeys After 2 Weeks in Space

Science Discipline
Bone Physiology

Investigator	Institute
S.B. Arnaud	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Hutchinson, T.	NASA Ames Research Center (ARC)
Bakulin, A.V.	Institute of Biomedical Problems
Steele, C.R.	Stanford University

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
Arnaud, S.B.; Hutchinson, T.; Bakulin, A.V.; and Steele, C.R.: Bending Stiffness of the Tibia in Young Rhesus Monkeys after Two Weeks in Space Aboard the Cosmos 2229 Biosatellite. Final Reports of the U.S. Experiments Flown on the Russian Biosatellite Cosmos 2229. J.P. Connolly, M.G. Skidmore, and D.A. Helwig, eds., NASA TM-110439, Apr. 1997, pp. 67–80.

Hutchinson, T.M.; Arnaud, S.B.; Bakulin, A.V.; Emery, J.: Right and Left Tibia EI (Bending Stiffness) Differences in Rhesus Monkeys (abstract). Aviation, Space, and Environmental Medicine, vol. 64, no. 5, May 1993, p. 453.

Objectives/Hypothesis
Localized demineralization has been documented in the proximal tibia of young monkeys after 2 weeks of spaceflight. It is not known whether this is the result of microgravity effects or of chair restraint during flight. It is also not known if the acquired mineral deficit in the localized area impairs the function of the whole tibia and its loading capability. The purpose of this experiment was to assess the effects of inactivity during chair restraint and spaceflight through the analysis of bending stiffness measurements obtained from intact flight and ground-control monkeys.

Approach or Method
Two male Rhesus monkeys were flown on the unmanned Russian biosatellite Cosmos for 11.5 days. Cross-sectional bone stiffness measurements (EI, Nm^2) were taken from the tibia of flight monkeys 7 days before launch, the day of launch, the day of landing, and 7, 20, and 30 days after landing. Measurements were also made on five ground-control monkeys in restraint chairs built to resemble biosatellite conditions. Measurements were made using the Mechanical Response Tissue Analyzer (MRTA). The MRTA applies a low-frequency vibration with a magnetic shaker to the center a long bone. The resonant response is used to compute bending stiffness.

Results
When all EI values were combined, EI was higher in the right tibia than the left. An average EI decrease of 33% was seen in ground-control monkeys after 2 weeks of chair restraint and was still 28% lower than basal one week later. Flight monkey #906 showed a maximum decrease of 24% in EI in the right tibia and a 37% decrease in EI of the left tibia on tests performed 20 days postflight and compared to tests performed 45 days postflight. Flight monkey #151, which was dehydrated and did not eat well during flight, showed a 7% decrease in EI of the right tibia 7 days postflight, and an increase of 4%, 20 days postflight. The left tibia showed a 35% increase in EI 7 days after recovery.

Cosmos 2229 / Bion 10

Launch Date 12/29/1992 **Landing Date** 1/10/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
DEXA Measurements: Cosmos 2229 Rhesus Flight Experiment

Science Discipline
Bone Physiology

Investigator	Institute
A. LeBlanc	Baylor College of Medicine

Co-Investigator(s)	Institute
Evans, H.	Baylor College of Medicine
West, S.	Baylor College of Medicine
Shackelford, L.C.	NASA Johnson Space Center (JSC)
Bakulin, A.V.	Institute of Biomedical Problems
Oganov, V.S.	Institute of Biomedical Problems
Rakhmanov, A.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
Zerath, E.; Novikov, V.; and LeBlanc, A.: Effects of Spaceflight on Bone Mineralization in the Rhesus Monkey. Journal of Applied Physiology, vol. 81, no. 1, July 1996, pp. 194–200.

Objectives/Hypothesis
Previous spaceflights have documented that significant bone and muscle atrophy occur during weightlessness. In addition to bone and muscle loss, renal stones may also form during flight. These effects may require that countermeasures be taken for their prevention. It is hypothesized that short-duration weightlessness will decrease bone remodeling and that this decrease will be apparent as a decrease in bone mineral immediately after flight or during the first few weeks following flight. Further, it is hypothesized that short-duration weightlessness will result in significant muscle atrophy that will be rapidly recovered following return to one G gravity. The objective of this experiment was to determine, using regional and whole body dual energy x-ray absorbtionetry (DEXA), if bone and lean body mass are reduced in the Rhesus monkey after exposure to short-duration weightlessness.

Approach or Method
Three groups of monkeys were used for this experiment; chaired control, caged control, and flight groups. DEXA scans were performed on flight monkeys at the following four times; 53 days before launch, and 3, 16, and 33 days after launch. Five body regions were statistically analyzed for changes in bone mineral density (BMD), bone mineral content (BMC), lean body mass, and body fat. For analysis, data for the chaired control group was combined with the caged control group, resulting in two groups, control and flight. The mean difference of the two groups was compared for each measurement type in each region.

Results
There was no evidence for bone loss through BMC or BMD values during the flight period. There was evidence that BMC and BMD may have increased during the reambulation period relative to control animals. Results show evidence of lean tissue loss in the arms and legs of flight monkeys as compared to controls. The total body change in lean tissue was not statistically significant. During the reambulation postflight period there was evidence of increased lean mass in flight animals relative to controls. There was no significant change in body fat measurements.

Launch Date 1/13/1993 **Landing Date** 1/19/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Spaceflight Changes in Skeletal mRNA

Science Discipline
Bone Physiology

Investigator	Institute
D.D. Bikle	Veterans Administration Medical Center

Co-Investigator(s)	Institute
Halloran, B.P.	Veterans Administration Medical Center
Harris, J.	Veterans Administration Medical Center
Morey-Holton, E.R.	NASA Ames Research Center

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control, Tail-Suspension Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Kostenuik, P.J.; Harris, J.; Halloran, B.; Turner, R.; Morey-Holton, E.R.; and Bikle, D.D.: Skeletal Unloading Causes Resistance of Osteoprogenitor Cells to Parathyroid Hormone and to Insulin-Like Growth Factor-I. Journal of Bone Mineral Research, vol. 14, 1999, pp. 21–31.

Bikle, D.D.; Morey-Holton, E.R.; Doty, S.B.; Currier, P.A.; Tanner, S.J.; and Halloran, B.P.: Alendronate Increases Skeletal Mass of Growing Rats During Unloading by Inhibiting Resorption of Calcified Cartilage. Journal of Bone and Mineral Research, vol. 9, no. 11, Nov. 1994, pp. 1777–1787.

Objectives/Hypothesis
Spaceflight can lead to osteopenia in developing rats through decreased bone formation, inhibiting mineralizartion, and delaying maturation. This may be a result of decreased osteoblast differentiation, decreased calcium to hydroxyproline ratio, or decreased osteocalcin levels in bone and serum as observed in previous spaceflight studies. Of interest in osteoblast differentiation is the observed increase in alkaline phosphatase messenger ribonucleic acid (mRNA) during matrix production and the subsequent increase in osteocalcin mRNA during mineralization. A decrease in mRNA levels of insulin-like growth factor and its receptor has also been observed. This turning on and off of gene expression may be a regulating factor in bone development and was the subject of this study.

Approach or Method
The femora, tibia, and the region from the midtibial plateau to the femoral metaphysis were taken from flight and ground control rats for analysis. Northern analysis was performed to measure the mRNA levels of alkaline phosphatase and osteocalcin. Solution hybridization/ribonuclease (RNase) protection analysis was performed to measure the IGF-I and IGF-IR mRNA levels.

Results
Spaceflight transiently increased mRNA levels for IGF-I, IGF-IR, and alkaline phosphatase but decreased the mRNA levels for osteocalcin. The ratio of alkaline phosphatase to osteocalcin mRNA levels was 2.2 times higher in flight rats as compared to control rats. The increase in mRNA for IGF-I and IGF-IR was not expected, as spaceflight leads to a reduction in bone formation. However studies have shown an increase in resistance to IGF-I as a response to microgravity. The observed mRNA increase for IGF-I may be a compensation to this resistance. The changes in osteocalcin and alkaline phosphatase mRNA levels are consistent with a shift towards decreased maturation. Data indicate skeletal unloading during spaceflight resets the pattern of gene expression in the osteoblast, giving it a less mature profile.

Launch Date 4/8/1993 **Landing Date** 4/17/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Cell Kinetic and Histomorphometric Analysis of Microgravitational Osteopenia

Science Discipline
Bone Physiology

Investigator / Institute
W.E. Roberts — Indiana University

Co-Investigator(s) / Institute
Garetto, L.P. — Indiana University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Jennermann, C. and Garetto, L.P.: A Comparison of Labeling Methods and Embedding Media for Localizing of S-Phase Osteogenic Cells. Journal of Histotechnology, vol. 20, 1997, pp. 39–44.

Objectives/Hypothesis
The main hypothesis of this experiment was that spaceflight blocks osteoblast formation; however, it rapidly recovers upon return to Earth. The study attempted to gain a better understanding of the way that physiological processes adapt both to microgravity and a return to Earth's environment. In order to achieve this, four goals were established: 1) to study, through the use of a specific marker, DNA synthesis that lead to preosteoblast cell proliferation (this method would yield new results that could build on previous research); 2) to confirm previous findings that suggested the presence of postflight inhibition of osteoblast formation; 3) to determine if the block to osteoblast formation is confined to specific areas of the skeleton; and 4) to determine how long it takes for osteoblast formation to recover after spaceflight.

Approach or Method
The incidence of osteoblast formation sites during a 9-day flight, as well as their rates of apposition at 4–6, 24, and 72 hours after spaceflight were studied. Rat fibroblast-like osteoblast precursor cells from both maxillary molar periodontal ligaments (PDLs) and mandibular condyle cells were analyzed using a nuclear morphometric assay. The PDL cells were placed in ~4M Hematoxylin and stained with eosin to obtain the relative number of preosteoblasts. The cell kinetics of osteoblast histogenesis at 4–6, 24, and 72 hours postflight were assessed to record the path of recovery of osteoblast production.

Results
Analysis of fibroblast-like osteoblast precursor cells in rat PDL yielded a statistically significant reduction in osteogenic precursor formation after a return from a microgravity environment. Production quickly returned to normal levels, and after 24 hours most cell populations were at preflight levels. These findings supported the hypothesis that microgravity inhibited osteoblast formation. However, analysis of the rat mandibular condyle cells showed that microgravity had not significantly affected osteoblast production. This was most likely due to the fact that the cells were growing rapidly, and thus the genetic need to produce new cells outweighed the effects of microgravity. Further study of adult mandibular condyle cells is needed and would be most relevant to human spaceflight, particularly of growing individuals.

STS-56 / PARE.03

Launch Date 4/8/1993 **Landing Date** 4/17/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Acute Adaptation of Bone to Spaceflight

Science Discipline
Bone Physiology

Investigator / Institute
E.R. Morey-Holton — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
Turner, R.T. — Mayo Clinic

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Delayed Synchronous Control, Tail Suspension Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Morey-Holton, E.R.; Whalen, R.T.; Arnaud, S.B.; and Van Der Meulen, M.C.: The Skeleton and Its Adaptation to Gravity. Handbook of Physiology: Environmental Physiology, vol. 1, 1996, pp. 691–720.

Wade, C.E.; Harper, J.S.; Daunton, N.G.; Corcoran, M.L.; and Morey-Holton, E.: Body Mass Change During Altered Gravity: Spaceflight, Centrifugation, and Return to 1G. Journal of Gravitational Physiology, vol. 4, no. 3, 1997, pp. 43–48.

Objectives/Hypothesis
Animals on Earth experience the constant loading of gravitational forces on their skeletal system. These forces are a factor in determining size, shape, and strength of bones. When growing bones are unloaded, maturation is delayed and growth rate is reduced. There may be an increase in size and mass but not in strength. The hypothesis of this experiment was that gravity is necessary for normal development of bone structure and, furthermore, that unloading causes defective bone growth. The expression of the skeletal growth factor TGF-b after exposure to microgravity was also examined.

Approach or Method
Flight rats were sacrificed 4, 24, and 72 hours postflight. Hematoxylin- and eosin-stained sections of the ligament were analyzed using a nuclear morphometric assay. Total cellular ribonucleic acid (RNA) was isolated from long bone periosteum and cancellous metaphysis and characterized by Northern analysis.

Results
Northern analysis showed messenger ribonucleic acid (mRNA) levels for the skeletal growth factor TGF-b reduced by 57% in the periosteum. However, reloading upon return to 1 G caused an increase to 309% of levels found in the ground controls by 24 hours postflight. TGF-b mRNA levels returned to normal by 72 hours postflight. No changes in mRNA levels were observed in the metaphysis. Nearly identical results were found in the simultaneous hindlimb suspension control. These results indicate both a depression in osteoblast precursor cell differentiation caused by microgravity and tissue-specific responses to dynamic weight bearing.

STS-57 / PSE.03

Launch Date 6/21/1993 **Landing Date** 7/1/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Effect of Weightlessness on Tissue Regeneration in Rodents

Science Discipline
Bone Physiology

Investigator	Institute
W.W. Wilfinger	Pennsylvania State University

Co-Investigator(s)	Institute
Kohn, S.R.	Space Dermatology Foundation

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Ambient Temperature Recorder (ATR)

Selected Publications
Grove, D.S.; Pishak, S.A.; and Mastro, A.M.: The Effects of a 10-Day Space Flight on the Function, Phenotype and Adhesion Molecule Expression of Splenocytes and Lymph Node Lymphocytes. Experimental Cell Research, vol. 219, no. 1, July 1995, pp. 102–109.

Staron, R.S.; Kraemer, W.J.; Hikida, R.S.; Reed, D.W.; Murray, J.D.; Campos, G.E.; and Gordon, S.E.: Comparison of Soleus Muscles From Rats Exposed to Microgravity for 10 Versus 14 Days. Histochemistry and Cell Biology, vol. 110, 1998, pp. 73–80.

Objectives/Hypothesis
Little is currently known about the effects of microgravity on tissue repair. In the era of a long-term human presence in space, the probability of minor injury requiring on-orbit treatment will increase. This experiment aimed to evaluate the effects of spaceflight on the histological and tensiometric properties of full thickness abdominal incisional skin wounds in the rat. A second experimental objective was to evaluate the effectiveness of basic fibroblast growth factor (FGF) and platelet-derived growth factor (bb-homodimer, PDGF) in promoting granulation tissue formation and collagen deposition.

Approach or Method
Flight and control rats received preflight abdominal implants of polyvinyl acetal sponge disks containing either recombinant human bFGF, recombinant PDGF-BB, or a placebo. Postflight, animals received an injection of hypothalamic-releasing hormones. After sacrifice, the sponges were removed and prepared for biochemical and histological analysis of DNA, protein, and collagen content. Histological organization, amount of visible collagen, and the resolution of hemorrhage at the infiltrating interface were also examined.

Results
Histological analysis showed that both bFGF and PDGF showed positive effects in the ground-control rats, but only immediate-release bFGF and delayed-release PDGF had significant, positive effects in the flight rats. This may be due to the 2-day launch delay of the Shuttle mission, which caused the growth factor to be released earlier during spaceflight than planned. Although cellular influx into the tissue space of placebo-treated sponges was unaffected by spaceflight, there was a significantly blunted response to either bFGF or PDGF-BB in flight animals. Microgravity significantly reduced wound collagen concentration regardless of the treatment group. The collagen concentration of granulation tissue in flight animals treated with bFGF was significantly less than in those treated with PDGF, but not significantly less than in the placebo treated group. These results show that a highly standardized wound repair process in young rats is significantly altered by spaceflight.

STS-58 / SLS-2

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Bone, Calcium, and Spaceflight

Science Discipline
Bone Physiology

Investigator	Institute
E.R. Morey-Holton	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Doty, S.B.	Columbia University
Roberts, W.E.	Indiana University
Vailas, A.C.	University of Wisconsin, Madison
Cann, C.E.	University of California, San Francisco

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Fox, R.A.; Corcoran, M.; Daunton, N.G.; and Morey-Holton, E.: Effects of Spaceflight and Hindlimb Suspension on the Posture and Gait of Rats. Amsterdam: Elsevier Science Publishers (International Congress Series), vol. 1070, 1994, pp. 603–606.

Durnova, G.; Kaplansky, A.; and Morey-Holton, E.: Histomorphometric Study of Tibia of Rats Exposed Aboard American Spacelab Life Sciences 2 Shuttle mission. Journal of Gravitational Physiology, vol. 3, no. 2, Sept. 1996, pp. 80–81.

Objectives/Hypothesis
The objectives of this experiment were: 1) to determine if exposure to microgravity causes a significant decrease in bone mineralization at the outer surface of limb bones within the first week of flight, and to assess those bone parameters causing or affected by this decrease; 2) to measure activity of osteoblast immediately postflight; 3) to determine if bone mineralization is restored following 2 weeks of recovery from spaceflight; 4) to determine total skeletal and site-specific bone mineralization rates, mineralization and resorption, as well as calcium absorption and excretion; 5) to relate effects of microgravity on bone to changes in calcium metabolism; and 6) to determine gut and renal responses during postflight recovery period.

Approach or Method
Body mass, blood pH, and urine volume were measured. Upon sacrifice, the vertebrae, maxillae, tibias, femurs, humeri, and calvaria were removed and processed. Bone samples were analyzed for bone mineralization rates, alkaline phosphatase activity, bone dimensions, osteoblast populations, matrix and mineral content, and biomechanics. Calcium and crosslink content was determined for the urine as well as bone samples. Bone samples were also analyzed under electron microscopy and 3-D X-ray topographic microscopic images.

Results
Ionic calcium and pH were similar in all groups at the end of the flight period, suggesting that any changes induced by flight had returned to normal prior to the time that the animals were sacrificed. All groups had similar bone length in both front and hindlimbs as well as in the jaw. Bone mineralization on the periosteal surface at the tibiofibular junction was suppressed in the flight rats during the flight period and did not return to normal until the second week of the recovery period. Alkaline phosphatase activity, a marker of bone matrix maturation, was suppressed in the endosteal osteoblast immediately postflight and at 2 weeks post-recovery. These data suggest that bone response to unloading and reloading may be different at different bone sites. Urinary collagen crosslinks were slightly decreased following flight, suggesting that resorption was not dramatically affected during the recovery period. Surprisingly, very few significant changes in bone were noted in these very young, rapidly growing rats (38 days old at launch).

STS-58 / SLS-2

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Cytochemistry and Biochemistry of Hydrolic Enzymes in Osteoclasts and Bones

Science Discipline
Bone Physiology

Investigator	Institute
G. Durnova	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Kaplansky, A.S.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Durnova, G.N.; Kaplansky, A.S.; Morey-Holton, E.R.; and Vorobeva, V.N.: Investigations of Tibial Bones of the Rats Exposed on Board Spacelab-2: Histomorphometric Analysis. Aviakom Ekologie Med, vol. 30, no. 1, 1996, pp. 21–26.

Durnova, G.; Kaplansky, A.; Morey-Holton, E.: Histomorphometric Study of Tibia of Rats Exposed Aboard American Spacelab Life Sciences 2 Shuttle Mission. Journal of Gravitational Physiology, vol. 3, no. 2, Sept. 1996, pp. 80–81.

Objectives/Hypothesis
Previous flight experiments demonstrated reduction of trabecular bone caused by inhibited neoformation and enhanced resorption. However, it was difficult to determine what changes in spongy bone were caused by spaceflight and what were caused by the stress of returning to the Earth's gravity. The objective of this experiment was to differentiate bone changes caused by microgravity from those caused by reentry factors.

Approach or Method
Proximal metaphyses of the tibiae were cut off and separated into equal portions. One half was decalcified and the other was dehydrated and embedded in metacrylate. Nondecalcified bones were used to detect osteoid and acid phosphatase and to measure bone neoformation and resoption levels. Spongy bone was examined histmorphometrically. The amount of spongy bone in the metaphysis was measured. In the primary and secondary spongiosa, the following parameters were determined: spongy bone volume, number of trabeculae, trabecular thickness, and space between trabeculae. In addition, osteoid surface and resorption surface in the secondary spongiosa were estimated. Tibia growth was measured indirectly on the basis of the primary spongiosa width, epiphyseal cartilage growth plate height, and area.

Results
No changes were seen in the growth plate or spongiosa parameters of flight rats (IF), however, rats sacrificed 5 hours after flight (F+0) had significantly lower parameters. There were no changes in the histomorphometric parameters of IF rat spongiosa, but F+0 rats exhibited a decrease in primary spongiosa due to a reduction in trabeculae. Flight rats sacrificed 14 days after landing had primary spongiosa volumes 23% higher than control rats. Secondary spongiosa of IF rats was decreased by 22%. An associated decrease in trabeculae caused the space between trabeculae to increase. The ratio of osteoid surface per bone surface was lower in flight rats than in ground controls. A visual examination of tibia preparations showed IF and F+0 had higher amounts of osteoclasts and exhibited characteristics of high resorptive capability. All results indicate that spaceflight causes changes characteristic of early stages of osteopenia. Bone resorption enhancement and mononuclear osteoclast precursors were a result of gravitational stress.

STS-58 / SLS-2

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Bone Cell Activity During Spaceflight and Recovery

Science Discipline
Bone Physiology

Investigator	Institute
E. Zerath	Centre d'Etudes et de Recherches de Medecine Aerospatiale (CERMA)

Co-Investigator(s)	Institute
Marie, P.J.	INSERM

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Zerath, E; Godet, D; Holy, X; Andre, C; Renault, S; Hott, M; and Marie, PJ: Effects of Spaceflight and Recovery on Rat Humeri and Vertebrae: Histological and Cell Culture Studies. Journal of Applied Physiology, vol. 81, no. 1, July 1996, pp. 164–171.

Objectives/Hypothesis
Microgravity associated with spaceflight has been shown in numerous experiments to be associated with marked skeletal changes, ranging from decrease in bone volume to alterations in biomechamical properties. However, little attention has been given to bone tissue recovery that follows spaceflight after return to Earth's gravity. In addition, the effect of microgravity on bone metabolism at the cell level has only been assessed by histological techniques; studies of osteoblastic cell numbers and differentiation in spaceflight animals have not been performed. This study investigated the effects of microgravity and subsequent recovery on trabecular bone morphology and compared histomorphometric parameters on caudal vertebrae with the behavior of vertebral osteoblastic cells in culture.

Approach or Method
Two groups of five rats were flown on SLS-2; one group was sacrificed after landing, the other after a recovery time equal to the flight (14 days). Histomorphometric measurements were made on caudel vertebrae, thoracic vertebrae and the left humeri. Lengths, volumes, and wet weights were measured. Trabecular volume, thickness, and spacing was measured using vertibral bodies. Osteoid thickness was measured. Osteoid, osteoblast, and osteoclast surfaces were measured. Caudel vertebral bone cells were isolated and cultured. Cultures were evaluated for cell growth and phenotype. The proliferation rate was determined by cell number. Cell alkaline phosphatase activity (ALP) and osteocalcin production, two parameters of osteoblast differentiation, were determined in confluent cells isolated from the caudal vertebrae.

Results
Humeral weight showed normal growth during the experiment and was unaffected by spaceflight or recovery from spaceflight. However, the spaceflight resulted in inhibition of static indexes of bone formation in humeral proximal metaphphysis and thoracic vertebral bodies. This was associated with a decrease in bone volume in humeral metaphysis. After 14 days of on Earth recovery, osteoblastic and osteoid surfaces returned toward normal and bone volume was normalized in humeri, whereas the static bone formation parameters were not restored on thoracic vertebrae. In addition, histological indexes of bone formation and osteoblastic cell growth in vitro were not affected by spaceflight in caudal verebrae. The rat humeri and thoracic and caudal vertebrae exhibit different patterns of response to spaceflight and subsequent on-Earth recovery, which could be due, at least in part, to the different loading pattern of these bones, and to differences in bone turnover rate.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Histomorphometric Analysis of Bone Tissue After Weightlessness Exposure and Recovery

Science Discipline
Bone Physiology

Investigator	Institute
C. Alexandre	Laboratoire de Biologie du Tissu Osseux

Co-Investigator(s)	Institute
Lafage-Proust, M.H.	Laboratoire de Biologie du Tissu Osseux
Vico, L.	Laboratoire de Biologie du Tissu Osseux

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Lafage-Proust, M.H.; Collet, P.; Dubost, J.M.; Laroche, N.; Alexandre, C.; and Vico, L.: Space-Related Bone Mineral Redistribution and Lack of Bone Mass Recovery after Reambulation in Young Rats. American Journal of Physiology, vol. 274, no. 2, pt. 2, Feb. 1998, pp. R324–R334.

Vico, L. and Alexandre, C.: Normalisation of Bone Cellular Responses Occurs between 7 and 14 Days of Simulated Weightlessness in Rats. Physiologist, supl., vol. 32, no. 1, 1989, pp. S25–S26.

Vico, L. and Alexandre, C.: Microgravity and Bone Adaptation at the Tissue Level. Journal of Bone Mineral Research, supl., vol. 7, no. 2, Dec. 1992, pp. S445–S447.

Objectives/Hypothesis
The characteristics of spaceflight-induced bone loss have been the subject of many studies. However, the mechanism of bone mass recovery on Earth is not well understood. Studies examining humans for up to 5 years after a Skylab mission showed that calcaneum bone mineral remained decreased. Additionally, rats from other missions showed incomplete bone mass recovery after a reambulation period longer than the flight itself. The purpose of this study was to examine histomorphometric changes of various bones after a 14-day exposure to weightlessness and after a 14-day recovery.

Approach or Method
After an appropriate time period, the rats were euthanized and the skull, last thoracic vertebra, right humerus, right femur, and right tibia were removed and fixed. The length of each bone was measured using calipers. Bone mineral density was assessed using a dual-energy X-ray absorptiometry in the humerus, femur, and tibia. Using X-ray microanalysis, Ca2+ and P concentrations were measured in the cancellous and cortical portions of the tibia, vertebrae, and parietal bones. Histomorphometric analysis (producing measurements of bone-mass parameters, bone-architecture parameters, osteoblastic activity, and osteoclastic activity of the tibial and humeral metaphyses) was also performed.

Results
Flight animals examined immediately postflight (R+0) showed detectable bone loss in the femoral metaphysis/epiphysis when compared to the appropriate synchronous control group. Osteoclastic surfaces area and number of cells were much higher in tibial secondary spongiosa of R+0 animals, while osteoblastic parameters remained relatively unchanged. Ca2+ and P in R+0 animals were lower in tibia secondary spongiosa and higher in calvaria as compared to synchronous controls. Bone mineral density was decreased in R+14 animals relative to vivarium controls, suggesting that the effects of flight were somewhat delayed. However, bone mineral density was greater in R+14 animals relative to R+0 animals, suggesting an active recovery process. Furthermore, Ca2+ and P concentrations in the tibia of R+14 animals remained below control levels after the reambulation process. Site-to-site differences were also observed. There were early and major changes in the tibia, less marked and delayed changes in the humerus, and minor changes in the greater trochanter of the femur.

STS-62 / PSE.04

Launch Date 3/4/1994

Landing Date 3/18/1994

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Role of the Immune System in Mediating Bone Turnover in Ovariectomized Rats in Microgravity

Science Discipline
Bone Physiology

Investigator	Institute
W.W. Wilfinger	Pennsylvania State University

Co-Investigator(s)	Institute
Hymer, W.C.	Pennsylvania State University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Ambient Temperature Recorder (ATR)

Selected Publications
Day, J.R.; Frank, A.T.; O'Callaghan, J.P.; and DeHart, B.W.: Effects of Microgravity and Bone Morphogenetic Protein II on GFAP in Rat Brain. Journal of Applied Physiology, vol. 85, 1998, pp. 716–722.

Staron, R.S.; Kraemer, W.J.; Hikida, R.S.; Reed, D.W.; Murray, J.D.; Campos, G.E.; and Gordon, S.E.: Comparison of Soleus Muscles From Rats Exposed to Microgravity for 10 Versus 14 Days. Histochemistry and Cell Biology, vol. 110, 1998, pp. 73–80.

Objectives/Hypothesis
The purpose of this experiment was to evaluate the effect of Bone Morphogenetic Protein (BMP) on the immune and skeletal systems in microgravity. Both of these systems are impaired simultaneously during prolonged exposure to weightlessness. Simultaneous impairment of these two systems has also been observed in some diseases, suggesting that their physiological controls may be linked. This experiment analyzed the effect of BMP on the bone, connective tissue, muscle, and lymphoid systems to determine if BMP slows or prevents immune system impairment and bone demineralization.

Approach or Method
Before launch, rats were implanted with six subcutaneous pellets each, containing either the protein or a placebo, and injected with the bone marker calcein. Postflight, animals were euthanized and turned over to the commercial partner team for biosample processing and analysis.

Results
No scientifically relevant results are available.

STS-59 / NIH.C1

Launch Date 4/9/1994

Landing Date 4/20/1994

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effects of Hypogravity on Osteoblast Differentiation

Science Discipline
Bone Physiology

Investigator	Institute
R.K. Globus	University of California, San Francisco

Co-Investigator(s)	Institute
Doty, S.B.	Hospital for Special Surgery

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat) cultured cells

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Space Tissue Loss-A Module

Selected Publications
Globus, R.K.; Moursi, A.; Zimmerman, D.; Lull, J.; and Damsky, C.: Integrin-Extracellular Matrix Interactions in Connective Tissue Remodeling and Osteoblast Differentiation. American Society of Gravitational Space Biology Bulletin, vol. 8, no. 2, Oct. 1995, pp. 19–28.

Objectives/Hypothesis
Weight bearing is essential for normal skeletal function. Without weight bearing, the rate of bone formation by osteoblasts decreases in the growing rat. The fundamental question of whether the defects in osteoblast function due to weightlessness are mediated by localized skeletal unloading or by systemic physiologic adaptation such as fluid shifts has not been answered. This study proposed to examine whether exposure of cultured rat osteoblasts to spaceflight inhibits cellular differentiation and impairs mineralization when isolated from the influence of both systemic factors and other skeletal cells.

Approach or Method
Osteoblasts were purified by collagenase digestion, plated on microcarrier beads in Petri dishes, then loaded after 5 days into polypropylene fiber cartridges. Cells were maintained for 2 days on continuous flow CellCo Units, then transferred to the Space Tissue Loss (STL) hardware and maintained on the unit for 4 days prior to launch. The flight duration was 11 days. Within 4 hours after landing flight cartridges were recovered. Differentiation was assessed by histological analysis with light and electron microscopy and by the measurement steady-state expression of messenger ribonucleic acid (mRNA) genes (alkaline, phosphatase, osteopontin, and osteocalcin) that mark progressive osteoblast differentiation. In order to measure metabolism of the cultures, glucose and lactate concentrations were measured 8 hours before the flight, during the time the cartridges were in the STL unit, and after the cartridges were recovered.

Results
Analysis under light microscopy revealed that the flight cultures had less cells per section, but were otherwise indistinguishable from the control cultures. In addition, ultrastructural analysis by electron microscopy showed that osteoblasts exposed to spaceflight possessed less well-organized rough endoplasmic reticulum/Golgi apparatus than ground controls. These results indicate that osteoblasts exposed to microgravity are less differentiated than control cells. After the omission of suspected defective culture from the statistical pool, glucose utilization and concomitant lactate production were significantly lower for flight cultures. These results indicate that spaceflight may inhibit energy metabolism and the protein-synthetic activity of osteoblasts.

STS-59 / NIH.C1

Launch Date 4/9/1994

Landing Date 4/20/1994

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Molecular and Cellular Analysis of Space Flown Myoblasts

Science Discipline
Bone Physiology

Investigator / Institute
D.A. Kulesh — Armed Forces Institute of Pathology

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat) L8 cell line

Ground-Based Controls
Synchronous Cultures

Key Flight Hardware
Space Tissue Loss-A Module

Selected Publications
Kulesh, D.A.; Anderson, L.H.; Wilson, B.; Otis, E.J.; Elgin, D.M.; Barker, M.J.; Mehm, W.J.; and Kearney, G.P.: Space Shuttle Flight (STS-45) of L8 Myoblast Cells Results in the Isolation of a Non-Fusing Cell Line Variant. Journal of Cellular Biochemistry, vol. 55, 1994, pp. 530–544.

Objectives/Hypothesis
Myoblast cells have been widely employed in conventional studies of biological processes because characteristics of intact muscle can be readily observed in these cultured cells. The purpose of this experiment was to investigate the effects of spaceflight on muscle by utilizing a well characterized myoblast cell line cultured in the Space Tissue Loss (STL) flight module. More specifically, this study aimed to: 1) determine the role of microgravity in regulating the proliferation and differentiation of various skeletal muscle myoblast cell lines, and 2) determine whether pheotypic changes are the direct result of microgravity-modulated gene expression.

Approach or Method
Cultures of L8 myoblast cells were monitored and prepared for loading into ground control and flight cartridges. During flight, cells were monitored for growth, contamination, and fusing. After landing, some cells were frozen and some plated in dishes for transport to the investigator's lab.

Results
Flight cultures, upon recovery, were found to have a low-level fungal contamination. The flight cultures were not viable.

STS-59 / NIH.C1

Launch Date 4/9/1994

Landing Date 4/20/1994

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Influence of Spaceflight on Bone Cell Cultures

Science Discipline
Bone Physiology

Investigator	Institute
W.J. Landis	Harvard Medical School and Children's Hospital

Co-Investigator(s)	Institute
Gerstenfeld, L.C.	Harvard Medical School and Children's Hospital
Toma, C.D.	University of Vienna Medical School

Research Subject(s)
Gallus gallus (White leghorn chicken) cultured cells

Ground-Based Controls
Basal Cartridges, Synchronous Cartridges

Key Flight Hardware
Space Tissue Loss-A Module

Selected Publications
Landis, W.J.; Hodgens, K.J.; Block, D.; Toma, C.D.; and Gerstenfeld, L.C.: Spaceflight Effects on Cultured Embryonic Chick Bone Cells. Journal of Bone and Mineral Research, vol. 15, no. 6, June 2000, pp. 1099–112.

Objectives/Hypothesis
The purpose of this experiment was to determine whether osteoblast cells would respond to the shift from normal gravity to microgravity. Specific attention was given to the effects of microgravity on the metabolic state of the cells, their molecular biological nature, biochemical characteristics and structural features. The hypothesis tested is that the vertebrate skeletal system undergoes adaptive changes in response to microgravity, and such changes will be apparent in measurements of cellular (RNA, proteins, and cytoskeletal elements) and extracellular constituents (collagen, mineral). Observations of these responses may provide insight as to how bones, and the skeleton in general, respond to microgravity.

Approach or Method
Cells from 14- or 17-day-old embryonic chick calvaria were grown in DME + 10% FBS; aliquots (~7x10E6) were mixed with 125 mg Cytodex microcarriers and inoculated in hollow fiber cartridges of artificial capillary culture units (Cellco, Inc.). Cartridge media were supplemented with 12.5 mcg/ml ascorbate and 10 mM beta-glycerophosphate before and during flight. Four cartridges containing cells committed to the osteoblast lineage (5 days of ascorbate prior to launch) and four with uncommitted cells (10 days ascorbate) were flown; the same number of cartridges with the same kinds of cells were used as ground controls. Basal cartridges containing either committed or uncommitted cells were terminated at launch and analyzed identically with flight and synchronous controls.

Results
A possible overall 1/3 to 1/2 reduction in total RNA was observed in flight compared to synchronous control cell groups. Decreased gene expression in flight compared to controls was observed for collagen and osteocalcin. Glucose and lactate measures were statistically similar among these cell groups. Electron microscopy showed matrix development for both committed and uncommitted cell flight groups, but less than that of respective controls. Uncommitted cell groups produced greater matrix than committed cells, and all these groups contained secreted collagen fibrils. In summary, the cells continued to be metabolically active, but they elaborated a less extensive extracellular matrix during spaceflight. The results suggest that microgravity exerts demonstrable effects on bone cells.

STS-66 / NIH.R1

Launch Date 11/3/1994

Landing Date 11/14/1994

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Effects of Gravity on the Attachment of Tendon to Bone

Science Discipline
Bone Physiology

Investigator	Institute
R.B. Johnson	University of Mississippi

Co-Investigator(s)	Institute
Zardiackas, L.D.	University of Mississippi
Tsao, A.K.	University of Mississippi
St. John, K.R.	University of Mississippi

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Johnson, R.B.: The Bearable Lightness of Being: Bones, Muscles, and Spaceflight. The Anatomical Record, vol. 253, no. 1, Feb. 1998, pp. 24–27.

Johnson, R.B.; Tsao, A.K.; St John, K.R.; Betcher, R.A.; Tucci, M.A.; and Benghuzzi, H.A.: Effects of Spaceflight on the Attachment of Tendons to Bone in the Hindlimb of Pregnant Rat. The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology, vol. 282, no. 2, Feb. 2005, pp. 147–56.

Objectives/Hypothesis
The objective of this experiment was to determine the structural damage of muscle-bone junctions in adult rats after spaceflight and to examine the potential for reversal of these structural damages upon return to Earth.

Approach or Method
In order to perform light microscopy on the left tibia, tissue was demineralized, dehydrated, and embedded in paraffin wax. Sections were cut, mounted, and stained with hematoxylin and eosin. Photographs were taken for computer analysis of quadriceps femoris, semimembranosus, semitendinosus, gracilis, popliteus, and tibialis anterior muscles. Muscle fiber size was determined for each muscle. Bone histomorphometric parameters were measured using photographs of cross sections of the tibia. The maximal diameter of each tendon was measured from tissue sections of tendon insertions into bone. The right tibia was analyzed by scanning electron microscopy. The origin and insertion of tendons and density of fibers of each muscle were studied. Bone porosity was quantified for each animal. For specific gravity analysis, bones were pulverized, cooled, and fractionated. The samples were then dessicated in ethanol to calculate density fractions.

Results
The data from this experiment showed significant increases in cortical porosity and periosteal resorption in the tibia and fibula of the flight subjects. It was observed that the fibula of the flight animals became thinner. In addition, there was significant endosteal resorption and a decrease in trabecular volume. The damage adjacent to attachments of the soleus and other anti-gravity muscles was the most serious. The findings indicate that spaceflight causes tibia and fibula atrophy at the site of muscle attachments, and this may lead to fracture when one returns to gravity.

STS-66 / NIH.C2

Launch Date 11/3/1994

Landing Date 11/14/1994

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effects of Microgravity on In Vitro Calcification

Science Discipline
Bone Physiology

Investigator	Institute
A.L. Boskey	Hospital for Special Surgery

Co-Investigator(s)	Institute
Doty, S.B.	Hospital for Special Surgery

Research Subject(s)
Gallus gallus (White leghorn chicken) embryos

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Space Tissue Loss-A (STL-A) Module

Selected Publications
None

Objectives/Hypothesis
Earlier observations of animals flown in microgravity have produced conflicting information about the nature and amount of the mineral formed in bone during flight in microgravity. One cause for these differences was that much of the mineral had been formed on Earth, and there was no way of knowing how much new mineral formed in microgravity. Because crystals formed in vitro in microgravity are generally larger and more perfect than those formed under similar conditions on Earth, it was hypothesized that the initial mineral crystals formed under physiologic control in microgravity would also be larger. The purpose of this experiment was to evaluate the initial mineral formed in flight and compare it to mineral formed in ground based controls. Because biologic mineralization is mediated both by the cells and the extracellular matrix, the second goal was to evaluate the effects of microgravity on the cells and matrix formed in this culture system.

Approach or Method
Cartilage cells were grown as micromass cultures and flown for 11 days; total time in culture was 16 or 19 days. All cells were fixed in 2-percent paraformaldehyde plus 0.5-percent glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4 for electron microscopy, histochemistry, and immunocytochemistry.

Results
No mineral was formed in either ground controls or flight experiment. There was increased cell proliferation in flight.

STS-63 / NIH.C3

Launch Date 2/3/1995

Landing Date 2/11/1995

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effects of Hypogravity on Osteoblast Differentiation

Science Discipline
Bone Physiology

Investigator	Institute
R.K. Globus	University of California, San Francisco

Co-Investigator(s)	Institute
Doty, S.B.	Hospital for Special Surgery

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Space Tissue Loss-A Module

Selected Publications
Malouvier, A.; Globus, R.K.; Doty, S.; Lull, J.; and Morey-Holton, E: Gravity Regulates Glucose and Lactate Metabolism in Cultured Osteoblasts. Amercian Society for Gravitational and Space Biology Bulletin, vol. 9, no. 1, Oct. 1995, p. 28.

Objectives/Hypothesis
Weight bearing is essential for normal skeletal function. Without weight bearing, the rate of bone formation by osteoblasts decreases in the growing rat. The fundamental question of whether the defects in osteoblast function due to weightlessness are mediated by localized skeletal unloading, or by systemic physiologic adaptation such as fluid shifts, has not been answered. This study proposed to examine whether exposure of cultured rat osteoblasts to spaceflight inhibits cellular differentiation and impairs mineralization when isolated from the influence of both systemic factors and other skeletal cells.

Approach or Method
Stock cultures of osteoblasts were plated onto tissue culture dishes. Cells were plated onto beads, then loaded into cartridges and maintained in CellCo units as in NIH.C1. Recovery of the samples after landing revealed obvious signs of bacterial contamination in two out of four flight cartridges and one out of four ground control cartridges. Later, rigorous media sterility tests conducted in the principal investigator's laboratory showed that all of the spent media samples collected both before and after transfer of the cartridges were harboring contamination at the end of the flight period.

Results
Control and flight cultures on NIH.C3 acquired a bacterial contamination in the course of the experiment. Analysis of spent media samples revealed that the cultures acquired contamination at the time of transfer from ground-based CellCo units to the Space Tissue Loss (STL) hardware. In general, light and electron microscopy did not show any significant differences in cell morphology between flight and ground control groups, and little evidence of collagen accumulation in either flight or ground control cultures. Northern analysis revealed that the cells expressed significant levels of messenger ribonucleic acid (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 differences were not observed in the amounts of glucose consumed and lactate produced between flight and ground control samples at the end of the flight period. However, given the problem of contamination, data acquired from this flight are not informative and conclusions about the effects of spaceflight cannot be drawn.

STS-63 / NIH.C3

Launch Date 2/3/1995

Landing Date 2/11/1995

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Molecular and Cellular Analysis of Space Flown Myoblasts

Science Discipline
Bone Physiology

Investigator / Institute
D.A. Kulesh — Armed Forces Institute of Pathology

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat) L8 cell line

Ground-Based Controls
Synchronous cell cartridges

Key Flight Hardware
Space Tissue Loss-A Module

Selected Publications
Kulesh, D.A.; Anderson, L.H.; Wilson, B.; Otis, E.J.; Elgin, D.M.; Barker, M.J.; Mehm, W.J.; and Kearney, G.P.: Space Shuttle Flight (STS-45) of L8 Myoblast Cells Results in the Isolation of Non-Fusing Cell Line Variant. Journal of Cellular Biochemistry, vol. 55, 1994, pp. 530–544.

Objectives/Hypothesis
Characteristics of intact muscle can be readily observed in cultured myoblasts. The purpose of this experiment was to investigate the effects of spaceflight on muscle, specifically to: 1) determine the role of microgravity in regulating the proliferation and differentiation of various skeletal muscle myoblast cell lines; and 2) determine whether phenotypic changes are the direct result of microgravity-modulated gene expression.

Approach or Method
Ground and flight media bags were prepared without added growth factors, and once they were determined to be satisfactory (without contamination), additional growth components (L-gluatamine, chick embryo extract and antibiotics) were added. Postflight, cultures were labeled in order to evaluate cell proliferation and differentiation. The cultures were analyzed on days 1, 2, 4, 6, and 8 for sarcomeric myosin, a and ß actin, desmin, vimentin, and titin immunofluorescent antibody staining patterns. The expression of genes related to myogenesis (MyoD, MRF4, myf-5, myogenin, and ID) were analyzed with Northern blotting and hybridization analysis.

Results
Cell nuclei labeling revealed that while microgravity does decrease the ability of myoblast cells to differentiate into myofibrils, it does not seem to affect their natural ability to cease proliferation when cultured to confluence in vitro. Both sarcomeric myosin and a-sarcomeric actin are actively expressed after L8 cells fuse to form myotubes. Little of either protein is seen prior to fusion. Spaceflown cells, however, fuse to a much lesser extent than do ground-based cultures. ß-actin has an opposite expression schedule. Expression is dramatically reduced within 2 days after confluence, regardless of whether fusion occurs. Therefore, it appears that while spaceflight significantly decreases the ability of L8 cells to fuse and differentiate into myotubes, the expression and assembly of sarcomeric myosin, a-sarcomeric actin, and ß-actin filaments remain unchanged in control cells and in those few spaceflown cells that progress to the fusing stage. However, more importantly, in those spaceflown cells that do not fuse, neither sarcomeric myosin nor a-sarcomeric actin filaments are detected.

STS-63 / NIH.C3

Launch Date 2/3/1995

Landing Date 2/11/1995

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effects of Microgravity on Bone Cell Cultures

Science Discipline
Bone Physiology

Investigator	Institute
W.J. Landis	Harvard Medical School and Children's Hospital

Co-Investigator(s)	Institute
Gerstenfeld, L.C.	Harvard Medical School and Children's Hospital

Research Subject(s)
Gallus gallus (White leghorn chicken) cultured cells

Ground-Based Controls
Basal Cartridges, Synchronous Cartridges

Key Flight Hardware
Space Tissue Loss-A Module

Selected Publications
Landis, W.J.: An Overview of Vertebrate Mineralization With Emphasis on Collagen-Mineral Interaction. Gravitational and Space Biology Bulletin, vol. 12, no. 2, May 1999, pp. 15–26.

Objectives/Hypothesis
This experiment was a continuation of the studies begun on the NIH.C1 flight, to examine the possible effects of spaceflight and microgravity on a number of aspects of osteoblast cell growth and gene expression. Parameters to be assessed include glucose and lactate content as a measure of cell metabolism; collagen expression, accumulation, and extracellular matrix assembly; presence of non-collagenous proteins, including osteopontin, bone sialoprotein, osteocalcin, and others; and a definition of spatial and temporal events of mineralization. The studies examine the hypothesis that unloading bone cells in spaceflight result in altered bone matrix production and mineral formation.

Approach or Method
Cells were obtained from 17-day-old embryonic chick calvaria, grown in Dulbecco's modified Eagle medium supplemented with 10% FBS and ultimately inoculated into hollow fiber cartridges of artificial capillary culture units (Cellco, Inc.). Unlike the protocol for NIH.C1, the cartridges were seeded with cells in the absence of microcarrier beads. After the 8-day flight, cells were removed from the cartridges and analyzed, along with the synchronous controls. Basal cartridge cells, terminated at launch, were identically studied.

Results
Measurements of the metabolic state of the flight, basal, and control cells showed that glucose was consumed completely and to the same measurable extent by all three groups of cells. Differences between metabolism of basal cells and either flight or control cells were interpreted as a possible effect induced by the feeding regime in the Space Tissue Loss (STL) unit. Osteopontin, bone sialoprotein, and osteocalcin appeared with reduced immunoreactivity in flight compared to control and basal cells. These results were consistent with the work from NIH.C1 showing down-regulation of collagen and osteocalcin gene expression during spaceflight. Adaptation of cultured bone cells during spaceflight was mediated in part by changes in non-collagenous proteins in addition to alterations in collagen.

STS-78 / LMS

Launch Date 6/20/1996 **Landing Date** 7/7/1996

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Role of Corticosteroids in Bone Loss During Spaceflight

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
T.J. Wronski	University of Florida

Co-Investigator(s)	Institute
Halloran, B.P.	VA Medical Center
Miller, S.C.	University of Utah

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM), Ambient Temperature Recorder (ATM)

Selected Publications
Wronski, T.J.; Halloran, B.P.; and Miller, S.C.: Role of Corticosteroids in Bone Loss During Spaceflight. In: Life and Microgravity Spacelab (LMS): Final Report. J.P. Downey, ed. NASA/CP-1998-206960, 1998, pp. 338–360.

Vajda, E.G.; Wronski, T.J.; Halloran, B.P.; Bachus, K.N.; and Miller, S.C.: Spaceflight Alters Bone Mechanics and Modeling Drifts in Growing Rats. Aviation, Space, and Environmental Medicine, vol. 72, no. 8, Aug. 2001, pp. 720–726.

Objectives/Hypothesis
Previous studies have shown that stress-induced corticosteroid hormones increase during spaceflight. Negative effects on bone, such as decreased bone formation, are also associated with these hormones. The objective of this study was to determine whether corticosteroid excesses contribute to the bone loss that occurs during spaceflight.

Approach or Method
Prior to the flight, male Sprague Dawley rats were anesthetized and subjected to bilateral adrenalectomy. Pellets made of cholesterol with dissolved corticosterone and aldosterone were implanted in each adrenalectomized rat. All rats were injected with a label to mark the bone forming surfaces. After the flight, serum samples of corticosterone and aldosterone concentrations were measured by radioimmunoassay techniques. The left tibia was frozen and subsequently used to measure dry and ash bone weights. Bone samples were sectioned, stained, and counterstained for measurements of cancellous bone volume (%), osteoclast surface (%), an index of cancellous bone resorption and osteoblast surface (%), and an index of cancellous bone formation. The right tibia underwent a polymerization process and was cut into cross sections. From the cross sections, cortical bone area, cortical width, and marrow area were measured.

Results
From the data in this study, it was found that rats in both the experiment and control populations had increased in body weight. This result indicated that the rats went through the flight well and were healthy. The flight group that did not undergo adrenalectomy showed significant increased adrenal gland weights. Thus, it suggests that the rats had corticosteroid excess during spaceflight. It was determined that the intact flight rats had normal bone mass, thus the objective of this experiment was not met.

STS-80 / NIH.C6

Launch Date 11/19/1996

Landing Date 12/7/1996

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Osteoblast Adhesion and Phenotype in Microgravity

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
R.J. Majeska — Mt. Sinai Medical Center

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Space Tissue Loss-A (STL-A) Module

Selected Publications
None

Objectives/Hypothesis
The objective of this experiment was to determine if spaceflight causes changes in the osteoblast consistent with a reduction in bone formation and increase in bone resorption by analyzing the biochemistry of the cells, the histochemistry of mineral, matrix, and cells, and adhesion plaque properties. The level of integration (cell vs. whole specimen) required for gravitational effects on bone was also examined.

Approach or Method
A permanent cell line derived from a rat osteosarcoma and stably exhibiting an osteoblast-like phenotype (ROS 17/2.8) was cultured on microcarrier beads and inoculated into 24 STL cartridges. During flight, fraction collection from the 12 flight and 12 ground control cartridges, followed by fixation with paraformaldehyde or Histochoice, occurred at L+3 hr, L+1d, L+2d, L+4d, L+7d, and L+10d. Postflight, cultures were assayed for lactate, an indicator of metabolic activity and cell viability.

Results
Upon examination, all cartridges showed a yellow color in the extra-fibril space occupied by cells and beads. This discoloration had not been observed in any previous experiments using the Cellmax culture systems. Analysis of lactate production in conditioned media samples showed considerable variation in lactate levels among replicates. Several samples from later time points showed a decline in lactate levels to near zero. These declines were presumably due to active degradation: control studies indicated that lactate levels in conditioned medium from ROS 17/2.8 cells remained essentially constant at 37°C for over 2 weeks. The yellow color was most likely caused by microbial contamination, which could account for variations in lactate levels in the conditioned media. The likely presence of contaminants in the samples unfortunately compromises any experimental findings.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Bone and Lean Body Mass Changes Following Spaceflight

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
L.C. Shackelford	NASA Johnson Space Center (JSC)
V.S. Oganov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Evans, H.	NASA Johnson Space Center (JSC)
Bakulin, A.V.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Oganov, V.S.; Bakulin, A.V.; Novikov, V.E.; and Murashko, L.M.: Change and Recovery of Bone Mass and Acoustic Properties of Rhesus Monkeys after Bion 11 Spaceflight. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S163-S168.

Objectives/Hypothesis
Loss of bone mineral mass (bone mineral density - BMD) due to microgravity exposure occurs primarily in the lumbar vertebrae, the proximal segment of the femoral bone, and the pelvic bone. BMD loss tends to develop in areas with a large percentage of trabecular (spongy) bone. BMD has not been observed to decrease in the upper body, such as the skull, arms, and ribs. No direct correlation between BMD and bone strength has been observed. This has made it difficult to predict the level of osteopenia an individual will experience during space flight. This experiment will examine: 1) whether exposure to microgravity causes changes in the mass and structure of trabecular and cortical bone; and 2) whether postflight changes in the skeletal bone status can be examined by measuring the ultrasound propagation speed.

Approach or Method
The DEXA method was used to measure BMD of the lumbar vertebrae and the tibial lower third. Measurements included a whole body scan (for body mineral content and BMD of different skeletal compartments), a lumbar spine scan, and a regional scan of both tibia bones. The speed of ultrasound propagation (SOS) was measured in eight zones of the tibia. Measurements were taken pre- and postflight in flight, ground control, and vivarium animals.

Results
Age-related increase in BMD was delayed in the flight animals. No absolute BMD loss was seen in the tibia; however, age-related BMD increase was delayed to a greater extent in the metabolically active trabecular bone compared to cortical bone. SOS measured before and after flight changed only slightly, indicating that exposure to microgravity decreased age-related bone development. However, bone changes observed may also have been associated with housing and training patterns. Compared to similar human data, the BMD gradient in the Bion monkeys remained essentially unchanged.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Complex Study of Micro and Macro Structure and Mineral Composition of Spongy Bone

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
V.S. Oganov — Institute of Biomedical Problem

Co-Investigator(s) / Institute
None

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications

Rodionova, N.V.; Oganov, V.S.; and Zolotova, N.V.: Ultrastructural Changes in Osteocytes in Microgravity Conditions. Advances in Space Research, vol. 30, no. 4, 2002, pp. 765-770.

Rodionova, N.V. and Oganov, V.S.: Morpho-functional Adaptations in the Bone Tissue Under the Space Flight Conditions. Journal of Gravitational Physiology, vol. 8, no. 1, Jul 2001, pp. P87-88.

Rodionova, N.V.; Shevel, I.M.; Oganov, V.S.; Novikov, V.E.; and Kabitskaya, O.E.: Bone Ultrastructural Changes in Bion 11 Rhesus Monkeys. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S157-161.

Objectives/Hypothesis
Exposure to microgravity leads to many changes in bone formation and growth. Diminished osteoblast production, osteoporotic signs, bone mass density loss, and reduced strength can occur. Though these effects can prove especially dangerous on long-term missions, little is known about the actual mechanisms by which bone loss occurs. The differentiation of osteoblasts and alteration of bone matrix in microgravity is not well understood. This study utilizes electron microscopy to examine bone changes in Bion 11 monkeys exposed to microgravity.

Approach or Method
Flight and control animals were anesthetized using isofluorethane and iliac crest samples were taken. Samples were fixed for 24 hours in 2% glutaraldehyde adjusted to pH 7.2 with paraformaldehyde in phosphate buffer. Fixed samples were rinsed three times with 70% ethanol, then postfixed in 1% OsO_4, dehydrated, and embedded in araldite. Ultrathin sections were cut, contrasted, and examined using transmission electron microscopy. Other samples were fixed in 2% glutaraldehyde, dried, gold plated, and examined using scanning electron microscopy. Observed osteoblasts were classified into four categories. Type 1 osteoblasts predominantly synthesize and secrete proteoglycans and alkaline phosphatase, and also accumulate and secrete Ca and P. Type 2 osteoblasts predominantly synthesize collagen proteins. Type 3 osteoblasts secrete accumulated protein and glycosaminoglycans. Type 4 osteoblasts are inactive.

Results
Bone structure in samples taken from the vivarium and flight control animals were similar. Osteogenesis was slow in the monkeys, compared to rats observed in previous studies. The flight monkeys' osteoblast synthetic rates were lower than the control monkeys. A small number of Type 2 and Type 3 osteoblasts versus a large number of Type 4 osteoblasts was observed, indicating a lower rate of collagen synthesis and secretion. Signs of asynchronicity of synthetic processes were not seen, which are typically observed in the norm. Signs of adaptive changes were seen in osteoblasts in several zones. These changes may be associated with osteoblast transformation into fibroblasts. The osteoblast changes observed in this experiment are consistent with the changes found by histomorphometric analysis of monkeys on the Cosmos 2044 flight. Osteocyte structure in flight animals was normal, but some osteocytes developed increased osteolysis. These changes were interpreted as bone adaptation to microgravity.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Bone Tissue and Cell Effects of Space Flight in Young Rhesus Monkey

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
E. Zerath	Centre d'Etudes et de Recherches de Medecine Aerospatiale (CERMA)

Co-Investigator(s)	Institute
Marie, P.J.	Hospital Lariboisiere

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Zerath, E.; Grynpas, M.; Holy, X.; Viso, M.; Patterson-Buckendahl, P.; and Marie, P.J.: Spaceflight Affects Bone Formation in Rhesus Monkeys: A Histological and Cell Culture Study. Journal of Applied Physiology, vol. 93, no. 3, Sep 2002, pp. 1047-1056.

Zerath, E.; Holy, X.; Andre', C.; Renault, S.; Noel, B.; Delannoy, P.; Hott, M.; and Marie, P.J.: Effects of Bion 11 14-day Space Flight on Monkey Iliac Bone. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S155-156.

Objectives/Hypothesis
Previous space flight experiments utilizing the rat have shown that bone loss during microgravity exposure is primarily due to diminished bone formation. Few histological studies have been done on non-human primates flown in space, and delays between reentry and surgery in those experiments prevented early post-flight cell activity studies. This experiment was the first to investigate bone cell activities in space-flown primates using histological and cell culture methods.

Approach or Method
Two flight monkeys and eight ground control monkeys were used in the experiment. Iliac bone biopsies were taken 4–5 months preflight and at recovery. The samples were processed for histological and cell culture analysis.

Results
A loss of bone mass was observed postflight in the iliac cancellous area. The bone volume to total volume ratio (BV/TV) was 35% lower postflight in the flight animals, while control animals did not show significant changes in this area. The osteoblast surface as a function of bone surface and osteoid thickness, indicators of osteoblast activity, were 40% and 18% lower, respectively, in flight monkeys postflight. Mineral apposition rate and mineral surface as a function of bone surface, mineralizing parameters, were 33% and 32% lower, respectively, in flight monkeys postflight. Osteoclast surface, referenced to bone surface, was unchanged in both flight and control animals. Overall, data suggests that space flight alters iliac bone formation in young rhesus monkeys.

STS-95 / NIH.C8

Launch Date 10/29/1998

Landing Date 11/7/1998

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effect of Spaceflight on Cartilage Cell Cycling and Differentiation

Science Discipline
Bone and Calcium Physiology
Cell and Molecular Biology

Investigator / Institute
S.B. Doty — Hospital for Special Surgery

Co-Investigator(s) / Institute
None

Research Subject(s)
Gallus gallus (White leghorn chicken) cartilage cells

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cell Culture Module (CCM)

Selected Publications
Doty, S.B.; Stiner, D.; and Telford, W.G.: The Effect of Spaceflight on Cartilage Cell Cycle and Differentiation. Journal of Gravitational Physiology, vol. 6, 1999, pp. P89–P90.

Objectives/Hypothesis
Although spaceflight has long been known to induce loss of bone and muscle, the exact mechanism of loss is not understood. This experiment studied the effect of microgravity conditions on the cell differentiation process, which appears to be the physiological event most affected by spaceflight. Cartilage cells from chick limb buds flown in space were studied to determine the impacts on the differentiated cell, the cell cycle and differentiation process, and cell death.

Approach or Method
Mesenchymal cells from chick limb buds were grown for 8 days preflight and then placed into Walter Reed Army Institute of Research (WRAIR) incubators. The incubators were divided into flight and ground-control groups, and the flight group was flown for 9 days. Cartilage samples were fixed with 70% ethyl alcohol and 2% sucrose at 2, 4, 7, and 9 days of flight. After flight, the samples were collected, divided, and analyzed using either flow cytometry or immunocytochemistry.

Results
After performing flow cytometry, the flight cells showed significantly higher Cyclin E, proliferating cell nuclear antigen (PCNA), and p27 protein content compared to ground controls, which indicates that the flight cells either: 1) contained more cells undergoing cell division; or 2) cells were blocked within the cycle and could not undergo further differentiation. Flight cells metabolized glucose to lactate continuously throughout the flight and possibly at a reduced rate compared to ground controls, although this difference was not statistically significance. From the immunostaining results, there were no differences when comparing number of cartilage nodules in cartridges from the flight and control groups. In conclusion, this experiment showed that spaceflight does have an effect on cell cycle activity in connective tissue cells. These results may provide insight relative to the effect of spaceflight on cell and tissue differentiation.

STS-108 / UF-1

Launch Date 12/5/2001

Landing Date 12/17/2001

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Skeletal Development in Embryonic Quail

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
S.B. Doty — Hospital for Special Surgery

Co-Investigator(s) / Institute
None

Research Subject(s)
Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls
Delayed Synchronous Control

Key Flight Hardware
Avian Development Facility (ADF)

Selected Publications
Doty, S.B.: Space Flight and Bone Formation. Materwiss Werksttech, vol. 35, no. 12, Dec. 2004, pp. 951–961.

Doty, S.B.; Vico, L.; Wronski, T.; and Morey-Holton, E.: Use of Animal Models to Study Skeletal Effects of Space Flight. Advances in Space Biology and Medicine, vol. 10, 2005, pp. 209–224.

Objectives/Hypothesis
Previous studies of limb and musculoskeletal development in quail eggs incubated onboard the Mir Space Station showed that there was a developmental perturbation in the early embryo that seemed to be corrected during later embryogenesis. From these results, the following objectives were identified: 1) to determine if fibronectin influences the cellular differentiation process and the subsequent formation of a mineralizable matrix; 2) to investigate the effect of microgravity on cell cycle changes and/or cell death (apoptosis); 3) to localize and compare the distribution of BMP-2, PTHrP, and alkaline phosphatase activity in the developing bone and cartilage, as affected by microgravity; and 4) to analyze the early mineral deposition by Fourier transform infrared microscopy (FTIRM) to determine chemical changes or content of the mineralization process occurring during exposure to microgravity.

Approach or Method
Light and electron microscopy were used, coupled to morphometric analysis of bone and cartilage, to evaluate limb development during spaceflight. The Avian Development Facility (ADF) contained a 1-G centrifuge so that some embryos were exposed to 1 G during flight, whereas some were maintained at 0 G. Immunohistochemistry was used to determine cellular function of cartilage and bone and evaluate any matrix change. Edax and FTIRM were used to analyze the mineral component of bone.

Results
Embryos at day 6 did not show any significant alteration in development among the four groups although the number of viable embryos was small. At day 12, the ADF Flight group maintained on the centrifuge at 1 G showed good embryo viability, increased body weight, and longer limbs, compared to the ADF Flight group at 0 G. No effects of flight were seen in the cartilage as measured by Type X collagen, or proteoglycan content. The day-12 embryos also did not show any flight effect on osteocalcin staining in the bone matrix. Immunostaining for Type I procollagen, which is found in the osteoblast's cytoplasm, showed greater content in the 1-G (no spin) ground control than either one of the two flight groups. This result would suggest that application of 1 G during flight does not prevent a decrease in osteoblast activity, and bone loss continues during flight due to reduced formation rates. The finding that the limbs were the same length among all groups would suggest that because the embryonic limbs are largely cartilage, spaceflight does not have an effect on the cartilage anlage formation. Thus, bone and cartilage may be differentially affected by spaceflight; a conclusion that requires supporting evidence from a longer duration spaceflight.

STS-108 / CBTM

Launch Date 12/5/2001 **Landing Date** 12/17/2001

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Commercial Biomedical Testing Module (CBTM)

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
T. Bateman — Clemson University

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Animal Enclosure Module (AEM) Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Harrison, B.C.; et al.: Skeletal muscle adaptations to microgravity exposure in the mouse. J Appl Physiology, vol. 95, no. 6, 2003, pp. 2462 -2470.

Pecaut MJ, et al.: Genetic models in applied physiology: selected contribution: effects of spaceflight on immunity in the C57BL/6 mouse. I. Immune population distributions. J Appl Physiology, vol. 94, no. 5, 2003, pp. 2085-2094.

Gridley, D.S.; et al: Genetic models in applied physiology: selected contribution: effects of spaceflight on immunity in the C57BL/6 mouse. II. Activation, cytokines, erythrocytes, and platelets. J Appl Physiology, vol. 94, no. 5, 2003, pp. 2095-2103.

Objectives/Hypothesis
Commercial Biomedical Testing Module: Effects of Osteoprotegerin on Bone Maintenance in Microgravity (CBTM) provides the capability to use the microgravity environment for evaluation of new pharmaceutical candidates in small mammals. Results may expedite the review of new pharmaceuticals for allowing immediate access to new disease treatments.

Osteoporosis is a debilitating disease that afflicts millions of people worldwide. One of the physiological changes experienced by astronauts during spaceflight is the accelerated loss of bone mass due to the lack of gravitational loading on the skeleton. This bone loss experienced by astronauts is similar to osteoporosis in the elderly population. Osteoprotegerin (OPG), a bone metabolism regulator, is being considered by the Food and Drug Administration (FDA) as a new treatment for osteoporosis.

Approach or Method
Laboratory mice were treated with either OPG or a placebo before launch. The mice were housed in an Animal Enclosure Module (AEM) designed specifically for spaceflight. This experiment provided a preclinical trial model to determine the effectiveness of OPG in treating bone loss.

Results
Mice exposed to microgravity exhibited a 15 - 20 percent decline in femur elastic strength and a 40 - 60 percent decrease in bone formation when compared to the controls. The femur elastic strength decline was caused by three mechanisms: reduced bone formation, increased bone resorption, and inhibition of mineralization. OPG treatment in mice exposed to microgravity nearly reversed the decline in strength and the increase in bone resorption found in untreated mice.

Foton / M3

Launch Date 9/14/2007

Landing Date 9/26/2007

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effect of Microgravity on the Morphology and Function of the Nervous System, Skeleton, and Endocrine Organs of the Gecko

Science Discipline
Bone Physiology

Investigator	Institute
E. Almeida	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Turner, R.T.	American Lake VA Medical Center
Hill, E.	NASA Ames Research Center (ARC)
Philips, J.	NASA Ames Research Center (ARC)

Research Subject(s)
Pachydactylus turneri (Gecko)

Ground-Based Controls
48-Hour Delayed Synchronous Control

Key Flight Hardware
BB boxes (provided by IMBP)

Selected Publications
Nikitin, V.B.; Proshchina, A.E.; Kharlamov, A.S.; Barabanov, V.M.; Krivova, J.S.; Godovalova, O.S.; Savelieva, E.S.; Makarova, A.N.; Gulimova, V.I.; Okshtein, L.L.; Naidenko, S.V.; Souza, K.A.; Almeida, E.A.C.; Ilyin, E.A.; and Saveliev, S.V.: Comparative Studies of the Thick-Toed Geckos After the 16 and 12 Days Spaceflights in Foton-M Experiments. Journal of Gravitational Physiology, vol. 15, no. 1, July 2008, pp. 285–288.

Objectives/Hypothesis
Reflight of Foton-M2 experiment to confirm results, improve research techniques, and expand the areas of inquiry based on the Foton-M2 results. The major objective of the Gecko experiment on Foton-M3 was to study systemic responses of geckos to microgravity. Because the flight offered an opportunity to examine *Pachydactylus turneri* geckos as a potential animal model for space research, the focus was on morphological and metabolic examinations of intact animals. In addition, plans included measuring Gecko cell proliferation rates in microgravity using BrdU delivered in drinking water, and conducting micro computer tomography of bone mineral dynamics in response to spaceflight.

Approach or Method
Five female geckos of the species *Pachydactylus turneri* were flown. Five delayed synchronous controls were put into a container, similar to the one used for the flight group. The temperature during Foton-M3 was 21–24.5°C. After the flight the animals were delivered to the laboratory ~13.5 hours after landing. The Geckos were euthanized and examined using traditional histology immunohistochemistry and X-ray microtomography.

Results
No major differences were found in heart, liver, small intestine, pancreas, and spleen of the flight animals compared to the controls. Geckos of the flight group showed some signs of metabolic loading of vestibular nuclei of myelencephalon but it is still in question whether it is connected with spaceflight factors or conditions of landing an following adaptation. Immunohistochemical analysis showed changes in several section of brain. These results show that even short orbital flight can cause some changes in the brain of vertebrates.

Examination of skeleton bones of M3 gekos mostly confirmed the data from M2, demonstrating that there was no major demineralization of skeleton bones and discovering changes are connected with redistribution of structure elements of cancellous bones in flight compared with control.

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Rodent Spine Deconditioning After 30 Days of Microgravity

Science Discipline
Bone Physiology

Investigator	Institute
A.R. Hargens	University of California, San Diego

Co-Investigator(s)	Institute
None	

Research Subject(s)
Mus musculus (B6.SJL-Ptprca Pepcb/BoyJ mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Bailey, J.F.; Hargens, A.R.; Cheng, K.K.; and Lotz, J.C.: Post-Spaceflight Recovery of Biomechanical Properties of Murine Intervertebral Discs. Gravitational and Space Biology, vol. 26, no. 2, Oct. 2012, pp. 38–47.

Zhang, B.; Cory, E.; Bhattacharya, R.; Sah, R.; and Hargens, A.R.: Fifteen days of microgravity causes growth in calvaria of mice. Bone. vol. 56, no. 2, Oct. 2013, pp. 290-5.

Objectives/Hypothesis
Deconditioning of the intervertebral discs and spinal tissues due to microgravity exposure poses a serious risk upon re-exposure to 1 G, as the data in identical twins exposed to 30-days simulated microgravity suggest. The hypothesis for this experiment was that nucleus pulposus swelling pressures in mouse spines would significantly decrease following 15- and 30-days of microgravity; additionally, that lack of gravitational loading on lumbar discs during microgravity leads to extracellular matrix damage and significantly reduces disc compression performance. Secondly, bone remodeling may occur in spaceflight as a response to skeletal unloading and head-ward fluid shifts, but while unloading causes significant loss of bone mass and density in legs of animals exposed to microgravity, increased blood and interstitial fluid flows may elicit an opposite effect in the head. In bones that normally do not bear weight, the hypothesis was that adaptation to microgravity can induce growth in skull-bone structures.

Approach or Method
Spine study: Spines were harvested from ground-based control mice and flight mice exposed to 15 days of microgravity. The spines were frozen and transported to University of California San Diego (UCSD) and University of California San Francisco (UCSF) for accepted methods of testing nucleus pulposus swelling pressure, lumbar disc dimensions, proteoglycan content, and spine biomechanics.

Methods: Eight C57BL/6 mice (16 weeks old) were divided into two groups: 1) exposed to microgravity conditions during a 15-day NASA mission, STS-131; and 2) ground-based controls.

Skull study: Flight and control mice calvariae were imaged on a micro-computed tomography scanner. A standardized rectangular volume was placed on the parietal bones of each calvaria for analyses, and three parameters were determined to measure bone growth: bone volume (BV), cross-sectional thickness (CsTh), and tissue mineral density (TMD).

Results
In biomechanical testing and morphology studies, a loss of disc height was documented, possibly due to degeneration of the discs, during 15 days of microgravity. In particular, a marked disc height loss at the L5/6 level and L6/S1, the lower lumbar level, was found. This parallels somewhat the high incidence of lumbar disc herniation in astronauts (Johnston et al., 2010). Additionally, decreased disc height at the posterior zone was documented in the study, suggesting increased lordosis. In the MRI analyses, the differences between the control and flight groups were not significant. To assess further the effects of microgravity on the spine, other means of examination (e.g., bone mineral density and trabecular analysis) are currently ongoing.

In the brain studies, a significant difference in water content was opposite to what was hypothesized. The control mice had more brain water than flight mice. Separately, the results indicate that microgravity causes adaptive growth in calvarial bones that do not normally bear weight. These findings suggest that fluid shifts accompanying microgravity may initiate bone remodeling independent of skeletal loading.

STS-131 / BSP

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Role of the p21/p53 Pathway in Spaceflight-Induced Tissue Degeneration

Science Discipline
Cell and Molecular Biology

Investigator	Institute
E. Almeida	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Globus, R.	NASA Ames Research Center (ARC)

Research Subject(s)
Mus musculus (B6.SJL-Ptprca Pepcb/BoyJ mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Blaber, E.A.; Dvorochkin, N.; Lee, C.; Alwood, J.S.; Yousuf, R.; Pianetta, P.; Globus, R.K.; Burns, B.P.; and Almeida, E.A.: Microgravity induces pelvic bone loss through osteoclastic activity, osteocytic osteolysis, and osteoblastic cell cycle inhibition by CDKN1a/p21. PLoS One, vol. 18, no. 8, 2013, pp. 1-15.

Objectives/Hypothesis
Spaceflight factors, including microgravity and radiation, have many detrimental effects on human physiology, including muscle and bone degradation, immune system dysfunction and other effects. The long-term progression of these physiological effects is still poorly understood and a serious concern for long-duration spaceflight missions.

This experiment tested the hypothesis that some of the degenerative effects of spaceflight may be caused in part by an inability of stem cells to proliferate and differentiate normally resulting in an impairment of tissue regenerative process. Furthermore, it was hypothesized that bone loss in space may be mediated by activation of the p53/p21 signaling network resulting in cell cycle arrest and/or apoptosis.

Approach or Method
For STS-131 BSP mouse tissues were received immediately post-landing along with recovered bone from the pelvis and femur as well as bone marrow. Bone samples were from adult skeletally mature mice that had not beet treated. The studies included microCT analysis of bone loss during spaceflight, and patterns of gene expression in bone tissue and bone marrow progenitor lineages following spaceflight. In addition, bone marrow cells were isolated, and were in vitro differentiated into osteoblastic and osteocytic lineages to determine the effects of spaceflight on the differentiation potential of adult stem cell progenitor lineages.

Results
Analyses found that spaceflight caused significant bone loss in the weight-bearing bones of mice with reduction in bone volume and decrease in bone thickness associated with increased osteoclastic activity. Along with rapid bone loss, alterations were also observed in the cell cycle characterized by an increase in the Cdkn1a/p21 cell cycle arrest molecule independent of Trp53. Overexpression of Cdkn1a/p21 was localized to osteoblasts lining the periosteal surface of the femur and chondrocytes in the head of the femur, suggesting an inhibition of proliferation in two key regenerative cell types of the femur in response to spaceflight. Additionally, overexpression of several matrix degradation molecules were found, including MMP-1a, 3 and 10, of which MMP-10 was localized to osteocytes within the shaft of the femur. This, in conjunction with an increase in osteocyte lacunae cross-sectional area, perimeter, and a decrease in circularity measured with 40-nm resolution, indicates a potential role for osteocytic osteolysis in the observed bone degeneration in spaceflight.

To further investigate the genetic response of bone to mechanical unloading in spaceflight, genome wide microarray analysis of total RNA isolated from the mouse pelvis using an Affymetrix GeneChip® Gene 1.0 ST Array System for Mouse was conducted. Preliminary results show that more than 6,000 genes had statistically significant alterations in spaceflight compared to ground controls. These included cell cycle arrest molecules p21, and p18, cell survival molecule Crbp1, and cell cycle molecules cyclin D1, and Cdk1. Additionally, results indicated alterations in molecular targets of cyclin D1 and Cdk4, senescence pathways resulting from abnormal laminin maturation, cell-cell contacts via E-cadherin, and several pathways relating to protein translation and metabolism. These alterations indicate significant impairment of normal cellular function in the mechanically unloaded environment of space and could provide important genetic insight into the observed deregulation of bone formation and resorption in space.

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effect of Weightlessness on the Tendon-to-Bone Insertion

Science Discipline
Cell and Molecular Biology

Investigator / Institute
S. Thomopoulos — Washington University

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (B6.SJL-Ptprca Pepcb/BoyJ mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Lee, K.S.; Lim, C.; Carr, J.A.; Gastwirt, R.F.; Thomopoulos S.; and Ward, S.R.: Effects of Microgravity on the Passive Mechanical Properties of Muring Skeletal Muscles. Transactions of the 57th Meeting of the Orthopaedic Research Society, Long Beach, Calif., 2011, vol. 36, paper 1665.

Lim, C.; Shen, H.; and Thomopoulos S.: Microgravity Leads to Degeneration of Murine Rotator Cuff Muscles. Transactions of the 57th Meeting of the Orthopaedic Research Society, Long Beach, Calif., 2011, vol. 36, paper 208.

Objectives/Hypothesis
The overall objective of this experiment was to examine the effect of prolonged weightlessness on the mechanical and biochemical properties of muscles, tendons, and their insertions into bone. Soft tissue injuries often occur at the sites of attachment of tendons and ligament to bone. The sensitivity of the musculoskeletal system to its mechanical environment may magnify the injury susceptibility of insertion sites during prolonged exposure to microgravity. Changes in loading across joints lead to changes in tissue mechanical, structural, and biochemical properties. Joint unloading results in a catabolic biologic state and subsequent tissue degeneration. Physiologic joint loading, on the other hand, results in an anabolic biologic state and maintenance of tissue properties. The negative effect of unloading has most clearly been demonstrated in bone. Less is known about the effect of weightlessness on joint soft tissues.

Approach or Method
Experiment animals: 16 mice in spaceflight and 16 mice housed at Kennedy Space Center (KSC) as ground controls. Left and right shoulders (humerus-rotator cuff-scapula units) and ankles (Achilles tendon-calcaneous bone) were removed from each mouse approximately 2 hours after landing, processed, and shipped to the laboratory. Quantitative polymerase chain reaction (qPCR) was performed on rotator cuff mouse muscles, tendons, insertions, and bones. Biomechanics was performed on mouse supraspinatus tendon-to-bone samples. Histology was performed on supraspinatus tendon-humeral head and Achilles tendoncalcaneous samples.

Results
The effect of microgravity on gene expression in the humerus:
Preliminary data demonstrated that bone resorption is increased (as indicated by increases in the ratio of RANKL and OPG gene expression) and osteoblast differentiation is decreased (as indicated by decreases in OSX gene expression). Surprisingly, adipogenesis was decreased due to the weightless environment (as indicated by decreases in PPARG gene expression). As expected, the mechanoresponsive gene COX2 was dramatically downregulated due to microgravity.

The effect of microgravity on the rotator cuff muscles:
Microgravity significantly downregulated the expression of both myogenic and adipogenic genes, MyoD and C/EBP**α,** and there was an increase in collagen type III (fibrosis marker).

The effect of microgravity on the rotator cuff tendons and their bony insertions:
Joint unloading due to a weightless environment led to similar changes, with significant increases in MMP-3 and MMP-13 messenger ribonucleic acid (mRNA) expression and significant decreases in tendon cross-sectional area.

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Response of Articular Cartilage to Microgravity

Science Discipline
Cell and Molecular Biology

Investigator / Institute
D. Fitzgerald — Oregon Health and Science University

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (B6.SJL-Ptprca Pepcb/BoyJ mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Fitzgerald, J. and Moscibrocki, C.: Activation of Stress Response Networks in Cartilage and Skeletal Muscle During Spaceflight. Orthopedic Research Society Annual Meeting, San Francisco, Calif., Feb. 4–7, 2012, Workshop 4 Handout, p. 3.

Objectives/Hypothesis
The objective of this experiment was to determine if the reduced biomechanical forces due to microgravity impair the ability of chondrocytes to maintain healthy articular cartilage, leading to increased cartilage breakdown; specifically whether the reduced mechanical forces associated with microgravity lead to osteoarthritis. Although the effects of microgravity on bone are well known and appear to be reversible, the effects of reduced mechanical forces on the cartilage surfaces of articulating joints are less well understood. Studies in humans and animals clearly demonstrate that osteoarthritis results from abnormal (both increased and decreased) mechanical forces on cartilage. Based on these findings, the hypothesis is that appropriate mechanical forces are critical for the health of articular cartilage. Following on from this, a further hypothesis is that the absence of significant biomechanical forces affects that ability of the chondrocyte to maintain healthy functioning cartilage. This may lead to accelerated cartilage breakdown and osteoarthritis.

Approach or Method
For the STS-131 mission, animals were subjected to 15 days of microgravity and the joints harvested three hours following landing. Femoro-tibial joints from four flight and four ground control animals were paraffin-embedded, sectioned and stained for proteoglycan using toluidine blue. Proteoglycan degradation is a key indicator of cartilage breakdown and one of the first stages in osteoarthritis. Stained sections were scored using a modified Mankin histological semi-quantitative grading system (see Research Plan for description of scoring system). There was no difference in toluidine blue staining between flight and ground articular cartilage (data not shown). We conclude that 15 days exposure to microgravity does not alter proteoglycan content of articular cartilage in mouse knee joints.

For RNA expression analyses, joint articular cartilage total RNA from the four flight and ground control samples were isolated. The mean total RNA yield per joint was 1.2 ug. Since the function of microRNAs (miRNAs) in cartilage has received substantial research attention and several appear essential to normal development and homeostasis each of the eight samples were subjected to miRNA microarray analysis using the Affymetrix Genechip miRNA array. Samples were also hybridized to the Affymetrix Murine Gene 1.0 ST GeneChip microarray for mRNA expression.

Results
miRNA data: Bioinformatic analysis of the miRNA data demonstrated that 10 miRNAs were upregulated ($p>0.001$) and 26 were downregulated in flight compared to control mice. Interestingly, miR-365, which was downregulated 1.4-fold, is a known mechanoresponsive microRNA in chondrocytes. It has been reported that miR-365 was upregulated following cyclical loading of chick sternal chondrocytes and that it plays a role in chondrocyte proliferation and differentiation. Furthermore, miR365 binds to and inhibits HDAC4, a negative regulator of chondrocyte hypertrophy. Our finding that miR-365 is downregulated in the reduced biomechanical unloading of spaceflight is consistent with a role in mechano-sensing. Reduced levels of miR-365 would be expected to reduce chondrocyte proliferation thereby contributing to an OA phenotype.
mRNA data: Thirteen mRNAs were upregulated >1.5-fold in flight compared to the ground control animals and one downregulated >1.5 fold. Of the 13 upregulated genes, 7 have been implicated in either osteoarthritis, rheumatoid arthritis or both. These are *Vdr, Nfkbia, Cited2, Traf6, Mt1, Txnip*, and *Cdkn1a*. The highest upregulated gene was *Vdr* which encodes the vitamin D receptor. Vdr is a key genetic and biochemical marker of osteoarthritis (OA). Several genetic association studies on large populations of OA patients have linked the *Vdr* gene locus with knee OA. The link between Vdr and OA is further highlighted by the finding that *Vdr* mRNA is upregulated in severe osteoarthritis. Consistent with this is the finding that, vitamin D, the Vdr ligand, upregulates matrix metalloproteinase (MMP) production. MMPs are the major class of matrix-degrading enzymes in cartilage.

STS-133 / BSP

Launch Date 2/24/2011 **Landing Date** 3/9/2011

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Role of the p21/p53 Pathway in Spaceflight-Induced Tissue Degeneration

Science Discipline
Cell and Molecular Biology

Investigator / Institute
E. Almeida — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Simulated Flight Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Blaber, E.A.; Dvorochkin, N.; Lee, C.; Alwood, J.S.; Yousuf, R.; Pianetta, P.; Globus, R.K.; Burns, B.P.; and Almeida, E.A.: Microgravity induces pelvic bone loss through osteoclastic activity, osteocytic osteolysis, and osteoblastic cell cycle inhibition by CDKN1a/p21. PLoS One, vol. 18, no. 8, 2013, p. e61372.

Objectives/Hypothesis
This study hypothesized that following spaceflight and upon reloading of mice at 1g, the gene expression changes related to tissue degeneration and unloading observed in STS-131 immediately after flight might be reversed, and that markers of bone formation and progenitor cell differentiation would gradually reappear.

Approach or Method
For STS-133 BSP, mouse tissues were received 1, 5, and 7 days post-landing along with recovered bone from the pelvis and femur, as well as bone marrow. Bone samples were from juvenile mice exposed to respiratory syncytial virus (RSV) infection shortly after landing and, therefore, may reflect responses to infection over the course of 7 days in addition to the effects of spaceflight and subsequent reloading at 1g. Sham infected mice were available, but the low n = 2 selected by the Biospecimen Sharing Program (BSP) did not allow their inclusion as statistically significant in this study. This study included microCT analysis of bone loss during spaceflight and subsequent recovery over 7 days, as well as patterns of gene expression in bone tissue and bone marrow progenitor lineages.

Results
Gene expression data collection is currently being conducted for bone and bone marrow, and data analysis. Preliminary results, however, suggest that some markers of gene expression associated with tissue degeneration and cell cycle arrest in space may still show elevation following reloading for 1–7 days, suggesting at least some cell cycle arrest effects of microgravity may persist upon reloading, with the caveat, of course, that these studies are conducted in an RSV infection background and in skeletally immature, growing mice.

STS-133 / BSP

Launch Date 2/24/2011 **Landing Date** 3/9/2011

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Rodent Tail and Brain Deconditioning after 10–15 Days of Microgravity

Science Discipline
Bone Physiology

Investigator	Institute
A.R. Hargens	University of California, San Diego

Co-Investigator(s)	Institute
None	

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Simulated Flight Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Bailey, J.F.; Hargens, A.R.; Cheng, K.K.; and Lotz, J.C.: Post-Spaceflight Recovery of Biomechanical Properties of Murine Intervertebral Discs. Gravitational and Space Biology, vol. 26, no. 2, Oct. 2012, pp. 38–47.

Objectives/Hypothesis
Prolonged exposure to microgravity during spaceflight is thought to adversely affect the human spine because disc herniation risk is increased post-spaceflight. The increased herniation risk is highest during the first post-spaceflight year, and gradually subsides thereafter. Consequently, it was hypothesized that the biomechanical properties of the intervertebral disc (IVD) deteriorate during spaceflight but then recover after acclimation to normal gravity.

Approach or Method
To test this hypothesis, the compressive creep properties of caudal intervertebral discs (IVDs) of murine subjects that had returned from a 13-day Shuttle mission (STS-133) were compared to those of ground-based control mice. Spaceflight (n = 6) and control (n = 10) groups consisted of 13-week-old BALB/c mice (11 weeks at launch). Mice were sacrificed +1 day, +5 days, or +7 days after the landing of STS -133. Disc height was measured in situ, and compressive creep rate was fit to a fluid transport model to determine disc biomechanical properties.

Results
Compared to controls, spaceflight mice had 12% lower disc height and 21% lower strain-dependence on swelling pressure. Biomechanical properties did not recover significantly over the 7-day-postflight period. Biomechanical properties of the murine caudal IVD were diminished by spaceflight, consistent with observations that prolonged exposure to microgravity increases disc herniation risk. These properties did not recover after short-term reacclimation to 1-G loading.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Assessment of Anti-Sclerostin Antibody as a Novel Anabolic Therapy for Prevention of Spaceflight-Induced Skeletal Fragility in Mice

Science Discipline
Bone Physiology

Investigator	Institute
M.L. Bouxsein	Beth Israel Deaconess Medical Center & Harvard Medical School

Co-Investigator(s)	Institute
Baron, R.	Beth Israel Deaconess Medical Center & Harvard Medical School
Rutkove, S.	Beth Israel Deaconess Medical Center & Harvard Medical School

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Sung, M.; Li, J.; Spieker, A.J.; Spatz, J.; Ellman, R.; Ferguson, V.L.; Bateman, T.A.; Rosen, G.D.; Bouxsein. M.; Rutkove, S.B.: Spaceflight and hind limb unloading induce similar changes in electrical impedance characteristics of mouse gastrocnemius muscle. J Musculoskelet Neuronal Interact, vol. 13, no. 4, Dec. 2013, pp. 405-11.

Objectives/Hypothesis
Hypothesis 1: In mice exposed to spaceflight, those treated with sclerostin antibody (Scl-Ab) have greater bone mass, microarchitecture, and strength compared to vehicle-treated mice. Secondarily, in mice exposed to hindlimb unloading for a similar duration as spaceflight, those Scl-Ab treated mice have greater bone mass, microarchitecture, and strength compared to vehicle-treated mice. Hypothesis 2: Compared to ground-based controls housed in an identical environment, mice exposed to spaceflight have decreased bone mass, microarchitecture, and strength; and increased serum and bone levels of sclerostin. Hypothesis 3: Mice exposed to spaceflight have altered electrical impedance signals in skeletal muscle that correlates with changes in muscle morphology and function.

Approach or Method
Aim 1: Determine ability of a sclerostin antibody to inhibit spaceflight-induced skeletal fragility in adult mice. Adult female mice were given a single injection of sclerostin antibody (Scl-Ab) or vehicle (VEH) just prior to a ~12-day spaceflight. Comprehensive musculoskeletal outcomes were obtained upon return to Earth and compared to those in ground-based mice exposed to identical housing and environmental conditions and treatments. The effects of Scl-Ab in mice exposed to a common ground-based analog for microgravity, hindlimb unloading (HLU), were also examined to determine whether the response to a bone anabolic therapy is similar in HLU and spaceflight. Aim 2: Determine effects of spaceflight on multiple metrics of skeletal fragility, and compare these to changes seen with hindlimb unloading. By comparing ground-based VEH-treated mice to VEH-treated mice exposed to spaceflight, prior studies of skeletal health in microgravity were expanded and insight gained into the material, structural, and biologic mechanisms of microgravity-induced skeletal fragility. Aim 3: Compare effects of spaceflight and hindlimb unloading on muscle atrophy using electrical impedance myography.

Results
Female, 9 week old, C57Bl/6 mice were assigned to one of four groups (15 mice/group) based on body mass and total body bone mineral density (BMD): vehicle-treated flight (VF), drug-treated flight (DF), vehicle-treated ground controls (VG), and drug-treated ground controls (DG). Mice were loaded in the AEM approximately 21 hours before launch and were dispositioned 3 to 7 hours after landing 12 days later. Primary in vivo outcome assessments included body weight, BMD, and serum markers of bone turnover. Primary ex vivo outcome assessments include bone microarchitecture, bone strength, and quantitative histomorphometry.

STS-135 / BSP

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Role of the p21/p53 Pathway in Spaceflight-Induced Tissue Degeneration

Science Discipline
Cell and Molecular Biology

Investigator	Institute
E. Almeida	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Globus, R.K.	NASA Ames Research Center (ARC)

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Blaber, E.A.; Dvorochkin, N.; Lee, C.; Alwood, J.S.; Yousuf, R.; Pianetta, P.; Globus, R.K.; Burns, B.P.; and Almeida, E.A.: Microgravity induces pelvic bone loss through osteoclastic activity, osteocytic osteolysis, and osteoblastic cell cycle inhibition by CDKN1a/p21. PLoS One, vol. 18, no. 8, 2013, p. e61372.

Objectives/Hypothesis
The objective for STS-135 was to confirm the results on p21 cell cycle arrest from the STS-131 experiment, and to expand the current findings to postflight effects on bone health. Recovering pelvis tissue from STS-135 was proposed because it would offer the best replication of STS-131 experimental results—the same mouse strain was used and the tissues were recovered immediately after landing, thus closely replicating the STS-131 experimental conditions—however these tissues were not provided by the Biospecimen Sharing Program (BSP) making direct comparisons impossible. Although the requested tissues were not provided, the metatarsals provided a good alternative model for a load-bearing set of bones for analysis. Metatarsal were used in organ culture assays to test some aspects of the original hypothesis in the proposal that spaceflight factors such as radiation affect the regenerative health of stem cells in bone.

Approach or Method
Following the STS-135 mouse BSP, we dissected and collected bone samples at Kennedy Space Center (KSC) and began analyzing the combined effects of microgravity and gamma irradiation on the p53 pathway in mouse metatarsals placed in organ culture immediately following spaceflight. Because of unfortunate errors in sample labeling and distribution, the number of replicate samples of the experimental tissues provided to BSP investigators was reduced. This impacted the ability to perform all of the planned analyses. However, due to the large number of metatarsal bones (10) in the feet of each animal, a slightly more limited, but still statistically significant, analysis was conducted of thickness, mineral content, and architectural differences in bones belonging to ground controls versus spaceflight, either non-irradiated or irradiated at 10 or 50 cGy.

Results
As a result it was found that cortical bone in metatarsals is sensitive to microgravity-induced mineral loss, with significant decreases in cortical thickness. Additional metatarsal bones were also demineralized and sectioned for immunohistochemical analysis of p53 pathway proteins and recovered total ribonucleic acid (RNA) for gene expression studies, which are currently ongoing. Conclusions from these studies are expected to determine if osteoclast maturation and recruitment to endosteal bone surfaces is subject to synergistic or additive effects of low-dose gamma irradiation and microgravity, and if activation of the p53 pathway by irradiation is modulated by exposure to microgravity.

STS-135 / BSP

Launch Date 7/8/2011

Landing Date 7/21/2011

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Gene Expression Alterations in Articular Cartilage, Skeletal Muscle, and Skin Exposed to Microgravity

Science Discipline
Cell and Molecular Biology

Investigator / Institute
D. Fitzgerald — Oregon Health and Science University

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
The overall objective of this experiment was to investigate alterations in gene expression in articular cartilage, skeletal muscle, and skin exposed to microgravity. The data from STS-131 animals demonstrated that in cartilage, muscle, and skin, genes that respond to different types of common stresses such as oxidative stress and genotoxic stress are also upregulated under conditions of microgravity. This was a completely novel finding and may explain some of the tissue-level effects of microgravity reported, such as muscle atrophy. This experiment examined the hypothesis that one effect of microgravity in these tissues is the activation of stress response genetic networks, and this has effects on cell proliferation and tissue maintenance.

Approach or Method
As was done with STS-131 samples, ribonucleic acid (RNA) was extracted using MirVana and the RNA assessed for quality. Samples were then assessed for gene expression differences using defined microarrays for Affymetrix. The tissue samples stored in formalin were used to examine microgravity-related changes in protein levels and were guided by the gene expression analyses.

Results
At the time of publication data analysis is still in progress.

STS-135 / BSP

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
The Effect of Weightlessness on the Tendon-to-Bone Insertion

Science Discipline
Cell and Molecular Biology

Investigator / Institute
S. Thomopoulos — Washington University

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
The overall objective of this proposal was to examine the effect of prolonged weightlessness on the biology of tendons and their insertions into bone. Samples from STS135 allowed us to verify results from STS131. These specimens also allowed us to perform additional assays to verify the hypotheses. Raman microprobe spectroscopy was performed at the tendon-to-bone insertion to determine the mineral gradient across the insertion.

Approach or Method
Experiment animals: 15 mice flew on this mission and 30 mice were housed at Kennedy Space Center (KSC) to serve as ground controls. Left and right shoulders (humerus-rotator cuff-scapula units) and ankles (Achilles tendon-calcaneous bone) were removed from each mouse approximately 2 hours after landing, processed, and shipped to the laboratory. Ongoing studies analyzing specimens from this mission include: biochemistry and proteomics on rotator cuff mouse muscles, micro-computed tomography on the humeral head bone, biomechanics on the supraspinatus tendon-to-bone insertion, and histology on supraspinatus tendon-humeral head and Achilles tendon-calcaneous samples.

Results
The effect of microgravity on the rotator cuff tendons and their bony insertions: Microgravity led to significant increases in MMP-3 and MMP-13 messenger ribonucleic acid (mRNA) expression and significant decreases in tendon cross-sectional area. These changes led to a surprising increase in the modulus and strength of the tendon-to-bone insertion. The stiffer tendon-to-bone insertion may be indicative of early tissue pathology.
The effect of microgravity on the humeral head bone: Preliminary data from STS-131 and STS-135 demonstrate that bone resorption is increased (as indicated by increases in the ratio of RANK-L to OPG gene expression) and osteoblast differentiation is decreased (as indicated by decreases in osterix and Runx-2 gene expression). Bone mineral density (BMD) and trabecular thickness (Tb.Th.) were decreased due to microgravity.
The effect of microgravity on the rotator cuff muscles: Microgravity significantly downregulated the expression of myogenic genes indicating atrophy/degeneration of the muscles due to microgravity. Downregulation of adipogenic genes indicated that degeneration due to microgravity may occur through different pathways than degeneration due to tendon and/or nerve injury in gravity conditions. An increase in collagen type III indicates muscle fibrosis. Preliminary proteomics results indicated that the expression of a large number of proteins was changed due to microgravity conditions. These changes included proteins related to muscle metabolism, muscle type, and fatty acid/glucose metabolism.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: BONE PHYSIOLOGY

Title of Study
Unloading-Driven Regulation of Eukaryotic Initiation Factor 2 (eIF2) and Genes linked to Integrated Stress Response in Mouse Bone Tissues

Science Discipline
Cell and Molecular Biology

Investigator / Institute
H. Yokota — Indiana University Purdue University Indianapolis

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
The objective of the proposed sub-project was to examine a molecular mechanism of gravitational unloading-driven bone loss, focusing on the role of phosphorylation of eIF2α and integrated stress responses (ISRs). It is known that diverse environmental stresses including radiation, oxidative stress, nutrient limitation, and stress to the endoplasmic reticulum induce integrated stress responses. The responses lead to a pro-survival pathway (rescue program) for alleviation of cellular injury or, conversely, an apoptotic pathway for removal of damaged cells. Gravitational unloading in space is a unique form of environmental stress, and the resulting physiological responses in connection to the phosphorylation of eIF2α and ISR are poorly understood.

Approach or Method
Three specific aims using a mouse hindlimb-suspension model and space-flown samples were used to test the hypothesis by using real-time polymerase chain reaction (PCR), Western blot analysis, enzymatic assays, and bone histology with pharmacological agents, and eIF2α-linked knockout mice.:

Aim 1: Evaluate phosphorylation of eIF2α and its kinases (e.g., PERK and GCN2) as well as translational efficiency in response to transient unloading.

Aim 2: Evaluate gene regulatory mechanisms linked to oxidative stress and stress to the endoplasmic reticulum (ER) in response to continuous unloading.

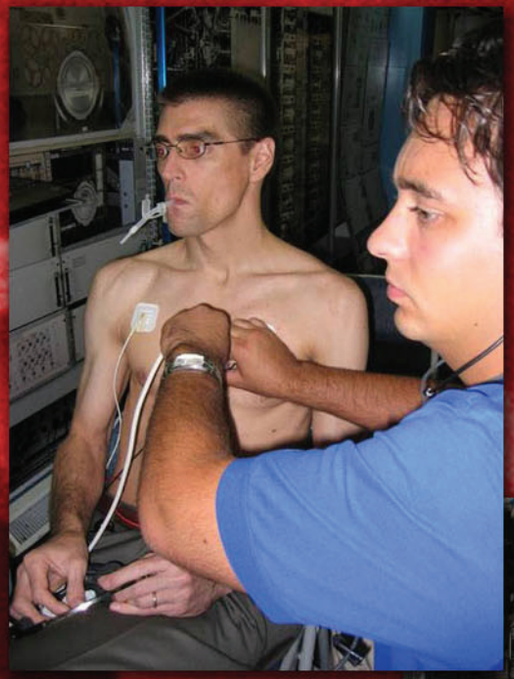
Aim 3: Examine the effects of administration of salubrinal (inhibitor of eIF2α phosphatase) on pro-survival signaling and bone metabolism.

In the first year: Aim 1 was completed; the second year was focused on Aim 2 with bone samples from STS-135, therefore the experiments were conducted using both space-flown samples and hindlimb suspended samples.

Results
STS-135 space-flown mice: Among seven distal tibia samples that were harvested 4 hours 20 minutes–7 hours 20 minutes after landing, the level of eIF2α-p was significantly reduced compared to two ground control samples. Unlike the ground hindlimb-unloaded samples, the reduction in the eIF2α-p level of space-flown samples was not restored, even 7 hours 20 minutes after landing.

Discussion of the current data: In summary, this study presented for the first time that the phosphorylation of eIF2α and the genes involved in ISR are modulated after 2 weeks of spaceflight. The ground-based loading and unloading control experiments suggested that the observed alterations in the level of eIF2α and the messenger ribonucleic acid (mRNA) levels in space-flown animals were potentially caused by the several hours between landing and tissue harvest. Loads linked to landing presumably applied stimulatory loading to mouse limbs and reduced ISR. Based on the ground experimental results, it is possible that the level of eIF2α and the expression levels of stress-sensitive genes during spaceflight might be substantially different from the samples harvested several hours after landing. To evaluate molecular events such as phosphorylation and transcriptional activation that may take place within an hour, it is necessary to harvest and freeze/store tissues on orbit.

Cardiovascular/Cardiopulmonary



Cardiovascular/Cardiopulmonary Introduction

Michael Delp, Ph.D., Florida State University

Approximately two-thirds of the human body mass is composed of water in the form of intracellular fluid, interstitial fluid, and blood plasma. On Earth, the head-to-foot gravity vector creates a fluid pressure gradient in humans when standing whereby fluid pressure in the feet can be more than double that at the level of the heart and head. When this gravity vector is removed, such as occurs during spaceflight, there is a resulting fluid shift toward the head that redistributes fluid pressures more evenly throughout the body. One organ system that is greatly affected by this fluid-pressure redistribution is the cardiovascular system. Until recently, the prevailing sentiment has been that these fluid shifts did little to jeopardize in-flight crew health or their ability to function normally. It was only upon return to Earth's gravitational influence that clinical health issues became manifest. Two of the primary postflight health issues associated with the cardiovascular system were the diminution of work capacity and a compromised ability to properly maintain arterial blood pressure during orthostatic stress. In fact, orthostatic hypotension was one of the first medical complications reported to occur in Project Mercury astronauts after only brief excursions into space, and the incidence of orthostatic hypotension among Shuttle and International Space Station (ISS) astronauts is now known to increase as the duration of time in space becomes greater.

Animal research sponsored by NASA Ames Research Center (ARC) has made vital contributions to our ability to fully understand the structural anatomy, biochemistry, and physiology underlying microgravity-induced orthostatic hypotension and cardiovascular deconditioning [Buckey, 1996]. Research in flight animals has shown that cardiac papillary fiber cross-sectional area is decreased, and that mitochondrial volume of left ventricular myocytes is lower with no change in the myosin isoform profile. These results suggest a deconditioning effect on cardiac muscle with possible changes in the morphology of cardiac myocytes that could compromise cardiac function [Philpott, 1987] [Cosmos 1887 / Bion 8, p. 118]; [Mednieks, 1991] [Cosmos 1887 / Bion 8, p. 119]; [Cosmos 2044 / Bion 9, p. 122-123]. Other work suggests that tissue perfusion may be altered as a result of the cephalad fluid shifts in microgravity, and that intrinsic vasomotor responsiveness of resistance arteries [Keil, 1994][Cosmos 2044 / Bion 9, p. 120] is altered so that their ability to contract and elevate peripheral vascular resistance is impaired [Hatton, 2002; Stabley, 2012; Behnke, 2013]. Further, vascular control mechanisms governing cerebral perfusion are altered in space [Taylor, 2013]. Research also confirms that spaceflight-induced hemolysis occurs in animals, and that this may be the result of fluid shifts and a corresponding hemoconcentration. This research provides important information regarding neurohormonal changes that occur in the body during spaceflight that can impact the function of the cardiovascular system [Mednieks, 1991][Cosmos 1887 / Bion 8, p. 119], as well as other organ systems in the body. Collectively, these studies provide important information regarding the effects of microgravity on the structure and function of heart muscle [Cosmos 2044 / Bion 9, p. 124] and vascular tissue, and the cellular composition of the blood to help elucidate mechanisms for cardiovascular deconditioning and orthostatic intolerance.

It is evident from these experimental findings that animal research is still needed in our pursuit to understand the biomedical effects of microgravity on human health and bodily function, and in particular that of the cardiovascular system. Our recent realization that in-flight crew health and safety may not be as unaffected by fluid shifts as originally supposed, indicates the need for further study.

For example, the relatively recent discovery of visual impairment among astronauts is a serious concern and has been hypothesized to be related to increases in intracranial pressure during flight. The likely involvement of the cerebral arterial, venous, and lymphatic circulations with putative increases in intracranial pressure has yet to be determined [Sandler, 1987] [Cosmos 1514 / Bion 6, p. 115], [Sandler, 1994] [Cosmos 1667 / Bion 7, p. 117]. The involvement of bone and marrow perfusion, fluid flow, and vascular coupling mechanisms in microgravity-induced bone loss, as well as the involvement of the lymphatic circulation in spaceflight-associated immunodepression, are two other areas where research is needed on the interaction of the cardiovascular system with organ systems [Cosmos 2044 / Bion 9, p. 121] that impact astronaut health. And as NASA considers space exploration beyond the Earth's magnetic field, the effects of the deep space radiation environment on cardiovascular health will necessitate animal research to discover their impact on the function of the heart and regional arterial, venous, and lymphatic circulations. These are but a few examples whereby animal research can help address the health issues that arise with human space exploration and continue to provide insight to their physiological mechanisms

List of referenced flight experiments:

Cosmos 1514 / Bion 6, H. Sandler, Cardiovascular Results from a Rhesus Monkey Flown Aboard the Cosmos 1514 Spaceflight, and Ground-Based Controls

Cosmos 1667 / Bion 7, H. Sandler, Effect of Microgravity on Blood Pressure and Flow in the Common Carotid Artery of Primates

Cosmos 1887 / Bion 8, D. Philpott, Morphological and Biochemical Examination of Heart Tissue: I. Effects of Microgravity on the Myocardial Fine Structure of Rats Flown on Cosmos 1887, Ultrastructure Studies

Cosmos 1887 / Bion 8, M. Mednieks, Morphological and Biochemical Examination of Heart Tissue: II. Cellular Distribution of Cyclic AMP-Dependent Protein Kinase Regulatory Subunits in Heart Muscle of Rats Flown on Cosmos 1887

Cosmos 2044 / Bion 9, D. Philpott, Morphological and Biochemical Examination of Heart Tissue: I. Ultra- structural Alterations in Rat Hearts After Cosmos 2044 Compared to Cosmos 1887

Cosmos 2044 / Bion 9, J.B. West, Histologic Examination of Lung Tissue

Cosmos 2044 / Bion 9, L. Keil, Measurement of Heart Atrial Natriuretic Peptide Concentrations

Cosmos 2044 / Bion 9, M. Goldstein, Morphological and Biochemical Examination of Heart Tissue: II. Cardiac Morphology After Conditions of Microgravity

Cosmos 2044 / Bion 9, M. Mednieks, Morphological and Biochemical Examination of Heart Tissue: III. Cyclic AMP Receptor Protein Distribution in Heart Muscle of Rats Flown on Cosmos 2044

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Behnke, B.J.; Stabley, J.N.; McCullough, D.J.; Davis, R.T.; Dominguez, J.M.; Muller-Delp, J.M.; and Delp, M.D.: Effects of Spaceflight and Ground Recovery on Mesenteric Artery and Vein Constrictor Properties in Mice. FASEB J., vol. 27, no. 1, Jan. 2013, pp. 399–409.

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.: Orthostatic Intolerance After Spaceflight. J. Applied Physiology, vol. 81, 1996, pp 7–18.

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Keil, L.C.; Evans, J.; Grindeland, R.; and Popova, I.: Natriuretic Peptide Content of Atria From Rats Exposed to 14 Days of Spaceflight. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 1. J.P. Connolly, R.E. Grindeland, and R.W. Ballard (Eds.), NASA TM-108802, 1994, pp. 374–379.

Mednieks, M.I.; Popova, I.A.; and Grindeland, R.E.: Cyclic-AMP Receptor Proteins in Heart Muscle of Rats Flown on Cosmos 1887. Aviation, Space, and Environmental Medicine, vol. 62, 1991, pp. 947–952.

Philpott, D.E.; Kato, K.; and Mednieks, M: Ultrastructure and Cyclic AMP Medicated Changes in Heart Muscle Under Altered Gravity Con-

ditions. J. Molecular and Cellular Cardiology, vol. 19, no. IV, 1987, p. S61.

Sandler, H.: Cardiovascular Results From a Rhesus Monkey Flown Aboard the Cosmos 1514 Spaceflight. Aviation, Space, and Environmental Medicine, vol. 58, no. 6, 1987, pp. 529–536.

Sandler, H.; Skidmore, M.; Hines, J.; Osaki, R.; Agasid, E.; MacKenzie, R.; Krotov, V.P.; Bazunova, E.G.; Belgorodsky, A.O.; Estratov, Y.A.; and Nazin, A.N.: Primate Cardiovascular Flight Experiment and Ground-Based Controls. Final Report of the U.S. Experiment Flown on the Soviet Biosatellite Cosmos 1667. J.W. Hines and M.G. Skidmore (Eds.), NASA T-108803, 1994, pp. 25–126.

Stabley, J.N.; Dominguez, J.M.; Dominguez, C.E.; Mora Solis, F.R.; Ahlgren, J.; Behnke, B.J.; Muller-Delp, J.M.; and Delp, M.D.: Spaceflight Reduces Vasoconstrictor Responsiveness of Skeletal Muscle Resistance Arteries in Mice. J. Applied Physiology, vol. 113, no. 9, Nov. 2012, pp. 1439–1445.

Taylor, C.R.; Hanna, M.; Behnke, B.J.; Stabley, J.N.; McCullough, D.J.; Davis, R.T.; Ghosh, P.; Papadopoulos, A.; Muller-Delp, J.M.; and Delp, M.D.: Spaceflight-Induced Alterations in Cerebral Artery Vasoconstrictor, Mechanical, and Structural Properties: Implications for Elevated Cerebral Perfusion and Intracranial Pressure. FASEB J., vol. 27, no.1, Jan. 2013, pp. 2282–2292.

Biosatellite III

Launch Date 6/28/1969 **Landing Date** 7/7/1696

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Monitoring Cardiovascular Function and Performance in the Primate Under Prolonged Weightlessness

Science Discipline
Cardiovascular Physiology

Investigator	Institute
J.P. Meehan	University of Southern California

Co-Investigator(s)	Institute
Rader, R.D.	University of Southern California

Research Subject(s)
Macaca nemestrina (Pig-Tailed Monkey)

Ground-Based Controls
Laboratory (Flight Backup Subjects)

Key Flight Hardware
Primate Life Support System; Primate Physiological Sensors

Selected Publications

Meehan, J.P. and Rader, R.D.: Cardiovascular Observations in the Macaca nemestrina Monkey in Biosatellite III. Physiologist, vol. 16, 1973, pp. 184-193

Meehan, J.P. and Rader, R.D.: Cardiovascular Observations of the Macaca nemestrina Monkey in Biosatellite III. BIOSPEX: Biological Space Experiments, NASA TM #58217, 1979, pp. 125.

Meehan, J.P. and Rader, R.D.: Monitoring Cardiovascular Function in the Primate Under Prolonged Weightlessness: Final Report. NASA CR -73498, 1970.

Objectives/Hypothesis
This portion of the primate mission was to determine the physiological effects of Earth orbit on a nonhuman primate, so as to provide insights into the possible hazards associated with long-term space flight, and to acquire information on basic physiological adjustments to extended weightlessness, particularly concern- ing the cardiovascular system. The basic premise was that in weightlessness, as a consequence of reduction of the gravitational effect on the long columns of blood in the body, pooling of blood in the large vessels would occur, and that a compen- satory mechanism would act to decrease this high blood volume. One indicator of this reflex is the pressure in the large vessels near the heart.

Approach or Method
Inflight vascular pressures were obtained by catheterization techniques. A low power pulsate infusion system maintained catheter patency, and amplification was incorporated to obtain signals compatible with Biosatellite III telemetry. Four indwelling catheters, two venous and two arterial, yielded redundant pressure measurements; additional redundance was obtained by connected tow transducers to each arterial catheter. One pair of electrodes provided electrocardiographic and respiratory information. Heparin pumps were used to keep the catheters clear. Inflight results were compared to the four ground control monkeys, plus the preflight baseline data from the flight monkey.

Results
There was a shift of blood volume to the heart; the flight subject experienced an immediate sustained increase in central venous pressure resulting from a central pooling of blood volume. The observed increase in atrial pressure of 2-3 cm water was large enough to provide a stimulus whereby urine level was initially main- tained at a high level, which coupled with a high evaporative fluid loss, produced an early dehydration, probably associated with electrolyte imbalance. Weightless- ness and hypothermia acted to shift blood volume centrally, which provided a strong drive for the reduction of blood volume. Restraint, unusual vestibular sensations, and the continuing polydipsia all acted to disturb the central mecha- nisms which might have acted to restore normal control and regulation of salt and water metabolism. Venous pressure started to fall on flight day five, while arterial pressure and heart rate were within physiologic limits until day eight, i.e. near the termination of the flight.

Cosmos 936 / Bion 4

Launch Date 8/13/1977

Landing Date 8/22/1977

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Effect of Weightlessness and Centrifugation (1xG) on Erythrocyte Survival in Rats Subjected to Prolonged Spaceflight

Science Discipline
Hematology

Investigator	Institute
H. Leon	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Landaw, S.A.	VA Hosiptal, Syracuse
Serova, L.V.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Centrifuged Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Leon, H. A.; Serova, L.V.; and Landaw, S. A.: Effect of Weightlessness and Centrifugation on Red Blood Cell Survival in Rats Subjected to Spaceflight. Aviation, Space, and Environmental Medicine, vol. 51, 1980, pp. 1091–1094.

Objectives/Hypothesis
With few exceptions, the loss of a variable quantity of red blood cell (RBC) mass has been a consistent finding in humans subjected to spaceflight. Past data suggest that mechanisms other than oxygen-induced hemolysis must be operative to cause this decrease in RBC mass. Analysis of Cosmos 782 data showed that random hemolysis was increased three-fold in the flight rats, and the mean potential life span was decreased about 5%. The present experiment was undertaken to verify the alterations seen in Cosmos 782 and to ascertain if the alterations could be attenuated by artificial gravity via an onboard centrifuge.

Approach or Method
Five rats were subjected to near weightlessness spaceflight and five rats were subjected to a 1-g force via an onboard centrifuge. Comparisons were made to ground-based vivarium, and synchronous stationary and centrifuged controls. Erythrocyte hemolysis and life span were evaluated by quantification of radioactive carbon monoxide exhaled in breath, which arises from the breakdown of the previously 2-14C glycine labeled hemoglobin.

Results
Considering only the output of 14CO during the first 3 days postflight, radioactivity was 60% higher in the flight-weightless than in the flight-centrifuged group. The mean, overall, RBC life span was significantly shortened in the latter group as a result of increased hemolysis, a difference even more pronounced if the one rat that suffered measurable injury is excluded from the data. Changes in hemolysis are also reflected in the portion of RBCs dying of senescence. The results support previous findings, wherein hemolysis was found to increase as a result of spaceflight. Unrelated to exogenous factors, this change appears specific to weightlessness, because it was attenuated by artificial gravity created by in-flight centrifugation. A possible initiator of the hemolysis is a cephalad fluid shift and subsequent hemoconcentration caused by the weightlessness.

Cosmos 1514 / Bion 6

Launch Date 12/14/1983

Landing Date 12/19/1983

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Cardiovascular Results from a Rhesus Monkey Flown Aboard the Cosmos 1514 Spaceflight, and Ground-Based Controls

Science Discipline
Cardiovascular Physiology

Investigator	Institute
H. Sandler	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Krotov, V.P.	Institute of Biomedical Problems
Stone, H.L.	NASA Ames Research Center (ARC)
Benjamin, B.	NASA Ames Research Center (ARC)

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Primate-BIOS: Cardiovascular Experiment Hardware; Combined Pressure/Flow (CPF) Cuff; Cosmos 1514 Russian Hardware Suite

Selected Publications
Sandler, H.: Cardiovascular Results From a Rhesus Monkey Flown Aboard the Cosmos 1514 Spaceflight. Aviation, Space, and Environmental Medicine, vol. 58, no. 6, 1987, pp. 529–536.

Korolkov, V.T.; Krotov, V.P.; Badakva, A.M.; Lobachik, V.T.; Truzhennikov, A.N.; Yushin, V.A.; Vacek, A.; and Sandler, G.: Circulation in Primates Exposed to Microgravity Aboard the Cosmos Biosatellites. Physiologist, supl., vol. 35, no. 1, 1992, pp. S245–S247.

Objectives/Hypothesis
This flight experiment was designed to determine whether blood pressure and flow relationships to the head change with weightlessness. The objectives of this experiment were: 1) to develop a conscious Rhesus monkey model to record carotid pressure and flow in a non-stressed animal; 2) to determine the effects of weightlessness on pressure-flow relationships to the head; and 3) to correlate cardiovascular findings with other simultaneously recorded physiologic information before, during, and after the flight.

Approach or Method
A single cylindrical probe (CPF Cuff) containing both flow and pressure transducers was chronically implanted around the left common carotid artery. Flow was measured using Doppler ultrasonic crystals and the continuous wave technique. Data collection before, during, and after flight consisted of 5 minutes of continuous recording every 2 hours. For pressure, each beat was analyzed with respect to value at peak systolic (maximum) and diastolic (minimum) levels. Systolic and diastolic pressures were determined by averaging all the values detected in respective intervals. Mean blood pressure was derived as the average (integrated under the curve) for the entire 20-beat or 20-second interval. Carotid blood velocity was calculated as the mean, integrated area under the curve, for each beat, representing the sum of systolic and diastolic flow periods.

Results
The prevailing heart rates during flight (80 bpm to 130 bpm) provide evidence that the animal model developed for this flight represents a normal (non-stressed) state for the monkey. Relative levels of blood flow velocity and blood flow per minute decreased during days two to three of flight and included a decrease in signal amplitude fluctuations, suggesting that there was significant stress on the animal during this period. On the third day, there was a relative increase in amplitude of 24-hour fluctuations for all parameters, signifying the development of circulatory adaptive processes and associated neuroendocrine mechanisms. In summary, the monkey demonstrated from the first hour of flight, an increase in blood pressure, a decrease in blood flow velocity to the head, and an increase in common carotid artery peripheral vascular resistance. Following this, compensatory mechanisms were invoked leading to rapid adaptation of regional hemodynamics in response to general hemodynamic changes. Cardiovascular system changes were maximal on day two of flight, with signs of adaptation appearing on days three to five of flight.

STS-51B / SL-3

Launch Date 4/29/1985 **Landing Date** 5/6/1985

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Animal Studies on Spacelab-3

Science Discipline
Cardiovascular Physiology

Investigator	Institute
R.E. Grindeland	NASA Ames Research Center (ARC)
P.X. Callahan	

Co-Investigator(s)	Institute
Berry, W.E.	NASA Ames Research Center (ARC)
Schatte, C.	NASA Ames Research Center (ARC)
Funk, G.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)
Saimiri scuireus (Squirrel monkey)

Ground-Based Controls
Simulated Flight Control Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Ray, C.A.; Vasques, M.; Miller, T.A.; Wilkerson, M.K.; and Delp, M.D.: Effect of Short-Term Microgravity and Long-Term Hindlimb Unloading on Rat Cardiac Mass Function. Journal of Applied Physiology, vol. 91, no. 3, Sept. 2001, pp. 1207–1213.

Objectives/Hypothesis
NASA Ames Research Center undertook the first of several missions using animals as model mammalian systems with which to delineate the fundamental mechanisms of physiological response to an environment; i.e., microgravity. The primary objective of the Spacelab-3 mission was to evaluate the ability of the Research Animal Holding Facility (RAHF) to maintain animals in a normal, laboratory environment in space. It also provided the opportunity to obtain preliminary data on animal health and well being in flight, as well as to sample selected physiological parameters that might be the focus of future flight experiments.

Approach or Method
Two monkeys and 2 groups of 12 rats each were flown in the primate and rodent cage modules of the RAHF, respectively. It was important to select an animal that grows normally, behaves normally, and is free from chronic stress. One group of rats was composed of 12-week-old adults and the other group was composed of juveniles about 8 weeks old. Four of the large rats were implanted with a transmitter, which permitted the continuous monitoring of electrocardiogram (EKG), heart rate, and deep-body temperature. Food and water consumption, activity, and intermittent photographic records were obtained automatically and temperature, humidity, and light cycles were all controlled via the RAHF. Animals spent 1 hour in the Spacelab prior to launch, and rodents were not sacrificed until 12 hours following recovery. More extensive studies were performed by various investigators on the rats, following biosample distribution.

Results
Data indicate that the RAHF was able to maintain both rats and monkeys in a relatively normal condition suitable for their use as experimental animals. Both monkeys ate less food and were less active in space than on the ground. One animal appeared to maintain relatively normal eating behavior throughout the mission, while the other showed abnormally low food consumption for the first 4 days followed by substantial recovery during the last 3 days. Videotape records were consistent with the conclusion that the latter animal may have suffered from space motion sickness. Heart rate monitored in flight rats was lower at all times, but its circadian rhythm was unchanged from that of preflight. Mean body temperature was not changed; however, its rhythm was increased in flight, suggesting that microgravity may cause body-temperature rhythm to become free-running. Growth parameters and several indices of chronic stress suggested that changes in bone and muscle were a result of exposure to microgravity per se and not an artifact resulting from adverse housing conditions. It is apparent that microgravity induces a wide range of physiological changes.

Cosmos 1667 / Bion 7

Launch Date 7/10/1985

Landing Date 7/17/1985

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Effect of Microgravity on Blood Pressure and Flow in the Common Carotid Artery of Primates

Science Discipline
Cardiovascular Physiology

Investigator	Institute
H. Sandler	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Krotov, V.P.	Institute of Biomedical Problems
Osaki, R.I.	NASA Ames Research Center (ARC)
Hines, J.	NASA Ames Research Center (ARC)

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cardiovascular Experiment Hardware-Mod 1; Combined Pressure/Flow (CPF) Cuff; Cosmos 1667 Russian Hardware Suite

Selected Publications

Korolkov, V.T.; Krotov, V.P.; Badakva, A.M.; Lobachik, V.T.; Truzhennikov, A.N.; Yushin, V.A.; Vacek, A.; and Sandler, G.: Circulation in Primates Exposed to Microgravity Aboard the Cosmos Biosatellites. Physiologist, supl., vol. 35, no. 1, 1992, pp. S245–S247.

Sandler, H.; Skidmore, M.; Hines, J.; Osaki, R.; Agasid, E.; MacKenzie, R.; Krotov, V.P.; Bazunova, E.G.; Belgorodsky, A.O.; Estratov, Y.A.; and Nazin, A.N.: Primate Cardiovascular Flight Experiment and Ground Based Controls. Final Report of the U.S. Experiment Flown on the Soviet Biosatellite Cosmos 1667. M.G. Skidmore and J.W. Hines, eds., NASA TM-108803, 1994, pp. 25–126.

Objectives/Hypothesis
The experiment was designed to study cardiovascular changes in non-human primates exposed to the microgravity environment. As the results of Cosmos 1514 indicated that significant cardiovascular changes occur during spaceflight, the study conducted aboard Cosmos 1667 was to strengthen this conclusion. An additional feature of the experiment included a postflight control study using the flight animal.

Approach or Method
One flight subject, along with one-half of the flight candidates, also received cardiovascular (CV) pressure and flow sensor implants (CPF Cuff), and respiration sensors. Because the CV flow and pressure transducers were subject to drift with variations in barometric pressure, a cross-calibration method was developed. Both flight monkeys were also instrumented to record neurophysiological parameters. Intermittent postural tilt tests were conducted before and after spaceflight and synchronous studies to simulate the fluid shifts associated with spaceflight.

Results
The experiment results support the conclusion derived from Cosmos 1514 that significant cardiovascular changes occur with spaceflight. The changes most clearly seen were rapid initial decreases in heart rate and further decreases with continued exposure to microgravity. The triggering mechanism appeared to be a headward shift in blood and tissue volume which, in turn, triggered adaptive cardiovascular changes. Dramatic increases in arterial pulse pressure may indicate the in-flight hemodynamic adjustments to this headward fluid shift. The adaptive responses could be a means to maintain adequate oxygen delivery to the brain under these circumstances. Adaptive changes took place rapidly and began to stabilize after the first 2 days of flight. However, these changes did not plateau in the animal by the last day of the mission, and heart rate and blood pressure did not demonstrate evidence of stabilization by the end of the flight.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Morphological and Biochemical Examination of Heart Tissue: I. Effects of Microgravity on the Myocardial Fine Structure of Rats Flown on Cosmos 1887–Ultrastructure Studies

Science Discipline
Cardiovascular Physiology

Investigator	Institute
D.E. Philpott	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Kato, K.	NASA Ames Research Center (ARC)
Sapp, W.	Tuskegee University
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS
Cosmos 1887 Russian Hardware Suite

Selected Publications
Philpott, D.E.; Kato, K.; and Mednieks, M.: Ultrastructure and Cyclic AMP-Medicated Changes in Heart Muscle Under Altered Gravity Conditions. Journal of Molecular and Cellular Cardiology, supl., vol. 19, 1987, p. S61.

Philpott, D.E.; Popova, I.A.; Kato, K.; Stevenson, J.; Miquel, J.; and Sapp, W.: Morphological and Biochemical Examination of Cosmos 1887 Rat Heart Tissue: Part I–Ultrastructure. FASEB Journal, vol. 4, no. 1, 1990, pp. 73–78.

Objectives/Hypothesis
Vascular deconditioning, which is an important medical problem, is recognized as one of the key concerns of spaceflight research. This loss of physiological performance of the circulatory system is accompanied by disuse muscle atrophy triggered by the reduced functional load of the musculoskeletal system in the microgravity environment. Animal models are often used to examine disuse atrophy because they are more amenable to morphological and biomedical research needed to unravel the mechanisms of muscle breakdown. Using these models, one could design preventive countermeasures. This study sought to expand previous research.

Approach or Method
Tissue was obtained for study of the effects of microgravity on the myocardium. The left ventricles of hearts from rats flown on the biosatellite were compared to the same tissue of synchronous and vivarium control animals using a minimum of 100 electron micrographs from each animal. Volume density of the mitochondria was determined by point counting, using 8x10 micrographs at a magnification of 27,500x.

Results
Structural changes seen in the flown rats included some loss of microtubules and fibrillar edema that may be linked to tissue breakdown, with a concomitant increase in osmotic pressure and fluid entry into the cells. Intermittent areas of missing protofibril (actin, myosin filaments) were observed in cross sections of flight tissue, which is indicative of muscle breakdown. Point counting of the mitochondria in the left ventricle resulted in a mean of 39.9 for the vivarium, 38.9 synchronous, and 32.5 for the flight tissue. It is clear that the volume density of the mitochondria in the flight group was reduced by a significant amount. Capillary alterations were also seen in the flight tissue in the form of numerous endothelial invaginations projecting into the lumen of the capillaries. The present data support the view that an optimum work load imposed on the heart (i.e., moderate physical exercise at normal Earth gravity) is essential for preservation of mitochondrial structure and function.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study

Morphological and Biochemical Examination of Heart Tissue: II. Cellular Distribution of Cyclic AMP-Dependent Protein Kinase Regulatory Subunits in Heart Muscle of Rats Flown on Cosmos 1887

Science Discipline

Cardiovascular Physiology
Endocrinology

Investigator	Institute
M.I. Mednieks	Northwestern University

Co-Investigator(s)	Institute
Popova, I.A.	Institute of Biomedical Problems
Serova, L.V.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Wistar rat)

Ground-Based Controls

Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS
Cosmos 1887 Russian Hardware Suite

Selected Publications

Mednieks, M.I.; Popova, I.A.; and Grindeland, R.E.: Cyclic-AMP Receptor Proteins in Heart Muscle of Rats Flown on Cosmos 1887. Aviation, Space, and Environmental Medicine, vol. 62, no. 10, 1991, pp. 947–952.

Mednieks, M.I.: Morphological and Biochemical Examination of Heart Tissue: Part II. Cellular Distribution of Cyclic Amp-Dependent Protein Kinase Regulatory Subunits in Heart Muscle of Rats Flown on Cosmos 1887. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 315–329.

Objectives/Hypothesis

The study was undertaken in order to gain insight into the mechanistic aspects of cardiac changes that both animals and humans undergo as a consequence of space travel. A number of physiologic changes, attributed to space travel conditions in experimental animals and humans, can be the consequence of increased circulating levels of catecholamine hormones. In this study, the cellular compartmentalization and biochemical localization of regulatory subunits (R-subunits) of cyclic AMP-dependent protein kinase (cAPK) of ventricle heart tissue obtained from space-flown rats were determined.

Approach or Method

Photoaffinity labeling with a 32P-8-azido analog of cyclic AMP, and electrophoretic separation of the proteins, was followed by autoradiographic identification of the subcellular fraction in which the labeled R subunits were localized. Similarly, antibodies to R-subunits were prepared and employed in an immunogold electron microscopic procedure to directly visualize cellular compartmentalization of the cAPK R-subunits.

Results

Results showed that protein banding patterns in both the cytoplasmic fraction and in a fraction enriched in chromatin-bound proteins showed some individual variability in tissues of different animals, but exhibited no changes that can be attributed to the flight. Examination of cellular localization of the isotopically labeled R-subunits of cAPK isotopes showed no change in the distribution of RI in either soluble or particulate fractions, whereas the presence of RII in the particulate subcellular fraction, as well as in regions of nuclear chromatin, was greatly decreased in tissues from rats in the flight group when compared to controls. These findings indicate that a major catecholamine hormone regulated mechanism in cardiac tissue is altered during some aspect of space travel.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Measurement of Heart Atrial Natriuretic Peptide Concentrations

Science Discipline
Cardiovascular Physiology
Endocrinology

Investigator	Institute
L.C. Keil	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Evans, J.	NASA Ames Research Center (ARC)
Grindeland, R.E.	NASA Ames Research Center (ARC)
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Laboratory Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Keil, L.; Evans, J.; Grindeland, R.; and Popova, I.: Natriuretic Peptide Content of Atria from Rats Exposed to 14 Days of Spaceflight. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 1, J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-108802, 1994, pp. 374–379.

Objectives/Hypothesis
Although fluid-electrolyte balance has not been determined during flight, postflight hormone measurement and salt water loading experiments indicate that rats also respond to microgravity by readjustment of their fluid-electrolyte requirements. Atrial natriuretic peptide (ANP) secretion is regulated primarily by atrial pressure. The purpose of this experiment was to determine if spaceflight exposure had an effect on the concentration of ANP.

Approach or Method
Acid extracts of atria were radioimmunoassayed for 1–28 rat atrial natriuretic peptide. Antibodies to ANP were developed in male, New Zealand rabbits after multiple injections of 1-28 ANP. The amount needed to displace 50% of the labeled hormone was 30.5 ±0.8 pictograms for a typical standard curve. Inter- and intra-assay coefficient of variation was 7.4 and 7.1%, respectively.

Results
Atria from the flight group contained significantly less ANP than the control groups, including the tail-suspended animals. Reduction of atrial ANP may reflect an increase in hormone secretion during flight. Flight animals may have experienced a rise in atrial pressure that provoked an increase in ANP secretion, or the reduced levels may represent some of the changes in hormonal regulation of fluid-electrolyte metabolism that occur during spaceflight. The tail-suspension model does not seem to be an appropriate model for simulating the effects of microgravity on ANP metabolism.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Histologic Examination of Lung Tissue

Science Discipline
Cardiovascular Physiology

Investigator	Institute
J.B. West	University of California, San Diego

Co-Investigator(s)	Institute
Elliott, A.	University of California, San Diego
Mathieu-Costello, O.	University of California, San Diego
Kaplansky, A.S.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Elliot, A.R.; Mathieu-Costello, O.; and West, J.B.: Lung Morphology Study. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 2, J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-108802, 1994, pp. 223–231.

Objectives/Hypothesis
Limited information is available regarding the effect of spaceflight on the respiratory system. Though animals as small as rats are not expected to undergo major cephalad fluid shifts during microgravity exposure as man does, tail-suspended rats do experience large cephalad shifts in body fluids. This study, through light and electron microscopy, compared the lung tissue of five space-flown rats to tissue from similarly maintained synchronous and tail-suspended animals.

Approach or Method
Within ten minutes of sacrifice, the left lungs were removed and immersed in 3% glutaraldehyde in 0.1 M phosphate buffer. Sections (5–6 μm) taken from a tissue slab cut perpendicular to the cranio-cadual axis just across the most caudal aspect of the hilum were stained with hemotoxin-eosin for light microscopy. Samples for electron microscopy were taken from the most ventral and dorsal aspects of the remaining lower lobe. Sections (1 μm and 50–70 nm), contrasted with uranyl acetate and bismuth subnitrate, were examined for peribronchial cuffing of pulmonary vessels, presence of alveolar edema, and general appearance of pulmonary capillaries and parenchyma.

Results
By the onset of dissection, flight animals were inactive and had reddish fluid drops on the tips of their noses, signs attributed to the stress induced by transition into Earth's gravity. No obvious evidence of perivascular cuffing was observed in any group. Red blood cells were seen in the lumen of major airways of all samples, with the least observed in vivarium controls. Pulmonary capillaries appeared more congested in flight animals, possibly related to increased hematocrit due to microgravity. The flight, tail-suspended, and synchronous animals that showed intra-capillary vesicles probably experienced pulmonary hyper-intensive episodes that could have induced a hemodynamic form of pulmonary edema.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Morphological and Biochemical Examination of Heart Tissue: I. Ultra-Structural Alterations in Rat Hearts After Cosmos 2044 Compared to Cosmos 1887

Science Discipline
Cardiovascular Physiology

Investigator	Institute
D.E. Philpott	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Popova, I.A.	Institute of Biomedical Problems
Kato, K.	NASA Ames Research Center (ARC)
Sapp, W.	Tuskegee University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Philpott, D.E. et al.: A Promising Drug for Prevention of Heart Deconditioning During Weightlessness in Rats. Proceedings of Electron Microscope Society, vol. 46, 1988, pp. 130–131.

Philpott, D.E.; Kato, K.; and Miguel, J.: Ultrastructural and Cellular Mechanisms in Myocardial Deconditioning in Weightlessness. Advances in Space Biology and Medicine, S.L. Bonting, ed., vol. 2, 1992, pp. 83–112.

Objectives/Hypothesis
Previous studies have concluded that when rats undergo a change from the weightless environment of space to Earth's gravity, the heart experiences an increase in load compared to weightlessness, manifesting in tissue anoxia and concomitant mitochondrial disorganization. One investigation of this sort flew on Cosmos 1887. This experiment repeats that study.

Approach or Method
The left ventricles of rats were dissected, dehydrated with ascending concentrations of acetone, infiltrated, embedded, sectioned, and stained for observation by an electron microscope. Volume density was determined by point counting using 240 micrographs (8x10) at a magnification of 27,500x. Capillary counts, using randomly selected open-grid squares in the microscope, were converted to counts per 600 square microns.

Results
The results were comparable to those in Cosmos 1887. Statistical analysis showed a significant reduction in flight mitochondria and an increase in glycogen and edema. It is possible that the edema may be linked to tissue breakdown, with concomitant increase in osmotic pressure and fluid reentry into the cells. Lipid accumulation was significant in flight animals as well as an increase in dense bodies. The increase in dense bodies is an indication of increased lysosomal activity, which is expected in muscle atrophy. There was also an increase in total free lysosomal enzyme activities in the ventricles, indicating the occurrence of active degradation. Although at present the basic mechanisms of cardiovascular deconditioning and recovery are not well understood, comparison with tail-suspension data suggests that fixation in space is necessary to obtain the complete picture of heart changes during flight.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Morphological and Biochemical Examination of Heart Tissue: II. Cardiac Morphology After Conditions of Microgravity

Science Discipline
Cardiovascular Physiology

Investigator	Institute
M. Goldstein	Baylor College of Medicine

Co-Investigator(s)	Institute
Schroeder, J.P.	Baylor College of Medicine
Edwards, R.J.	Baylor College of Medicine
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Goldstein, M.A.; Edwards, R.J.; and Schroeter, J.P.: Cardiac Morphology After Conditions of Microgravity During Cosmos 2044. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S94–S100.

Objectives/Hypothesis
The objectives of this study were to: 1) compare papillary muscle and ventricular muscle, 2) determine if there is a change in heart cell size in rat hearts exposed to microgravity, and 3) assess concomitant ultrastructural change.

Approach or Method
Cross-sectional areas of heart cell profiles in light microscope sections were measured for papillary and ventricular muscle samples. Electron microscopy was used to correlate general morphological features in the capillaries with features observed in the larger samples taken from the light microscopy. Stereological analysis and optical diffraction techniques were used for quantitative electron microscopy studies.

Results
Endothelial cell surfaces of capillaries with extended projections and elongated marginal folds were observed in papillary muscle from all groups, but were most pronounced in flight animals. Stereological analysis of papillary muscles revealed increased mitochondrial density values for flight and tail-suspended rats, mitochondrial-to-myofibril ratios showing the same trend. Optical diffraction studies revealed normal A and Z band spacings. In conclusion, cardiac morphology is affected by spaceflight, and a continuing concern for a compensated adaptation of the heart to microgravity is warranted.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study

Morphological and Biochemical Examination of Heart Tissue: III. Cyclic AMP Receptor Protein Distribution in Heart Muscle of Rats Flown on Cosmos 2044

Science Discipline

Cardiovascular Physiology
Endocrinology

Investigator	Institute
M.I. Mednieks	University of Chicago

Co-Investigator(s)	Institute
Grindeland, R.E.	NASA Ames Research Center (ARC)
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Mednieks, M.I.; Popova, I.: and Grindeland, R.E.: Photoaffinity Labeling of Regulatory Subunits of Protein Kinase A in Cardiac Cell Fractions of Rats. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S101–S106.

Objectives/Hypothesis

The effect of space travel on the cardiovascular system has been investigated to determine if measurable changes could be noted, and if such changes might influence the health of the individual. The present study was undertaken in order to gain insight into a specific biochemical mechanism that may be associated with cardiac changes that experimental animals undergo as a consequence of travel in space, the mechanism in question being cyclic AMP-receptor reactions in the cell particulate fraction.

Approach or Method

Tissue homogenates in addition to the subcellular fractions were aziado-labeled and analyzed for total cyclic AMP-binding analysis. Photoaffinity labeling of regulatory subunits (RI and RII) of cyclic AMP dependent protein kinase was carried out to measure cyclic AMP binding protein activities. Polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulfate, was carried out using a conventional size as well as a mini-gel apparatus.

Results

Cyclic AMP binding protein activities were decreased in heart tissue of flight rats. Densitometric analyses showed a significant decrease of RII in the particulate cell fraction extract. The photoaffinity labeling of soluble fraction was unaffected, as previously observed on Cosmos 1887. A negative correlation resulted when incorporation of total counts of aziado-labeling was based on body weights, while no changes were seen when total label was calculated on the basis of adrenal gland weights. Factors that influence body weight changes therefore may alter hormone response, while changes in a relatively minor aspect of cyclic AMP mediated reactions may have a metabolic effect on an organismic level.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study

Morphological and Biochemical Examination of Heart Tissue: IV. Altered Myosin Expression in Rat Ventricular Muscle

Science Discipline

Cardiovascular Physiology

Investigator	Institute
D.B. Thomason	University of Tennessee

Co-Investigator(s)	Institute
Booth, F.W.	University of Texas Health Center
Baldwin, K.M.	University of California, Irvine
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Thomason, D.B.; Morrison, P.R.; Oganov, V.; Ilyina-Kakueva, E.; Booth, F.W.; and Baldwin, K.M.: Altered Actin and Myosin Expression in Muscle During Exposure to Microgravity. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S90–S93.

Thomason, D.B. and Booth, F.W.: Atrophy of the Soleus Muscle by Hindlimb Unweighting. Journal of Applied Physiology, vol. 68, 1990, pp. 1–12.

Objectives/Hypothesis

Microgravity is known to produce cardiovascular deconditioning, changes that may result in part from a regulation of cardiac muscle gene expression at the level of translation. However, little is known about the possible role of transcriptional regulation in the control of the heart during spaceflight. The purpose of this study was to examine the potential role of transcriptional regulation of myosin expression experienced by rats flown in space.

Approach or Method

Total RNA was extracted from the frozen, powdered muscle with the guanidinium isothiocyanate-cesium chloride method. Varying amounts of total ribonucleic acid (RNA) from each sample were electrophoresed on an RNA denaturing gel, probed with a 32P-labeled oligonucleotide probe specific for the beta-myosin heavy chain messenger ribonucleic acid (mRNA), and exposed to x-ray film that was subsequently scanned densitometrically. Statistical differences between the flight and control groups were determined by analysis of covariance for the heavy-chain mRNA expression and mapping of composite densitometric scans.

Results

No differences between the flight and control groups were observed in the myosin protein isoform profile of the cardiac muscle samples. Beta-myosin heavy-chain mRNA expression was also not statistically different. In part, this lack of difference may be artifactual, because on a relative basis, many other species of contractile protein and RNA may also be changing in content, as demonstrated by the covariance mapping, where there were clearly differences identified by the probe between flight and control hearts. Therefore, the cardiovascular deconditioning that is observed may begin early during exposure as a change in transcriptional regulation of gene expression.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Erythroid Colony Formation In-Vitro and Erythropoietin Determinations

Science Discipline
Hematology

Investigator	Institute
R.D. Lange	University of Tennessee

Co-Investigator(s)	Institute
Michurina, T.	Institute of Developmental Biology
Vacek, A.	Institute of Developmental Biology
Khrushchev, N.G.	Institute of Biophysics, Czechoslovakia

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Lange, R.D. et al.: Rat Hematological Parameters Following Flight on Soviet Biosatellite Cosmos 2044. Proceedings Cosmos Biosatellites: Experimental Results, Theories and Hypotheses, Leningrad, U.S.S.R., August 12–15, 1991, pp. 197–198.

Objectives/Hypothesis
In a continuing investigation of the pathogenesis of "space anemia," three red blood cell studies were performed on five flight rats and compared to controls. The bone marrow cell differentials, clonal bone marrow studies of red blood cell colony formation, and plasma erythropoietin determinations were performed, and compared to previous studies of these three parameters.

Approach or Method
Bone marrow smears were made at the landing site; received slides were stained, and bone marrow cells were counted (500 cell differential counts were performed) and classified. For erythroid clonal studies, tibial bone marrow plugs were cultured in Petri dishes; colonies were scored by the ability of hemoglobin-containing cells to reduce 2,7-diaminofluorene. Frozen plasma (0.2 ml) was used for erythropoietin determinations, utilizing a commercial kit.

Results
While some minor variations were found, there were no essential differences in bone marrow differential counts or erythropoietin levels of flight or tail-suspended animals as compared to controls. In studies of colony formation, there was a marked increase in the number of CFU-e colonies in frozen flight bone marrow, while no such increase was noted in the "fresh" cultures prepared in Moscow. As with previous studies, no pattern of erythropoiesis has emerged, and the rat may or may not be a valid model to study the decrease in red blood cell mass that occurs in humans exposed to microgravity.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Histologic Examination of Lung Tissue

Science Discipline
Pulmonary Physiology

Investigator	Institute
J.B. West	University of California, San Diego

Co-Investigator(s)	Institute
Mathieu-Costello, O.	University of California, San Diego
Elliott, A.	University of California, San Diego

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Delayed Synchronous Basal Control, Synchronous Control, Delayed Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)
Animal Enclosure Module (AEM)

Selected Publications
Prisk, G.K.; Elliott, A.R.; Guy, H.J.B.; and West, J.B.: Inhomogeneity of Pulmonary Perfusion During Sustained Microgravity on Spacelab SLS-1. Journal of Applied Physiology, vol. 76, 1994, pp. 1730–1738.

Dalton, B. P.; Jahns, G.; Meylor, J.; Hawes, N.; Fast, T.M.; Zarow, G. Spacelab Life Sciences-1 Final Report. NASA TM-4706. Aug. 1995.

Objectives/Hypothesis
Limited information is available regarding the effect of microgravity on the lung, though several functional aspects of the respiratory system have been shown to be extremely sensitive to microgravity exposure. Human studies have shown a headward fluid shift during weightlessness. Pulmonary blood flow and alveolar ventilation become more uniform. It is hypothesized that exposure to changes in gravitational forces could potentially induce pathological changes in the lung related to abnormal lung fluid balance, altered pulmonary capillary hemodynamics, and possible pulmonary hypertension. The objective of this experiment was to examine the effects of microgravity exposure on lung ultrastructure and relate the changes in lung histology, if any, to alterations in lung physiology.

Approach or Method
Lungs were removed from the animals within 10 minutes of decapitation. One lung from each animal was fixed in a glutaraldehyde fixative. Samples for electron microscopy were taken from the most ventral and dorsal aspects of a tissue slab cut perpendicular to the cranio-caudal axis of the lung. Tissue samples were rinsed overnight in 0.1M phosphate buffer adjusted to 350 Osm, then dehydrated, rinsed, and embedded in Araldite. Sections were also cut from two tissue blocks selected randomly from each lung site (dorsal/ventral), stained, and examined by light microscopy. Ultrathin sections were examined with an electron microscope. Samples were examined for peribronchial cuffing of smaller pulmonary vessels, the presence of alveolar edema, and general appearance of the pulmonary capillaries and lung parenchyma. The ultrastructure of the blood-gas barrier was also examined by electron microscopy.

Results
Only minor changes were noted in any of the lung tissues examined. Six of the animals had very rare intraalveolar fragments of debris. In all cases, these were limited to one to three total fragments in the section examined. In two of the animals (animals 2 and 7) a single hair fragment was noted within an alveolus. In the remaining animals, the debris was limited to rare, tiny, sharp, somewhat crystalline shards that were not identifiable as to specific type of material due to the very small size and limited amount of material present. Congestive changes consistent with decapitation were most prominently noted in animals 1, 2, 3, 5 and 10, while rare to mild peribronchiolar accumulations of small numbers of lymphocytes and rare plasma cells were noted in animals 4, 5, 6, 7 and 10.

In summary, there were no distinctive features separating any one group of animals clearly from another group.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Regulation of Erythropoiesis During Spaceflight

Science Discipline
Hematology
Immunology

Investigator	Institute
A.T. Ichiki	University of Tennessee

Co-Investigator(s)	Institute
Jones, J.B.	University of Geogia

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)
General Purpose Transfer Unit (GPTU)

Selected Publications
Allebban, Z.; Gibson, L.A.; Lange, R.D.; Jago, T.L.; Strickland, K.M.; Johnson, D.L.; and Ichiki, A.T.: Effects of Spaceflight on Rat Erythroid Parameters. Journal of Applied Physiology, vol. 81, no. 1, July 1996, pp. 117–122.

Congdon, C.C.; Gibson, L.A.; Lange, R.D.; Allebban, Z.; Kaplansky, A.; Strickland, K.M.; Jago, T.L.; Johnson, D.L.; and Ichiki, A.T.: Lymphatic Tissue Changes in Rats Flown on SLS-2. Journal of Applied Physiology, vol. 81, no. 1, July 1996, pp. 172–177.

Objectives/Hypothesis
Experiment results from SLS-1 showed a decrease in the number of Epo-responsive total bone marrow progenitors in flight rats compared to the ground controls. SLS-2 allowed further investigations into this study with the addition of two features; the injection of recombinant human Epo (rhEpo) to examine its affect on progenitor cells in microgravity and the in-flight collection of samples. The objective was to assess peripheral blood and bone marrow erythroid parameters.

Approach or Method
Blood samples were taken at scheduled times from the tail vein. Automated blood cell counts were performed twice and results were averaged for each sample. Reticulocytes were counted both manually from slides and through flow cytometry. Erythroid cultures were assayed with various combinations of rhEpo and in the absence of rhEpo. A 200 cell differential count was performed on bone marrow smears. On dissection days, cardiocentesis was performed and serum was sampled. Epo was measured using commercial radioimmunoassay kits.

Results
No significant changes were seen in peripheral blood erythroid elements. Nonadherent bone marrow cells taken from rats on flight day 13 had a lower number of recombinant rat interleukin-3 (rrIL-3) responsive cells and a lower number of (rrIL-3) plus rhEpo-responsive blast forming unit erytroid (BFU-e) colonies than ground controls. There was a slight increase in the number of rhEo plus rrIL-3 responsive BFU-e colonies on landing day. Flight rats stimulated with rhEpo or rhEpo plus rrIL-3 showed an increase in the number of erytroid colony forming units and a decrease in BFU-e colonies 9 days after flight. Results indicate that spaceflight affects rat bone marrow progenitor cells but has little affect on peripheral blood erythroid parameters.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Regulation of Blood Volume During Spaceflight

Science Discipline
Hematology

Investigator	Institute
C.P. Alfrey	Methodist Hospital

Co-Investigator(s)	Institute
Driscoll, T.B.	Baylor College of Medicine
Nachtman, R.G.	Krug International
Udden, M.M.	Baylor College of Medicine

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Alfrey, C.P.; Driscoll, T.; Nachtman, R.G.; and Udden, M.M.: Regulation of Blood Volume During Spaceflight. SLS-2 180-Day Report, May 1994, pp. 2-47–2-56.

Objectives/Hypothesis
Human adaption to spaceflight is accompanied by a loss of red blood cell mass (RBCM), a loss of plasma volume (PV), and a decrease in total blood volume. This leads to a decrease in gravity-dependent space below the heart. In this study, rats were examined to determine whether similar hemodynamic changes occur, making them a suitable subject for study. SLS-1 marked the first time PV and RBCM were measured pre and postflight. SLS-2 allowed for repeat determinations and the first opportunity for in-flight assessment of erytropoiesis. An additional in-flight experiment tested the ability of the rat bone marrow to respond to a bolus of erythropoietin, the major hormone controlling erythropoiesis in animals and man.

Approach or Method
Seven days prior to launch, PV and RBCM was determined by isotopic dilution of 125I labeled albumin and 51Cr labeled red blood cells, respectively. On fight day 6, five rats (group A) were given 125I labeled albumin and 59Fe injections. Samples were taken 10 minutes later to determine PV. Samples to determine 59Fe incorporation into RBCs were taken 24 hours later and on landing day (9 days later). The remaining 10 rats (group B and C) were given 59 Fe injections on flight day 9. Group B was also given a 200 U injection of erythropoietin at this time. Upon landing, RBCM and PV were measured for all flight rats and ground controls. 59 Fe incorporation into red blood cells was also determined.

Results
RBCM showed an increase in both the spaceflight animals and ground control animals, but less of an increase in flight animals. When this increase was normalized for growth (flight animals had a lower growth rate than ground controls) a decrease was seen in the flight animals RBCM. Plasma volume increased in both flight and ground control animals. The PV increase was greater in ground control animals although not statistically different. 59Fe incorporation was lower in flight animals but again not statistically different. Animals that received erythropoietin injections had higher levels of iron incorporation. Incorporation was not statistically significant, indicating erythropoisis is stimulated normally under conditions of microgravity.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Molecular Pharmacology of Alpha-1 Adrenoreceptors and Calcium Channels in Rat Vascular Myocytes

Science Discipline
Cardiovascular Physiology

Investigator	Institute
C. Mironneau	Universite de Bordeaux II

Co-Investigator(s)	Institute
Mironneau, J.	Universite de Bordeaux II
Rakotoarisoa, L.	Universite de Bordeaux II
Neuilly, G.	Ingenieur d'etudes INSERM
Sayet-Colombet, L.	Allocataire de recherche MRE

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Morel, J.L.; Boittin, F.X.; Halet, G.; Arnaudeau, S.; Mironneau, C.; and Mironneau, J.: Effect of a 14-Day Hindlimb Suspension on Cytosolic Ca2+ Concentration in Rat Portal Vein Myocytes. American Journal of Physiology, vol. 273, no. 6, pt. 2, Dec. 1997, pp. H2867–H2875.

Neuilly, G.; Sayet, I.; and Mironneau, C.: Influence of Spaceflight, Hindlimb Suspension, and Venous Occlusion on Alpha 1-Adrenoceptors in Rat Vena Cava. Journal of Applied Physiology, vol. 78, no. 5, May 1995, pp. 1882–1883.

Objectives/Hypothesis
The cardiovascular system adapts successfully to upper body fluid shifts by increasing heart rate, blood pressure, and total peripheral vascular resistetance and by decreasing venous pressure. These adaptive responses to fluid shift in spaceflight lead to a severe increase in heart rate and low blood pressure upon return to Earth. There have been few studies examining microgravity's effect on contractile properties of smooth muscles and the cellular and molecular alterations that control vascular tone. The objectives of this experiment were to look at changes in contractile response to norepinephrine of the vena cava, especially the $alpha_{1B}$ adrenoreceptors, and to determine the mechanism by which the adrenoreceptors are altered.

Approach or Method
Vena cava were removed from flight rats and cut into longitudinal strips. Specific binding to adrenoceptors was measured by incubating the strips in various concentrations of (^{3}H) prazosin and determining radioactivity after disolving strips in NaOH. Isometric contraction was measured in an experimental chamber using circular strips taken from the venae cavae. The maximum contractile response was determined using 30 micromolar norepinephrine. All other contractions were expressed as a percentage of maximal contraction. The inhibition of contractile response due to the binding of prazosin was also determined.

Results
A decrease in contractile strength in response to norepinephrine was found in flight rats. (^{3}H) prazosin binding affinity was reduced, indicating a reduction in specific affinity to $alpha_1$ adrenoreceptors. These data indicate that the reduction in contractile strength is due to a decrease in sensitivity of adrenoreceptors rather than a decrease in the number of adrenoreceptors. Ground control studies show a similar decrease in sensitivity through sustained activation of protein kinase C. This effect was not seen in the presence of an inhibitor of protein kinase C. This implies that desensitization of adrenoreceptors due to microgravity may be dependent on increased protein kinase C activity.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
ANP, Pro-ANP, and mRNA Distribution in Rat Heart During a Spaceflight

Science Discipline
Cardiovascular Physiology

Investigator	Institute
C. Gharib	Universite de Montpiler

Co-Investigator(s)	Institute
Fagette, S.	Université de Lyon
Fareh, J.	Université de Lyon
Somody, L.	Université de Lyon
Gauquelin, G.	Université Claude-Bernard
Koubi, H.	Centre National Recherche Scientifique
Viso, M.	Centre National d'Etudes Spatiales

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Cage Control, Asynchronous Tail Suspension Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Fagette, S.; Somody, L.; Koubi, L.; Fareh, J.; Viso, M.; Gharib, C.; and Gauquelin, G.: Central and Peripheral Noradrenergic Responses to 14 Days of Spaceflight (SLS-2) or Hindlimb Suspension in Rats. Aviation, Space, and Environmental Medicine, vol. 67, no. 5, 1996, pp. 458–462.

Objectives/Hypothesis
Previous studies show that spaceflight induces a wide variety of changes in biological systems, including the cardiovascular and neuroendocrine systems. Cardiovascular deconditioning occurs upon return to Earth. The regulation of blood pressure is partially carried out by the noradrenergic cell groups of the brain. However, the involvement of these systems is still unknown. Data provided by SLS-1 experiments on central and peripheral catecholamines could be the result of acute stress occurring in the animals. The purpose of this experiment was to reevaluate the data retrieved from SLS-1 and compare those data with those obtained in simulated microgravity experiments (hindlimb-suspended rats).

Approach or Method
The flight and control groups were divided into two groups: one group examined upon recovery (R+0) and the other 9 days later (R+9). For the ground-based simulation experiment, the tail-suspended and control animals were divided into analogous groups. Rats were anesthetized with metofane and blood was taken through cardiocentesis. The animals were then sacrificed by decapitation. The brain, kidneys, and heart were removed and prepared for analysis. Thick serial frontal sections were taken to analyze the noradrenergic cell groups (A1, A2, A5, and A6) of the central nervous system. Brain and peripheral tissue samples were treated with perchloric acid. The brain and tissue extraces were analyzed with liquid chromatography for norepinephrine (NE) content. The protein content of peripheral tissues was measured with the Bradford method.

Results
There was no significant difference of the norepinephrine (NE) contents in the A1, A2, A5, and A6 cell groups between the flight and vivarium control groups. In the peripheral noradrenergic system, neither the cardiac atria and ventricles nor kidneys showed significant differences after spaceflight. The tail-suspended group, when compared to controls, showed similar results. In the central nervous system, there was no significant difference between the NE levels in the A1, A2, A5, and A6 groups. In the peripheral noradrenergic system, neither the cardiac atria and ventricles nor kidneys showed significant differences after tail suspension.

Launch Date 11/19/1996 **Landing Date** 12/7/1996

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Calcium Metabolism and Vascular Function in Rats After Spaceflight

Science Discipline
Cardiovascular Physiology

Investigator	Institute
D. McCarron	Oregon Health Sciences University

Co-Investigator(s)	Institute
Hatton, D.C.	Oregon Health Sciences University
Roulle, C.	Oregon Health Sciences University
Xue, H.	Oregon Health Sciences University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat) spontaneously hypertensive

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM), Ambient Temperature Recorder (ATM)

Selected Publications
Hatton, D.C.; Yue, Q.; Chapman, J.; Xue, H.; Dierickx, J.; Roullet, C.; Coste, S.; Roullet, J.B.; and McCarron, D.A.: Blood Pressure and Mesenteric Resistance Arterial Function After Spaceflight. Journal of Applied Physiology, vol. 92, no. 1, Jan. 2002, pp. 13–17.

Hatton, D.C.; Yue, Q.; Dierickx, J.; Roullet, C.; Otsuka, K.; Watanabe, M.; Coste, S.; Roullet, J.B.; Phanouvang, T.; Orwoll, E.; Orwoll, S.; and McCarron, D.A.: Calcium Metabolism and Cardiovascular Function After Spaceflight. Journal of Applied Physiology, vol. 92, no. 1, Jan. 2002, pp. 3–12.

Objectives/Hypothesis
The inability of a vessel to increase resistance in the face of an orthostatic challenge is most likely associated with the problem of orthostatic intolerance. In animals that are exposed to simulated weightlessness, vascular contraction and regional blood flow is altered. The animal's ability to perform the necessary hemodynamic adjustments that are necessary to counter a sudden change in cardiac output may therefore be compromised.

Approach or Method
Twenty-one 1-day-old male spontaneously hypertensive rats (SHR) were placed on either high-calcium (2.0%) or low-calcium (0.2%) diets. Indirect systolic blood pressure was measured at 5 and 7 weeks. The seven rats from the low-calcium diet group with the highest blood pressure, as well as the seven rats from the high-calcium group with the lowest pressure, were selected for the flight portion of the experiment. They were placed in two animal enclosure modules (AEMs), separated by diet, and flown on the 18-day STS-80 mission. After landing, indirect systolic blood pressure was measured. Then the rats were anesthetized and direct arterial blood pressure was determined with a catheter that was inserted into the carotid artery. The animal was exsanguinated and the mesenteric vascular bed was collected. First, normalized media thickness and lumen diameter were determined. Then the vessel segment was set to its initial length and media thickness and axial length were measured. Next, the normalized length was measured with the vessel stretched to 90% of the diameter that it would have with an intraluminal pressure of 100 mmHg. Each vessel was then subjected first to a challenge with 100 mmol/l KCl three times and then to a challenge with 100 mmol/l KCl plus 10 micromol/l norepinephrine twice and its contractile response was measured.

Results
Anesthetic conditions were found to be associated with differences in blood pressure between flight and control animals. The flight groups' vascular contraction was found to be attenuated, and relaxation to acetylcholine was diminished. However, there was no difference in relaxation due to sodium nitroprusside between the two groups. Analysis suggests an alteration in endothelial function that may be associated with increased synthesis and release of nitric oxide. Overall, results point to the hypothesis that the problem of increasing vascular resistance in the mesenteric vascular bed is due to the animals' inability to divert blood flow from the viscera. Thus, decreased vessel responsiveness in visceral vascular beds may contribute to orthostatic intolerance.

Bion 11

Launch Date 12/24/1996 **Landing Date** 1/7/1997

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Hematology, Cytological Composition of Bone Marrow and Standard Blood Parameters

Science Discipline
Cardiovascular Physiology
Immunology, Infection, and Hematology

Investigator	Institute
T. Burkovskaya	Institute of Biomedical Problems

Co-Investigator(s)	Institute
None	

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Burkovskaya, T.E. and Korolkov, V.I.: Hemopoiesis in Bone Marrow of Monkeys After Spaceflight. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S129-S134.

Objectives/Hypothesis
The effects of the space flight environment upon blood are not well understood. Rhesus monkeys have the same hemopoietic type (myeloid) as humans, and therefore are useful in the study of cytological components of bone marrow after microgravity exposure. This experiment measured hematological parameters in rhesus monkeys after a 14-day space flight.

Approach or Method
Under ketamine anesthesia, bone marrow samples were drawn from the proximal metaphysis of the humerus before flight and after landing. Bone marrow hemopoiesis was measured as cell counts in 1 ml of marrow and cytological composition. A minimum of 500 cells were counted in the myelogram. Blood tests were performed to measure erythrocyte sedimentation rate, hematocrit, hemoglobin, erythrocytes, reticulocytes, and total and differential leukocytes. Proliferative, maturing, and mature pools of neutrophils, and early and mature erythrokaryocytes were measured. The ratio of early erythroblasts to total erythrokaryocytes was used to determine the rate of erythropoiesis. After the postflight ground control studies at R+17 and R+45, hematological tests were performed according to the flight protocols.

Results
Data were collected from three Bion missions: Bion 9, 10, and 11. Preflight, animals received antibiotic treatment following bioimplantation. This may have impacted preflight blood data. Postflight data were compared with data collected at the last stage of preparation before flight. After both the flight and the R +45 control study, stress reactions were manifested through increased neutrophils and decreased lymphocyte and eosinophils in the blood. These stress reactions are due to such factors as decreased motor activity (restraint) and examinations, as well as microgravity exposure. Space flight led to diminished erythropoiesis and granulocytopoiesis in the bone marrow, indicated by a reduced reticulocyte count in the blood and a reduced population of early erythroid elements and proliferative neutrophils in the bone marrow, observed at R+1. Upon return to 1 G, stimulated erythropoiesis and granulocytopoiesis were indicated by a greater number of dividing neutrophils, eosinophils, early generations of erythrokaryocytes, and a higher reticulocyte count in the blood. Decreased lymphoid population in the bone marrow was only observed after space flight, not after the ground control studies.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Fluid Compartments, Hemocirculation and Erythrocyte Function in Monkeys after Space Flight

Science Discipline
Cardiovascular physiology

Investigator / Institute
V.I. Lobachik — Institute of Biomedical Problems

Co-Investigator(s) / Institute
None

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Lobachik, V.I., Chupushtanov, S.A.; Pischulina, G.N.; and Nosovsky, A. M.: The Effect of a 14-day Flight on Body Fluids in Primates. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S135-137.

Objectives/Hypothesis
Study of the fluid compartments of the body can give insight into the structure and function of organs and tissues, as well as their microcirculation. Evidence indicates that interstitial fluid, circulating blood volumes, and arterial and venous capacity remain constant under normal conditions. This experiment measured the volumes of fluid compartments and circulating blood before and after microgravity exposure. The results may help to gain an insight into the changes seen in different muscle types during space flight.

Approach or Method
Using low doses of bromine and radioisotopes, the following parameters were measured: total body water, intracellular fluid, extracellular fluid, plasma volume, interstitial fluid, blood volume, and red blood mass. Measurements were taken preflight and 36 hours postflight. Although during this time the animals were provided with fluids ad libitum, this is not believed to be a problem, since previous studies in humans have shown that a dehydrated body is unable to compensate rapidly for fluid loss, even when fluids are consumed in large quantities. Since approximately 100 days separated the pre- and postflight measurements, growth-control measurements were made in four vivarium animals. The parameters were also measured in R+45 restraint control animals.

Results
Flight monkeys showed a decrease in all parameters compared to the preflight level, replicating the findings from previous primate flights. During the 100 days, the fluid volumes of the growing animals increased. However, the parameters expressed as a percent of body mass decreased, as is seen when both lean and adipose body mass increase. ECF increased to a greater extent than ICF, suggesting that the animals' metabolic activity decreased as they grew. Similar age-related changes can be assumed to have taken place in the flight animals. Therefore, when compared to the control data, flight data were found to be less significant. ECF decreased to a greater degree (11% and 8% in the two monkeys, due to a 13% and 12% decline in interstitial fluid) than ICF, a change that could be unfavorable for the support of cell structures. These changes are inadequate at 1 G, leading to diminished motor activity upon return to earth. After recovery, the measured parameters returned to normal levels. Similar but less significant trends were observed in the R+45 restraint control animals.

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Regional Arterial Remodeling Induced by Microgravity

Science Discipline
Cardiovascular Physiology

Investigator / Institute
M. Delp — University of Florida

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (B6.SJL-Ptprca Pepcb/BoyJ mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Stabley, JN, JM Dominguez, CE Dominguez, FR Mora Solis, J Ahlgren, BJ Behnke, JM Muller-Delp, and MD Delp. Spaceflight reduces vasoconstrictor responsiveness of skeletal muscle resistance arteries in mice. J Appl Physiol., vol. 113, no. 9, Nov. 2012, pp. 1439-1445.

Behnke, B.J.; Stabley, J.N.; McCullough, D.J.; Davis, R.T.; Dominguez, J.M.; Muller-Delp, J.M.; and Delp, M.D.: Effects of Spaceflight and Ground Recovery on Mesenteric Artery and Vein Constrictor Properties in Mice. FASEB Journal, vol. 27, no. 1, Jan. 2013, pp. 399–409.

Objectives/Hypothesis
It is now evident that the shift of fluid toward the head and the unloading of postural muscles induced in the rodent hindlimb unloading model and during exposure to microgravity, alter the mechanical forces exerted on resistance arteries, the vessels responsible for regulating tissue blood flow and arterial blood pressure. Tail suspension in rats can lead to atrophy of these peripheral arteries and a reduced ability to vasoconstrict and maintain normal blood pressure.

The purpose of this proposal was to determine whether the fluid shifts and reduced activity of postural muscles induced by microgravity similarly alter rodent arterial vessel structure and some key cellular signaling pathways: does spaceflight result in smooth muscle atrophy of skeletal muscle and mesenteric arteries?

Approach or Method
On the STS-131 flight, physiological responses in mesenteric and gastrocnemius muscle resistance arteries were obtained in 11 control and 11 flight animals. These were sufficient numbers of animals to provide complete and high-quality data.

The mesenteric and gastrocnemius muscle resistance arteries were isolated and placed in cold buffer solution at Kennedy Space Center (KSC). The arteries were then transported to the University of Florida where they were mounted on glass pipettes for in vitro experimentation. Wall thickness and lumen diameter were measured using video microscopy. The resistance arteries were further studied to determine physiological responses to vasoconstrictor agonists working through different mechanisms.

Results
Results in the mesenteric and gastrocnemius muscle resistance arteries demonstrate that microgravity attenuates both the magnitude and the rapidity of vasoconstrictor responses to norepinephrine, a receptor-mediated vasoconstrictor mechanism, as well as to potassium chloride (KCl), a non-receptor vasoconstrictor mechanism. Furthermore, vasoconstrictor responses to caffeine, an intracellular calcium-release mechanism through ryanodine receptors, were also attenuated in mesenteric resistance arteries after spaceflight. Analysis of messenger ribonucleic acid (mRNA) expression of ryanodine receptors in mesenteric and gastrocnemius arteries demonstrated depressed expression in the flight animals relative to controls mice. These microgravity-induced changes in arterial vasoconstriction occurred in the absence of any gross structural changes in artery wall thickness or maximal interluminal diameter, or by any alterations in the passive mechanical properties of the vessels, as indicated by an unaltered passive pressure-diameter response.

Launch Date 2/24/2011 **Landing Date** 3/9/2011

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Regional Arterial Remodeling Induced by Microgravity

Science Discipline
Cardiovascular Physiology

Investigator / Institute
M. Delp — University of Florida

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Simulated Flight Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Behnke, B.J.; Stabley, J.N.; McCullough, D.J.; Davis, R.T.; Dominguez, J.M.; Muller-Delp, J.M.; and Delp, M.D.: Effects of Spaceflight and Ground Recovery on Mesenteric Artery and Vein Constrictor Properties in Mice. FASEB Journal, vol. 27, no. 1, Jan. 2013, pp. 399–409.

Objectives/Hypothesis
Previous findings with STS-131 mice demonstrated that vasoconstrictor responses of mesenteric arteries were diminished by spaceflight. The purpose of this study was to determine if norepinephrine-evoked vasoconstriction is reversible within 1 week of return to Earth's gravitational influences.

Approach or Method
On the STS-133 flight, physiological responses in mesenteric resistance arteries were obtained in 10 control and 2 flight animals at R+1, 2 flight animals at R+5, and 2 flight animals at R+7. The mesenteric arteries were isolated and placed in cold buffer solution at Kennedy Space Center (KSC). The arteries were then transported to the University of Florida where they were mounted on glass pipettes for in vitro experimentation.

Results
Norepinephrine-evoked constriction of mesenteric arteries was significantly lower in the spaceflight R+1 group relative to that from ground-control mice, and qualitatively similar to results obtained immediately after spaceflight in STS-131 mice. However, after 5 and 7 days of ground recovery, mesenteric artery vasoconstriction was not different between groups.

Physiological/Science Discipline: CARDIOVASCULAR/CARDIOPULMONARY

Title of Study
Regional Arterial Remodeling Induced by Microgravity

Science Discipline
Cardiovascular Physiology

Investigator / Institute
M. Delp — University of Florida

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Behnke, B.J.; Stabley, J.N.; McCullough, D.J.; Davis, R.T.; Dominguez, J.M.; Muller-Delp, J.M.; and Delp, M.D.: Effects of Spaceflight and Ground Recovery on Mesenteric Artery and Vein Constrictor Properties in Mice. FASEB Journal, vol. 27, no. 1, Jan. 2013, pp. 399–409.

Taylor, C.R.; Hanna, M.; Behnke, B.J.; Stabley, J.N.; McCullough, D.J.; Davis, R.T.; Ghosh, P.; Papadopoulos, A.; Muller-Delp, J.M.; and Delp, M.D.: Spaceflight-induced alterations in cerebral artery vasoconstrictor, mechanical, and structural properties: Implications for elevated cerebral perfusion and intracranial pressure. FASEB Journal, vol. 27, no.1, Jan. 2013, pp. 2282-2292.

Objectives/Hypothesis
Two important questions were addressed in these studies. First, does microgravity enhance vasoconstriction of cerebral arteries, which could contribute to inadequate cerebral perfusion and postflight orthostatic intolerance? Previous studies with hindlimb unloaded rats demonstrate simulated microgravity enhances cerebral artery vasoconstrictor properties. And second, previous results from STS-131 and STS-133 experiments have shown that vasoconstrictor properties of arteries from the mesentery and gastrocnemius muscle are diminished by spaceflight. This experiment addressed whether this impairment is limited to the arterial circulation, or whether the venous circulation is likewise impaired.

Functional and structural studies were conducted on cerebral arteries and mesenteric veins to address whether the impairment of smooth muscle function occurs cerebral circulation, or is limited to the arterial circulation, i.e., whether smooth muscle cells on the venous side of the circulation are likewise affected.

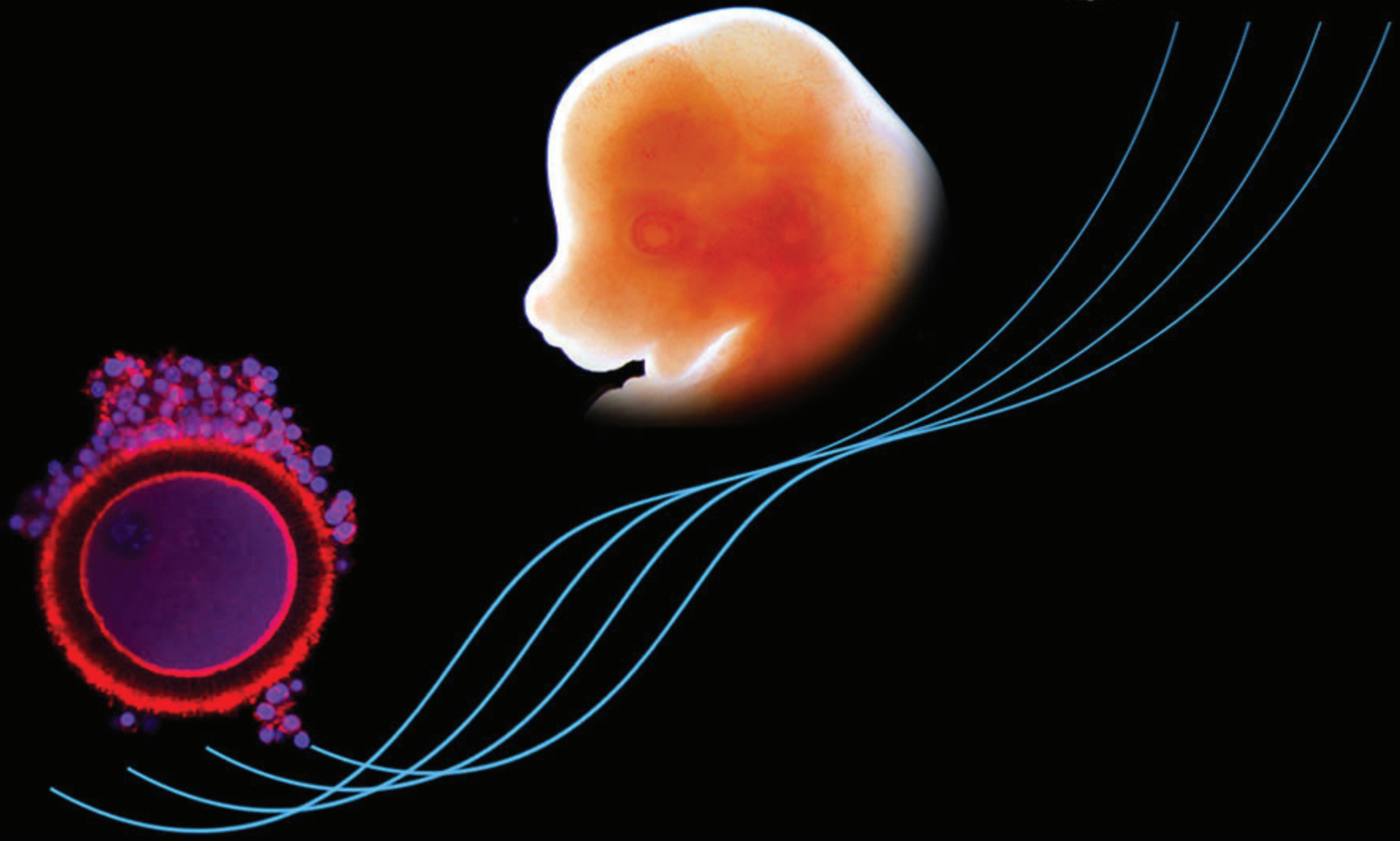
Approach or Method
Basilar and posterior communicating arteries were isolated for in vitro and mechanical testing. Myogenic vasoconstrictor responses and passive pressure-diameter responses were obtained in basilar arteries from 12 control and 7 flight animals. Vascular stiffness, as characterized via nanoindentation, was determined in posterior communicating arteries from 12 control and 7 flight animals. For mesenteric veins, norepinephrine dose response curves were obtained from 9 control and 3 flight animals.

The cerebral arteries and mesenteric veins were isolated and placed in cold buffer solution at Kennedy Space Center (KSC) and transported to the University of Florida. Basilar arteries and mesenteric veins were mounted on glass pipettes to determine constrictor properties, as well as wall thickness and maximal lumen diameter via video microscopy.

Results
In the basilar arteries, myogenic vasoconstrictor tone was lower in flight animals relative to that in ground-control mice. Passive pressure-diameter responses were also greater in basilar arteries from flight mice, indicating greater distension of these cerebral arteries. Structural analysis demonstrated that basilar artery wall thickness and maximal diameter were unaffected by spaceflight. Posterior communicating arteries were also less stiff in mice flown on the space shuttle.

Venoconstriction to norepinephrine was lower in veins from spaceflown mice. Mesenteric vein luminal diameter also increased as a function of increasing intraluminal pressure in the presence of 10-4 M NE in both groups. However, intraluminal diameter was greater at each pressure in veins from the spaceflight group.

Developmental & Reproductive Biology



Developmental and Reproductive Biology Introduction

April E. Ronca, Ph.D., NASA Ames Research Center

A major goal of space biology research is to broaden scientific understanding of how the Earth's constant gravitational force (1g) affects living organisms. To this end, studying reproduction and development of various species in the microgravity of space promises to uncover exciting new insights into how gravity shaped life on Earth, with potential translational relevance to human health. In all, NASA Ames Research Center, in collaboration with other space research programs, has developed and flown over 50 experiments focused on this topic. These studies involved an impressive variety of species, viz., insects, fish, amphibians, aves, and mammals, characterized by vastly differing features and adaptations that vary in number and complexity [Marthy, 2003].

The inaugural developmental space biology experiments (with frog eggs and fruit fly larvae) were flown as part of the Gemini and Biosatellite programs spanning the years 1965–1967. In 1975, in parallel with the Apollo/Soyuz mission, the first U.S. participation in the Soviet Space Program "Bion" was marked by the launch of Cosmos 782 from Plesetsk. This unmanned satellite carried various plants and animals including fruit flies (Drosphila melanogaster) [Cosmos 782 / Bion 3, p. 147] and minnow embryos (Fundulus heteroclitis) [Cosmos 782 / Bion 3, p. 146] into space before landing 20 days later in Siberia. In these experiments, it was determined that fly development across the life span is relatively unaffected by spaceflight, with the exception of impaired adaptation to the gravity vector [Miquel and Souza, 1991]. Notably, this early foray into developmental space biology incorporated the "gold standard" control—a small, short-arm centrifuge, contributed by the Russian team, via which flight specimens were exposed to an Earth-normal (1-g) gravity load in space. In subsequent spaceflight studies of invertebrates, Mike Wiederhold observed gravity-induced changes in the size of the otolith organs in space-reared snails [Wiederhold, 1997] [STS-90 / Neurolab, p. 360]. Eberhard Horn reported that the gravity sensors of crickets reared in space developed normally, however significant alterations were observed in neuronal sensitivity in crickets [STS-90 / Neurolab, p. 352] in space. He reported similar findings following exposure of crickets to 3-g hypergravity [Horn, 2002]. Similar results were reported in space-reared rats by Jacqueline Raymond [Dememes, 2001] [STS-90 / Neurolab, p. 357].

For over a century, scientists have questioned whether gravity is required for normal embryonic development [Roux, 1884] [Morgan, 1904]. On Earth, amphibian eggs are spawned in a random orientation but undergo "rotation of fertilization" that determines the polarity of the embryonic axis and allows dorsal structures to develop on the side of the egg uppermost in the gravitational field. In 1992, Ken Souza, Steven Black, and Richard Wassersug conducted the Frog Embryology Experiment on the Space Shuttle Spacelab-Japan mission [STS-47 / SL-J, p. 152]. This landmark investigation demonstrated for the first time that gravity is not required for ovulation, fertilization, pattern formation, and maturation of frogs (Xenopus laevis) to a free-swimming stage. The tadpoles that developed in microgravity also failed to locate an air/water interae and thus failed to inflate their lungs, creating a barrier to development beyond metamorphosis. Subsequent studies of Medaka fish and salamanders provide further evidence of successful vertebrate mating, external fertilization, and hatching in space [Ijiri, 2003] [Dournon, 2003] [STS-78 / LMS, p. 187]. Space-born fish possess the normal complement of germ cells, and subsequently produced offspring at 1g. Characteristic development of chick and quail embryos also occurs in space [Jones,

1992]. Collectively, these findings provide good evidence that certain key aspects of vertebrate reproduction and development are largely gravity independent, adaptive, and plastic.

A total of six pioneering missions yielded the first insights into whether mammals can reproduce and develop in space. To determine if mammalian mating and conception can occur in microgravity, the unmanned Russian biosatellite Cosmos 1129, flown in 1979, provided the opportunity for rats to mate during flight [Cosmos 1129 / Bion 5, p. 148]. Following launch, a partition separating male and female rats was removed during spaceflight. No pregnancies were realized at recovery in either flight or ground-control rats, precluding clear interpretations [Keefe, 1985]. In 1983, Cosmos 1514 carried the first experiments with pregnant rats led by Luba Serova, Dick Keefe, and Jeff Alberts, on a 4.5-day mission, returning the rats to Earth prior to the expected time of birth [Cosmos 1514 / Bion 6, p. 150]. This landmark mission established proof-of-concept that mammalian pregnancy can proceed in the microgravity of space. After a hiatus of more than a decade, NASA and the National Institutes of Health (NIH) jointly sponsored two secondary payloads of pregnant dams and pups on the Space Shuttle STS-66 (1994) and STS-70 (1995) missions, NIH.Rodent (R)1 and R2, respectively. Sixteen international and domestic science teams analyzed the behavior, morphology, neurobiology, and physiology of 10 pregnant rats and their offspring launched at mid-gestation and landed 24–48hrs before the expected time of birth. Following recovery, dams had uncomplicated, successful vaginal deliveries and nursed their young [Ronca, 2000]. Notably, R1 and R2 [STS-66 / NIH.R1] [STS-70 / NIH.R2] were model cooperative space biology efforts that fostered novel cross-disciplinary interactions among scientists from diverse areas of expertise and led to numerous published reports describing reproductive, neural, vestibular, locomotor, immune, musculoskeletal, and circadian factors in dams and offspring [Ronca, 2003].

In 1996 and 1998, two Space Shuttle missions [STS-72 / NIH. R3, p. 174] and [STS-90 / Neurolab] carried nursing rat dams and their litters into space for 9 and 16 days, respectively. Intriguing results were obtained from studies of sensorimotor, neural, muscular, vestibular, cardiovascular, and cognitive processes [Buckey, 2003] [Ronca, 2003]. Kerry Walton reported that space-reared postnatal rats exhibited enduring deficits in the "righting reflex," a species characteristic response whereby rodents orient their bodies against the gravity vector when placed on their backs [Walton, 1997] [STS-90 / Neurolab, p. 299]. These findings suggest there is a critical gravity-dependent period for motor development. Danny Riley reported decreased muscle fiber growth in the soleus muscle, increased sensitivity to muscle reloading injury, reduced growth of motor neuron terminals, and reduced ability of corresponding nerves to utilize oxygen [Buckey, 2003] [STS-90 / Neurolab, p. 298], suggesting that normal nerve and muscle growth depends upon gravitational loading during formative phase(s) of development. Ken Kosik observed no change in spatial learning or memory mediated by the hippocampus—the "navigation center" of the brain [Temple, 2002] [STS-90 / Neurolab, p. 353]. Tsuyoshi Shimizu observed significant differences between the pressure receptors in the cardiovascular systems in Flight versus Control rats [Shimizu, 1999] [STS-90 / Neurolab, p. 359]; Ken Baldwin reported impaired antigravity muscle growth and changes in the types of proteins used in that muscle [Adams, 2000a and 2000b] [STS-90 / Neurolab, p. 297].

The NIH.R3 and Neurolab missions also yielded some important "lessons learned" [Ronca, 2003] [Maese, 2002]. Obstacles to effective maternal care and feeding of the young were imposed by the weightless of space combined with limitations of the an-

imal habitats for supporting developing rodents. This led to significant mortality and feeding difficulties in the youngest infant rats flown on these two missions (NIH.R3, 5 day old; Neurolab, 8 day old), requiring intervention by the astronauts [Maese, 2002]. Similarly, quail fledglings hatched on the Russian Space Station Mir were unable to regulate their body position in space, requiring cosmonaut assistance to accomplish feeding [Jones, 1992] [Mir 19 / Incubator]. The primary "lessons learned" from the NIH.R3 and Neurolab efforts are: (1) mammalian mothers and their young comprise a biological system that is exquisitely sensitive to changes in gravity, especially during the early postnatal period when infants are dependent for their survival upon maternal care (requiring sustained close proximity between mothers and young); (2) flight conditions and hardware, including caging, food delivery, and waste removal, exert extraordinary influences on the animals' health and development, and are therefore vital considerations for flight experiments; (3) the existing studies provide single "snapshots" of mating, and prenatal and postnatal development, however the current literature on reproduction and development in space is minimal, limiting speculation about the possibility that intricate and complex phases of reproduction and development in mammals can occur in space. For example, no mammal has yet given birth in space. For further discussion, see [Ronca, 2003] [Maese, 2002].

A major unknown is whether mammals can reproduce in space. Near the close of the Shuttle Era, Joseph Tash studied the effects of spaceflight on reproductive organs of female mice in three missions [STS-131, p. 419] [STS-133, p. 420] [STS-135, p 421]. Female adult mice that were cycling at launch exhibited spaceflight-induced cessation of cycling, loss of corpora lutea, and significantly reduced estrogen receptor mRNA levels in the uterus [Holets, 2014]. These data demonstrate clear impacts of the space environment on female reproductive organs, however the persistence of these changes following short-term exposures has not been determined. There have been no comparable studies of male mouse reproductive changes in space. But, male rats exposed to 6 weeks of simulated microgravity (using Hindlimb Unloading (HLU)) showed severe testicular and epididymal degeneration including massive testicular apoptosis 6 months later. These effects were postulated to occur because of (a) chronic testicular hyperthermia, (b) invasion of inflammatory cells, and/or (c) catastrophic apoptosis, leading to aspermatogenic dysfunction [Tash, 2002]. Systematic and detailed studies using the ISS and ground-based analogues are warranted to determine effects of the space environment, including microgravity, radiation, and stress, on the gonads and reproductive function in males and females. Future studies in this area are vital for establishing a robust program of mammalian reproductive, developmental space biology research to determine how gravity affects structure and function, and for informing and supporting astronaut health.

Translational Relevance

The field of developmental space biology provides unique perspectives on biomedical concerns on Earth and in space. The omnipresent gravity force on Earth stimulates vestibular organs and exerts major influences on physiology and health. Developmental analysis is especially useful for studying gravity-induced changes in structure and function. It is widely recognized that activity-dependent processes and experience play important roles in the establishment of neural architecture and function [Hubel and Wiesel, 1972]. The vestibular system is one of the first to develop in the vertebrates. Researchers are using space biology approaches in studies of vestibular development, equilibrium, and locomotion. Fetuses with vestibular disorders have an increased incidence of breech birth. Babies born after a sustained period of

maternal bed rest during pregnancy crave vestibular simulation, and require more rocking to be quieted. Gravity alters body-weight regulation and metabolism. Changes in body mass and energy balance induced by gravitational loading may provide important clues regarding developmental origins of childhood and adult obesity. Recent gravitational biology research is addressing prenatal contributions to the establishment of body weight regulation in later life. These are central issues in the field of epigenetic developmental programming of adult disease, and can be used to gain important new insights into health, disease, and aging. Multiple lines of convergence suggest that spaceflight is a model for aging [Vernikos, 2012] based on the several rapid, and sometimes profound, aging effects observed that usually recover postflight. The population of the U.S. is living longer than ever before, with a nearly 30-year increase in life span in the past century. It is predicted that the number of people over 85 will double by the year 2020, and that by 2050, over 20 percent of the population will be over 65. Vestibular and equilibrium disorders are frequent symptoms of aging, related to "multisensorial decay," age-related conditions, Parkinson's disease, and other degenerative conditions. Due to vestibular and proprioceptive dysfunction, the elderly suffer disastrous falls from which many never recover. Understanding the developmental origins of how gravity shapes the brain and body function holds immense potential for application to health problems encountered on Earth.

Spaceflight poses a number of potential reproductive health risks for men and women related to microgravity, radiation, and stress (including sleep disruption). With overall increased durations of contemporary space missions, a deeper understanding of reproduction-related responses and adaptations to the space environment is warranted to minimize risks and ensure healthy aging of the men and women who travel into space [Ronca, 2014]. To date, just 15 percent of U.S. astronauts have been women, but the numbers are increasing, as evidenced by women achieving parity with men for the first time in the 2013 NASA Astronaut Class. There is a strong need for detailed and systematic studies of both sexes.

Despite the consideration that, in the distant future, human reproduction is likely to occur in space, the current literature base is insufficient, limiting speculation about the possibility that intricate and complex phases of reproduction in mammals—including mating, fertilization, implantation, placentation, embryogenesis, organogenesis, prenatal and postnatal development, birth, lactation, and suckling—can occur in space.

Conclusion

Reproduction and development are hallmarks of a species' ability to adapt to a novel environment. Developmental space biology research has major translational relevance to life on Earth. Advancing knowledge of the role of gravity in the normal development of cells and systems will harness new information that can translate into benefits for humans on Earth. Because developing cells and systems undergo remarkable changes at a rapid pace, effects of environmental factors, such as gravity, on developmental sequences are amplified and observed at far shorter timescales than in adulthood where effects may be difficult to see and study. Reproductive and developmental space biology, scientific disciplines spearheaded by NASA Ames Research Center, have a promising future. Intriguing questions remain involving internal fertilization, placentation, birth, trajectories of postnatal development, and cross-generational phenotypic heritability. Through egg-to-egg-to-egg investigations using a combination of time-honored, modern biology approaches, and cutting-edge research, solving the exciting mysteries of how gravity shaped life on Earth and how we adapt to space are on the horizon.

List of referenced flight experiments:

Cosmos 782 / Bion 3, J. Keefe, Killifish Development in Zero-G on Cosmos 782

Cosmos 782 / Bion 3, J. Miquel, Effects of Weightlessness on the Embryonic Development and Aging of Drosophila;

Cosmos 1129 / Bion 5, J. Keefe, Rat and Quail Ontogenesis

Cosmos 1514 / Bion 6, J.R. Alberts, Early Postnatal Development of Rats Exposed In Utero to Microgravity

Mir 19 / Incubator, S. Doty, Skeletal Development in Long Duration Spaceflight

STS-47 / SL-J, K.A. Souza, Effect of Weightlessness on Development of Amphibian Eggs

STS-66 / NIH.R1, J.R. Alberts, Spaceflight Effects on Mammalian Development

STS-70 / NIH.R2, J.R. Alberts, Spaceflight Effects on Mammalian Development

STS-72 / NIH.R3, K.D. Walton, Rodent Dam/Neonate Animal Enclosure Module Nursing Facility Development Experiment (E122A)

STS-78 / LMS, D. Wolgemuth, Development of the Fish Medaka in Microgravity

STS-90 / Neurolab, D.Riley, The Effects of Microgravity on Neuromuscular Development

STS-90 / Neurolab, E.Horn, Development of an Insect Gravity Sensory System in Space

STS-90 / Neurolab, J.Raymond, Microgravity and Development of Vestibular Circuits

STS-90 / Neurolab, K.Kosik, Neuronal Development Under Conditions of Space Flight

STS-90 / Neurolab, K.M. Baldwin, Neural Thyroid Interaction on Skeletal Isomyosin Expression in Zero-G

STS-90 / Neurolab, K.Walton, Effects Of Microgravity On Postnatal Motor Development

STS-90 / Neurolab, M.L. Wiederhold, Development of Vestibular Organs in Microgravity

STS-90 / Neurolab, T.Shimizu, Development of the Aortic Baroreflex Under Conditions of Microgravity

STS-131 / J. Tash, Long Term Space Flight Impacts on Female Reproductive Health

STS-133 / J. Tash, Long Term Space Flight Impacts on Female Reproductive Health

STS-135 / J. Tash, Long Term Space Flight Impacts on Female Reproductive Health

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Cosmos 782 / Bion 3

Launch Date	Landing Date
11/25/1975	12/15/1975

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Weightlessness on the Embryonic Development and Aging of *Drosophila*

Science Discipline
Developmental Biology

Investigator	Institute
J. Miquel	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Philpott, D.E.	NASA Ames Research Center (ARC)
Lundgren, P.R.	NASA Ames Research Center (ARC)
Binnard, R.	NASA Ames Research Center (ARC)

Research Subject(s)
Drosophila melanogaster (Fruit fly)

Ground-Based Controls
Synchronous Control, Laboratory Control

Key Flight Hardware
Cosmos Centrifuge, Cosmos Fruit Fly Container

Selected Publications

Miquel, J. and Souza, K.A.: Gravity Effects on Reproduction, Development, and Aging. Advances in Space Biology and Medicine, vol. 1, 1991, pp. 71–89.

Miquel, J.: Comparison Between the Weightlessness Syndrome and Aging. Space Gerontology, NASA CP-2248, 1982, pp. 1–8.

Miquel, J.; Philpott, D.E.; Lundgren, P.R.; Binnard, R.; and Turnbill, C. E.: Effects of Weightlessness on the Embryonic Development and Aging of *Drosophila*. Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 782, S.N. Rosenzweig and K.A. Souza, eds., NASA TM-78525, 1978, pp. 382–409.

Objectives/Hypothesis
All living organisms have evolved under the influence of Earth's gravity, and, therefore, it is generally assumed that gravity has played a role in shaping structure and function. Previous laboratory work has documented that the aging process of *Drosophila* is strikingly sensitive to altered environments, such as temperature changes and rotation in a clinostat. This study focused on the aging process of fruit flies that developed and spent their first days of life in space.

Approach or Method
The experimental *Drosophila* population was exposed to weightlessness, while an equal number of flies housed in a centrifuge at approximately 1 g served as in-flight controls. Synchronous and vivarium groups served as ground controls. All flies were individually weighed in a Cahn electric balance. Other parameters included: vitality, as expressed by negative geotaxis and mating; external morphology and age-associated degenerative changes, as demonstrated by gross photography and scanning electron microscopy; glycogen content of the thorax to estimate muscle energy reserve; and life span.

Results
Apparently, the development of *Drosophila* was insensitive to weightlessness and the aging processes were not influenced, except for a slight reduction in the amount of lipofuscin present in the midgut and Malpighian tubules, the tubular glands of excretory function. The only detrimental effect seemed to be a decrease in the negative geotaxis and mating. It is likely that this decreased mating ability (almost half in both flight groups) was the result of injury to the wing structures (which play a crucial role in *Drosophila* mating), as the consequence of acceleration or other flight stresses unrelated to weightlessness. Otherwise, the weightless flies were identical to controls in all parameters investigated.

Cosmos 782 / Bion 3

Launch Date 11/25/1975

Landing Date 12/15/1975

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Killifish Development in Zero-G on Cosmos 782

Science Discipline
Developmental Biology

Investigator	Institute
J.R. Keefe	University of Louisville

Co-Investigator(s)	Institute
Scheld, H.W.	University of Houston
Boyd, J.F.	Northrop Services, Inc.
Fuller, P.M.	University of Louisville

Research Subject(s)
Fundulus heteroclitus (Killifish)

Ground-Based Controls
"Dish," Synchronous Control, Rotated Control, U.S. Control Eggs

Key Flight Hardware
Cosmos Fundulus Chambers, Cosmos Centrifuge

Selected Publications
Scheld, H.W.; Boyd, J.F.; Bozarth, G.A.; Conner, J.A.; Eichler, V.B.; Fuller, P.M.; Hoffman, R.B.; Keefe, J.R.; Kuchnow, K.P.; Oppenheimer, J.M.; Salinas, G.A.; and von Baumgarten, R.J..: Killifish Hatching and Orientation Experiment MA-161. Apollo-Soyuz Test Project, NASA TM-X-58173, 1976, pp. 19-1–19-14.

Fuller, P.M. and Keefe, J.R.: Support of ASTP/ Kosmos Fundulus Embryo Development Experiment: Final Report. NASA CR-151816, 1977.

Objectives/Hypothesis
The *Fundulus* embryogenesis experiment was the third in a series of experiments to assess the possible effects of the space environment upon developing organisms. On the Skylab-3 and Apollo-Soyuz missions, juvenile fish initially exhibited obvious disorientation reactions (swimming rapidly in loops and circles), but over a period of several days in orbit, they gradually adapted to weightlessness and to dependence on visual cues, while space-hatched fry exhibited no disorientation. The major refinement in this experiment was the use of a 1-g control centrifuge.

Approach or Method
Experimental treatment groups of 500 embryos were comprised of groups of 100 eggs from the each of the 5 nominal developmental stages. Specimens were kept in Polyethylene bags each containing 50 embryos of a given age and 23-ml sterile filtered 21% Instant Ocean. Treatments included flight stationary, an onboard 1-g centrifuge, and various ground control experiments. The primary data yielded by the experiment was in the form of fixed material for light and electron microscopic analysis, focusing on the vestibular and other sensory regions. Light orientation, rotating striped drum, and geotaxis tests were employed postflight.

Results
Postflight testing of procedures and materials indicates that the probable cause of the high incidence of anomalous development lies in the tape used to label the plastic bags. All morphologically normal hatchlings though, exhibited a typical fright-diving response, and there were no apparent differences among treatments with respect to the diving response. Likewise, behavioral testing ascertained that development in weightlessness of *Fundulus*, from the earliest exposure achievable, had no radical effect upon the vestibular function. Microscopic observations indicated a generally better health in flight animals, with the possible exceptions of those aspects of development where gravity is required as a cue for establishment of polarity or as a reference stimulus for sensory development.

Cosmos 936 / Bion 4

Launch Date 8/13/1977

Landing Date 8/22/1977

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Weightlessness on the Genetic and Aging Process of *Drosophila melanogaster*

Science Discipline
Developmental Biology

Investigator	Institute
J. Miquel	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Philpott, D.E.	NASA Ames Research Center (ARC)

Research Subject(s)
Drosophila melanogaster (Fruit fly)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Fruit Fly Container

Selected Publications

Miquel, J.; Philpott, D.E.; Lundgren, P.R.; Binnard, R.; and Turnbill, C. E.: Effects of Weightlessness on the Embryonic Development and Aging of Drosophila. Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 782, S.N. Rosenzweig and K.A. Souza, eds., NASA TM-78525, 1978, pp. 382-409.

Miquel, J.; and Souza, K.: Gravity Effects on Reproduction, Development and Aging. Advances in Space Biology and Medicine, vol. 1, 1991, pp. 71–97.

Miquel, J.: Comparison Between the Weightlessness Syndrome and Aging. Space Gerontology, NASA CP-2248, 1982, pp. 1–8.

Objectives/Hypothesis
The main purpose of the U.S.S.R. portion of this experiment was to study the genetic effects of near-weightlessness, while the U.S. contribution was concerned with the developmental and aging process. This investigation was intended to be a follow-up to the *Drosophila* experiment flown previously on Cosmos 782, which suggested that the development of *Drosophila* was not appreciably affected by the lack of gravity.

Approach or Method
Two age groups of mature flies (7 and 26 days at launch) were used in order to compare the effects of microgravity on insects that were at the peak of their vitality, with others already shown to exhibit senescent loss of vigor and tissue disorganization. Investigations included: determination of vitality, as expressed in negative geotaxis and mating; study of the external morphology using a scanning electron microscope; investigation of age-associated changes in the muscle and other tissues by transmission electron microscopy on material fixed in glutaraldehyde; and life span determination on 70 flies from Cosmos-flown and synchronous populations.

Results
The transmission electron microscopic study demonstrated the presence of normal mitochondria both in flies that developed in space and in those that were exposed to microgravity as young and middle-aged imagoes. On the other hand, the amount of glycogen granules in the wing were strikingly lower in the Cosmos-flown young flies than in their ground controls. Regarding embryonic development, these findings are in agreement with previous research and observations on Cosmos 782, suggesting that near-weightlessness does not seriously interfere with the developmental processes of *Drosophila*. However, the reduced vitality and the short life span manifested by the flies that were exposed to hypogravity during the first days of their imaginal life suggest that the aging process may be accelerated during spaceflight. These effects may be similar to those of other life-shortening environmental parameters, such as moderately raised oxygen tensions and high ambient temperature.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Rat and Quail Ontogenesis

Science Discipline
Developmental Biology

Investigator / Institute
J.R. Keefe — BioSpace Incorporated

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Wistar rat)
*Coturnix coturnix (*Japonica quail)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Keefe, J.R.: Rat and Quail Ontogenesis. Final Reports of U.S. Rat Experiments Flown on the Soviet Satellite Cosmos 1129. M.R. Heinrich and K.A. Souza, eds., NASA TM-81289, 1981, pp. 325–362.

Objectives/Hypothesis
The flight of Cosmos 1129 attempted to provide information with respect to potential effects of spaceflight on mammalian fertilization, implantation, and embryonic development. The principal objectives of this study were: 1) to determine the capability of a selected mammalian species to undertake reproductive processes, including copulation, fertilization, placentation, and embryogenesis during spaceflight exposure; 2) to separate potential spaceflight factors from indirect factors due to stress; and 3) to demonstrate the capability of avian embryos to carry out normal embryogenesis during spaceflight.

Approach or Method
Both flight and synchronous rat groups were provided a dual-chambered mating cage. Fertility in test animals was ensured by successful preflight breeding. The divider separating male and female rats was removed on the second day of flight. Seventeen days after recovery, flight and control females were laparotomized and the uteri and ovaries photographed. “Triangular implantation sites” and “yellow bodies” (corpora lutea) were tallied by gross inspection. Two flight females and flight males were mated postflight.

Although all of the quail eggs were adversely impacted by an in-flight failure of the incubator humidifier, several embryos were able to progress to a developmental stage equivalent to that of a control 10- to 12-day embryo. Postflight, representative samples of the dead embryos were fixed in Bouin’s solution and examined by light microscopy.

Results
None of the flight or synchronous rat females gave birth as a result of breeding that may have occurred during the flight phase of the experiment. Flight males have subsequently sired litters from both vivarium and post-operative flight females. One flight and two synchronous females have since produced viable litters with a normal size and sex ratio. Pups from these litters demonstrate normal morphological development. The basic questions concerning mammalian copulation, insemination, fertilization, implantation, placentation, and embryogenesis still remain unanswered.

Based upon examination of the external features and analyses of the one quail flight embryo received, development under conditions of spaceflight appeared to be normal. The drop in relative humidity to a level of 23–25% for a period over 6 days must have led to a dehydration of the eggs and an increase in the fragility of the shell.

Cosmos 1514 / Bion 6

Launch Date 12/14/1983

Landing Date 12/19/1983

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Early Postnatal Development of Rats Exposed In Utero to Microgravity

Science Discipline
Developmental Biology

Investigator	Institute
J.R. Alberts	Indiana University

Co-Investigator(s)	Institute
Keefe, J.R.	Case Western Reserve University
Serova, L.V.	Institute of Biomedical Problems
Apanasenko, Z.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Mothers w/Pups, Synchronous Mothers w/Pups

Key Flight Hardware
Cosmos Primate-BIOS
Cosmos 1514 Russian Hardware Suite

Selected Publications
Serova, L.V. et al.: Growth and Development of Newborn Rats During Their First Month of Life. Ontogenez Mlekopitayuschikh v Nevesomosti (Ontogenesis of Mammals in Microgravity). O.G. Gazenko, ed., Nauka Press, 1988, pp. 82–88.

Alberts, J.R.; Serova, L.V.; Keefe, J.R.; and Apanasenko, Z.: Early Postnatal Development of Rats Gestated During Flight of Cosmos 1514. Physiologist, supl., vol. 28, no. 6, 1985, pp. S81–S82.

Objectives/Hypothesis
The last trimester of the rat's gestational period is a stage of formation and differentiation of sensory and motor systems vital to early survival and postnatal maturation. As part of a broad research program designed to examine the adequacy of maternal care following spaceflight and evaluate the postnatal development of the infant rats, this study represents a preliminary appraisal of the functional status of sensory and motor systems in the infant rat. It charts some of the fundamental landmarks of maturation during the first 2 to 3 weeks of postnatal life.

Approach or Method
Five pregnant rats flown on the biosatellite (embryonic days 13 to 18) were allowed to complete their pregnancies after recovery. These pups and their mothers were then examined in a comprehensive, quantitative study of sensory and behavioral development. A microcomputer system monitored the cages 24 hours/day and recorded (each 15 minutes) the number and duration of maternal visits to the nest. Pups were inspected each day at the time of weighing to note their general appearance and check for landmark events. Sensory tests were performed to demonstrate the presence of functional responsiveness in selected modalities.

Results
Four of the flight females had normal deliveries of live offspring postflight. The postpartum cycle of lactation and maternal behavior was displayed by all dams. Olfactory, tactile, and vestibular perceptions were functional at birth. High frequency (40 KHz) auditory detection appeared impaired, and there were signs of possible vestibular supersensitivity. In contrast to data derived from flight animals sacrificed at recovery, the anatomical and functional picture presented by this study suggest that the 5-day interval between recovery and birth may have constituted an ontogenetically significant period of readaptation to gravity, during which time compensatory alterations in development removed or repaired perturbations exerted by the space environment.

Cosmos 1514 / Bion 6

Launch Date 12/14/1983

Landing Date 12/19/1983

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study

Developmental Morphology of the Eye, Vestibular System, and Brain in 18-Day Fetal and Newborn Rats Exposed In Utero to Null Gravity During the Flight of Cosmos 1514

Science Discipline

Developmental Biology

Investigator	Institute
J.R. Keefe	Case Western Reserve University

Co-Investigator(s)	Institute
Alberts, J.R.	Indiana University
Krasnov, I.B.	Institute of Biomedical Problems
Serova, L.V.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Wistar rat)

Ground-Based Controls

Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Primate-BIOS
514 Russian Hardware Suite

Selected Publications

Keefe, J.R.; Krasnov, I.B.; Alberts, J.R.; and Serova, L.V.: Effects of Fetal Period Null-Gravity Exposure on Wistar Rats During the Flight of Cosmos 1514. Physiologist, vol. 28, 1981, p. 262.

Keefe, J.R.; Alberts, J.R; Krasnov, I.B.; and Serova, L.V.: Developmental Morphology of the Eye, Vestibular System, and Brain in 18-Day Fetal and Newborn Rats Exposed In Utero to Null Gravity During the Flight of Cosmos 1514. Final Reports of U.S. Monkey and Rat Experiments Flown on the Soviet Satellite Cosmos 1514. R.C. Mains and E.W. Gomersall, eds., NASA TM-88223, 1986, pp. 189–279.

Objectives/Hypothesis

If it is assumed that female mammals demonstrate adaptive physiological responses to null-gravity exposure similar to flown males, then questions of transplacental expression of a variety of systemic effects from null-gravity exposure become highly significant in evaluating the results of developmental studies under altered gravity. Such an initial study of both direct and potentially indirect transplacental impacts of null-gravity on neural structures during the relatively stable midlife period represents the main thrust of this experiment.

Approach or Method

Five pregnant rats (embryonic days 13 to 18) flown on the biosatellite were sacrificed at the recovery site, and the fetal heads were preserved for morphological studies. Five remaining females were allowed to proceed with normal term delivery, with selected newborns sacrificed at litter culling and postnatal days 15 and 30. All heads were opened along the dorsal midline of the skull, prior to immersion fixation in modified Karnovsky biostabilizer. Analysis focused on the cerebral hemispheres, the peripheral vestibular apparatus, and vision organs.

Results

Examinations of fetal specimens revealed an effect of spaceflight exerted upon the normal developmental progression of neuronal maturation, reflected in the following aspects of development: 1) the comparative overall systemic immaturity of the flight specimens most evident in ocular, vestibular, and cortical structures; 2) the presence of abnormal mitotic figure in or near neuronal regions displaying high levels of neuroblast generation and migration along sustentacular elements, such as the neuronal retina and cerebral plates; and 3) the apparent volume disturbances reflected in ocular, vestibular, cochlear, and ventricular cavities, as well as disturbances in the development of their neuronal and non-neuronal complements. No such differences could be detected in newborn, or 15- and 30-day-old pups. It is uncertain whether these effects are the result of direct exposure to the null-gravity environment or an expression of the spaceflight environment acting directly upon the dam, and indirectly, via the placenta, upon the developing fetus.

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Microgravity-Induced Weightlessness on *Aurelia* Ephyra Differentiation and Statolith Synthesis

Science Discipline
Developmental Biology

Investigator / Institute
D.B. Spangenberg — Eastern Virginia Medical School

Co-Investigator(s) / Institute
None

Research Subject(s)
Aurelia (Jellyfish)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control

Key Flight Hardware
Jellyfish kit
Refrigerator/Incubator Module (R/IM)

Selected Publications
Spangenberg, D.B.: Developmental Studies of Aurelia (Jellyfish) Ephyrae Which Developed During the SLS-1 Mission. Advances in Space Research, vol. 14, no. 8, 1994, pp. 239–247.

Spangenberg, D.B.: Graviceptor Developed in Space and on Earth. Advances in Space Research, vol. 14, no. 8, 1994, pp. 317–325.

Objectives/Hypothesis
Aurelia polyps and ephyrae were exposed to microgravity for 9 days as part of the SLS-1 mission. The purpose of this experiment was to study the effects of microgravity on the development of ephyrae from polyps; the development of the graviceptors (rhopalia) of ephyrae; the formation or demineralization of statoliths of rhopalia; and the swimming/pulsing behavior of ephyrae.

Approach or Method
Polyps were induced to strobilate at 28°C, using iodine or thyroxine, at 48 hours (L-48h) and 24 hours (L-24h) before launch, and 8 hours after liftoff (L+8h). Some ephyrae that formed in space were fixed in space on mission day 8, while others were fixed postflight. Postflight, light, and electron scanning microscope examinations were performed.

Results
The number of ephyrae formed per polyp were slightly higher in the L+8h groups compared to those induced at L-24h and L-48h. On Earth, iodine is used by jellyfish to synthesize jellyfish-thyroxine (Jf-T4), which is necessary for ephyra production. Because iodine-treated polyps gave rise to ephyrae in space, it appears that jellyfish are able to synthesize Jf-T4 in space. The two groups of polyps not given the inducer still formed ephyrae in space presumably due to enhanced Jf-T4 synthesis, utilization, or accumulation. Morphologically, ephyrae that developed in space were very similar to those that developed on Earth. Quantitation of arm numbers revealed that there were no significant differences between space- and Earth-developed ephyrae. Pulsing abnormalities, however, were found in greater numbers (18.3%) than in Earth-developed controls (2.9%). These abnormalities suggest abnormal development of the graviceptors, the neuromuscular system, or a defect in the integration between systems in these microgravity-sensitive animals.

Launch Date 9/12/1992 **Landing Date** 9/20/1992

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effect of Weightlessness on Development of Amphibian Eggs

Science Discipline
Developmental Biology

Investigator	Institute
K.A. Souza	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Black, S.	Reed College
Wassersug, R.	Dalhousie University
Ross, M.D.	NASA Ames Research Center (ARC)

Research Subject(s)
Xenopus laevis (Frog)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control

Key Flight Hardware
Frog Embryology Unit (FEU)
General Purpose Work Station (GPWS)
General Purpose Transfer Unit (GPTU)

Selected Publications
Black, S.; Larkin, N.; Jacqmotte, N.; Wassersug, R.; Pronych, S.; and Souza, K.: Regulative Development of Xenopus Laevis in Microgravity. Advances in Space Research, vol. 17, nos. 6/7, 1996, pp. 209–217.

Pronych, S.P.; Souza, K.A.; Neff, A.W.; and Wassersug, R.J.: Optomotor Behaviour in Xenopus Laevis Tadpoles as a Measure of the Effect of Gravity on Visual and Vestibular Neural Integration. Journal of Experimental Biology, vol. 199, 1996, pp. 2689–2701.

Fejtek, M.; Souza, K.; Neff, A.; and Wassersug, R.: Swimming Kinematics and Respiratory Behaviour of Xenopus Laevis Larvae Raised in Altered Gravity. Journal of Experimental Biology, vol. 201, 1998, pp. 1917–1926.

Objectives/Hypothesis
On Earth the animal-vegetal axis of amphibian eggs will rotate upon fertilization to align with the gravitational field. While this is not a requirement for normal development, it does play a role in determining the polarity of the embryonic axis. In addition, eggs inclined with respect to gravity develop dorsal structures on the uppermost surface from the gravitational field. The objective of this experiment was to determine whether gravity is required for normal embryonic development. Prolific *Xenopus laevis* females were selected for flight.

Approach or Method
Frogs were injected subcutaneously with human chorionic gonadotropin 18 hours into flight. Eggs were collected, fertilized, and inserted into chambers, half of which were incubated in the Frog Environmental Unit (FEU) centrifuge at 1g and half were incubated in the FEU at microgravity. Fifty hours into the flight, the temperature was raised to 21 and held there to increase the rate of development. Some embryos were fixed in flight and sectioned and stained postflight. Live embryos were received by the laboratory within 3.5 hours and staged. Normality was assessed by applying criteria of the dorso-anterior index to determine the extent of dorso-anterior differentiation. Optomotor behavior of the tadpoles was determined based on their tendency to track a moving stimulus.

Results
Ovipositon occured in all frogs within 16 hours of hormone injection. Both centrifuge and microgravity groups had high fertilization rates. Embryos at the two-cell stage showed a cleavage furrow in the normal position for both groups. There were no gross abnormalities in gastrulae, but embyos developing in microgravity had thicker blastocoel roofs. In addition, the blastopore lip formed at a slightly more vegetal latitude in the microgravity group than the 1g group. Despite these differences, development to the neurula stage was unimpaired. All fixed neurula and tadpoles appeared normal. Flight tadpoles had stronger optomotor responses than control tadpoles. Because there were no gravitational clues as to the direction of the water/air interface, the tadpoles may have compensated with visual information, thus increasing the strength of their optomotor response. This difference dissappeared by 9 days postflight.

Launch Date 7/8/1994 **Landing Date** 7/23/1994

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Microgravity on *Aurelia* Ephyra Behavior and Development

Science Discipline
Developmental Biology

Investigator / Institute
D.B. Spangenberg — Eastern Virginia Medical School

Co-Investigator(s) / Institute
None

Research Subject(s)
Aurelia (Jellyfish)

Ground-Based Controls
Delayed Synchronous Control, Synchronous Control

Key Flight Hardware
NIZEMI
Refrigerator/Incubator Module (R/IM)
Temperature Recording System-Modification 1 (ATR-4)

Selected Publications
Spangenberg, D.B. and Lattanzio, F.: 1994 Computer-Assisted Videoanalysis of Pulsing/Swimming Behavoir of Aurelia Ephyrae From the SLS-1 Mission. American Society for Gravitational and Space Biology Bulletin, vol. 6, no. 1, 1994, p. 48.

Objectives/Hypothesis
Aurelia polyps and ephyrae were exposed to microgravity and inflight centrifugation during the IML-2 mission. This experiment was designed to study the effects of microgravity on the development of ephyrae from polyps, the development of the graviceptors (rhopalia) of ephyrae, the formation or demineralization of statoliths of rhopalia, and the swimming/pulsing behavior of ephyrae.

Approach or Method
Polyps were induced to strobilate at 28°C, using iodine or thyroxine. Some ephyrae that formed in space were fixed in space on mission day 8, while others were fixed postflight. Swimming behavior in flight and under in-flight centrifugation was observed through in-flight video. Postflight, light, and electron scanning microscope examinations were performed.

Results
The number of ephyrae formed per polyp were slightly higher in the L+8h groups as compared to those induced at L-24h and L-48h. On Earth, iodine is used by jellyfish to synthesize jellyfish-thyroxine (Jf-T4), which is necessary for ephyra production. Because iodine-treated polyps gave rise to ephyrae in space it appears that jellyfish are able to synthesize Jf-T4 in space. The two groups of polyps not given the inducer still formed ephyrae in space presumably due to enhanced Jf-T4 synthesis, utilization, or accumulation. Morphologically, ephyrae that developed in space were very similar to those that developed on Earth. Quantitation of arm numbers revealed that there were no significant differences between space and Earth-developed ephyrae. Pulsing abnormalities, however, were found in greater numbers (18.3%) than in Earth-developed controls (2.9%). These abnormalities suggest abnormal development of the graviceptors, the neuromuscular system, or a defect in the integration between systems in these microgravity-sensitive animals.

STS-66 / NIH.R1

Launch Date 11/3/1994 **Landing Date** 11/14/1994

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Spaceflight Effects on Mammalian Development

Science Discipline
Developmental Biology

Investigator / Institute
J.R. Alberts — Indiana University

Co-Investigator(s) / Institute
Ronca, A. — Indiana University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)
Temperature Recording System-Modification 1 (ATR-4)

Selected Publications
Alberts, J.R. and Ronca, A.E.: Rat Pregnancy and Parturition Survive Spaceflight Challenge: New Consideration of Developmental Consequences. Journal of Gravitational Physiology, vol. 4, no. 2, July 1997, pp. P55–P58.

Ronca, A.E. and Alberts, J.R.: Altered Vestibular Function in Fetal and Newborn Rats Gestated in Space. Journal of Gravitational Physiology, vol. 4, no. 2, July 1997, pp. 63–66.

Objectives/Hypothesis
The purpose of this study was to observe the in-flight activity of pregnant dams in order to obtain quantitative comparisons of the frequency and duration of definable species-typical behavior patterns in microgravity, with special attention to activities that can affect subsequent maternal behavior and fetal development.

Approach or Method
Upon landing, each dam was videotaped for 1 minute and then transferred to surgery for unilateral hysterectomy (G20/21). Following three rounds of vestibular testing, each fetus was tested for tactile sensitivity. Between recovery day and gestational day 23, remaining flight dams were observed and videotaped to determine any quantitative changes in activity profiles as readaptation to 1G proceeded. Dams were allowed to deliver naturally. On recovery day 0, pups were tested for symmetrical and asymmetrical labyrinthine stimulation. On recovery day 1, pups were used for heart rate studies of vestibular responsivity to tilt and to tactile stimulation. On recovery day 2, pups were tested for head nystagmus responses to rotation. On recovery day 3, pups were tested for symmetrical and asymmetrical labyrinthine stimulation.

Results
The flight dams observed on in-flight video records displayed seven more times rolling movement than controls, probably due to the increased number of surfaces available in microgravity. Immediately postflight, flight dams in general moved less. Flight dams had uncomplicated and successful vaginal deliveries and had similar size litters. However, flight dams had significantly more lordosis contractions, probably related to spaceflight muscle atrophy. Pups had no difference in the success or latency of the righting response, indicating the flight groups retained the ability to orientate themselves with respect to gravity. Flight pups tested on recovery day 3 had a diminished water righting response, but flight pups tested on recovery day 5 could not be distinguished from control animals. Flight pups showed the same response as controls during the rotary stimulus but were less likely to perform the postrotary righting response.

STS-66 / NIH.R1

Launch Date 11/3/1994

Landing Date 11/14/1994

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effect of Microgravity on Epidermal Development in the Rat

Science Discipline
Developmental Biology

Investigator	Institute
S.B. Hoath	Children's Hospital Medical Center

Co-Investigator(s)	Institute
Hussain, A.	FDA Office of Pharmaceutical Sciences
Pickens, W.L.	Children's Hospital Medical Center

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)
Temperature Recording System-Modification 1 (ATR-4)
Biorack US3 Experiment Hardware: Cell Chambers and Assemblies

Selected Publications
Wickett, R.R.; Nath, V.; Tanaka, R.; and Hoath, S.B.: Use of Continuous Electrical Capacitance and Transepidermal Water Loss Measurements for Assessing Barrier Function in Neonatal Rat Skin. Skin Pharmacology, vol. 8, no. 4, 1995, pp. 179–185.

Objectives/Hypothesis
The overall goal of this study was to examine the effects of prolonged weightlessness on epidermal development in the late gestation Sprague-Dawley rat. Specifically: 1) exposure of the pregnant rat to microgravity during late gestation will diminish transplacental (maternal-fetal) calcium transport leading to decreases in total epidermal and dermal calcium content; 2) microgravity will lead to mild intrauterine somatic growth retardation and a diminution in the rate of stratum corneum formation (rate of programmed cell death); and 3) microgravity will lead to formation of a stratum corneum envelope with decreased DC electrical resistance and increased permeability to triated water.

Approach or Method
Epidermis and whole skin (epidermis + dermis) were harvested from ground-control fetal rats and fetal rats exposed to conditions of spaceflight during midgestation. Morphological studies of skin development included transmission and scanning electron microscopy and determination of stratum corneum layer formation following alkaline expansion of cryopreserved tissue specimens. Tissues of fetal and term animals were assayed for calcium content by atomic absorption spectrophotometry. Transport studies of water across whole skin and determination of skin electrical properties were measured on cryopreserved sections. Current voltage profiles for skin excised from newborn rat pups were generated between –10 microamps to +10 microamps.

Results
Pregnancy in the Sprague-Dawley rat can be maintained under the adverse conditions of spaceflight and readaptation to terrestrial gravity. No evidence of increased fetal wastage or somatic growth retardation was observed. Vaginal delivery can be achieved following short-term (3 days) readaptation to terrestrial conditions. Epidermal barrier development in the late gestational fetal rat appears to be advanced under the conditions examined. Fetal skin calcium levels are increased following development under conditions of microgravity. Neonatal epidermal calcium levels are decreased following short term readaptation to terrestrial gravity. Morphologically, the epidermal barrier is advanced by 12–24 hours. Measurement of water flux and electrical resistance of the skin support the hypothesis of a better epidermal barrier in the flight animals compared to ground controls.

STS-66 / NIH.R1

Launch Date 11/3/1994 **Landing Date** 11/14/1994

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Microgravity and Placental Development

Science Discipline
Developmental Biology

Investigator / Institute
R.H. Renegar — East Carolina University

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Renegar, R.H.; Owens, C.R.; and Whitehead, D.: Morphological and Functional Parameters of Placentas From Rat Dams flown on the NIH. R1 Study. American Society for Gravitational and Space Biology Bulletin, vol. 9, no. 1, 1995, p. 98.

Objectives/Hypothesis
The role of gravity in proper placental development has not been previously studied and could be necessary to successful mammalian reproduction. The objective of this experiment was to assess the effect of microgravity on placental growth and development of the rat, with the specific aim of:
1) determining the morphology of the placenta and decidua, and their organizational relationship immediately upon return from space; and 2) assessing the functional differentiation of trophoblast cells of the labyrinth and trophospongial regions of the placenta by comparing the expression of specific developmentally expressed proteins.

Approach or Method
Between 3 and 6 hours following recovery, one uterine horn was removed from each of the flight and control group animals. After removing the fetuses from the horns, placenta were weighed and prepared for morphological evaluation and measurement of hormone expression. Expression of two forms of placental lactogen (PL) was measured using complementary DNA (cDNA) probes specific to the hormones.

Results
Morphological studies showed that cross-sectional area of the total placenta, trophospongium and labyrinth, and placental wet weight were not different among flight and control groups. However, percent dry weight for the asynchronous control animals was less ($p < 0.05$) than that for the other groups. Concentration of DNA in the placenta was also less in the asynchronous controls. Placental concentrations of RNA and protein were not different among flight and control groups. Hybridization with the cDNA probes showed that the quantity of PL-I, PL-II, and the ratio PL-II:PL-I were not different among the groups. Spaceflight during gestation days 9–20 does not seem to influence placental growth, development, or function.

STS-66 / NIH.R1

Launch Date 11/3/1994

Landing Date 11/14/1994

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Skeletal Development

Science Discipline
Bone Physiology

Investigator / Institute
L.V. Serova — Institute of Biomedical Problems

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Serova, L.V.; Natochin, Y.V.; Nosovsky, A.M.; Savelyev, S.V.; Chelnaya, N.A.; Shakmatova, E.I.; and Fast, T.: Effect of Weightlessness on the Mother-Fetus System (Results of Embyrological Experiment NIH-R1 Aboard the "Space Shuttle") (in Russian). Aviakosm Ekolog Med, vol. 30, no. 6, 1996, pp. 4–8.

Serova, L.V.: Simulation Models of Weightlessness in Mammalian's Developmental Program. Journal of Gravitational Physiology, vol. 5, no. 1, July 1998, pp. 127–128.

Objectives/Hypothesis
Previous experiments have revealed that an adult organism may well remain functional, and a developing fetus may also develop normal functions in a space environment. However, changes were also observed in space-flown fetuses and animals. In this experiment it was assumed that a longer flight and exposure to microgravity may aggravate these changes. The purpose of this experiment was to assess the effect of microgravity on skeletal development in fetuses of rats exposed to microgravity during gestation days 9 through 20.

Approach or Method
The skeletal development of pups that developed in space was investigated using one fetus from each flight, synchronous, and vivarium laparatomized group. Fetuses were fixed in 100% ethanol, bleached in 0.7% KOH, and stained with alizarine red; bones were dissected and measured in a binocular lens.

Results
The mean fetal mass in the flight and control fetuses did not differ significantly; however, the mean weight of the vivarium laparatomized fetus was 2.143 g, versus 2.469 g of the synchronous control, and 2.394 g of the flight fetus ($p < 0.002$). The body weight was in correlation with the ossification areas that were the lowest in the vivarium controls. The mandibles of the 20-day fetuses of the flight group were 7% longer than those in the vivarium controls and 3% longer than in the synchronous group. The sizes of the forelimb bones (humerus, radius, and ulna), as well as the number of ossification sites in the foot, were identical in the flight and synchronous rats, being greater than in the vivarium controls. The scapulae of the flight and synchronous animals were identical and larger than in the vivarium controls. The clavicles of the flight fetuses were 6% longer than those in the synchronous controls. No significant differences were found in the length of the femur, tibia, and fibula bones, or in the number of ossification sites in the foot between the flight and synchronous fetuses.

STS-66 / NIH.R1

Launch Date 11/3/1994

Landing Date 11/14/1994

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Developmental Abnormalities

Science Discipline
Developmental Biology

Investigator	Institute
L.V. Serova	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Saveliev, S.V.	nstitute of Human Morphology
Besova, N.V	nstitute of Human Morphology

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Saveliev, S.: The Effect of Weightlessness on Developing Neuro-Endocrine System in Rats (in Russian). Aerospace and Environmental Medicine, vol. 32, no. 2, 1998, pp. 31–36.

Serova, L.V.; Natochin, Y.V.; Nosovsky, A.M.; Savelyev, S.V.; Chelnaya, N.A.; Shakmatova, E.I.; and Fast, T.: Effect of Weightlessness on the Mother-Fetus System (Results of Embyrological Experiment NIH-R1 Aboard the “Space Shuttle”) (in Russian). Aviakosm Ekolog Med, vol. 30, no. 6, 1996, pp. 4–8.

Objectives/Hypothesis
Previous experiments revealed that an adult organism may well remain functional and a developing fetus may also develop normal functions in a space environment. However, changes were also observed in space-flown fetuses and animals. In this experiment it was assumed that a longer flight and exposure to microgravity may aggravate these changes. The purpose of this experiment was to perform a morphological and histological examination of rat pups that developed during the mother's exposure to microgravity during gestation days 9 through 20.

Approach or Method
Developmental abnormalities were studied in newborns obtained on the 22nd/23rd gestational day. We were provided with a newborn pup from the flight and synchronous rats and from both vivarium controls. After isoflurance anesthesia, the newborn pups were fixed in Bouin’s solution. The fixed pups were examined and observed defects were measured subjectively. The surface skin area was measured with a digitizer attached to a personal computer (PC). Pup volume was determined according to the method of ethanol displacement. Serial sections of the pup were stained and examined for any changes with the aid of computer analysis.

Results
Histological examinations of the sensors of the flight animals did not reveal any abnormalities. The pups that developed in space showed disintegration of neurons in various brain compartments (cortex, hippocampus, and spinal cord). The change was similar to that observed in porencephalia but was less significant than in disease. None of the synchronous controls showed signs of neuronal disintegration. Most pups from the flight group developed an enhanced differentiation of both thyroid cells, secreting thyroid hormones, C-cells, and parathyroid cells. Those pups showed decreases in the size of the epiphysis and adenohypophysis and in the number of pinealocytes, as well as spatial rearrangement of trabeculae in the adenohypophysis.

Mir 19 / Incubator 2

Launch Date 6/27/1995 **Landing Date** 9/11/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Skeletal Development in Long-Duration Spaceflight

Science Discipline
Developmental Biology

Investigator / ***Institute***
S.B. Doty — Hospital for Special Surgeries

Co-Investigator(s) / ***Institute***
None

Research Subject(s)
Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
Doty, S.B.: Space Flight and Bone Formation. Materwiss Werksttech, vol. 35, no. 12, Dec. 2004, pp. 951–961.

Objectives/Hypothesis
The objective of this experiment was to study embryogenesis and cellular differentiation of endochondral and intramembranous bone formation, as affected by long-term spaceflight.

Approach or Method
This experiment was performed twice, once during Mir 18 (returning on STS-71) and once during Mir 19 (returning on STS-74). Fertilized eggs were carried to the Mir Space Station aboard a Russian Progress (227) supply vessel, and placed in an incubator supplied by the Institute for Biomedical Problems. On days 7, 10, 14, and 16, eight eggs were fixed and stored for return to Earth.

Results
During both runs of the experiment, internal incubator temperature was higher than nominal throughout the experiment, and most of the quail embryos failed to develop as expected, resulting in low science return. During Mir 19, 10 embryos developed past 7 days and 4 embryos developed to an appropriate age. No viable results were obtained.

Mir 19 / Incubator 2

Launch Date 6/27/1995

Landing Date 9/11/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effect of Microgravity on Afferent Innervation

Science Discipline
Developmental Biology
Neurophysiology

Investigator / Institute
C. Fermin — Tulane University

Co-Investigator(s) / Institute
None

Research Subject(s)
Coturnix coturnix (Japonica quail)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications

Fermin, C.D.; Martin, D.S.; and Hara, H.: Color Threshold and Ratio of S100b, MAP5, NF68/200, GABA and GAD.I. Distribution in Inner Ear Afferents. Cell Vision, vol. 4, no. 5, 1997, pp. 280–295.

Fermin, C.D.; Lychakov, D.; Campos, A.; Hara, H.; Sondag, E.; Jones, T.; Jones, S.; Taylor, M.; Meza-Ruiz, G.; and Martin, D.S.: Otoconia Biogenesis, Phylogeny, Composition and Functional Attributes. Histology and Histopathology, vol. 13, no. 4, 1998, pp. 1103–1154.

Hara, H.; Chen, X.; Hartsfield, J.F.; Hara, J.; Martin, D.; and Fermin, C. D.: Chicken (*Gallus Domesticus*) Inner Ear Afferents. Primary Sensory Neuron, vol. 2, no. 4, 1998, pp. 253–274.

Objectives/Hypothesis
The objective of this experiment was to determine the effects of microgravity on connectivity of afferent neurons and inner ear hair cells and vestibular nuclei neurons, and characterize changes in innervation patterns of inner ear afferent and efferent neurons.

Approach or Method
This experiment was performed twice, once during Mir 18 (returning on STS-71) and once during Mir 19 (returning on STS-74). Fertilized eggs were carried to the Mir Space Station aboard a Russian Progress (227) supply vessel and placed in an incubator supplied by the Institute for Biomedical Problems. On days 7, 10, 14 and 16, eight eggs were fixed and stored for return to Earth. The branching pattern and morphology of the afferent terminals in one ear was analyzed under light and electron microscopy. The brainstem and opposite ear were sectioned and analyzed immunohistochemically for neurofilament (NF) content, the S100ß protein, and synthesizing and degrading enzymes for neurotransmitters gamma-amino-butyric acid (GABA), and acetylcholine (ACh). The branching patterns of afferents inside the epithelia were observed with NF staining. Changes in GABA and ACh staining suggest changes in the afferent and efferent system respectively. The utricle-lateral canal ampulla (ULC) was dissected and observed with electron microscopy in order to evaluate synaptic density.

Results
During both runs of the experiment, internal incubator temperature was higher than nominal throughout the experiment, and most of the quail embryos failed to develop as expected, resulting in low science return. During Mir 19, 10 embryos developed past 7 days and 4 embryos developed to an appropriate age. After sharing viable specimens with other researchers, this experiment received only three partially intact flight specimens, preventing statistically significant analysis. However, analysis of ground control embryos was conducted, providing normative data for future flight experiments.

Mir 19 / Incubator 2

Launch Date 6/27/1995

Landing Date 9/11/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Weightlessness on Vestibular Development of Quail

Science Discipline
Developmental Biology
Neurophysiology

Investigator	Institute
B. Fritzsch	Creighton University

Co-Investigator(s)	Institute
Bruce, L.L.	Creighton University

Research Subject(s)
Coturnix coturnix (Japonica quail)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
None

Objectives/Hypothesis
The objective of this experiment was to determine the effects of microgravity on the development of connections between the gravistatic receptors and the brainstem in quail raised in microgravity. The long-range importance of this research is to find out whether or not there is a critical phase during development of the vestibular system in which appropriate stimuli are needed to fine tune synaptogenesis. These data are crucial for future long-range space explorations that require multi-generation flights.

Approach or Method
This experiment was performed twice, once during Mir 18 (returning on STS-71) and once during Mir 19 (returning on STS-74). Fertilized eggs were carried to the Mir Space Station aboard a Russian Progress (227) supply vessel and placed in an incubator supplied by the Institute for Biomedical Problems. On days 7, 10, 14, and 16, eight eggs were fixed and stored for return to Earth.

Results
During both runs of the experiment, internal incubator temperature was higher than nominal throughout the experiment, and most of the quail embryos failed to develop as expected, resulting in low science return. During Mir 19, 10 embryos developed past 7 days and 4 embryos developed to an appropriate age. No viable results were obtained.

Mir 19 / Incubator 2

Launch Date 6/27/1995

Landing Date 9/11/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Weightlessness on Quail Eye Development

Science Discipline
Developmental Biology

Investigator / Institute
G. Conrad — Kansas State University

Co-Investigator(s) / Institute
None

Research Subject(s)
Coturnix coturnix (Japonica quail)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
Barrett, J.E.; Wells, D.C.; Paulsen, A.Q.; and Conrad, G.W.: Embryonic Quail Eye Development in Microgravity. Journal of Applied Physiology, vol. 88, 2000, pp. 1614–1622.

Barrett, J.E.; Wells, D.C.; and Conrad, G.W.: Pretreatment Methods to Improve Nerve Immunostaining in Corneas From Long-Term Fixed Embryonic Quail Eyes. Journal of Neuroscience Methods, vol. 92, 1999, pp. 161–168.

Objectives/Hypothesis
The objective of this experiment was to determine if microgravity affects the ultrastructural development of the cornea in quail.

Approach or Method
This experiment was performed twice, once during Mir 18 (returning on STS-71) and once during Mir 19 (returning on STS-74). Fertilized eggs were carried to the Mir Space Station aboard a Russian Progress (227) supply vessel and placed in an incubator supplied by the Institute for Biomedical Problems. On days 7, 10, 14, and 16, eight eggs were fixed and stored for return to Earth.

Results
During both runs of the experiment, internal incubator temperature was higher than nominal throughout the experiment, and most of the quail embryos failed to develop as expected, resulting in low science return. During Mir 19, 10 embryos developed past 7 days and 4 embryos developed to an appropriate age. No viable results were obtained.

Mir 19 / Incubator 2

Launch Date 6/27/1995

Landing Date 9/11/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study

Hypogravity's Effect on the Life Cycle of Japanese Quail

Science Discipline

Developmental Biology

Investigator | ***Institute***

P. Hester | Purdue University

Co-Investigator(s) | ***Institute***

None

Research Subject(s)

Coturnix coturnix (Japonica quail)

Ground-Based Controls

Asynchronous Control

Key Flight Hardware

Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications

Hester, P.Y. and Boda, K.: Egg Rotation During Avian Embryogenesis. American Society for Gravitational and Space Biology Bulletin, vol. 11, 1997, p. 28.

Hester, P.Y.; Orban, J.I.; Sabo, V.; and Boda, K.: Egg Rotation During Avian Embryogenesis. Folia Veterinaria, supl., vol. 42, 1998, pp. S67–S72.

Objectives/Hypothesis

The objective of this experiment was to determine if quail embryos exposed to microgravity use minerals from the egg shell in the same manner as embryos on Earth.

Approach or Method

This experiment was performed twice, once during Mir 18 (returning on STS-71) and once during Mir 19 (returning on STS-74). Fertilized eggs were carried to the Mir Space Station aboard a Russian Progress (227) supply vessel and placed in an incubator supplied by the Institute for Biomedical Problems. On days 7, 10, 14, and 16, eight eggs were fixed and stored for return to Earth.

Results

During both runs of the experiment, internal incubator temperature was higher than nominal throughout the experiment, and most of the quail embryos failed to develop as expected, resulting in low science return. During Mir 19, 10 embryos developed past 7 days and 4 embryos developed to an appropriate age. No viable results were obtained.

Mir 19 / Incubator 2

Launch Date 6/27/1995

Landing Date 9/11/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Expression of Contractile Protein Isoforms in Microgravity

Science Discipline
Developmental Biology
Muscle Physiology

Investigator / Institute
P. Anderson — Duke University

Co-Investigator(s) / Institute
None

Research Subject(s)
Coturnix coturnix (Japonica quail)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
None

Objectives/Hypothesis
The objective of this experiment was to determine the effects of microgravity on developmentally programmed expression of Troponin T and I isoforms known to regulate cardiac and skeletal muscle contraction.

Approach or Method
This experiment was performed twice, once during Mir 18 (returning on STS-71) and once during Mir 19 (returning on STS-74). Fertilized eggs were carried to the Mir Space Station aboard a Russian Progress (227) supply vessel and placed in an incubator supplied by the Institute for Biomedical Problems. On days 7, 10, 14, and 16, eight eggs were fixed and stored for return to Earth.

Results
During both runs of the experiment, internal incubator temperature was higher than nominal throughout the experiment, and most of the quail embryos failed to develop as expected, resulting in low science return. During Mir 19, 10 embryos developed past 7 days and 4 embryos developed to an appropriate age.

Mir 19 / Incubator 2

Launch Date 6/27/1995

Landing Date 9/11/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Avian Blood Vessel Formation in Space

Science Discipline
Developmental Biology

Investigator	Institute
P. Lelkes	University of Wisconsin Medical School

Co-Investigator(s)	Institute
Unsworth, B.R.	Marquette University

Research Subject(s)
Coturnix coturnix (Japonica quail)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
Henry, M.K.; Unsworth, B.R.; Sychev, V.; Guryeva, T.S.; Dadasheva, O.A.; Piert, S.J.; Lagel, K.E.; Dubrovin, L.C.; Jahns, G.C.; Boda, K.; Sabo, V.; Samet, M.M.; and Lelkes, P.I.: Launch Conditions Might Affect the Formation of Blood Vessels in the Quail Chorioallantoic Membrane. Folia Veterinaria, supl., vol. 42, 1998, pp. S25–S31.

Objectives/Hypothesis
The objective of this experiment was to determine whether prolonged spaceflight causes a change in the pattern of vascularization during the development and maturation of the chorioallantoic membrane (CAM).

Approach or Method
This experiment was performed twice, once during Mir 18 (returning on STS-71) and once during Mir 19 (returning on STS-74). Fertilized eggs were carried to the Mir Space Station aboard a Russian Progress (227) supply vessel and placed in an incubator supplied by the Institute for Biomedical Problems. On days 7, 10, 14, and 16, eight eggs were fixed and stored for return to Earth. Postflight, vivarium, aysnchronous, and flight egg shells were opened longitudinally, and the CAM was carefully removed. The CAM was then analyzed under autoflourescent bright light microscopy to evaluate arterial blood vessel density and diameter.

Results
During both runs of the experiment, internal incubator temperature was higher than nominal throughout the experiment, and most of the quail embryos failed to develop as expected, resulting in low science return. During Mir 19, 10 embryos developed past 7 days and 4 embryos developed to an appropriate age. Statistically significant analysis of flight was animals was impossible. However, comparative analyses between vivarium control and simulated ground-based groups were viable. Blood vessel density was significantly lower in simulated subjects when compared to the vivarium subjects. Only small vessel density was affected suggesting that the forces imposed on the simulated group affected normal angiogenesis.

Mir 19 / Incubator 2

Launch Date 6/27/1995

Landing Date 9/11/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Fecundity of Quail in Spacelab Microgravity

Science Discipline
Developmental Biology

Investigator	Institute
B. Wentworth	University of Wisconsin, Madison

Co-Investigator(s)	Institute
Wentworth, A.L.	University of Wisconsin, Madison

Research Subject(s)
*Coturnix coturnix (*Japonica quail)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
None

Objectives/Hypothesis
The objective of this experiment was to assess the effects of microgravity on arrangement, normal development, and primordial germ cell (PGC) migration in the gonads of Japanese quail embryos, and to determine the effects of microgravity on normal respiratory function.

Approach or Method
This experiment was performed twice, once during Mir 18 (returning on STS-71) and once during Mir 19 (returning on STS-74). Fertilized eggs were carried to the Mir Space Station aboard a Russian Progress (227) supply vessel and placed in an incubator supplied by the Institute for Biomedical Problems. On days 7, 10, 14, and 16, eight eggs were fixed and stored for return to Earth.

Results
During both runs of the experiment, internal incubator temperature was higher than nominal throughout the experiment, and most of the quail embryos failed to develop as expected, resulting in low science return. During Mir 19, 10 embryos developed past 7 days and 4 embryos developed to an appropriate age. No viable results were obtained.

Mir 19 / Incubator 2

Launch Date 6/27/1995 **Landing Date** 9/11/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

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

Science Discipline
Developmental Biology
Neurophysiology

Investigator / Institute
T. Shimizu — University of South Florida

Co-Investigator(s) / Institute
None

Research Subject(s)
*Coturnix coturnix (*Japonica quail)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
Shimizu, T.: Effects of Weightlessness on the Avian Visuo-Vestibular System: Immunohistochemical Analysis. NASA Technical Memorandum 4751, June 1996, pp. 81–82.

Shimizu. T.: Effects of Weightlessness on the Avian Visuo-Vestibular System: Immunohistochemical Analysis. NASA Technical Memorandum 4801, May 1997, pp. 66–67.

Objectives/Hypothesis
The objective of this experiment was to investigate the fundamental effects of gravity deprivation on the visuo-vestibular system in birds by measuring distribution of neurochemicals in quail raised in microgravity.

Approach or Method
This experiment was performed twice, once during Mir 18 (returning on STS-71) and once during Mir 19 (returning on STS-74). Fertilized eggs were carried to the Mir Space Station aboard a Russian Progress (227) supply vessel and placed in an incubator supplied by the Institute for Biomedical Problems. On days 7, 10, 14, and 16, eight eggs were fixed and stored for return to Earth.

Results
During both runs of the experiment, internal incubator temperature was higher than nominal throughout the experiment, and most of the quail embryos failed to develop as expected, resulting in low science return. During Mir 19, 10 embryos developed past 7 days and 4 embryos developed to an appropriate age. No viable results were obtained.

STS-70 / NIH.R2

Launch Date	Landing Date
7/13/1995	7/22/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Spaceflight Effects on Mammalian Development

Science Discipline
Developmental Biology

Investigator	Institute
J.R. Alberts	Indiana University

Co-Investigator(s)	Institute
Ronca, A.	Indiana University
Burden, H.	East Carolina University
Plaut, K.	University of Vermont

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Cage Control, Delayed Synchronous Control

Key Flight Hardware
Ambient Temperature Recorder (ATR)
Animal Enclosure Module (AEM)

Selected Publications
Ronca, A.E. and Alberts, J.R.: Altered Vestibular Function in Fetal and Newborn Rats Gestated in Space. Journal of Gravitational Physiology, vol. 4, no. 2, July 1997, pp. 63–66.

Alberts, J.R. and Ronca, A.E.: Rat Pregnancy and Parturition Survive Spaceflight Challenge: New Consideration of Developmental Consequences. Journal of Gravitational Physiology, vol. 4, no. 2, July 1997, pp. P55–P58.

Objectives/Hypothesis
The development of sensory systems has been shown to be affected by stimulation. It is hypothesized that exposure to microgravity reduces the stimulation of the developing fetal vestibular system and alters early vestibular functions in fetal rats. The purpose of this experiment was to explore that hypothesis, as well as to study the effects of spaceflight on the behavior and physiology of pregnancy, labor, delivery, and the onset of postnatal care, especially with respect to lactation.

Approach or Method
Vestibular development was studied prenatally and postnatally. During flight, a kinematic analysis of dams was conducted using daily videorecordings of their activity. Postflight, prenatal fetuses were externalized from the uterus while keeping umbilical and placental connections to the dam intact. Electrocardiogram (EKG) electrodes were implanted in the embryos and EKG was monitored during vestibular perturbations of a 10 s, 70° tilt. Postnatally, pups were placed supine in and out of water and videorecorded in order to evaluate their standard righting response and water immersion righting response. Pups were also subjected to rotary stimulation to evaluate their responses to horizontal rotation. Rat pregnancy and parturition were also studied. Animals were recorded in flight to observe the behavior of the dams. Immediately postflight, dams were placed in a glass chamber and videotaped before and after parturition.

Results
Flight fetuses responded more dramatically than the controls to vestibular perturbations. Pups had no difference in the success or latency of the righting response indicating the flight groups retained the ability to orientate themselves with respect to gravity. Flight pups tested on recovery day 3 had a diminished water righting response, but flight pups tested on recovery day 5 could not be distinguished from control animals. Flight pups showed the same response as controls during the rotary stimulus, but were less likely to perform the post-rotary righting response. Results from the pregnancy and parturition portion of this study are as follows: the flight dams displayed seven more times rolling movement than controls, probably due to the increased number of surfaces available in microgravity. Immediately postflight, flight dams in general moved less. Flight dams had uncomplicated, successful vaginal deliveries, and had similar size litters. However, flight dams had significantly more lordosis contractions probably related to spaceflight muscle atrophy.

STS-70 / NIH.R2

Launch Date 7/13/1995 **Landing Date** 7/22/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Microgravity on Bone Development

Science Discipline
Bone Physiology

Investigator / Institute
N.C. Partridge — St. Louis University

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)
Ambient Temperature Recorder (ATR)

Selected Publications
Davis, B.A.; Sipe, B.; Gershan, L.A.; Fiacco, G.; Lorenz, T.C.; Jeffrey, J.J.; and Partridge, N.C.: Collagenase and Tissue Plasminogen Activator Production in Developing Rat Calvariae: Normal Progression Despite Fetal Exposure to Microgravity. Calcified Tissue International, vol. 63, 1998, pp. 416–422.

Objectives/Hypothesis
As shown in previous studies, the development and mineralization of new bone decreases in microgravity environments. Other studies have shown that bone resorption is not affected by exposure to microgravity. Thus, the bone forming cells, osteoblasts, have been implicated in the decrease of bone mass. Osteoblasts secrete the neural proteinases collagenase, and tissue plasminogen activator (tPA) that are crucial to the process of bone mineralization. This study examined whether the expression of collagenase and tPA in rats of various ages is affected by prenatal exposure to microgravity.

Approach or Method
Pups prenatally exposed to microgravity were delivered and prepared at various ages. Frozen blocks of bone from the pups were then sectioned for immunohistochemistry analysis. The antiserum used for collagenase was a monospecific polyclonal rabbit antiserum raised against rat osteoblastic procollagenase. The antiserum used for immunolocalization of tissue plasminogen activator was raised in rabbits against purified rat insulinoma tissue plasminogen activator. Stained sections were photographed and examined to determine distinct differences in the appearance or developmental pattern of the calvariae. The specificity of the tPA and collagenase antibodies was established with Western Blot and Zymogram analyses. Thickness of the bone matrix was then analyzed with photoimagery.

Results
Staining for collagenase was present at all ages, and revealed little difference between the flight and the control animals. Tissue plasminogen staining also showed no significant differences between flight and control animals. Staining revealed that tPA localized to blood vessels, and that collagenase is localized to endocranial areas that are actively being modeled and in the matrix. This result suggest that tPA and collagenase are not produced by the same population of cells. Photoimagery analysis supported the results obtained with immunohistochemistry, and showed that the calvariae thickness was similar for control and flight animals. The relatively brief exposure of the pups to microgravity might account for the lack of difference noted in this study.

STS-70 / NIH.R2

Launch Date	Landing Date
7/13/1995	7/22/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Neuromuscular Development and Regulation of Mysoin Expression

Science Discipline
Muscle Physiology

Investigator	Institute
S.C. Bodine-Fowler	University of California, San Diego

Co-Investigator(s)	Institute
None	

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Ambient Temperature Recorder (ATR)
Animal Enclosure Module (AEM)

Selected Publications
Talmadge, R.J.; Roy, R.R.; Bodine-Fowler, S.C.; Pierotti, D.J.; and Edgerton, V.R.: Adaptations in Myosin Heavy Chain Profile in Chronically Unloaded Muscles. Basic and Applied Myology, vol. 5, no. 2, 1995, pp. 117–137.

Objectives/Hypothesis
The objectives of this experiment were: 1) to examine the time-course of myosin heavy chain expression and the development of adult fiber types in hindlimb muscles that have been exposed to microgravity during embryonic development; and 2) to determine if the embryonic system requires gravity in order to establish normal differentiation of muscle fiber types.

Approach or Method
Tissues from dams, gestation-day-20 fetuses, and pups at ages 1, 3, 7, 10, 14, 21, and 35 days were obtained following flight on STS-70. Launch occurred on gestation day 11, and landing was on gestation day 20. Hindlimbs and muscles from fetuses and pups were sectioned and labeled using antibodies for myogenin, MyoD, and myosin heavy chain isoforms for embryonic, neonatal, type I, type Ia, type Ib, and type Ix.

Results
In soleus and medial gastrocnemius muscles from the dams, no differences were found between flight and control animals in mean fiber area or myosin heavy chain expression.

STS-70 / NIH.R2

Launch Date 7/13/1995 **Landing Date** 7/22/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Spaceflight on the Development of the Circadian Timing System

Science Discipline
Neurophysiology

Investigator	Institute
C.A. Fuller	University of California, Davis

Co-Investigator(s)	Institute
Murakami, D.M.	University of California, Davis
Hoban-Higgins, T.M.	University of California, Davis

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Ambient Temperature Recorder (ATR)
Animal Enclosure Module (AEM)

Selected Publications

Hoban-Higgins, T.M.; Murakami, D.M.; Fermin, C.; and Fuller, C.A.: Development of Circadian Rhythms of Body Temperature and Activity in Sprague-Dawley Rat Pups. Society for Neuroscience, vol. 21, 1995, p. 955.

Murakami, D.M.; Tang, I.H.; Hoban-Higgins, T.M.; and Fuller, C.A.: The Development of the Photic Induction of c-Fos in the Retina and SCN. Society for Neuroscience, vol. 21, 1995, p. 956.

Murakami, D.M.; Tang, I.H.; Hoban-Higgins, T.M.; and Fuller, C.A.: The Effect of Spaceflight on Retino-Hypothalamic Development. Journal of Gravitational Physiology, vol. 4, 1997, p. 67.

Objectives/Hypothesis
The objective of this study was to determine the effect of spaceflight on retinal development, development of the suprachiasmatic nucleus (SCN), the circadian pacemaker of the circadian timing system (CTS), maturation of the retinohypothalamic pathway, and the development and maturation of circadian rhythms.

Approach or Method
Retinas were dissected, sectioned, and mounted on slides, counterstained, and analyzed for thickness of the inner-plexiform, inner-nuclear, outer-nuclear, and outer-plexiform layers. Differences in the initiation and maturation rate of laminar thickness were compared between animal groups. Coronal sections were made through the hypothalamus and mounted on slides that were counterstained and analyzed for mean soma diameter of neurons within the SCN. Coronal sections through the SCN were examined for cytochrome oxidase (CYOX) staining both within the SCN and in the surrounding regions at each age. As a provocative test of SCN function, animals from each group were either exposed to a light pulse (LP) or not (NLP). Sections through the SCN and surrounding hypothalamus were immunohistochemically stained for c-Fos reactive neurons. Brain sections containing c-Fos-labeled neurons were mounted, counterstained, and examined by light microscopy to determine differences in the number of immunoreactive SCN neurons between LP and NLP animals at the age that the RHT projection becomes functional.

Results
Histological examination of the retina revealed no differences in development between the Flight (FLT) and Flight-Delayed Synchronous (FDS) retina at G20, PN1, PN3, and PN8. The pattern of cFos activation within the SCN of the G20 animals indicated that the FLT group was significantly delayed from the FDS group at this age. These differences disappeared by PN1. There was no difference between groups in the responsiveness of the SCN to light stimulation; a robust response was present by PN5.

STS-69 / NIH.C4

Launch Date 9/7/1995 **Landing Date** 9/18/1995

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Osteoblast Adhesion and Phenotype in Microgravity

Science Discipline
Bone Physiology

Investigator ***Institute***
R.J. Majeska — Mount Sinai Medical Center

Co-Investigator(s) ***Institute***
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Space Tissue Loss unit-A (STL)

Selected Publications
None

Objectives/Hypothesis
The purpose of this experiment was to investigate: 1) whether microgravity induces changes in osteoblastic cells similar to those caused by bone resorption-stimulating agents like parathyroid hormone (PTH), and 2) whether those phenotypic changes are associated with alterations in cell shape and/or adhesive interactions with the extracellular matrix.

Approach or Method
A permanent cell line derived from a rat osteosarcoma and stably exhibiting an osteoblast-like phenotype (ROS 17/2.8) was cultured on microcarrier beads and inoculated into 24 Space Tissue Loss (STL) cartridges. At times ranging from 6 hours to 10 days post-launch, samples of conditioned medium were collected from pairs of cartridges from the 12 flight and 12 ground control groups, and the cartridges were perfused with fixative. Upon recovery after landing, the medium was assayed for lactate (an indicator of metabolic activity and cell viability), alkaline phosphatase (an osteoblast marker enzyme), cyclic adenosine monophosphate (cAMP) (produced by osteoblastic cells in response to PTH) and prostaglandin E2 (a locally produced mediator of bone turnover). The cells were examined by phase contrast and fluorescence microscopy to assess shape changes, cytoskeletal organization, and interactions with their substrata.

Results
Measurements of lactate, alkaline phosphatase, cAMP and PGE2 indicated the presence of viable cells expressing an osteoblastic phenotype; however, no differences were found in any of these parameters between the flight and ground samples. Microscopic examination of the beads indicated that a loss of cells occurred early after inoculation into cartridges in flight and ground samples alike, followed apparently by slow recovery and growth. No gross differences were found in cell shape between flight and ground samples.

STS-72 / NIH.C5

Launch Date 1/11/1996 **Landing Date** 1/20/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
The Effects of Micro-Gravity on In Vitro Calcification

Science Discipline
Bone and Calcium Physiology

Investigator	***Institute***
A.L. Boskey	Hospital for Special Surgery

Co-Investigator(s)	***Institute***
Binderman, I.	Hospital for Special Surgery
Doty, S.B.	Hospital for Special Surgery

Research Subject(s)
Gallus gallus (White leghorn chicken) cells

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Space Tissue Loss-A (STL-A) Module

Selected Publications
None

Objectives/Hypothesis
Biomineralization is regulated by a number of factors during both development and disease. Cell culture can be used to study mineralization and how cells react to it. Various events in endochronal bone development can be studied using micro-mass cell cultures of mesenchymal cells. Micro-mass cultures can therefore be used to test the hypothesis that bone loss in microgravity is due to improper cell maturation instead of defective matrix mineralization.

Approach or Method
Stage 21–24 chick limb bud mesenchymal cells were plated in micro-mass culture. Cultures at 9, 10, and 17 days of age were placed in computer-operated cartridges and flown on STS-72.

Results
Due to a hardware malfunction in the STL-A, the flight experiment was never initiated, and no data was obtained from the flight cultures. In two earlier flight experiments, mesenchymal cells flown in hypogravity proliferated but failed to differentiate and form mineralized matrices. Because results were unable to be confirmed, there are no publications.

STS-72 / NIH.R3

Launch Date	Landing Date
1/11/1996	1/20/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Behavior of Postnatal Rats After Space Flight

Science Discipline
Developmental biology

Investigator	Institute
K. Walton	New York University Medical Ctr
D.A. Riley	Medical College of Wisconsin

Co-Investigator(s)	Institute
None	

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM), Ambient Temperature Recorder (ATM)

Selected Publications
Walton, K.; Heffernan, C.; Sulica, D.; and Benavides, L.: Changes in Gravity Influence Rat Postnatal Motor System Development: From Simulation to Space Flight. Gravitational and Space Biology Bulletin, vol. 10, no. 2, June 1997, pp. 111–118.

Walton, K.: Postnatal Development Under Conditions of Simulated Weightlessness and Space Flight. Brain Research, Brain Research Reviews, vol. 28, nos. 1–2, Nov. 1998, pp. 25–34.

Objectives/Hypothesis
This study makes use of neonatal mammals in order to study the effects of microgravity on the development of the nervous system. Altering gravity during development provides a noninvasive technique by which the influence of motor activity on the development of the nervous system may be studied. Neonates offer a unique and valuable model for experimentation because they possess critical periods of development during which an organism's development is most sensitive to environmental changes.

Approach or Method
The flight experiment consisted of six groups of ten neonates. The groups were housed in six nursing facilities that were contained within three Animal Enclosure Modules (AEM) and flown in shuttle middeck lockers. After landing, the P8 and P15 litters were studied for 14 days. Swimming ability was measured daily by placing the animal in an aquarium and videotaping them for 2 minutes. Strokes that propelled the animal forward were counted and hindlimb angles were measured using the Peak5 Motion Measurement System. The free walking capabilities of the animals were also measured by videotaping their walking and analyzing their step cycle with the Peak System. Finally, surface righting ability was quantified using frame by frame analysis.

Results
Results demonstrate that the development of the nervous system in microgravity is altered for both nonweightbearing activities, such as swimming, as well as for weight-bearing activities. Although microgravity has more of an effect on those functions that require weight-bearing. These results clearly show that environmental factors, such as gravity, affect the development of the nervous system. The data further supports the hypothesis that developing organisms have sets of overlapping critical periods.

STS-76 / Biorack 1

Launch Date 3/22/1996 **Landing Date** 3/31/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Microgravity Effects on Bone Cell Gene Expression

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
M. Hughes-Fulford	University of California, San Francisco

Co-Investigator(s)	Institute
None	

Research Subject(s)
Mus musculus (Mouse cells) osteoblasts

Ground-Based Controls
Delayed Synchronous Control

Key Flight Hardware
Biorack, Passive Thermal Conditioning Unit (PTCU), Ambient Temperature Recorder (ATM)

Selected Publications
Lewis, M.L. and Hughes-Fulford, M.: Regulation of Heat Shock Protein Message in Jurkat Cells Cultured Under Serum-Starved and Gravity-Altered Conditions. Journal of Cellular Biochemistry, vol. 77, no. 1, Apr. 2000, pp. 127–134.

Hatton, J.P.; Pooran, M.; Li, C.F.; Luzzio, C.; and Hughes-Fulford, M.: A Short Pulse of Mechanical Force Induces Gene Expression and Growth in MC3T3-E1 Osteoblasts Via an ERK 1/2 Pathway. Journal of Bone Mineral Research, vol. 18, no. 1, Jan. 2003, pp. 58–66.

Objectives/Hypothesis
Astronauts on long-term missions suffer continuous calcium loss and degradation of skeletal weight-bearing bone mass. Bone loss has been attributed to a decrease in the rate of bone formation, which is possibly caused by both direct and indirect effects of microgravity. Other effects of microgravity include caphalic fluid shifts, loss of muscle mass, space motion sickness, anemia, reduced immune response, and loss of fluid and electrolytes. Systemic or hormonal changes in body chemistry, or the cellular response to a lack of stress due to gravity, could contribute to these conditions. These experiments attempted to ascertain the mechanisms that cause these gravity-related changes at the cellular level.

Approach or Method
About 120,000 to 200,000 cells of an osteoblast cell line, clonally derived from embryonic mouse calvaria, (MC3T3-E1), were plated for flight experiments. These plates were grown in 10% serum containing alpha minimal essential medium (a-MEM), and then the plunger units were held in the shuttle middeck for 17 hours before launch. A modified guanidinium thicyanate method was used to extract the cultured osteoblast cells. Agarose gel electrophoresis was used to identify polymerase chain reaction bands. The bands were then photographed and scanned at 400 dots per inch (dpi) for quantification.

Results
Comparison of data from the GR, 0-G, and 1-G cells leads to several conclusions. First, it showed that early RNA induction, in conjunction with sera activated growth, was not affected by microgravity. Second, the data supported the conclusion that microgravity affected early protein translation through the reduction of early protein synthesis. Third, the 0-G cells were shown to have a significant change in cell structure, which may impede the formation of osteoblasts. Overall, the study highlighted the importance of having a 1-G control on board to examine the effect of microgravity on the living cells. Overall, the experiment supports the hypothesis that microgravity affects osteoblast morphology, gene expression, and prostaglandin content.

NASA 2 / Incubator 3

Launch Date 3/22/1996

Landing Date 9/25/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Expression of Contractile Protein Isoforms in Microgravity

Science Discipline
Developmental Biology
Muscle Physiology

Investigator	Institute
P. Anderson	Duke University

Co-Investigator(s)	Institute
None	

Research Subject(s)
Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
None

Objectives/Hypothesis
Normal function of cardiac and skeletal muscle depends heavily on the regulatory contractile proteins troponin T and troponin I. In both cardiac and skeletal muscle, a change in the expression of isoforms of these two proteins occurs during development. In turn, these isoforms alter the property of muscle myofibrils. Currently, the mechanisms behind the changes in isoform expression are unknown. It is hypothesized that microgravity will alter troponin isoform expression. Using microgravity to alter the expression of these isoforms may help to reveal the systems that alter gene expression and messenger ribonucleic acid (mRNA) processing in cardiac and skeletal muscle on Earth. Understanding the basic processes controlling cardiac and skeletal muscle may prove useful in the treatment of heart disease and other medical problems.

Approach or Method
Analysis concentrated on cardiac troponin T isoform expression. Hearts were isolated from embryos fixed in space and in ground experiments at ages 7, 10, 14, and 16 days. Ribonucleic acid (RNA) was purified from individual embryonic hearts for gene expression analysis by reverse transcriptase-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE) experiments.

Results
Initial experiments using RT-PCR primers based on chicken cardiac troponin T cDNA sequences were unsuccessful. Subsequently, primers based on human cardiac troponin T sequences successfully generated a quail cardiac troponin T cDNA. However, it lacked the 5´ sequence encoding the region of alternative splicing. The inclusion or exclusion of this sequence is the basis of the two cardiac troponin T isoforms expressed in the avian heart, and therefore the missing 5´ sequence is essential to testing the effects of microgravity on isoform expression and cardiac function. Rapid amplification of the cDNA 5´ was used to attempt to generate the region of alternative splicing. This approach was not successful. However, results demonstrate that sufficient RNA can be isolated from individual quail embryonic hearts to successfully perform RT-PCR, supporting future studies of gene regulation in microgravity.

NASA 2 / Incubator 3

Launch Date 3/22/1996

Landing Date 9/25/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Skeletal Development in Long Duration Spaceflight

Science Discipline
Developmental Biology

Investigator / Institute
S.B. Doty — Hospital for Special Surgeries

Co-Investigator(s) / Institute
None

Research Subject(s)
Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
Doty, S.B.: Space Flight and Bone Formation. Materwiss Werksttech, vol. 35, no. 12, Dec. 2004, pp. 951–961.

Doty, S.B.; Vico, L.; Wronski, T.; and Morey-Holton, E.: Use of Animal Models to Study Skeletal Effects of Space Flight. Advances in Space Biology and Medicine, vol. 10, 2005, pp. 209–224.

Objectives/Hypothesis
Previous studies have shown that the mammalian musculoskeletal system is very sensitive to mechanical loading and weight bearing. The objectives of this experiment were: 1) to determine the stage of limb development among quail embryos and hatchlings subjected to spaceflight relative to age-matched controls; 2) to use histochemistry, immunocytochemistry, morphometry, and electron microscopy to further describe any changes in limb development as a result of spaceflight; 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 by a more direct conversion of mesenchymal cells into bone forming cells; and 4) to analyze for mineral content of bone and calcifying cartilage.

Approach or Method
During dissection, measurements of the tibia and femur were carried out with calipers. These measurements were later verified using Faxitron (X-ray) images of the whole limb. Faxitron images were also made of wing samples, and used for measurement of size variations between groups. Wings were dissected into longitudinal and cross sections, embedded in Spurr's resin, and sectioned for light microscopy and image analysis for variation in bone density. Mandibles were prepared as described above for light microscopy. The tibia was analyzed for Ca/P ratios, relative trabecular bone versus compact bone, cartilage content per bone, growth plate size, and cellular morphology of osteoblasts and osteoclasts. The femur was prepared and used for electron microscopy.

Results
No significant change was seen in limb length at E10 (embryonic day 10), E14, and E16. However, light and electron microscopy showed that the degree of bone formation was less in the flight group compared to the controls. No noticeable developmental differences were observed by days E14 and E16. The Ca/P ratio in mandibular bones between controls and flight samples showed a reduced ratio for the flight mandibles at E10, but the difference had disappeared by day E16. Electron microscopy of E7 embryos showed less collagen matrix and less mineral deposited at the cartilage:bone interface of the tibia and femur in the flight samples compared to the controls. Poor fixation of the E14 and E16 embryos inhibited microscopic analysis; however, a subtle reduction in bone development was found in the early developmental periods in microgravity.

NASA 2 / Incubator 3

Launch Date 3/22/1996

Landing Date 9/25/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study

Fecundity of Quail in Spacelab Microgravity

Science Discipline

Developmental Biology

Investigator	***Institute***
B. Wentworth	University of Wisconsin

Co-Investigator(s)	***Institute***
Wentworth, A.L.	University of Wisconsin

Research Subject(s)

Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls

Asynchronous Control

Key Flight Hardware

Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications

None

Objectives/Hypothesis

In all vertebrates, the germinal cells (future sperm and eggs) must migrate from outside the embryo to the gonads, where they proliferate and differentiate to form spermatogonia and oogonia. The objectives of this experiment were to assess the effects of microgravity on arrangement, normal development, and primordial germ cell (PGC) migration in the gonads of Japanese Quail embryos, and to determine the effects of microgravity on normal respiratory function. The fundamental question being addressed was whether complete normal embryogenesis of the Japanese quail can be accomplished in microgravity. Basic knowledge of the role that Earth's gravity and space microgravity have on cell and tissue migration during embryo development differentiation is vital for long-term reproductive studies in space.

Approach or Method

Quail eggs were fertilized and laid at 1-G on Earth, and held at 16°C until placed into the incubator on Mir. Eggs were incubated for 16 days, starting incubation 6 days post lay. Histological, histochemical, and immunochemical analyses were performed on lung and reproductive tissue.

Results

Gross morphological development appeared to indicate that between 33% of the E-16 (fixation age in days) and 59% of the E-3 to E16 flight embryos developed at a normal rate in space microgravity. Gross abnormalities were observed in 13% of the flight embryos. None of the laboratory or synchronous controls showed abnormalities. Approximately 33% of the E-16 embryos showed normal male and female sexual development, as well as the ability to initiate breathing. Although it must be tested, the ability to hatch is presumed from these data. One difficulty that may be encountered in the hatching process of embryos developed in space is that some of the flight embryos had their head oriented in the small end of the egg, which may have impeded their hatching ability. A question that still must be answered is whether the initial development of the embryo (which, in this experiment, occurred on Earth) can proceed successfully in microgravity.

NASA 2 / Incubator 3

Launch Date 3/22/1996

Landing Date 9/25/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Weightlessness on Vestibular Development of Quail

Science Discipline
Developmental Biology
Neurophysiology

Investigator	Institute
B. Fritzsch	Creighton University

Co-Investigator(s)	Institute
Bruce, L.L.	Creighton University

Research Subject(s)
Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
None

Objectives/Hypothesis
Previous studies suggest that birds raised in microgravity may have difficulties orienting themselves in space, up to a point where they may not be viable. This may be due to abnormal formation of connections between the developing ear and the developing brain. This experiment examined the effect of long-term exposure to microgravity on the development of inner ear connections in quail embryos; whether gravity is essential for the normal development of connections between the gravistatic ear and the brain.

Approach or Method
The use of the lipophilic dye DiI was attempted in order to analyze the central projection of vestibular end organs such as the saccule, lagena, and utricle, and to compare this with non-gravity-sensing end organs such as the angular accelerometers of the semicircular canals. Unfortunately, bad breeding success combined with incomplete fixation of the petrous bone led to improper diffusion and nonspecific dye spreading. As an alternative, immunohistochemical analysis was attempted, using an antibody against acteylated tubulin. However, insufficient fixation also prevented this from leading to scientifically relevant data. Thick section analysis of hair cell numbers and degree of maturation was also attempted, but the low yield of ears (a total of three) precluded drawing of scientifically relevant conclusions.

Results
No scientifically relevant results are available.

NASA 2 / Incubator 3

Launch Date 3/22/1996

Landing Date 9/25/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Weightlessness on Quail Eye Development

Science Discipline
Developmental Biology

Investigator
G. Conrad

Institute
Kansas State University

Co-Investigator(s)
None

Institute

Research Subject(s)
Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
Barrett, J.E.; Wells, D.C.; Paulsen, A.Q.; and Conrad, G.W.: Embryonic Quail Eye Development in Microgravity. Journal of Applied Physiology, vol. 88, 2000, pp. 1614–1622.

Barrett, J.E.; Wells, D.C.; and Conrad, G.W.: Pretreatment Methods to Improve Nerve Immunostaining in Corneas From Long-Term Fixed Embryonic Quail Eyes. Journal of Neuroscience Methods, vol. 92, 1999, pp. 161–168.

Objectives/Hypothesis
Because of constant intraocular pressure (IOP) during development, the cornea of the eye becomes differentially bulged outward. The component cells of the cornea secrete an extracellular matrix known as the stroma, made up of collagen fibrils and proteoglycans. Eventually, the cornea becomes transparent, and more densely innervated than any other region on the surface of the body. Corneas from chicken embryos flown in microgravity were shown to have more cellular processes in the outer region of the stroma. The purpose of this experiment was to test if the same abnormalities are observed in quail exposed to microgravity during their development.

Approach or Method
Five eyes were removed from E16 (embryonic day 16) flight birds, 13 eyes from E16 laboratory control birds, and 9 eyes from E16 synchronous control birds. Eyes from postflight control groups were also studied. Eye weight, eye diameter (dorsal/ventral and nasal/temporal), and cornea diameter were measured. Corneal transparency was documented by photographing a fine wire mesh viewed through the corneas. After the corneas were removed, the scleral ossical ring was measured for bone numbers and orientation. Corneal tissue was stained immunohistochemically for observation of nerve growth patterns.

Results
No significant differences were found in the physical parameter measurements between the flight and original control animals. However, corneal diameter differences between the flight and the postflight controls were observed, as well as differences between the postflight control groups. No differences in corneal clarity were observed between the flight and control groups. Overall, no obvious disturbance of eye development occurred from exposure to microgravity.

NASA 2 / Incubator 3

Launch Date 3/22/1996 **Landing Date** 9/25/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Hypogravity's Effect on the Life Cycle of Japanese Quail

Science Discipline
Developmental Biology

Investigator / Institute
P. Hester — Purdue University

Co-Investigator(s) / Institute
None

Research Subject(s)
Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
Orban, J.I.; Piert, S.J.; Guryeva, T.S.; and Hester, P.Y.: Calcium Utilization by Quail Embryos During Activities Preceding Space Flight and During Embrygenesis in Microgravity Aboard the Orbital Space Station Mir. Journal of Gravitational Physiology, vol. 6, 1999, pp. 33–41.

Hester, P.Y.; Orban, J.I.; Sabo, V.; and Boda, K.: Egg Rotation During Avain Embryogenesis. Folia Veterinaria, supl., vol. 42, 1998, pp. S67–S72.

Objectives/Hypothesis
A long-term goal of studying avian development in space is to achieve a complete life cycle of Japanese quail in microgravity. If this can be achieved, quail could serve as a high-protein supplementary food source for astronauts on long-duration missions. Understanding the basic biological processes involved in avian development in microgravity is necessary for achieving this goal.

Approach or Method
Eggshells from flight and control quail embryos (from days 3, 7, 10, 14, and 16 of incubation) were analyzed for shell mineral content. The shells were cleaned of egg contents, rinsed, dried, and ashed. Samples were analyzed for calcium, phosphorous, and magnesium contents using an Inductively Coupled Plasma Atomic Emission Spectrometer (ICPAES). A series of studies were also conducted to determine how activities preceding spaceflight and during launch affect embryonic development. Quail eggs were subjected to the launch dynamics of vibration of hyper-g (centrifugation), vibration, or a combination of the two. Eggs were also tested for survivability in the refrigerator stowage kit which was used to transport the eggs to space, and in varied incubator temperatures with and without launch dynamics.

Results
Flight embryos used significantly less calcium from the shell when compared to both synchronous and laboratory controls. Spaceflight conditions seemed to interfere with the 16-day-old quail embryos' uptake of calcium from the shell. However, because synchronous control embryos at day 16 had calcium levels that did not differ statistically from those in spaceflight, the effect may have been due to the high incubator temperature (39 to 40°C) rather than microgravity exposure. In general, calcium utilization by developing embryos increased with age of incubation, with the largest increase occurring at day 16 of incubation. Launch dynamics had no effect on calcium uptake from the eggshell by developing embryos.

NASA 2 / Incubator 3

Launch Date 3/22/1996

Landing Date 9/25/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study

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

Science Discipline

Developmental Biology
Neurophysiology

Investigator	Institute
T. Shimizu	University of South Florida

Co-Investigator(s)	Institute
None	

Research Subject(s)

Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls

Asynchronous Control

Key Flight Hardware

Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications

Shimizu, T.: Effects of Weightlessness on the Avian Visuo-Vestibular System: Immunohistochemical Analysis. NASA Technical Memorandum 4751, June 1996, pp. 81–82.

Shimizu. T.: Effects of Weightlessness on the Avian Visuo-Vestibular System: Immunohistochemical Analysis. NASA Technical Memorandum 4801, May 1997, pp. 66–67.

Objectives/Hypothesis

The purpose of this experiment was to study the fundamental effects of gravity deprivation on the visuo-vestibular system of Japanese quail (*Coturnix coturnix japonica*). In particular, the distributions of various neurochemicals during development were analyzed by using immunohistochemical techniques. Development of the visual brain was also studied by measuring the volume of the structure called the optic tectum.

Approach or Method

On specified days, eggs were removed from the onboard Mir incubator, and the shells were cracked in a plastic bag filled with a fixative solution of 4% paraformaldehyde. All bags were stored at ambient temperature until return to Earth. Forebrains of four embryos from the flight group, two fixed on embryonic day 14 (E14) and two on day E16, were compared to samples from the ground-based control group. Tissues were frozen, cut in a cryostat, and processed with antibodies against 14 neurochemicals, which are known to exist in the avian and mammalian visuo-vestibular systems.

Results

For the samples that had adequate fixation, the results showed relatively consistent staining patterns for several neurochemicals, such as a calcium-binding protein (CB) and an enzyme for acetylcholine, which are important markers for avian sensory development. Although the positive staining was already visible in the visuo-vestibular system of the E14s and clearly detected in the E16s of the flight group, the number of stained cells appeared to be fewer, and the staining more faint, than stained cells in the control group. The avian optic tectum is the major retinorecipient structure with well-developed laminations. These layers were clearly stained with Nissl staining in all groups. Portions of the optic tectum of some samples were either damaged or detached due to an incomplete fixation, and thus no accurate measurement was possible for these samples. With the collaboration of Dr. Dmitri Lytchakov (Russian Academy of Sciences), a few cases were observed with an abnormal development of the eyes and optic tectum under the microgravity condition. For instance, there were cases with retarded or asymmetrical development of the eyes and tectum. These results indicate that microgravity significantly affects the embryogenesis of the avian brain in terms of morphology and chemistry. The number of subjects and tissue fixation methods need to be improved to confirm these observations.

NASA 2 / Incubator 3

Launch Date 3/22/1996

Landing Date 9/25/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Avian Blood Vessel Formation in Space

Science Discipline
Developmental Biology

Investigator	Institute
P. Lelkes	University of Wisconsin Medical School

Co-Investigator(s)	Institute
Unsworth, B.R.	Marquette University

Research Subject(s)
Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
Henry, M.K.; Unsworth, B.R.; Sychev, V.; Guryeva, T.S.; Dadasheva, O.A.; Piert, S.J.; Lagel, K.E.; Dubrovin, L.C.; Jahns, G.C.; Boda, K.; Sabo, V.; Samet, M.M.; and Lelkes, P.I.: Launch Conditions Might Affect the Formation of Blood Vessels in the Quail Chorioallantoic Membrane. Folia Veterinaria, supl., vol. 42, 1998, pp. S25–S31.

Objectives/Hypothesis
The initiation and maturation of the vasculature is an essential process during embryonic development. Previous studies have shown that birds that, as embryos, were exposed to microgravity during spaceflight, exhibit developmental anomalies that might be related to (or caused by) delayed or improper vascular development. The area vasculosa, the region of blood island formation and forerunner of the chorioallantoic membrane (CAM), was reportedly deformed in some quail embryos that had developed in space. Other studies have shown that specific cellular events that may be key to neovascularization seem to be affected by microgravity. Based on these studies, the hypothesis of this experiment was that the developmental anomalies observed in the past might be related to, or caused by, delayed or improper vascular development.

Approach or Method
Vessels and immunostain endothelial cells were counted in histological preparations using specific antibodies (anti-vWF and QH1). The extent of extracellular matrix protein deposition was assessed by immunohistochemistry and correlated with the degree of vascularization, using computer-based image analysis. In situ hybridization was used to assess the cellular source for extracellular matrix proteins. Vascular morphology was delineated by en face bright field/fluorescence microscopy.

Results
Development of the vasculature in the CAMs in the flight samples, as inferred from vessel density and vessel size, seemed retarded, as compared to the laboratory controls. Both a diminished number of small vessels for the day 14 and 16 embryos, and a delay in the time point of the peak of angiogenic activity around day 10, was observed. However, because only 3 time points (days 7, 10, and 14) were available and the sample size was limited, no definitive conclusions can be drawn. More space-flown samples are needed to come to a definitive conclusion. A postflight control group exposed to mechanical forces of a simulated shuttle launch did not show significant impairment of vascular development.

NASA 2 / Incubator 3

Launch Date 3/22/1996

Landing Date 9/25/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effect of Microgravity on Afferent Innervation

Science Discipline
Developmental Biology
Neurophysiology

Investigator / Institute
C. Fermin — Tulane University

Co-Investigator(s) / Institute
None

Research Subject(s)
Coturnix coturnix (Japonica quail)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Quail Incubator, Fundamental Biology Kit Hardware

Selected Publications
Fermin, C.D.; Lychakov, D.; Campos, A.; Hara, H.; Sondag, E.; Jones, T.; Jones, S.; Taylor, M.; Meza-Ruiz, G.; and Martin, D.S.: Otoconia Biogenesis, Phylogeny, Composition and Functional Attributes. Histology and Histopathology, vol. 13, no. 4, 1998, pp. 1103–1154.

Fermin, C.D.; Martin, D.S.; and Hara, H.: Color Threshold and Ratio of S100b, MAP5, NF68/200, GABA and GAD.I. Distribution in Inner Ear Afferents. Cell Vision, vol. 4, no. 5, 1997, pp. 280–295.

Objectives/Hypothesis
This experiment aimed to determine the effects of microgravity on connectivity of afferent neurons, inner ear hair cells, and vestibular nuclei neurons, as well as characterize changes in innervation patterns of inner ear afferent and efferent neurons.

Approach or Method
Tissues were examined, microdissected, postfixed with 10% formalin, embedded in paraffin, and cut at 10 mm thick. Sections with comparable inner ear structures from embryonic days 3, 7, and 10 were placed on the same slide and stained together for anti-S100b or anti-NF68/200. All incubations were done at room temperature in a humidity chamber. Two negative controls were used: 1) omission or replacement of the primary antibody with an unrelated antiserum, and 2) pre-absorption of antibody when the substrate was available. The hues, saturation, and intensity (HSI) of reaction products was used to calculate the relative concentration of the antibody in the tissue sections.

Results
Due to improper fixation, E3 (embryonic day 3) and E7 embryos did not stain with the neurofilament antibody. In E10 embryos, 77% of vestibular ganglion cells stained positive with anti-neurofilament in synchronous controls, whereas 90% of those counted from selected sections of the flight group were positive. This difference could be due to fixation artifact. The surface area of the vestibular neurons of the synchronous embryos was 202 square micra, whereas the surface area of the flight was 169 square micra. The anti-neurofilament reacted with its antigenic sites of the sensory inner ear structures at E10, but failed to recognize similar sites in E3 and E7 specimens. Positive staining in the E7 embryos was expected. The staining patterns of the neurofilament antibody was not always the same in all three groups when stained together, suggesting that there were marked differences in the mode of fixation and/or initial preparatory conditions for specimens in each group.

STS-77 / NIH.C7

Launch Date 5/19/1996

Landing Date 5/29/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
The Effects of Microgravity on In Vitro Calcification

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
A.L. Boskey — Hospital for Special Surgery

Co-Investigator(s) / Institute
None

Research Subject(s)
Gallus gallus (White leghorn chicken) cells

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Space Tissue Loss-A (STL-A) Module

Selected Publications
None

Objectives/Hypothesis
Biomineralization is regulated by a number of factors during both development and disease. Cell culture can be used to study mineralization and how cells react to it. Various events in endochronal bone development can be studied using micro-mass cell cultures of mesenchymal cells. Micro-mass cultures can therefore be used to test the hypothesis that bone loss in microgravity is due to improper cell maturation instead of defective matrix mineralization.

Approach or Method
Stage 21–24 chick limb bud mesenchymal cells were plated in micro-mass culture. Cultures at 9, 10, and 17 days of age were placed in computer-operated cartridges and flown on STS-77. Cartridges from both the flight and ground-control portions were fixed at 0, 2, 4, 6, 8, or 10 days after launch and at recovery. To determine mineral content, ethanol was used to fix the samples used for Fourier transform infrared microscopy (FTIRM). Aldehyde containing fixative was used for those samples to be examined with electron microscopy (EM).

Results
Younger cells subjected to microgravity appeared to proliferate, however they did not differentiate to form mature cartilage nodules, though cells in the ground control did mature. Older cells in which mineralization had already begun showed a change in Ca:DNA ratio when compared to the ground controls. These findings suggest that younger cells exposed to microgravity do not initiate mineralization properly. Though older cells exposed to microgravity produce less mineral than ground controls, some mineral proliferation does occur. These data demonstrate that mature cells are required to produce initial mineral deposits. Further, these findings suggest that bone loss in microgravity could be due to a combination of increased cell proliferation and decreased new mineral formation.

STS-77 / ARF-01

Launch Date 5/19/1996 **Landing Date** 5/29/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Aquatic Research Facility-1

Science Discipline
Developmental Biology

Investigator	Institute
H. Schatten	University of Wisconsin

Co-Investigator(s)	Institute
Chakrabarti, A.	University of Missouri-Columbia

Research Subject(s)
Sea urchin, painter (*Lytechinus pictus* and Strongylocentrotus purpuratus) egg and embryo

Ground-Based Controls
1 G On-Orbit Centrifuge Control

Key Flight Hardware
Aquatic Research Facility (ARF)

Selected Publications
Schatten, H.; Chakrabarti, A.; Levine, H.G.; and Anderson, K.: Utilization of the Aquatic Research Facility and Fertilization Syringe Unit to Study Sea Urchin Development in Space. Journal of Gravitational Physiology, vol. 6, no. 2, Oct. 1999, pp. 43–53.

Schatten, H.; Chakrabarti, A.; Taylor, M.; Sommer, L.; Levine, H.; Anderson, K.; Runco, M.; and Kemp, R.: Effects of Spaceflight Conditions on Fertilization and Embryogenesis in the Sea Urchin Lytechinus Pictus. Cell Biology International, vol. 23, no. 6, 1999, pp. 407–415.

Objectives/Hypothesis
The objectives of the experiment were: 1) to study the effects of microgravity on cytoskeletal organization and calcium metabolism during fertilization and early development in the sea urchin model system; and 2) to study calcium-dependent sperm incorporation, calcium-triggered cortical granule exocytosis, membrane fusion, and cytoskeletal organization within eggs and embryos fertilized and cultured in space.

Approach or Method
Methods were developed to study the effects of microgravity on early development in sea urchins in the Canadian Space Agency's Aquatic Research Facility (ARF). The ARF payload provided light, temperature control, automated fixation capability, and a 1-G on-orbit centrifuge control. Eggs and embryos of either the sea urchin species *Lytechinus pictus* or *Strongylocentrotus purpuratus* were loaded into Standard Container Assemblies (SCAs) that comprised the experimental aquaria (33 mL volume) contained within the ARF. A newly developed Fertilization Syringe Unit (FSU) was used to achieve in-flight fertilization capability. Fixative solutions were preloaded into fixation blocks maintained adjacent to the SCAs and injected at preselected time points, resulting in final (diluted) concentrations of either 0.5% or 2% glutaraldehyde (depending upon embryonic stage). Calcium and cytoskeletal events were investigated within sea urchin embryos that were cultured in space under both microgravity and 1-g conditions. Embryos were fixed at time-points ranging from 3 hours to 8 days after fertilization. Investigative emphasis was placed on: (1) sperm-induced calcium-dependent exocytosis and cortical granule secretion, (2) membrane fusion of cortical granule and plasma membranes; (3) microfilament polymerization and microvilli elongation; and (5) embryonic development into morula, blastula, gastrula, and pluteus stages.

Results
Light, scanning, and transmission electron microscopy determined that all desired embryonic and cell division stages (16-cell stage, blastula, gastrula, and pluteus) were preserved using the experimental protocols and fixation capability provided by the ARF/FSU system.

Launch Date 6/20/1996 **Landing Date** 7/7/1996

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Development of the Fish Medaka in Microgravity

Science Discipline
Neurophysiology
Developmental Biology

Investigator	Institute
D. Wolgemuth	Columbia University

Co-Investigator(s)	Institute
Phillips, C. R.	Bowdoin College

Research Subject(s)
Oryzias latipes (Medaka fish)

Ground-Based Controls
Nonsynchronous Control, Synchronous Control

Key Flight Hardware
Space Tissue Loss-B (STL-B) Module

Selected Publications
Wolgemuth, D.J.; Herrada, G.; Kiss, S.; Cannon, T.; Forsstrom, C.; Pranger, L.A.; Weismann, W.P.; Pearce, L.; Whalon, B.; and Phillips, C. R.: Vertebrate Development in the Environment of Space: Models, Mechanisms, and Use of Medaka. American Society for Gravitational and Space Biology Bulletin, vol. 10, no. 2, 1997, pp. 97–109.

Objectives/Hypothesis
The goal of these experiments was to determine the effect of microgravity on the early development of the fish Medaka. There were two objectives for this flight series. The primary objective was to assess the effects of microgravity on different stages of development and to ascertain whether the relevant developmental questions can be addressed at the gross morphological level or if the issues involve more subtle questions about regulation at the molecular and cellular levels. The secondary objective was the assessment of the utility of flight hardware with the capabilities to perform embryological studies.

Approach or Method
In this experiment, thirty-six Medaka embryos were used to examine the effects of microgravity on embryogenesis. One advantage of fish Medaka embryos was their clear appearance, because it allowed direct observation by video-microscopy. Video sequences, video tape, and digital images were all utilized to monitor development of the embryos. All of the embryos have been embedded and sectioned. Some embryos were fixed and returned to Earth for further analysis. However, two groups of embryos were returned to Earth unfixed in order to investigate their postflight development and reproductive abilities.

Results
In comparison to the ground controls, the flight group did not display any gross morphological abnormalities. An important observation from the experiment was the overall normal development of the flight animals. Data from the study also indicated that animals exposed to microgravity during embryogenesis retain their ability to reproduce. Furthermore, it was determined that the flight group's progeny was fertile as well.

STS-81 / Biorack 2

Launch Date 1/12/1997 **Landing Date** 1/22/1997

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Effects of Microgravity on Bone Cell Gene Expression (OSTEO)

Science Discipline
Bone and Calcium Physiology

Investigator	Institute
M. Hughes-Fulford	University of California, San Francisco

Co-Investigator(s)	Institute
None	

Research Subject(s)
*Mus musculus (*MC3T3 mouse) osteoblasts

Ground-Based Controls
Delayed Synchronous Control

Key Flight Hardware
Biorack, Passive Thermal Conditioning Unit (PTCU), Ambient Temperature Recorder (ATM)

Selected Publications
Hughes-Fulford, M. and Gilbertson, V.: Osteoblast fibronectin mRNA, protein synthesis and matrix is not changed after long term exposure to microgravity. FASEB J, vol. 13, 1999, pp. S121-128.

Objectives/Hypothesis
Astronauts on long-term missions suffer continuous calcium loss and degradation of skeletal weight-bearing bone mass. Bone loss has been attributed to a decrease in the rate of bone formation, which is possibly caused by both direct and indirect effects of microgravity. Systemic or hormonal changes in body chemistry, or the cellular response to a lack of stress due to gravity, could contribute to this condition. Osteoblastic cells must properly attach to the extracellular matrix (ECM) in order to grow normally. Fibronectin (FN), a cell surface adhesion protein, mediates the attachment of various cell types to other components of the ECM and is a known factor in cell shape and growth regulation. This experiment dealt with the effects of microgravity on the FN message, protein synthesis, and matrix organization, and whether these effects influenced bone formation.

Approach or Method
About 120,000 to 200,000 cells of an osteoblast cell line, clonally derived from embryonic mouse calvaria (MC3T3-E1), were plated for flight experiments. These plates were grown in 10% serum containing alpha minimal essential medium (a-MEM), and then the plunger units were held in the shuttle middeck for 17 hours before launch. A modified guanidinium thicyanate method was used to extract the cultured osteoblast cells. Agarose gel electrophoresis was used to identify polymerase chain reaction bands. The bands were then photographed and scanned at 400 dots per inch (dpi) for quantification.

Results
Analysis of transcriptional and translational control of FN suggests that aberrations in the FN portion of the ECM due to microgravity do not cause cell morphology changes. Further extrapolation indicates that loss of osteoblast growth in spaceflight is not due to changes in FN. However, other alterations may be related to FN binding and receptor-mediated signaling changes in microgravity. Samples fixed for immunofluorescence localization studies showed that microgravity did not affect FN synthesis, that FN protein had been exported out of the cell, and that the cell matrix was organized normally. Furthermore, 1-G on-controls, ground controls, and samples activated in microgravity had indistinguishable regulation of FN. Overall, these data suggest that changes in FN regulation do not directly affect changes in cell shape, bone matrix formation, and loss of bone growth in microgravity.

STS-81 / Biorack 2

Launch Date 1/12/1997 **Landing Date** 1/22/1997

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Microgravity and Signal Transduction Pathways in Sperm

Science Discipline
Developmental Biology

Investigator / Institute
J. Tash — University of Kansas Medical Center

Co-Investigator(s) / Institute
None

Research Subject(s)
Strongelocentrotus pupuratus (Sea urchin) sperm

Ground-Based Controls
Asynchronous Control, Lab Control

Key Flight Hardware
Biorack, Passive Thermal Conditioning Unit (PTCU), Ambient Temperature Recorder (ATM)

Selected Publications
Tash, J.S. and Bracho, G.E.: Microgravity Alters Protein Phosphorylation Changes During Initiation of Sea Urchin Sperm Motility. FASEB Journal, supl., vol. 13, 1999, pp. S43–S54.

Tash, J.S.; Kim, S.; Schuber, M.; Seibt, D.; and Kinsey, W.H.: Fertilization of Sea Urchin Eggs and Sperm Motility are Negatively Impacted Under Low Hypergravitational Forces Significant to Space Flight. Biology of Reproduction, vol. 65, no. 4., Oct. 2001, pp. 1224–1231.

Objectives/Hypothesis
Future long-term space exploration and habitation missions require an understanding of sperm motility and fertilization and how these processes are affected by microgravity. Previous research shows stimulation of sperm motility in microgravity. This effect could be supported by increased phosphorylation of flagellar proteins.

Approach or Method
S. purpuratus sperm was collected and combined to form a uniform mixture. Prior to launch, the sperm was loaded into cassettes contained in type I Biorack containers. At 19 hours mission elapsed time (MET), the cassettes were removed from their 5°C passive thermal conditioning unit (PTCU) and were equilibrated to the 22°C environment. After 50 minutes, the sperm sluice was opened and the sperm was injected into the culture chambers that contained HSW (sperm activating buffer). After either 0, 30, or 60 seconds of activation, the fixation sluice was opened and the fixative injected to terminate motility and phosphorylation. These were then placed in the –20°C freezer for the remainder of the mission. Ground controls were conducted 2 hours after the experiment to account for anomalies in the experiment on the Shuttle orbiter. Other ground controls were adjusted for launch vibration and/or acceleration. After landing, all samples were warmed and centrifuged. Western blotting of nitrocellulose membranes was performed. Sperm motility was videotaped and analyzed using digital motion analysis software.

Results
Analysis of the experimental data demonstrates that microgravity significantly stimulates sperm motility. Further, these results demonstrate a strong correlation between phosphorylation of flagellar phosphoproteins and sperm motility activation. The aconeme-bound phosphoprotein FP130 appeared to be the main target of changes in signal transduction pathways due to microgravity. The temporal pattern of FP130 phosphorylation in microgravity is altered. Because FP130 is also the target of speract (a chemotactic egg peptide), further study into the effect of microgravity on fertilization is necessary.

STS-84 / Biorack 3

Launch Date 5/5/1997 **Landing Date** 5/24/1997

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Microgravity and Signal Transduction Pathways in Sperm

Science Discipline
Endocrinology
Developmental Biology

Investigator / Institute
J. Tash — University of Kansas Medical Center

Co-Investigator(s) / Institute
None

Research Subject(s)
Lytechinus pictus (Sea urchin) sperm

Ground-Based Controls
Delayed Synchronous, Lab Control

Key Flight Hardware
Biorack, Passive Thermal Conditioning Unit (PTCU)

Selected Publications
Tash, J.S. and Bracho, G.E.: Microgravity Alters Protein Phosphorylation Changes During Initiation of Sea Urchin Sperm Motility. FASEB Journal, supl., vol. 13, 1999, pp. S43–S54.

Objectives/Hypothesis
Future long-term space exploration and habitation missions require an understanding of sperm motility and fertilization and how these processes are affected by microgravity. Previous research shows stimulation of sperm motility in microgravity. This effect could be supported by increased phosphorylation of flagellar proteins.

Approach or Method
L. pictus sperm was collected and combined to form a uniform mixture. Prior to launch, the sperm was loaded into cassettes contained in type I Biorack containers. At 19 hours mission elapsed time (MET), the cassettes were removed from their 5°C passive thermal conditioning unit (PTCU) and were equilibrated to the 22°C environment. After 50 minutes, the sperm sluice was opened and the sperm was injected into the culture chambers that contained HSW (sperm activating buffer). After either 0, 30, or 60 seconds of activation, the fixation sluice was opened and the fixative injected to terminate motility and phosphorylation. These were then placed in the –20°C freezer for the remainder of the mission. Ground controls were conducted 2 hours after the experiment to account for anomalies in the experiment on the Shuttle orbiter. Other ground controls were adjusted for launch vibration and/or acceleration. After landing, all samples were warmed and centrifuged. Western blotting of nitrocellulose membranes was performed. Sperm motility was videotaped and analyzed using digital motion analysis software.

Results
Analysis of the experimental data demonstrates that microgravity significantly stimulates sperm motility. Furthermore, these results demonstrate a strong correlation between phosphorylation of flagellar phosphoproteins and sperm motility activation. The aconeme-bound phosphoprotein FP130 (flagellar phosphoprotein) appeared to be the main target of changes in signal transduction pathways due to microgravity. The temporal pattern of FP130 phosphorylation in microgravity is altered. Because FP130 is also the target of speract (a chemotactic egg peptide), further study into the effect of microgravity on fertilization is necessary.

STS-84 / Biorack 3

Launch Date 5/5/1997 **Landing Date** 5/24/1997

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Microgravity Effects on Bone Cell Gene Expression

Science Discipline
Bone and Calcium Physiology

Investigator / Institute
M. Hughes-Fulford — University of California, San Francisco

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (MC3T3 mouse) osteoblasts

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Biorack, Passive Thermal Conditioning Unit (PTCU)

Selected Publications
Hughes-Fulford, M.; Tjandrawinata, R.; Fitzgerald, J.; Gasuad, K.; and Gilbertson, V.: Effect of Microgravity on Osteoblast Growth Activation: Analysis of Transcription, Translation and Morphology in ESA Biorack Flight STS-76. Biorack on Spacehab by Brinkmann and Brillouet; M. Perry, ed. European Space Agency ISBN 92-9092-484-5, 1999.

Objectives/Hypothesis
Astronauts on long-term missions suffer continuous calcium loss and degradation of skeletal weight-bearing bone mass. Bone loss has been attributed to a decrease in the rate of bone formation, which is possibly caused by both direct and indirect effects of microgravity. Systemic or hormonal changes in body chemistry or the cellular response to a lack of stress due to gravity could contribute to this condition. Osteoblastic cells must properly attach to the extracellular matrix (ECM) in order to grow normally. Fibronectin (FN), a cell surface adhesion protein, mediates the attachment of various cell types to other components of the ECM and is a known factor in cell shape and growth regulation. This experiment addressed whether the FN message, protein synthesis, and matrix organization affect bone formation.

Approach or Method
About 120,000 to 200,000 cells of an osteoblast cell line, clonally derived from embryonic mouse calvaria (MC3T3-E1), were plated for flight experiments. These plates were grown in 10% serum containing alpha minimal essential medium (a-MEM), and then the plunger units were held in the shuttle middeck for 17 hours before launch. A modified guanidinium thicyanate method was used to extract the cultured osteoblast cells. Agarose gel electrophoresis was used to identify polymerase chain reaction bands. The bands were then photographed and scanned at 400 dots per inch (dpi) for quantification.

Results
Analysis of transcriptional and translational control of FN suggest that aberrations in the FN portion of the ECM due to microgravity do not cause cell morphology changes. Further extrapolation indicates that loss of osteoblast growth in spaceflight is not due to changes in FN. However, other alterations may be related to FN binding and receptor-mediated signaling changes in microgravity. Samples fixed for immunofluorescence localization studies showed that microgravity did not affect FN synthesis, that FN protein had been exported out of the cell, and that the cell matrix was organized normally. Furthermore, 1-G on-controls, ground controls, and samples activated in microgravity had indistinguishable regulation of FN. Overall, these data suggest that changes in FN regulation do not directly affect changes in cell shape, bone matrix formation, and loss of bone growth in microgravity.

STS-93 / NIH.B1a

Launch Date 7/23/1999

Landing Date 7/27/1999

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Synaptogenesis in Microgravity

Science Discipline
Developmental Biology
Neurophysiology

Investigator / Institute
H. Keshishian — Yale University

Co-Investigator(s) / Institute
None

Research Subject(s)
Drosophila melanogaster (Fruit fly)

Ground-Based Controls
Not relevant due to experiment failure in flight

Key Flight Hardware
Commercial Generic Bioprocessing Apparatus (CGBA)

Selected Publications
None

Objectives/Hypothesis
Gravity plays a key role in the development of many biological systems. This study used the embryos and larvae of *Drosophila melanogaster* (fruit flies) to examine the effects of microgravity on the development and formation of neural connections between specific motorneurons and their target cells in muscle fibers. Specifically, the ability of neurons and their targets to maintain connectivity during development in a microgravity environment were examined.

Approach or Method
Both embryos and larvae at different stages in development were prepared, examined with fluorescent microscopy, and stored at 11°C in order to arrest development. Specimens were housed on petri plates containing a culture medium in the incubator containment module (ICM) and launched on the STS-93 mission. The petri dishes were housed in seven Group Activation Packs (GAPs) within the ICM. At specific time points in flight, each GAP was heated to 25°C to restart development for a set duration. Prior to landing and recovery, the GAPs were cooled to 11°C to halt development. Cell-specific Green Fluorescent Protein (GFP) expression methodologies were used to analyze the data; this allowed targeting errors and subtle defects to be detected in a large sample set.

Results
Hardware malfunction during flight caused the results to be indeterminate. Specifically, significant problems occurred with the Bioserve incubators. This same experiment was reflown on space mission STS-106.

Launch Date 9/8/2000 **Landing Date** 9/19/2000

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Synaptogenesis in Microgravity

Science Discipline
Developmental Biology
Neurophysiology

Investigator / Institute
H. Keshishian — Yale University

Co-Investigator(s) / Institute
None

Research Subject(s)
Drosophila melanogaster (Fruit fly)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Commercial Generic Bioprocessing Apparatus (CGBA)

Selected Publications
None

Objectives/Hypothesis
The effects of spaceflight on nervous system development and neuromuscular synapse formation were tested in a model genetic organism, *Drosophila melanogaster*. Specific objectives were: 1) to utilize the organism's genetic expression of a highly fluorescent mutant form of Green Fluorescent Protein (GFP) to visualize singly identified motoneurons and muscle targets, as well as all aspects of the nervous system development; and 2) to examine the critical developmental times of *Drosophila* embryos and larvae for the effects of microgravity during spaceflight.

Approach or Method
Both embryos and larvae at different stages in development were prepared, examined with fluorescent microscopy, and stored at 11°C in order to arrest development. Specimens were housed on petri plates containing a culture medium in the incubator containment module (ICM) and launched on the STS-93 mission. The petri dishes were housed in seven Group Activation Packs (GAPs) within the ICM. At specific time points in flight, each GAP was heated to 25°C to restart development for a set duration. Prior to landing and recovery, the GAPs were cooled to 11°C to halt development. Cell-specific Green Fluorescent Protein (GFP) expression methodologies were used to analyze the data; this allowed targeting errors and subtle defects to be detected in a large sample set.

Results
Preliminary results indicated that, although the CGBA hardware operated successfully, there were unexpected temperature drifts above the planned temperature in two of the seven containers. While ground tests were completed for comparison to the in-flight samples, final data analysis has not been released.

Launch Date 4/5/2010

Landing Date 4/20/2010

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
The Role of Artificial Gravity in Promoting Tissue-Regenerative Matrix-Integrin-Kinase Cell Signaling

Science Discipline
Cell and Molecular Biology

Investigator	Institute
E. Almeida	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Globus, R.K.	NASA Ames Research Center (ARC)

Research Subject(s)
Mus musculus (Mouse) embryonic stem cells

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cell Culture Module (CCM)

Selected Publications
Almeida, E.A.C.: Spaceflight reduces the tissue regenerative potential of stem cells by decreasing proliferation and increasing early differentiation. ASCB 50th Annual Meeting, San Francisco. Molecular Biology of the Cell. vol. 21, no. 24, 2010, p. 2494/B1069.

Blaber, E.; Finkelstein, H.; Dvorochkin, N.; Globus, R.K.; Burns, B.P.; and Almeida, E.A.C.: Spaceflight reduces the wound healing potential of stem cell derived keratinocytes by decreasing migration. 27th Annual ASGSB Meeting/32nd Annual International Society for Gravitational Physiology Meeting, San Jose, CA. Program and Abstracts. 2011, p. 52.

Objectives/Hypothesis
The work proposed here was centered on the hypothesis that somatic stem cells responsible for tissue regeneration may require the mechanical stimulation of gravity to proliferate and differentiate, and to regenerate tissues at normal rates, and therefore exposure to microgravity may slow down tissue regenerative processes based on adult stem cell progenitors. Results from spaceflight experiments with amphibian (newt) tail regeneration during the Foton M2 and Foton M3 missions suggest that spaceflight significantly retards tissue regeneration by interfering with newt tail blastema stem cell progenitor proliferation and differentiation. These findings suggest that normal long-term homeostatic tissue regenerative processes in mammals may also be sensitive to the absence of gravity-generated forces in space, and that this may cause tissue degeneration. This study sought to gather further evidence about the role of gravity-generated forces in promoting tissue regeneration and the molecular mechanisms by which gravity in stem cells is translated into proliferation and differentiation.

Approach or Method
The Cell Culture Module (CCM) hardware was used to differentiate mouse embryonic stem cells in spaceflight and in ground controls, using an experimental model based on the formation of embryoid bodies containing various differentiated tissue lineages and cell types. Three bioreactors were recovered with cells fixed during spaceflight for messenger ribonucleic acid (mRNA)/gene expression analysis, and three maintained live cells for cell biology assays conducted postflight.

Results
Experimental analysis showed that spaceflight cell cultures fixed in space did not express tissue lineage markers characteristic of differentiation and retained elevated stem cell markers, versus ground-control cultures that expressed tissue markers indicative of normally differentiated embryoid bodies and lacked stem cell markers. In ground controls, a variety of gene markers of tissue differentiation quantified using real-time quantitative polymerase chain reaction (qPCR) appeared normally as differentiated embryoid bodies. These markers included specific genes for endoderm, mesoderm, ectoderm, and specific cell types within these tissues. Additionally, the disappearance of stem cell markers was observed in these controls. However in spaceflight samples, nearly of 80% of the tissue lineage markers characteristic of embryoid body differentiation were not detected, and stem cell markers remained elevated. Measurements of cell number, DNA content, and glucose consumption also revealed a slight decrease in initial cell proliferation in space, but no changes in cell viability or apoptosis. Finally, live cells recovered in adhesion outgrowth assays from postflight embryoid bodies in spaceflight bioreactors, when cultured, showed greater potential for de novo differentiation of contractile cardiomyocyte colonies, suggesting greater numbers of undifferentiated progenitors were present. The results suggest that spaceflight inhibited normal stem cell differentiation and maintained the stem cell potential of the differentiating cultures, and support the hypothesis that the tissue regenerative potential of stem cells may be decreased during spaceflight. Further gene expression analysis and cell biology studies are currently underway.

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Protein Expression in Salivary Glands: Effects of Extended Spaceflight

Science Discipline
Cell and Molecular Biology

Investigator	Institute
M.I. Mednieks	University of Connecticut Health Center

Co-Investigator(s)	Institute
Hand, A.R.	University of Connecticut Health Center, School of Dental Medicine

Research Subject(s)
Mus musculus (B6.SJL-Ptprca Pepcb/BoyJ mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Mednieks, M.; Khatri, A.; Rubenstein, R.; Burleson, J.; and Hand, A.R.: Microgravity alters the expression of salivary proteins. Oral Health Dental Management, vol. 13, no. 2, 2014, pp. 211-216.

Objectives/Hypothesis
Physiological responses to environmental stress such as travel in space alter cellular signal processing of hormone action, and are reflected in saliva. These secretory proteins have been identified using biochemical tests and histologic analyses. The earlier studies were carried out using rat tissues whereas in the present missions mice were used.

The initial aim was to determine if the same responses occur in both animal models and to test the hypothesis that regulation of specific secretory protein expression is altered by spaceflight. The overall objective was to design biochemical tests to measure physiologic responses using biomarkers in saliva as indices. The ease of collecting saliva instead of blood and urine during spaceflight makes it possible to measure hormone changes experienced by Astronauts and Cosmonauts in microgravity.

Approach or Method
Salivary glands from flight animals and ground-based controls were prepared for biochemical (Western and Northern blotting and Microarray analyses) and morphological (light and electron microscopy and immunohistochemistry and immunocytochemistry) experiments to determine changes in ultrastructure, and in the expression and intracellular localization of proteins that are markers for particular cell types in the salivary glands and are indicators of specific cell functions. Several of the proteins have been previously shown to respond to a variety of environmental stimuli, including spaceflight, specifically, the type II regulatory subunit of cyclic AMP-dependent protein kinase (PKA RII) and alpha amylase, and others. The total number of mice in this experiment was 16; 8 flight mice housed in Animal Enclosure Modules (AEMs), and 8 control mice housed in a ground-based AEM.

Results
Protein expression is considerably altered during spaceflight and demonstrated by electrophoresis, Western Blotting and immunocytochemistry. Significant changes were seen in the major salivary secretory proteins. In the parotid gland, PKA RII, amylase, and PRP showed a decrease, PSP was unchanged, and DCPP showed an increase in the flight animals compared to controls. A cellular enzyme (PDE) was decreased in flight animals. In the submandibular gland, NGF was significantly increased, whereas EGF was decreased, although not significantly ($p = 0.08$). In the sublingual gland, mucin (MUC19) and PSP were significantly increased, whereas PKA RII and DCPP were unchanged. These results indicate that the responses to microgravity are specific to the gland, cell, and protein. Proteins from heart muscle showed variability in the banding patterns of individual animals, as well as differences between flight and controls. The ultrastructure of salivary glands is not significantly altered during spaceflight. Preliminary findings from STS-133 show similar results.

Launch Date 2/24/2011 **Landing Date** 3/9/2011

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
Protein Expression in Salivary Glands: Effects of Extended Spaceflight

Science Discipline
Cell and Molecular Biology

Investigator / Institute
M.I. Mednieks — University of Connecticut Health Center

Co-Investigator(s) / Institute
Hand, A.R. — University of Connecticut Health Center, School of Dental Medicine

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Simulated Flight Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Mednieks, M.; Khatri, A.; Rubenstein, R.; Burleson, J.; and Hand, A.R.: Microgravity alters the expression of salivary proteins. Oral Health Dental Management, vol. 13, no. 2, 2014, pp. 211-216.

Objectives/Hypothesis
The overall objectives were the same for all three missions (STS-131, -133, and -135). Namely that microgravity alters hormone action, and the changes play out in salivary glands and are reflected in saliva which, in turn, can be an accessible biofluid for biochemical tests. This study was designed to measure responses at various times after landing to determine if the observed changes return to normal upon return to Earth. This information can be used to design a test kit to measure responses using saliva. The ease of collecting saliva instead of blood and urine during spaceflight make it possible to measure hormone changes experienced by astronauts and cosmonauts at microgravity.

Approach or Method
Salivary glands from flight animals and ground-based controls were prepared for biochemical (Western and Northern blotting and Microarray analyses) and morphological (light and electron microscopy and immunohistochemistry and immunocytochemistry) experiments to determine changes in ultrastructure and in the expression and intracellular localization of proteins that are markers for particular cell types in the salivary glands and/or are indicators of specific cell functions. Several of the proteins have been previously shown to respond to a variety of environmental stimuli including spaceflight.

Results
The initial findings from STS-133 agree with those of STS-131. A greater number of animals were tested in STS-133 and the results are consistent between flights, increasing their validity and the significance of the statistical analyses. In addition, it was determined that on day 5 of recovery several of the secretory protein values that had been affected by spaceflight returned to those of earth-based controls. Data show the decreased levels of RII and IgA 1 day after recovery (R+1), and the return of these values to control levels by day 5 (R+5). For immunoglobulin A (IgA), there was a significant decrease in the flight (F) animals, $p < 0.025$, but on day R+5 the difference between the F and the AEM controls (C2) was not significant ($p = 0.2$). For RII, the decrease was even more dramatic with a $p < 0.001$ and the return to control values complete at R+5. Microarray analyses of ribonucleic acid (RNA) of the flight and comparable control samples to identify other proteins in salivary glands that are affected by spaceflight are in progress. There are multiple secretory proteins that have been shown to be markers of specific physiologic functions. Testing of salivary proteins provides a valuable ongoing clinical picture of individuals traveling in space.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study
The Role of Artificial Gravity in Promoting Tissue-Regenerative Matrix-Integrin-Kinase Cell Signaling

Science Discipline
Cell and Molecular Biology

Investigator	Institute
E. Almeida	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Globus, R.K.	NASA Ames Research Center (ARC)

Research Subject(s)
Mus musculus (Mouse) embryonic stem cells

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cell Culture Module (CCM)

Selected Publications
Almeida, E.A.C.: Spaceflight reduces the tissue regenerative potential of stem cells by decreasing proliferation and increasing early differentiation. ASCB 50th Annual Meeting, San Francisco. Molecular Biology of the Cell. vol. 21, no. 24, 2010, p. 2494/B1069.

Blaber, E.; Finkelstein, H.; Dvorochkin, N.; Globus, R.K.; Burns, B.P.; and Almeida, E.A.C.: Spaceflight reduces the wound healing potential of stem cell derived keratinocytes by decreasing migration. 27th Annual ASGSB Meeting/32nd Annual International Society for Gravitational Physiology Meeting, San Jose, CA. Program and Abstracts. 2011, p. 52.

Objectives/Hypothesis
As with STL, the experiments conducted on STL2 were centered on the specific hypothesis that somatic stem cells responsible for tissue regeneration may require the mechanical stimulation of gravity to proliferate and differentiate, and to regenerate tissues at normal rates, and that therefore, exposure to microgravity may slow down tissue regenerative processes based on adult stem cell progenitors. In STL significant microgravity effects were found on the ability of stem cells to differentiate using a stem cell to embryoid body model of tissue regeneration. STL 2 sought to complement previous studies by differentiation of a single cell type, keratinocytes, from mouse embryonic stem cells, and to replicate results on differentiation markers and tissue regeneration.

Approach or Method
The Cell Culture Module (CCM) hardware was used to differentiate mouse embryonic stem cells in spaceflight and in ground controls, using an experimental model based on the differentiation of a single tissue cell type. As in STL, three bioreactors were recovered with cells fixed during spaceflight for ribonucleic acid (RNA) for gene expression analysis, and three maintained live cells for cell biology assays conducted postflight.

Results
Preliminary experimental analysis shows that keratinocytes differentiated in spaceflight expressing various keratins in the cytoskeleton, however cells in cultures recovered from flight did not migrate normally, as required in keratinocyte-based wound closure. Gene expression is currently being analyzed to determine if markers characteristic of differentiation appear normally. The results so far suggest that spaceflight inhibited the migratory ability of microgravity-differentiated cultures, and also support the hypothesis that the tissue regenerative potential of stem cells may be decreased during spaceflight.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

Title of Study

Protein Expression in Salivary Glands: Effects of Extended Spaceflight

Science Discipline

Cell and Molecular Biology

Investigator / ***Institute***

M.I. Mednieks — University of Connecticut Health Center

Co-Investigator(s) / ***Institute***

Hand, A.R. — University of Connecticut Health Center, School of Dental Medicine

Research Subject(s)

Mus musculus (Mouse)

Ground-Based Controls

Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware

Animal Enclosure Module (AEM)

Selected Publications

Mednieks, M.; Khatri, A.; Rubenstein, R.; Burleson, J.; and Hand, A.R.: Microgravity alters the expression of salivary proteins. Oral Health Dental Management, vol. 13, no. 2, 2014, pp. 211-216.

Objectives/Hypothesis

Using Biochemical tests and histologic analyses, it has been determined that specific salivary proteins were affected in salivary gland tissues and cells in mice flown on the space shuttles. Secretory proteins have specific responses to microgravity: some increase, some decrease, others remain unchanged. A ribonucleic acid (RNA) microarray analysis to determine which proteins are altered by travel in space would indicate the nature of the observed biochemical and histological analyses. Ultimately, mouse and human gene sequence homology analysis will contribute to precise identification of protein markers that can be utilized in the design of a saliva testing device.

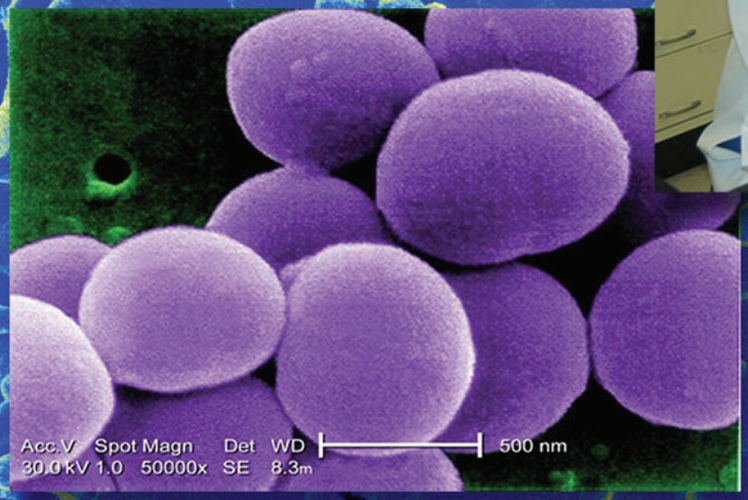
Approach or Method

Seven flight and eight control mouse parotid gland samples were tested by microarray analysis. The service included extraction and isolation of RNA applying quality control of RNA samples using Agilent BioAnalyzer and NanoDrop Mouse OneArray Hybridization Service: Single Color Labeling/3 Replications Hybridizations/Scanning/Signal Extractions and Normalization. Additionally, Western blotting and morphological (light and electron microscopy and immunohistochemistry and immunocytochemistry) experiments are in progress to determine changes in ultrastructure and in the expression and intracellular localization of proteins that are markers for particular cell types in the salivary glands and/or are indicators of specific cell functions. The number of animals in this mission were 37: 7 flight Animal Enclosure Module (AEM), 15 control AEM, and 15 vivarium controls.

Results

Microarray analyses of parotid gland samples from mice flown on STS-135 and ground control mice showed changes in the expression of genes associated with the cyclic AMP signaling pathway in the flight animals. The expression of type II PKA regulatory subunit (RII), cyclic AMP specific phosphodiesterase 4A, and adrenergic receptor beta 2A was decreased, while that of adenylyl cyclase 3, A-kinase anchoring protein 13, and cyclic AMP-specific phosphodiesterase 4D was increased. Changes in protein expression in flight animals, as determined by electron microscopic immunogold labeling, were similar to those observed for STS-131. The morphology of the glands from flight animals was similar to controls, however an increase in endocytic activity of duct cells was observed.

Immunology



Immunology Introduction

Stephen Keith Chapes, Ph.D., M.P.H., Kansas State University

NASA's official "Immune" program experiments began with Skylab, the first human-populated space station, in 1973. It coincided with important discoveries regarding the complexities of the immune response, which grew in logarithmic fashion from 1975–2011, as did our understanding of the function of the immune system in space and reduced gravity. In 1975, immunologists were just unraveling the complexity of immunoglobulin gene structure and regulation [Rabbitts and Forster, 1978], were about ready to describe the making of hybridomas [Kohler et al., 1976; Kohler and Milstein, 1976], and were still a few years away from describing the structure of the T cell antigen receptor [Haskins et al., 1983]. All of these concepts became important for the development of the experiments done at the end of the shuttle era. In 1975, the results of the first human space station-flight experiments were being revealed [Kimzey et al., 1975; Kimzey et al., 1976] and the international collaborations between the former USSR and the USA were beginning. Although the public's attention was captured by the Apollo-Soyuz docking in July 1975, in November, Bion 3 flew rats in a 20-day biosatellite flight [Mandel and Balish, 1977] [Cosmos 782 / Bion 3, p. 205]. This flight was significant in that it was the formal beginning of the use of rodents by NASA scientists to understand how the immune system changes in response to spaceflight. Mandel and Balish (1977) saw considerable variation in lymphocyte responses, and these studies reflected the continued issue present even through the shuttle program-reproducible experimental conditions. Unlike experiments done on Earth where laboratory conditions can be kept consistent, every spaceflight has different conditions. Even the Immune 1 and 2 flights [STS-60 / Immune.1, p. 215] [STS-63 / Immune.2, p. 217], which were closely parallel to one another, had significant methodological differences because of flight-specific conditions [Chapes et al., 1999b]. Therefore, it has taken longer to sort out many of the observations we have made about the immune system. Nevertheless, significant progress has been made.

Bion 3 was the first of several USSR–USA collaborative flights on Cosmos biosatellites to share rodent samples. With Bion 3 rodent samples, Lisbeth Kraft was able to show that lymphoid tissues did respond to spaceflight [Kraft, 1978] [Cosmos 782 / Bion 3, p. 206]. This was a common finding among the rodent flights that followed on subsequent Cosmos biosatellites and shuttle flights. Indications of changes in hematopoiesis were seen during the flights of Bion 8 [Sonnenfeld et al., 1990] and Bion 9 [Sonnenfeld et al., 1992] [Cosmos 2044 / Bion 9, p. 208]. These flights were significant because 1) flight rats were directly compared to hindlimb-unloaded rats on the ground, and 2) for the significant collaborative effort and logistical planning needed to recover the tissues. The novel concept of tissue-specific immune responses arose from these studies [Nash and Mastro, 1992] and has become well ingrained in the immunological literature since then [Kunkel and Butcher, 2002]. Bion 10, at the end of 1992, continued a long line of Soviet flights [Lesnyak et al., 1993] that included Rhesus monkeys as the subjects of the research. Results showed that bone marrow responses to granulocyte-macrophage colony stimulating factor (GM-CSF) were again affected by spaceflight, thus the impact of spaceflight on hematopoiesis in primates elevated the significance of this finding as a human health concern [Sonnenfeld, 2003] [Cosmos 2229 / Bion 10, p. 212].

During the 1990s we began to investigate the mechanisms by which spaceflight might affect processes important for immunological health, such as lymphocyte proliferation [Cogoli, 1981]. Spaceflights Immune 1–3 were designed to investigate whether Interleukin 2 [Chapes et al., 1999b] or insulin-like growth factor 1 [Chapes et al., 1999a] could impact the effects of spaceflight on

the immune system. Miller began finding changes in cytokine secretion postflight in the PARE.02 mission in 1993 [Miller et al., 1995] [STS-54 / PARE.02, p. 213], and the Biorack 3 experiment in 1997 suggested that some T cell markers of activation are directly impacted by spaceflight, and cytoskeletal disruption may affect T cell activation [Hashemi et al., 1999] [STS-84 / Biorack 3, p. 219]. This was a breakthrough experiment because the cellular responses in microgravity were directly compared to control cells also in the space station on a 1-g centrifuge. Understanding T cell as well as cytokine regulation and balance is important to normal health. Disruption of the cytokine balance can change resistance to opportunistic microbes [Flynn et al., 1995; Liang et al., 2006; Pie et al., 1997; Rudner et al., 2007; Song et al., 2005; Yang et al., 1996] or change susceptibility to autoimmune disease [Hori et al., 2003; Moudgil and Choubey, 2011]. The hope is if we can understand these regulatory systems, maybe we can combat autoimmune diseases [Haque, 2014].

The SLS-1 spaceflight on STS-40 in June 1991 was a landmark event for gravitational biology. This dedicated spaceflight focused on multiple biological systems including several immunological and hematological systems. Changes in peripheral white blood cells [Allebban et al., 1994] and NK cells [Konstantinova et al., 1995] were noteworthy, as was the initial reassurance that antioxidant mechanisms were not significantly impaired [Popova et al., 1995] [STS-40 / SLS-1, p. 210]. However, the dissection of rats during the SLS-2 in October 1993 generated even more enthusiasm because it allowed for the discrimination of spaceflight effects from landing effects on immune cells in the peripheral blood [Ichiki et al., 1996] [STS-58 / SLS-2, p. 128]. In addition, T cell responses were depressed when collected during spaceflight [Lesnyak et al., 1996], which indicated that T cell impairment was not a landing effect. The NIH.R1 flight, in November 1994, was remarkable because pregnant rats were flown and pups were born within 24 to 48 hours following return to Earth [STS-66 / NIH. R1, p. 154]. This was the first direct comparison of hematopoiesis between dams and pups from a spaceflight and reinforced the observations that spaceflight affected hematopoiesis [Sonnenfeld et al., 1998].

The new millennium brought us a powerful tool: the ability to examine genome-wide changes in host responses. The Leukin experiment took advantage of this technology and was taken to the International Space Station on the Soyuz TMA-9 in September 2006 [21P / Leukin, p. 221]. The results indicated that T cell transcriptional activation was inhibited compared to 1-g controls. In particular, Rel/NF-kB, CREB, and SRF transcripts were significantly lower in flight samples compared to onboard 1-g centrifuge controls [Chang et al., 2012]. The FIT experiment on STS-121 in 2006 used the power of the Drosophila molecular-genetic tool kit to examine both host responses and development [Marcu et al., 2011] [STS-121 / FIT, p. 220]. In the absence of the complicating factors of acquired immunity, the investigators found depressions both in the levels of innate immune phagocytosis and in the transcript levels of genes important for development. The BoneMac experiment on STS-126 in 2008 also used transcriptional arrays to look at the impact of spaceflight on the differentiation and growth of bone marrow macrophages [STS-126 / BONEMAC, p. 222]. Indeed, there were significant changes in bone marrow cells in response to spaceflight at both the cell surface level and at the transcript level [Ortega et al., 2012].

The end of the shuttle era, with flights STS-131, 133, and 135, came just as some of the most exciting immunological experiments were being performed. The Mouse Immune 1 and 2 flights examined long-standing questions about the impact of space on memory T cell responses [STS-131 / Mouse Immune 1, p. 223]

and how spaceflight affects the host's ability to respond to an infection [STS-133 / Mouse Immune 2, p. 224]. Spaceflight impacts the host's ability to respond to a respiratory virus (Respiratory Syncytial Virus) infection by down-regulating cytokines that would inhibit viral growth (e.g., IFNα/β) and increase immunoregulatory IL-17. On STS-135, it was discovered that spaceflight had a greater impact on CD4+ T cells than CD8+ T cells [STS-135, p. 227]. Monocytes tended to make more TNFα, made significantly more IL-6, and CD86+ cells were diminished [Hwang et al., 2011]. These studies are also relevant to host health. As stated previously, the subpopulations of T cells controls the activation of the immune system.

From 1975–2011 we learned that we could perform complex measurements of spaceflight experimental samples; we also began an era where manipulation of the samples in space was possible. We have used model organisms from various kingdoms (from dipterans to mammals), and in many cases the physiological experiments, e.g., Cosmos 1887 [Sonnenfeld et al., 1990] and Cosmos 2044 [Sonnenfeld et al., 1992] were complemented and supported by cell biology experiments [Ortega et al., 2012].

Translational Application

It is clear that spaceflight affects the differentiation of cells from the bone marrow, and the phenotypic changes we see in immune cell populations may translate into impaired host defenses or the activation of inappropriate immune responses that lead to autoimmune dysfunction. The aging population of the United States foreshadows the probability of many Americans being bedridden and "unloaded" much like our space experimental subjects. Therefore, the NASA immune experiments will directly translate to human health.

List of referenced flight experiments:

Cosmos 782 / Bion 3, A. Mandel, Effect of Spaceflight on Cell-Mediated Immunity

Cosmos 782 / Bion 3, L. Kraft, Results of Histological Examination of Inguinal Lymph Nodes

Cosmos 2044 / Bion 9, G. Sonnenfeld, Effect of Spaceflight on Level and Function of Immune Cells: I. Immunology Studies

Cosmos 2229 / Bion 10, G. Sonnenfeld, Rhesus Monkey Immunology Study

STS-40 / SLS-1, I.A. Popova, Lipid Peroxidation and Antioxidant Defense System

STS-54 / PARE.02, E. Miller, Influence of Spaceflight on the Production of Interleukin-3 and Interleukin-6 by Rat Spleen and Thymus Cells

STS-58 / SLS-2, A.T. Ichiki, Regulation of Erythropoiesis During Spaceflight

STS-60 / Immune.1, R. Zimmermann, Ability of Polyethylene Glycol Interleukin-2 (PEG-IL2) to Counteract the Effect of Spaceflight on the Rat Immune System

STS-63 / Immune.2, R. Zimmerman, Confirmation of Ability of Polyethylene Gloycol Intereuken-2 (PEG-IL2) to Counteract the Effect of Spaceflight on the Rat Immune System

STS-66 / NIH.R1, G. Sonnenfeld, Effect of Spaceflight on Development of Immune Responses

STS-84 / Biorack 3, C. Sams, Effect of Microgravity on Lymphocyte Activation: Cell-Cell Interaction and Signaling

STS-121 / FIT, S. Bhattacharya, Fungal Pathogenesis, Tumorigenesis, and Effects on Host Immunity in Space (FIT)

STS-126 / BONEMAC, S. Chapes, Differentiation of Bone Marrow Macrophages in Space (BONEMAC)

STS-131 / Mouse Immune 1, M. Hughes-Fulford, Antigen-Specific CD4+T Cell Priming and Memory Response During Spaceflight

STS-133 / Mouse Immune 2, R. Garofalo, Mouse Immunology-2

STS-135, C. Sams, Examination of Splenic and Thymic Immune Function in Mice

21P / Leukin, A. Cogoli, Role for the Interleukin-2 Receptor in Signal Transduction (Leukin)

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Chang, T. T.; Walther, I.; Li, C. F.; Boonyaratanakornkit, J.; Galleri, G.; Meloni, M. A.; Pippia, P.; Cogoli, A.; and Hughes-Fulford, M.: The Rel/NF- B pathway and transcription of immediate early genes in T cell activation are inhibited by microgravity. Journal of Leukocyte Biology, vol. 92, no. 6, 2012, pp. 1133-1145.

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Cosmos 782 / Bion 3

Launch Date 11/25/1975

Landing Date 12/15/1975

Physiological/Science Discipline: IMMUNOLOGY

Title of Study

Effect of Spaceflight on Cell-Mediated Immunity

Science Discipline

Immunology

Investigator	***Institute***
A.D. Mandel	NASA Ames Research Center (ARC)

Co-Investigator(s)	***Institute***
Balish, E.	University of Wisconsin

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent Cages

Selected Publications

Lesnyak, A.T.; Sonnenfeld, G.; Rykova, M.P.; Meshkov, D.O.; Mastro, A.; and Konstantinova, I.: Immune Changes in Test Animals During Spaceflight. Journal of Leukocyte Biology, vol. 54, no. 3, 1993, pp. 214–226.

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Mandel, A.D.; and Balish, E.: Effect of Spaceflight on Cell-Mediated Immunity. Aviation, Space, and Environmental Medicine, vol. 48, 1977, pp. 1051–1057.

Objectives/Hypothesis

It has been demonstrated that cell-mediated immune reactions in mammals are thymus-dependent, and that thymus-derived or thymus-dependent lymphocytes (T-cells) are responsible for the reactions of cellular immunity. Rats flown in Cosmos 605 have shown changes in the size of thymus and spleen, suggesting that spaceflight might have a noticeable effect on the cellular aspect of immune responsiveness. This study examined the effect of spaceflight on cell-mediated immunity by examining rats infected with Listeria monocytogen.

Approach or Method

Both flight and control groups were immunized with 106 formalin-killed Listeria suspended in Freund's Complete Adjuvant 5 days prior to flight. Following recovery, lymphocyte cultures were prepared from spleens of all rats, and cultured in vitro in the presence of Listeria antigens, phytohemagglutinin, concanavalin A, and purified protein derivative (PPD), and measured for their uptake of 3H-thymidine. The uptake of 3H-thymidine by the lymphocytes of immunized flight rats in the presence of specific antigen was compared with those of immunized ground controls. A Student's T test was used to assess the significance of the data.

Results

The lymphocytes of all rats gave a blastogenic response to phytohemagglutinin and concanavalin A. Although individual rats varied considerably, all flight and immunized control rats gave a blastogenic response to the Listeria antigens and PPD. With several mitogens the lymphocytes of flight rats showed a significantly increased response over the controls. If indeed the increased immune response to PPD in space can be confirmed, it suggests a practical application of the space environment for immunotherapy of tumors. Thus, the data do not support a hypothesis of detrimental effect of spaceflight on cell-mediated immunity, even suggesting an opposite effect.

Cosmos 782 / Bion 3

Launch Date 11/25/1975

Landing Date 12/15/1975

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Results of Histological Examination of Inguinal Lymph Nodes

Science Discipline
Immunology

Investigator / Institute
L. Kraft — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Kraft, L.M.: Results of Histological Examination of Inguinal Lymph Nodes: Supplementary Report. Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 782. S.N. Rosenzweig and K.A. Souza, eds., NASA TM-78525, 1978, pp. 227–231.

Objectives/Hypothesis
This study examined the effect of spaceflight on cell-mediated immunity by examining the inguinal lymph nodes of rats infected with a formalin killed culture of Listeria monocytogenes suspended in Complete Freund's Adjuvant prior to flight. Supplementing a larger study, this experiment attempted to obtain more information on how weightlessness affects cells responsible for the development of a specific immunity.

Approach or Method
Inguinal lymph nodes of all rats were received in neutral 10% formalin. They were processed for paraffin embedding, and sections 6 μm thick were stained with hematoxylin and eosin, pyronin-methyl green, and picrofuchsin. Six of the rats were from the vivarium control group (normal laboratory conditions), six were of the synchronous control group (simulated flight), and six were of the flight group.

Results
The most outstanding differences between the flight and ground-control groups were: 1) marked, widespread depletion of lymphocytes resulting in much larger pale zones than in controls, and 2) the occurrence of numerous foci of pyknotic and necrotic cells together with variable amounts of dust-like debris. The striking increase in the number of necrotic cells, together with evidence of phagocytosis of some cellular debris, is thought to be due to the multitude of stressful conditions of prolonged spaceflight. Precisely how the increased destruction of lymphoid cells fits with other experimental results remains an open question for future studies.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Effect of Spaceflight on Levels and Function of Immune Cells

Science Discipline
Immunology

Investigator	Institute
A.D. Mandel	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Sonnenfeld, G.	University of Louisville
Taylor, G.R.	NASA Johnson Space Center (JSC)
Fuchs, B.B.	Institute of Human Morphology

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS
Cosmos 1887 Russian Hardware Suite

Selected Publications
Sonnenfeld, G.; Mandel, A.D.; Konstantinova, I.V.; Taylor, G.R.; Berry, W.D.; Wellhausen, S.R.; Lesnyak, A.T.; and Fuchs, B.B.: Effect of Spaceflight on Levels and Activity of Immune Cells. Aviation, Space, and Environmental Medicine, vol. 61, 1990, pp. 648–653.

Konstantinova, I.V.; Sonnenfeld, G.; Schaffar, L.; and Mastro, A.: Results of Immunological Experiments Aboard the Cosmos Biosatellites and Problems in Space Immunology. Physiologist, supl., vol. 35, no. 1, 1992, pp. S220–S221.

Objectives/Hypothesis
The purpose of this experiment was to begin a systematic attempt to define the range of immunological parameters affected by spaceflight. Two different areas were chosen for study. First, the effect of spaceflight on the ability of cells to respond to an external immunological stimulus was determined. Second, the effects of spaceflight on the expression of cell surface markers of spleen and bone marrow cells were examined. These markers represent various immunologically important cell populations; an alteration in the percentage of cells expressing the markers could result in an alteration of the immunological function.

Approach or Method
In the first experiment, rat bone marrow cells were examined in Moscow for their response to colony stimulating factor-M. In the second experiment, rat spleen and bone marrow cells were stained with a variety of antibodies directed against cell surface antigenic markers. These cells were analyzed using an autofluorograph interfaced with a computer system.

Results
The results of the studies indicate that bone marrow cells from flight rats showed a lower response to colony stimulating factor than did bone marrow from control rats. There was a higher percentage of spleen cells from flight rats staining positively for pan-T-cell, suppressor-T-cell, and innate interleukin-2-receptor antigens than from control animals. In addition, a higher percentage of cells that appeared to be part of the myelogenous population of bone marrow cells from flight rats stained positively for surface immunoglobulin than did equivalent cells from control rats. This experiment presents additional data to indicate that spaceflight, even of a relatively short duration, affects certain parameters of the immune system. Through this study, it was possible to demonstrate some specific cell populations that appear to be affected by spaceflight.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Effect of Spaceflight on Level and Function of Immune Cells:
I. Immunology Studies

Science Discipline
Immunology

Investigator	Institute
G. Sonnenfeld	University of Louisville

Co-Investigator(s)	Institute
Mandel, A.D	NASA Ames Research Center (ARC)
Konstantinova, I.V.	Institute of Biomedical Problems
Berry, W.D.	University of Louisville

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Rykova, M.P.; Sonnenfeld, G.; Lesnyak, A.T.; Taylor, G.R.; Meshkov, D.O.; Mandel, A.D.; Medvedev, A.E.; Berry, W.D.; Fuchs, B.B.; and Konstantinova, I.V.: Effect of Spaceflight on Natural Killer Cell Activity. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S196–S200.

Sonnenfeld, G.; Mandel, A.D.; Konstantinova, I.V.; Berry, W.D.; Taylor, G.R.; Lesnyak, A.T.; Fuchs, B.B.; and Rakhmilevich, A.L.: Effect of Spaceflight on Immune Responses: Bone Marrow Cell Response to Colony Stimulating Factor and Leukocyte Subsets. Physiologist, supl., vol. 35, no. 1, 1992, pp. S222–S223.

Objectives/Hypothesis
The purpose of the immunology studies was to continue the systematic attempt to define the range of immunological parameters affected by spaceflight. The experiments were designed to allow repetition and expansion of studies from a previous flight (Cosmos 1887), with the addition of an antiorthostatic suspension study for direct comparison with spaceflight effects.

Approach or Method
The first portion of this experiment involved the ability of cells to respond to colony stimulating factor-granulocyte/monocyte (CSF-GM). For colony stimulating factor assays, 1x105 bone marrow cells were suspended with 10% fetal bovine serum and antibiotics containing 3% agar. The second set of studies involved the expression of cell surface markers (T-cell markers, B-cell markers, natural killer cell markers, and interleukin-2 receptors) of both spleen and bone marrow cells. Populations stained with fluorescein-labeled antibodies were analyzed using a flow cytometer. In addition, natural killer cell levels were also examined.

Results
Bone marrow cells from flight and tail-suspended (TS) rats had reduced response to CSF-GM, demonstrating that response to a recombinant DNA-derived cytokine affecting both monocyte and granulocyte cell populations in bone marrow was compromised by spaceflight. Spleen cells from flown rats showed increased percentages of pan leukocyte, helper-T, and suppressor-cytotoxic-T-cells, while TS samples had a different pattern of markers. In bone marrow lymphocytic cell population, the percentage of anti-asialo GM-1 bearing, interleukin-2 receptor bearing, pan-T, and helper-T cells was increased after flight, while TS samples again had a different pattern. This shows that suspension is useful for modeling spaceflight effects of functional immune responses, but not adequate for modeling spaceflight effects on cell population distribution. Additionally, specific natural killer cell subpopulations were depressed after spaceflight, while others were not, indicating lack of a general blunting of natural killer cell responses.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: IMMUNOLOGY

Title of Study

Effect of Spaceflight on Level and Function of Immune Cells:
II. Proliferation and Cytokines

Science Discipline

Immunology

Investigator	Institute
A.M. Mastro	Pennsylvania State University

Co-Investigator(s)	Institute
Konstantinova, I.V.	Institute of Biomedical Problems
Fuchs, B.B.	Institute of Human Mophology
Taylor, G.R.	NASA Johnson Space Center (JSC)

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Nash, P.V.; Bour, B.A.; and Mastro, A.M.: Effect of Hindlimb Suspension Simulation of Microgravity on In Vitro Immunological Responses. Experimental Cell Research, vol. 195, 1991, pp. 353–360.

Nash, P.V.; Konstantinova, I.V.; Fuchs, B.B.; Rakhmilevich, A.L.; Lesnyak, A.T.; and Mastro, A.M.: Effect of Spaceflight on Lymphocyte Proliferation and Interleukin-2 Production. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S186–S190.

Objectives/Hypothesis

There is ample evidence from the study of human peripheral blood lymphocytes following spaceflight that many astronauts have an impairment of mitogen-induced T-cell proliferation. Cosmos 2044 afforded the first opportunity to study spaceflight effects on the proliferation of T-cells and B-cells from rat lymph nodes. This study was also designed to investigate potential mechanisms involved in any absorbed T-cell deficiency.

Approach or Method

Lymphocytes from the superficial inguinal lymph nodes of space-flown rats were tested for proliferation in response to polyclonal activators. Lymph node cells (LNC) were cultured with T-cell or B-cell mitogens, phorbol ester, and calcium ionophore, or T-cell mitogen and the lymphokines interleukin-1 or interleukin-2 (IL-1, IL-2). Lymphocytes were incubated with concanavalin A (Con A), a T-cell mitogen, and tested for IL-2 production.

Results

The proliferation of rat LNC stimulated with polyclonal T or B mitogens was unaffected by the spaceflight. The proliferation of rat LNC T-cells from flight rats were more responsive to Con A and IL-2, and Con A and IL-1, than were vivarium controls. However, proliferation of flight rat lymphocytes was not greater than synchronous controls, so the increase does not appear to be associated with spaceflight. Results indicate there may have been a trend toward heightened responsiveness, but this was not confirmed statistically. Both flight and suspended LNC produced more IL-2 on average than did controls.

Launch Date 6/5/1991

Landing Date 6/14/1991

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Lipid Peroxidation and Antioxidant Defense System

Science Discipline
Immunology

Investigator	Institute
I.A. Popova	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Markin, A.A.	Institute of Biomedical Problems
Zhuravleva, O.A.	Institute of Biomedical Problems
Merrill, A.H.	Emory University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Popova, I.A.; Merrill, A.; Markin, A.A.; and Zhuravleva, O.A.: Lipid Peroxidation and Antioxidant Protection System. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, p. 32.

Objectives/Hypothesis
The system of lipid peroxidation reacts regularly to adverse effects, and results in enhancement of lipid peroxidation and buildup of LPO products in blood and tissues. Cosmos experiments have suggested a short-term stress effect, rather than any long-term adverse effects, is responsible for these changes. This experiment studied the effect of microgravity and other spaceflight factors on the system of lipid peroxidation and antioxidant defense of tissues.

Approach or Method
In the liver (right lobe), kidney (left), and skeletal muscle (quadriceps femoralis), in myocardial homogenates, and in blood plasma, the content of lipid peroxidation products—dienic conjugates, malonic dialdehyde, Schiff's bases, and the main lipid antioxidant, tocopherol—were determined. Also, the plasma total antioxidant activity was measured, and in tissue homogenates the activities of antioxidant enzymes, superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase were determined.

Results
It was found that the complex of spaceflight factors did not significantly influence the system of antioxidant protection and the intensity of lipid peroxidation. Changes of lipid peroxidation and antioxidant defense parameters in skeletal muscle and myocardium appeared only in rats sacrificed nine days postflight, reflecting the existence of functional tension in these tissues as a response to gravitation stress during readaptation to terrestrial conditions. There were no significant changes of investigated parameters in blood plasma; thus, whole free radical processes of rats were compensated during the postflight period.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Effect of Spaceflight Factors on the Functional Activity of Immune Cells

Science Discipline
Immunology

Investigator	Institute
I.V. Konstantinova	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Lesnyak, A.T.	Institute of Biomedical Problems
Rykova, M.P.	Institute of Biomedical Problems
Meshkov, D.O.	Institute of Biomedical Problems
Markin, A.A.	Institute of Biomedical Problems

Research Subject(s)
*Rattus norvegicus (*Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Konstantinova, I.V.; Lesnyak, A.T.; Lange, R.D.; Sonnenfeld, G.; Leon, H.; Rykova, M.P.; Meshkov, D.O.; Markin, A.A.; and Orlova, T.G.: Effect of Spaceflight Factors on the Functional Activity of Immune Cells. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, pp. 33–34.

Objectives/Hypothesis
The objective of this experiment was to further investigate the mechanisms underlying disorders in the immune system in microgravity. The ability to produce hormonal factors was also examined in spleen cultures.

Approach or Method
Rat spleen cells were examined for their proliferative response to concanavalin-A (0.1, 1.0, and 10.0 μg/ml) and interleukin-2 (2 U/ml) stimulation in 48-, 72-, and 96-hour cell cultures. Alpha- and gamma-interferon were assayed by a microplaque reduction on vesicular stomatitis virus on L-cells. The tumor necrosis factor was determined by cytotoxicity to tumor cells. Natural cytotoxicity, using cell line K-562, was determined in bone marrow and spleen samples.

Results
T-cell activity did not change in R+0 animals, increased in the R+9 group (in unstimulated cell cultures, and cultures stimulated with interleukin-2 and optimal and high concentrations of concanavalin-A), and decreased in the T+0 group (in unstimulated cell cultures, and in cultures with low concanavalin-A concentrations and short time incubation). Results indicate that spleen and bone marrow natural killer activity was increased in cultures in R+0, R+9, and S+0 (spleen cells only). There was an increase of spleen natural killer cell activity in cultures of K-562 target cells (in T+0 a small increase was noted). Bone marrow cell activity decreased slightly in R+0 animals. Compared to control rats, Alpha-interferon production was unaffected. Gamma-interferon activity was not diminished after flight or tail-suspension. There was an increase of tumor necrosis factor alpha production after flight. Interleukin-2 and tumor necrosis factor beta activity were decreased in the T+0 group only.

Cosmos 2229 / Bion 10

Launch Date 12/29/1992

Landing Date 1/10/1993

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Rhesus Monkey Immunology Study

Science Discipline
Immunology

Investigator	Institute
G. Sonnenfield	University of Louisville

Co-Investigator(s)	Institute
Davis, S.	University of Louisville Schools of Medicine and Dentistry
Taylor, G.R.	NASA Johnson Space Center (JSC)
Mandel, A.D.	NASA Ames Research Center (ARC)
Lesnyak, A.T.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
Lesnyak, A.T.; Sonnenfeld, G.; Rykova, M.P.; and Konstantinova, L.: Immune Changes in Test Animals During Spaceflight. Journal of Leukocyte Biology, vol. 54, Sept. 1993, pp. 214–226.

Sonnenfeld, G.; Davis, S.; Taylor, G.R.; Mandel, A.D.; Konstantinova, I.V.; Lesnyak, A.; Fuchs, B.B.; Peres, C.; Tkackzuk, J.; and Schmitt, D.A.: Effect of Space Flight on Cytokine Production and Other Immunologic Parameters of Rhesus Monkeys. Journal of Interferon and Cytokine Research, vol. 16, no. 5, May 1996, pp. 409–415.

Objectives/Hypothesis
This experiment on the Cosmos 2229 mission was designed to begin to determine the suitability of the Rhesus monkey as a surrogate for humans in space immunology research. Two tests were carried out to examine the effects of spaceflight on the capacity to resist infection. One tested the responsiveness of Rhesus bone marrow cells to recombinant human granulocyte/macrophage colony stimulating factor (GM-CSF). The other test used blood and bone marrow cells to examine microgravity-induced changes in staining patterns against a variety of antibodies.

Approach or Method
Tissue samples for preflight studies were taken 1.5 months prior to flight. Samples were also taken at various times from 1 to 12 days postflight. Two types of tissue samples were taken: peripheral blood and bone marrow. Bone marrow cells were exposed to recombinant human GM-CSF, incubated for 7 days, and examined for colony growth. Bone marrow and blood cells were also exposed to one of eight different antibody treatments. Before exposure, bone marrow cells were suspended in supplemented McCoy's media with 10% fetal bovine serum (FBS), centrifuged, and separated from the supernatant. Blood cells were lysed, centrifuged, resuspended, centrifuged again, and separated from the supernatant. After exposure to the antibody stains, the cells were fixed and analyzed to determine the presence of antigenic markers.

Results
The exposure of the two Rhesus monkeys to microgravity resulted in inhibition of the response of bone marrow cells to GM-CSF and depression of the percentage of peripheral blood and bone marrow leukocyte antibody markers. B cells were less affected than T cells. The parameters tested in the study appeared to return towards a more normal level by 3 days postlanding, but experienced a second drop by 12 days postlanding. Postflight testing could not be ruled out as a contributor to the second drop in immunological response. The results from Cosmos 2229 differed from previous flights, which had utilized rats as the experimental organism, indicating a species effect on immunological response to spaceflight.

Launch Date 1/13/1993 **Landing Date** 1/19/1993

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Influence of Spaceflight on the Production of Interleukin-3 and Interleukin-6 by Rat Spleen and Thymus Cells

Science Discipline
Muscle Physiology

Investigator / Institute
E.S. Miller — University of Louisville

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Sonnenfeld, G. and Miller, E.S.: Space Flight and Humoral and Cellular Immunity of Animals. Physiologist, supl., vol. 36, no. 1, 1993, pp. S68–70.

Miller, E.S.; Koebel, D.A.; and Sonnenfeld, G.: Influence of Spaceflight on the Production of Interleukin-3 and Interleukin-6 by Rat Spleen and Thymus Cells. Journal of Applied Physiology, vol. 78, no. 3, Mar. 1995, pp. 810–813.

Objectives/Hypothesis
Spaceflight is known to affect the immune system at the regulatory cytokine network level. These effects may cause astronauts and mammals to be more susceptible to health problems. Interleukin-3 (IL-3) is a colony stimulationg factor (CSF) and interleukin-6 (IL-6) can serve as a lymphocyte proliferator. Both are important in elements of the immune system. The purpose of this study was to examine the effects of spaceflight on the activity of interleukin-3 and interleukin-6.

Approach or Method
After 7 days of spaceflight, the spleen and thymus were taken from rats and assayed for the ability to secrete IL-3 and IL-6. Cells from the spleen and thymus were incubated with either concanavalin A or the monocyte/macrophage activator lipopolysaccharide to stimulate IL-3 and IL-6 production. To assay for IL-3, IL-3 colony stimulating factor dependent cell line 32D was used. To assay for IL-6, IL-6 dependent cell line 7TD1 was used.

Results
Production of IL-3 was higher in the spleen and thymus cells harvested from flight rats as compared to ground controls. IL-6 production was higher in the thymus cells of flight rats, but not in the spleen cells. These results show that spaceflight can enhance the production of cytokines in the immune system. The immunomodulatory factor responsible for this enhancement is not understood through this study.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: IMMUNOLOGY

Title of Study

Effect of SLS-2 Spaceflight on Immunological Parameters of Rats: Immunity Mediators

Science Discipline

Immunology

Investigator	***Institute***
I.V. Konstantinova	Institute of Biomedical Problems

Co-Investigator(s)	***Institute***
Lesnyak, A.T.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Synchronous Control, Vivarium Control

Key Flight Hardware

Research Animal Holding Facility (RAHF)

Selected Publications

Lesnyak, A.; Sonnenfeld, G.; Avery, L.; Konstantinova, I.; Rykova, M.; Meshkov, D.; and Orlova, T.: Effect of SLS-2 Spaceflight on Immunologic Parameters of Rats. Journal of Applied Physiology, vol. 81, no. 1, July 1996, pp. 178–182.

Objectives/Hypothesis

It has been demonstrated that exposure of animals and humans to microgravity caused immune alterations detected immediately after flight. Immune changes mainly consisted of decreases in the proliferative activity of T lymphocytes, cytotoxic activity of natural killer cells, and production of cytokines. The objectives of this experiment were to: 1) study the effects of spaceflight on the kinetics of lymphocyte proliferation; 2) study the effects of spaceflight on the activity of natural killer cells; and 3) study the effects of spaceflight on the production of cytokines such as interleukin-1, interluekin-2, interferon-alpha, interferon-gamma, tumor necrosis factor alpha, and tumor necrosis factor beta. SLS-2 marks the first time dissections were performed in space, thus making it possible to study cell mediated immunity without having to consider landing stresses.

Approach or Method

Spleen and bone marrow cells were used for the study. Lymphocyte proliferation activity and natural killer cytotoxicity were measured using RPMI-1640 media. Proliferative activity of concanavalin A, phytohemagglutinin and interleukin-2 stimulated spleen T cells were measured in terms of DNA synthesis after 48, 72, and 96 hours of cultivation. Activity of natural killer cells found in spleen and bone marrow was assayed in YAC-1 and K-562 cultured target cells labeled with 51Cr and (heavy) Uridine. Cytokines were assesed in supernatant fluids of cultivated spleen and bone marrow cells. IL-1 and IL-2 were expressed as units per milliliter. Interferon activity was measured in cultured murine L cells with respect to the suppresion of the cytopathic effect of murine encephalomyocarditis virus. Tumor necrosis factors were measured with respect to their cytopathic effect on L929 cells.

Results

T lymphocyte activity of rats dissected in spaceflight was significantly decreased compared to controls. Cell proliferation rate in rats dissected immediately after landing did not decrease, whereas that in rats dissected at R+14 increased. The activity of spleen natural killer cells was reduced in response to 51Cr labeled target cells during flight and after flight. At R+14, their activity returned to normal. In bone marrow, the activity of natural killer cells did not vary significantly. The production of IL-1 and IL-2 tumor necrosis factor alpha and beta in spleen cell cultures of the flight rats was reduced. At R+0, interferon alpha and gamma levels were diminished. In summary, cell mediated immunity in rats was significantly suppressed during flight. The time course variation of immune parameters after flight suggests that the changes may truly indicate a response of the immune system to spaceflight conditions that could increase over time.

STS-60 / Immune.1

Launch Date 2/3/1994 **Landing Date** 2/11/1994

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Ability of Polyethylene Glycol Interleukin-2 (PEG-IL2) to Counteract the Effect of Spaceflight on the Rat Immune System

Science Discipline
Immunology

Investigator	Institute
R. Zimmermann	Chiron Corporation

Co-Investigator(s)	Institute
Sonnenfeld, G.	Carolinas Medical Center
Chapes, S.K.	Kansas State University, BioServe
Ballard, R.W.	NASA Ames Research Center (ARC)
Goldwater, D.	NASA Ames Research Center (ARC)

Research Subject(s)
*Rattus norvegicus (*Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Ambient Temperature Recorder (ATR)

Selected Publications
Chapes, S.K.; Simske, S.J.; Sonnenfeld, G.; Miller, E.S.; and Zimmerman, R.J.: Effects of Spaceflight and PEG-IL-2 on Rat Physiological and Immunological Responses. Journal of Applied Physiology, vol. 86, no. 6, June 1999, pp. 2065–2076.

Chapes, S.K.: Lessons From Immune 1-3: What Did We Learn and What Do We Need to Do in the Future? Journal of Gravitational Physiology, vol. 11, no. 2, July 2004, pp. 45–48.

Objectives/Hypothesis
Because of the suppression of the immune system in otherwise healthy subjects during spaceflight, it offers a unique opportunity to study the effects of biological substances without the complications of illnesses normally present in immunosuppressed subjects. Polyethylene Glycol-Interleukin-2 (PEG-IL-2) is an immunological mediator and enhancer developed and manufactured by Chiron Corp. The purpose of this experiment, in conjunction with the Immune.2 experiment, was to confirm and define the ability of PEG-IL-2 to prevent or ameliorate the detrimental effects of spaceflight on immune responses of rats.

Approach or Method
Half of the animals in each group were injected intravenously with PEG-IL-2 2 to 3 hours before transfer to the Animal Enclosure Modules. Upon recovery, blood samples were collected and analyzed for corticosterone concentration with radioimmunoassay. Macrophage (M-CSF) and granulocyte-macrophage stimulating factor (GM-CSF) dependent macrophage colony formation from bone marrow cells were assayed. Lymphocytes were obtained from the spleen and lymph nodes and assayed to determine cell proliferation rates and the secretion of cytokines, M-CSF, interleukin-6 (IL-6), interferon-g (IFN-g), and transforming growth factor beta (TGF-ß). Peritoneal macrophages were assayed for secretion of TNFa and IL-6.

Results
Results of this experiment are joined with the Immune.2 experiment. Few immunological parameters were consistent across the Immune.1 and Immune.2 experiments, making conclusive observations difficult. Inconsistencies between the two experiments prevented any conclusive evidence concerning the effectiveness of the PEG-IL-2 treatment. Some of the animals flown were found to have damage to their tails upon recovery; however, compared to the animals in the same group, these changes did not correlate with any of the parameters measured. While there were the expected trends in the Immune.1 experiments, the control animals did not exhibit as many of the flight-related changes as were anticipated. The control group in the Immune.2 study had more dramatic changes associated with spaceflight. These differences may have been due to the fact that flight animals in the Immune.2 experiment were exposed to significantly higher ambient temperatures than animals in Immune.1.

Launch Date 11/3/1994

Landing Date 11/14/1994

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Effect of Spaceflight on Development of Immune Responses

Science Discipline
Immunology

Investigator / Institute
G. Sonnenfeld — Carolinas Medical Center

Co-Investigator(s) / Institute
Miller, E.S. — Harrington Cancer Center

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Sonnenfeld, G.; Foster M.; Morton, D.; Bailliard, F.; Fowler, N.A.; Hakenewerth, A.M.; Bates, R.; and Miller, E.S. Jr.: Spaceflight and Development of Immune Responses. Journal of Applied Physiology, vol. 85, no. 4, Oct. 1998, pp. 1429–1433.

Taylor, G.R.; Konstantinova, I.; Sonnenfeld, G.; and Jennings, R.: Changes in the Immune System During and After Spaceflight (review article). Advances in Space Biology and Medicine, vol. 6, 1997, pp. 1–32.

Objectives/Hypothesis
The objectives of this experiment were: 1) to evaluate the influence of spaceflight on the maternal-fetal interface; 2) to evaluate the influence of spaceflight on the development of immune system cells; 3) to evaluate the influence of spaceflight on the development of functional activities of immune system cells; 4) to evaluate the influence of spaceflight on the development of immunoregulation; 5) to evaluate the influence of spaceflight on the development of immune responsiveness to cytokines; and 6) to evaluate the influence of spaceflight on the ability to resist microbial infection.

Approach or Method
Total antibody titer and percent antibody isotype were determined in the plasma of the mother and progeny animals. Flow cytometric analysis of the percentages of leukocyte populations were performed on spleen, blood, and bone marrow tissues. Splenocytes and thymocytes were stimulated with the T cell mitogen concanavalin-A to evaluate blastogenic potential in progeny animals. Profiles of a broad spectrum of splenic and thymic cytokine activities were conducted at the genetic level as revealed by polymerase chain reaction (PCR). Bone marrow cells were incubated in the presence of macrophage-colony stimulating factor (M-CSF) to stimulate the differentiation of these cells into macrophages. Splenic macrophages harvested from progeny animals were analyzed for their ability to phagocytize pathogenic bacteria.

Results
The response of bone marrow cells to M-CSF, leukocyte blastogenesis, and cytokine production was altered in cells obtained from flight dams. Contrastingly, the response of bone marrow cells to M-CSF as well as leukocyte blastogenesis was not altered in cells taken from fetuses and pups from flown animals. Leukocyte subset analysis showed that flown dams, fetuses, and pups were altered. Cytokine production was reduced in flight pups, while immunoglobulin levels were not altered in offspring after spaceflight. This data indicates that there may be differential regulation in the development of immune responses.

STS-63 / Immune.2

Launch Date 2/3/1995 **Landing Date** 2/11/1995

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Confirmation of Ability of Polyethylene Glycol Interleuken-2 (PEG-IL2) to Counteract the Effect of Spaceflight on the Rat Immune System

Science Discipline
Immunology

Investigator	Institute
R. Zimmerman	Chiron Corporation

Co-Investigator(s)	Institute
Sonnenfeld, G.	Carolinas Medical Center
Chapes, S.K.	BioServe, Kansas State University
Goldwater, D.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Ambient Temperature Recorder (ATR)

Selected Publications
Chapes, S.K.; Simske, S.J.; Sonnenfeld, G.; Miller, E.S.; and Zimmerman, R.J.: Effects of Spaceflight and PEG-IL-2 on Rat Physiological and Immunological Responses. Journal of Applied Physiology, vol. 86, no. 6, June 1999, pp. 2065–2076.

Chapes, S.K.: Lessons From Immune 1-3: What Did We Learn and What Do We Need to Do in the Future? Journal of Gravitational Physiology, vol. 11, no. 2, July 2004, pp. 45–48.

Objectives/Hypothesis
Because of the suppression of the immune system in otherwise healthy subjects during spaceflight, it offers a unique opportunity to study the effects of biological substances without the complications of illnesses normally present in immunosuppressed subjects. Polyethylene Glycol-Interleukin-2 (PEG-IL-2) is an immunological mediator and enhancer developed and manufactured by Chiron Corp. The purpose of this experiment, in conjunction with an Immune.1 experiment, was to confirm and define the ability of PEG-IL-2 to prevent or ameliorate the detrimental effects of spaceflight on immune responses of rats.

Approach or Method
Half of the animals in each group were injected intravenously with PEG-IL-2 2 to 3 hours before transfer to the Animal Enclosure Modules. Upon recovery, blood samples were collected and analyzed for corticosterone concentration with radioimmunoassay. Macrophage stimulating factor (M-CSF) and granulocyte-macrophage stimulating factor (GM-CSF) dependent macrophage colony formation from bone marrow cells were assayed. Lymphocytes were obtained from the spleen and lymph nodes and assayed to determine cell proliferation rates and the secretion of cytokines, M-CSF, interleukin-6 (IL-6), interferon-g (IFN-g), and transforming growth factor beta (TGF-ß). Peritoneal macrophages were assayed for secretion of TNFa and IL-6.

Results
The results of this experiment are joined with the Immune.1 experiment. Few immunological parameters were consistent across the Immune.1 and Immune.2 experiments, making conclusive observations difficult. Significant changes in bone parameters were observed in Immune.2 but not Immune.1. The inconsistencies between the two experiments prevented any conclusive evidence concerning the effectiveness of the PEG-IL-2 treatment. Several factors may have played a role in these discrepancies. Some of the animals flown as a part of the Immune.1 experiment were discovered to have various degrees of damage to their tails (necrosis, loss, and gangrenous tissues). Additionally, flight animals in the Immune.2 experiment were exposed to significantly higher ambient temperatures than animals in Immune.1. These data illustrate some of the issues that can arise when small numbers of animals are studied under flight conditions that are difficult to reproduce from one flight to the next.

STS-77 / Immune.3

Launch Date 5/19/1996 **Landing Date** 5/29/1996

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Confirmation of Ability of Sustained-Release Insulin-Like Growth Factor I (IGF-I) to Counteract the Effect of Space Flight on the Rat Immune and Skeletal Systems

Science Discipline
Immunology
Bone and Calcium Physiology

Investigator	Institute
R. Zimmerman	Chiron Corporation

Co-Investigator(s)	Institute
None	

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM), Ambient Temperature Recorder (ATM)

Selected Publications
Chapes, S.K.; Simske, S.J.; Sonnenfeld, G.; Miller, E.S.; and Zimmerman, R.J.: Effects of Space Flight and PEG-IL-2 on Rat Physiological and Immunological Responses. Journal of Applied Physiology, vol. 86, no. 6, June 1999, pp. 2065–2076.

Bateman, T.A.; Zimmerman, R.J.; Ayers, R.A.; Ferguson, V.L.; Chapes, S.K.; and Simske, S.J.: Histomorphometric, Physical, and Mechanical Effects of Spaceflight and Insulin-like Growth factor-I on Rat Long Bones. Bone, vol. 23, no. 6, Dec. 1998, pp. 527–535.

Objectives/Hypothesis
The microgravity that accompanies spaceflight, as well as other concomitant environmental factors, alter the effectiveness of astronauts' immune systems and bone homeostasis. The cytokine insulin-like growth factor (IGF-1) promotes growth in peripheral tissue, affects marrow blood cells that develop into immune cells, and is an important factor in erythropoiesis. IGF-1 has been shown to stimulate bone cell component synthesis as well as reduce protein degradation. This study investigated the possibility of utilizing IGF-1 to counter the detrimental effects of spaceflight on both the immune and the skeletal systems.

Approach or Method
Four days prior to launch, 2-ml Alzet osmotic pumps were subcutaneously inserted into the dorsal region of 12 male Sprague-Dawley rats. Six of the rats received 10 mg/kg per day of IGF-1, while the other six received an equal volume of saline. All rats were injected with 20 mg/kg of oxytetracycline 1 day before launch. The animals were then placed in two Animal Enclosure Modules (AEMs), with six animals per module, and flown on the 10-day STS-77 mission. After landing, animals were weighed and euthanized. Ocular reflex test, cardiac puncture, and exsanguination were then performed. Blood samples were analyzed to determine complete cell counts and corticosterone content. Macrophage colony stimulating factor (M-CSF)-dependent macrophage colony formation was assayed from bone marrow cells, and the stimulation index of the lymphocytes was calculated. Bone marrow cells were also assayed for secretion of IL-6, TNF-a, and TGF-b.

Results
Results support the hypothesis that IGF-1 has a beneficial effect on immune cells by relieving at least some of the effects of microgravity, though spaceflight appeared to inhibit the physiological effects of IGF-1. During the spaceflight, IGF-1 was also shown to have some beneficial anabolic effects on bone.

STS-84 / Biorack 3

Launch Date 5/5/1997

Landing Date 5/24/1997

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Effect of Microgravity on Lymphocyte Activation: Cell-Cell Interaction and Signaling

Science Discipline
Immunology

Investigator / Institute
C. Sams — NASA Johnson Space Center (JSC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Homo sapiens (human) T-cell

Ground-Based Controls
Clinostat Control

Key Flight Hardware
Biorack, Passive Thermal Conditioning Unit (PTCU)

Selected Publications
Hashemi, B.B.; Penkala, J.E.; Vens, C.; Huls, H.; Cubbage, M.; and Sams, C.F.: T Cell Activation Responses are Differentially Regulated During Clinorotation and in Spaceflight. FASEB Journal, vol. 13, no. 14, Nov. 1999, pp. 2071–2082.

Crucian, B. and Sams, C.: Immune System Dysregulation During Spaceflight: Clinical Risk for Exploration-Class Missions. Journal of Leukocyte Biology, vol. 86, no. 5, Nov. 2009, pp. 1017–1018.

Objectives/Hypothesis
During T-cell activation, the interactive coupling of signal transduction and cytoskeletal elements plays a crucial role. Cell structure and cytoskeletal formation is crucial for proper T-cell formation. This study examined human peripheral T-cells and how microtubule architecture and activation-induced polarization of the Microtubule Organizing Center (MTOC) are affected by microgravity.

Approach or Method
Bead immobilized anti-CD3 or a combination of anti-CD3 and CD28 were used to activate human T-cells during flight.

Results
Analysis of T-cells activated in microgravity and others activated in 1g showed that both formed cell-bead aggregates. After being stained for Tubulin, both sets of samples were analyzed using Confocal Fluorescence Microscopy. T-cells in 1g, when activated with bead-mAb, showed a reorganization of tubulin architecture, as well as a reorientation of the MTOC in the direction of the cell-bead contact site due to a strong polarizing signal. In contrast, polarization of cells stimulated in microgravity was inhibited. This was evidenced by the more random orientation of the MTOC with relation to the cell-bead contact site. This demonstrates that the T-cell's change in cytoskeletal structure in microgravity inhibits its activation.

Launch Date 7/4/2006 **Landing Date** 7/17/2006

Physiological/Science Discipline: IMMUNOLOGY

Title of Study

Fungal Pathogenesis, Tumorigenesis, and Effects on Host Immunity in Space (FIT)

Science Discipline

Immunology

Investigator	***Institute***
S. Bhattacharya	NASA Ames Research Center (ARC)

Co-Investigator(s)	***Institute***
Kimbrell, D.	University of California, Davis

Research Subject(s)

Drosophila melanogaster (Fruit fly)

Ground-Based Controls

Asynchronous Control

Key Flight Hardware

Fungal Immunity (FIT) Fly Kit

Selected Publications

Marcu, O.; Lera, M.P.; Sanchez, M.E.; Levic, E.; Higgins, L.A.; Shmygelska, A.; Fahlen, T.F.; Nichol, H.; and Bhattacharya, S.: Innate Immune Responses of *Drosophila Melanogaster* are Altered by Spaceflight. PLoS One, vol. 6, no. 1, 2011, p. e15361.

Inan, O.T.; Marcu, O.; Sanchez, M.E.; Bhattacharya, S.; and Kovacs, G.: A Portable System for Monitoring the Behavioral Activity of *Drosophila*. Journal of Neuroscience Methods, vol. 202, no. 1, 2011, pp. 42–52.

Objectives/Hypothesis

The overall goal of the FIT experiment was to gain further insight into immune system function after exposure to the spaceflight environment, and to study the cellular and molecular biological responses of the immune system to postflight microbial infections.

Approach or Method

The payload was carried in a vented foam tray and located in a middeck locker. The FIT payload consisted of: 1) ten vented FIT Fly Kits (one fly cassette containing a food tray housed in a European Space Agency (ESA) vented Type 1 Container per kit); 2) one FIT Fungus Kit (two fungus tubes (10 ml) that are housed in an ESA vented Type 1 Container); 3) one RAM Kit (one Passive Radiation Dosimeter stored in an ESA vented Type 1 Container); 4) one Hobo data logger; and 5) five Platform Kits (one Food Tray Change Out Platform carrying one filled food tray per kit).

Results

There were several observed changes in the innate immune function of *Drosophila melanogaster* after spaceflight. Larval phagocytosis efficiency was reduced after flight compared to ground controls. The number of actively phagocytosing hemocytes was lower in flight compared to ground, and the capacity of phagocytosing cells to internalize bacteria was also reduced after spaceflight. Hemocyte counts per larva, and the size of larvae, were reduced in space-reared larvae compared to ground controls. While flight-reared adult flies inoculated postflight with live *E. coli* were able to clear the bacterial infection fairly efficiently, the pattern of gene expression changes in response to infection were different between space-flown adults and the analogous ground-control samples.

Humoral immunity genes were downregulated in larvae after spaceflight. Several genes related to immunity were differentially expressed in space-reared adult flies and larvae as shown by microarray. Gene expression data were also matched to correlate with the observed phenotypes of reduced phagocytosis function and reduced plasmatocyte count in larvae.

21P / Leukin

Launch Date 9/18/2006

Landing Date 4/21/2007

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Role for the Interleukin-2 Receptor in Signal Transduction (Leukin)

Science Discipline
Immunology

Investigator	Institute
A. Cogoli	Tahitian Park

Co-Investigator(s)	Institute
Hughes-Fulford, M.	University of California, San Francisco

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
1 G on Board Centrifuge Control

Key Flight Hardware
Kubik Incubator

Selected Publications
Paulsen, K.; Thiel, C.; Timm, J.; Schmidt, P.M.; Huber, K.; Tauber, S.; Hemmersbach, R.; Seibt, D.; Kroll, H.; Grote, K.H.; Zipp, F.; Schneider-Stock, R.; Cogoli, A.; Hilliger, A.; Engelmann, F.; and Ullrich, O.: Microgravity-Induced Alterations in Signal Transduction in Cells of the Immune System. Acta Astronautica, vol. 67, nos. 9–10, Nov.–Dec. 2010, pp. 1116–1125.

Objectives/Hypothesis
Early studies of Apollo astronauts returning to earth showed blunted immune responses. The nearly total loss of T-cell activation at the cell level in microgravity was discovered in a Spacelab experiment in 1983 and was confirmed later by 19 experiments in space. These studies indicated that inhibition of early IL-2R expression is probably one of the causes of such loss of activation. The objective of the experiment was to analyze the pathway of mitogenic signal transduction in T-cells in true microgravity, as well as at variable G levels between 0 and 1xg. Identification of the signaling pathways sensitive to gravity is crucial to understanding impaired immune function in spaceflight, which remains a critical obstacle to long-duration space exploration.

Approach or Method
The main experimental approach consisted of the activation of cultures of purified peripheral blood cells with T-cell mitogens in simulated microgravity. The specific IL-2R subunit messenger ribonucleic acid (mRNA) was quantitatively determined with the reverse transcriptase-polymerase chain reaction (RT-PCR) technology, the insertion of IL-2R in the membrane was visualized by immunofluorescence, and its secretion in the supernatant was measured by immunoassay. Signal transduction was furthered analyzed by determining the early expression of oncogenes.

The experiment, LEUKIN, was launched from Baikonur, Kazakhstan on September 18th, 2006. This experiment investigated human T cell activation by ConA and anti-CD28 during spaceflight compared to an onboard 1 g control. Cells were fixed during spaceflight after 1.5 hours of activation and mRNA were preserved or analysis with gene microarrays.

Results
After 1.5 hours of activation many genes involved in binding activity, signal transduction, and transcription were downregulated in orbital microgravity compared to 1 g controls. Compared to 4 hours of activation in Random Positioning Machine vectorless gravity, a greater proportion of genes involved in signal transduction and transcription were affected. Interestingly, at the 4-hour time point, expressions of more structural molecules were affected. These findings showed that at an early timepoint after activation (1.5 hours), pathways of signal transduction and transcription were already inhibited in T cells in spaceflight, resulting in significant downstream effects on function.

Launch Date 11/14/2008

Landing Date 11/20/2008

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Differentiation of Bone Marrow Macrophages in Space (BONEMAC)

Science Discipline
Immunology

Investigator / Institute
S.K. Chapes — Kansas State University

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (C57BL/6J mouse bone marrow cells)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
luid Processing Apparatus (FPAs), Group Activation Pack (GAP), Commercial Generic Bioprocessing Apparatus (CGBA)

Selected Publications
Ortega, M.T.; Lu, N.; and Chapes, S.K.: Evaluation of In Vitro Macrophage Differentiation During Space Flight. Advances in Space Research, vol. 49, May 2012, pp. 1441–1455.

Ortega, M.T.; Pecaut, M.J.; Gridley, D.S.; Stodieck, L.S.; Ferguson, V. L.; and Chapes, S.K.: Shifts in Bone Marrow Cell Phenotypes Caused by Space Flight. Journal of Applied Physiology, vol. 106, no. 2, Feb. 2009, pp. 548–555.

Objectives/Hypothesis
The goal of the BoneMac spaceflight experiment was to determine how C57BL/6J mouse macrophage differentiation was suppressed during spaceflight by: 1) testing the hypothesis that colony stimulating factor-1 (CSF-1) receptor expression in progenitor cells was decreased; 2) assessing effects of the spaceflight environment on differentiation mechanisms (this involved studying cell phenotypes, messenger ribonucleic acid (mRNA) expression, and cytokine expression during progenitor cell differentiation); and 3) determining which differentiation processes are affected by examining cell surface phenotypes and gene expression.

Approach or Method
Primary murine bone marrow cells from the humeri, femor, and tibia of adult C57BL/6J mice were used for this experiment. The collected cells were suspended in growth medium supplemented with macrophage CSF-1 and loaded into the primary chambers of 48 Fluid Processing Apparatus (FPAs). Formalin was loaded into the secondary chambers of 32 FPAs; 6.0 M guanidinium isothiocyanate (GITC) was loaded into the secondary chambers of 8 FPAs, and growth medium plus CSF-1 (nonpreserved) was loaded into the secondary chambers of 8 FPAs. A total of 6 Group Activation Packs (GAPs) were loaded with 8 FPAs each. After loading, the cells were placed into the Commercial Generic Bioprocessing Apparatus (CGBA) (37°C) to start incubation, pre-launch. In flight, one set of BONEMAC GAPs were operated on flight day 6 (FD6), and a second set of GAPs were operated on flight day 15 (FD15) to allow the bone cells to differentiate for different time durations. Post-flight, the FPAs were processed and analyzed. A ground control maintained at Space Life Sciences Laboratory Facility (SLSL) followed the same protocols and timeline as the flight samples.

Results
Mouse bone marrow cells were differentiated in the presence of recombinant macrophage colony stimulating factor (rM-CSF) for 14 days during the flight of space shuttle Space Transportation System (STS)-126. The hypothesis, that the receptor expression for M-CSF, c-Fms was reduced, was tested. Flow cytometry was used to assess molecules on cells that were preserved during flight to define the differentiation state of the developing bone marrow macrophages, including CD11b, CD31, CD44, Ly6C, Ly6G, F4/80, Mac2, and c-Fos, as well as c-Fms. In addition, RNA was preserved during the flight and was used to perform a gene microarray. There were significant differences in the number of macrophages that developed in space compared to controls maintained on Earth. There were also significant changes in the distribution of cells that expressed CD11b, CD31, F4/80, Mac2, Ly6C and c-Fos. However, there were no changes in c-Fms expression and no consistent pattern of advanced or retarded differentiation during spaceflight. A pattern of transcript levels were also found that would be consistent with a relatively normal differentiation outcome but increased proliferation by the bone marrow macrophages that were assayed after 14 days of spaceflight. There also was a surprising pattern of spaceflight influence on genes of the coagulation pathway. These data confirm that a spaceflight can have an impact on the in vitro development of macrophages from mouse bone marrow cells of mice.

STS-131 / Mouse Immune 1

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Antigen-Specific CD4+ T Cell Priming and Memory Response During Spaceflight (Mouse Immunology)

Science Discipline
Immunology

Investigator	Institute
M. Hughes-Fulford	Northern California Institute for Research and Education

Co-Investigator(s)	Institute
Chung, T.	Unversity of California, San Francisco

Research Subject(s)
Mus musculus (B6.SJL-Ptprca Pepcb/BoyJ mouse)

Ground-Based Controls
Asynchronous Control, 48-Hour Delayed Asynchronous Control, 48-Hour Delayed Animal Enclosure Module (AEM) Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Chang, T.T.; Walther, I.; Li, C.F.; Boonyaratanakornkit, J.; Galleri, G.; Meloni, M.A.; Pippia, P.; Cogoli, A.; and Hughes-Fulford, M.: The Rel/NF-ϰB Pathway and Transcription of Immediate Early Genes in T Cell Activation are Inhibited by Microgravity. Journal of Leukocyte Biology, vol. 92, no. 6, Dec. 2012, pp. 1133–1145.

Hughes-Fulford, M.; Meissler, J.; Aguayo, E.T.; Globus, R.; Aguado, J.; and Candelario, T.: Hyperoxia inhibits T cell activation in mice. In NASA Human Research Program Investigators' Workshop, Houston, TX, February 14-16, 2012, Abstract 4136.

Objectives/Hypothesis
This experiment tested whether initial specific activation of T-cells is intact and whether memory T-cell function is maintained during spaceflight to determine resistance to infections and acquired immune responses.

Approach or Method
Transgenic T-cells specific for a model protein were transferred into recipient mice with implanted mini-pumps that released the model protein along with noninfectious purified bacterial cell wall components. This model allowed T-cell stimulation to be delayed until after mice acclimated to spaceflight to ensure that T-cell activation was initiated in microgravity. NaÔve (previously unactivated) T-cells were transferred to determine whether responses to new immunological challenges (e.g., new infections) were intact. Also, previously activated T-cells that developed into memory cells were transferred to determine whether robust secondary responses could be elicited during spaceflight.

Results
Ribonucleic acid (RNA) recovery from the activated ground and flight samples was successful.

Preliminary reverse transcription polymerase chain reaction (RT-PCR) results showed lowered T-cell activation in the flown animals and, therefore, complete and successful analysis of the gene arrays and pathways operational for the ground and spaceflight samples are expected in continuing studies.

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Mouse Immunology-2

Science Discipline
Immunology

Investigator / Institute
R. Garofalo — University of Texas Medical Branch at Galveston (UTMB)

Co-Investigator(s) / Institute
Ivanciuc, T. — University of Texas Medical Branch at Galveston (UTMB)

Research Subject(s)
Mus musculus (Mouse) viral pathogens

Ground-Based Controls
Simulated Flight Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
The goal of the Mouse Immunology-2 experiment was to discover what triggers and leads to an increased susceptibility to an infection. The overarching hypothesis was that combinations of several factors, from microgravity to stress, affect the human immune system during spaceflight, resulting in an impaired antiviral response in the respiratory tract. Three specific hypotheses that were tested: 1. Spaceflight or ground conditions such as antiorthostatic suspension leads to an altered host response against respiratory syncytial virus (RSV); 2. This "deficient" antiviral response is characterized by a defect in both innate immunity, including interferon (IFN) type I production in the respiratory tract, and T cell-mediated immune response; and 3. The central events that cause this defect in antiviral immunity are the impaired trafficking and function of dendritic cells (DC) in the respiratory mucosa.

Approach or Method
Specific Aim 1: Characterize RSV replication in the lung and nasal turbinates, disease severity, and histopathology of the airways. These experiments were conducted in space-flown mice that were infected with RSV immediately after return to Earth.
Specific Aim 2: Determine the profile of IFN-α/β and innate cytokine response to viral infection in bronchoalveolar lavage (BAL) and nasal tissue, as well as other proteins by 2DE gel and Matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF). RNA was extracted from lung and thymus tissue for genomics studies. BAL and lymph nodes cells were characterized by flow cytometry. These studies conducted in mice as in Aim1.
Specific Aim 3: In the hindlimb unloading model: characterize viral replication in the lung and nasal turbinates, disease severity, and histopathology of the airways using both RSV and hMPV infection models. These studies were conducted in mice in the hindlimb unloading model.
Specific Aim 4: Profile IFN-α/β and innate cytokine response to infection in BAL and nasal tissue, and characterize dendritic cell migration and function in the lung and nasal tissue.

Results
The purpose of this study was to investigate the effects of spaceflight on immune function against a common viral pathogen, Respiratory Syncytial Virus (RSV), using an experimental murine model of infection. Eleven-week-old female BALB/c mice were assigned to one of three groups: spaceflight (FLT), Animal Enclosure Module (AEM), and vivarium (VIV). FLT animals were flown on the Space Shuttle Discovery (STS-133) and subjected to 12.8 days of microgravity. Two hours after return to earth, animals from all groups were infected with RSV or phosphate-buffered saline (PBS). Mice were serially sacrificed (days 1, 5, and 7 post-infection), and samples were collected for virus quantification. RSV infection was associated with similar degree of body weight loss in all groups, but disease severity was scored higher in the FLT group. Viral replication in the lungs was higher in FLT mice compared with the AEM and VIV infected mice. BAL cells revealed that neutrophils represented up to 45% of cells in the FLT mock-infected mice. RSV infection induced a significant elevation of MCP-1 at day 1 post-infection in the FLT group ($p < 0.01$). The concentrations of IFN-α/β in BAL were significantly higher in RSV-infected FLT group ($p < 0.001$). These findings suggest that immune parameters are influenced by relatively short exposure to spaceflight environment, changes that may become significant during long-term space missions.

STS-135 / BSP

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Effect of Spaceflight on Lung Gene and Protein Expression Profiles

Science Discipline
Cell and Molecular Biology

Investigator / Institute
R. Garofalo — University of Texas Medical Branch (UTMB) at Galveston

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
Analyses was performed to identify the set of lung genes that differed in expression between C57Bl/6 mice flown on Space Shuttle Atlantis, STS-135, and BALB/c mice from Space Shuttle Discovery, STS-133 missions. In a second analysis, C57Bl/6 and BALB/c mice pulmonary gene expression levels were compared with ground-control groups**.** Further study of these genes may yield specific candidates for subsequent clinical investigation for factors influencing pulmonary response to the spaceflight condition.

Approach or Method
Strain comparison of gene expression data: Analyses was completed to identify the set of lung genes that differed in expression between C57Bl/6 mice flown on STS-135 and BALB/c mice from STS-133 missions. In a second analysis, C57Bl/6 and BALB/c mice pulmonary gene expression levels were compared with ground control groups**.**

Altered expression of proteins in lung: A proteomics approach was applied using high-resolution two-dimensional gel electrophoresis (2DE) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) to evaluate relative changes in protein abundance in response to spaceflight.

Histopathological changes in lung: Characterization of the lung disease in C57Bl/6 and BALB/C mice from the flight and ground-control groups was assessed by histological analysis. Lung histological modifications were correlated with the transcriptional up-regulation of pro-inflammatory mediators by microarray analysis.

Results
At the time of publication data analysis is still in progress.

STS-135 / BSP

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Evaluation of Changes in Thymus and Spleenocytes After Spaceflight

Science Discipline
Immunology

Investigator / Institute
M. Hughes-Fulford — Northern California Institute for Research and Education

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
The samples requested from STS-135 were used to examine further changes in thymus and immune cells of flown mice. Excellent results were obtained from the initial experiment on STS-131, however, more sample was needed for follow-up to fully complete the study. First, more data was required on the thymus, e.g., protein composition of the thymus may be altered in spaceflight because a difference in the texture of the thymus was noted, but the cause was unknown. The thymus was sent out for fat analysis; key protein content analysis of the organ, as well as verifying the changes in ribonucleic acid (RNA) induction were performed in house. Secondly, spleen samples were requested to verify RNA T-cell induction data collected on STS-131, as well as collection of protein from the spleens to investigate target proteins suggested from the STS-131 astro mice.

Approach or Method
The objective of this study was to evaluate the effect of spaceflight on early T-cell activation. C57BL/6J female mice were exposed to microgravity for 15 days on STS-131. Splenocytes were harvested within 2–3 hours postflight, and cells isolated and activated (~9 hours after landing) with Concanavalin A and antiCD28 for 2.5 hours. Cells were harvested (11.5 hours post landing) and stored in RNAprotect (Qiagen) at –80°C. Total RNA was isolated from the activated samples with RNeasy (Qiagen).

Results
At the time of publication data analysis is still in progress. Due to limited initial sample size our results were not statistically complete. However flight results on STS-131 were very successful, and the results are being prepared for publication.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: IMMUNOLOGY

Title of Study
Examination of Splenic and Thymic Immune Function in Mice

Science Discipline
Immunology
Metabolism and Nutrition

Investigator	Institute
C. Sams	NASA Johnson Space Center (JSC)

Co-Investigator(s)	Institute
Crucian, B.	NASA Johnson Space Center (JSC)
Actor, J.	University of Texas, Houston
Hwang, S.-A.	University of Texas, Houston

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
The objective of this experiment was to determine effect of short-duration spaceflight on immune function in a murine model (immunocyte distribution, function, cytokine production profiles).

Approach or Method
To determine the effect of short-duration spaceflight on immune function in a murine model, murine splenocytes were stimulated immediately post-shuttle flight (14 days). The splenocytes were stimulated with T-cell or monocyte-specific mitogens or toll-like receptor agonists. Phenotype and cell function measurements were performed by flow cytometry. Production of adaptive (IFNg, IL2, IL-4, IL-10, and IL-17a) and innate (TNFa, IL-6) cytokines was determined by cytometric bead array. Postflight data were compared to ground-control splenocytes from age-matched mice.

Results
The CD4+ population decreased postflight with no concurrent decrease in CD8+ cells from shuttle mice postflight. Following T-cell-specific stimulation, a significant reduction in production of IL-10 was observed. Following monocyte-specific stimulation, a significant increase in IL-6 was observed, and TNFa production was trending towards elevation. Splenocytes also showed significant postflight increase in bead uptake, increased Class I expression, and increased TNF-a and IL-6 production in response to TLR-2 (zymosan) and TLR-4 (LPS) agonists. Dendritic cells showed markedly decreased levels of MHC I and CD86 after stimulation with TLR agonists. Stimulation of T-cells with signals that bypassed secondary activation processes increased expression of the IL-2R-alpha chain (CD25), consistent with increase in regulatory T-cell function.

These data indicate that alterations in splenocytes phenotype, function, and cytokine production patterns are evident following spaceflight. The pattern suggests that some innate immune functions are possibly enhanced, whereas some adaptive immune parameters may be inhibited. Follow-up human and in-flight studies will determine if a clinical risk related to immune dysregulation exists for astronauts.

Microbial Growth & Virulence

Microbial Growth and Virulence Introduction

Mark Ott, NASA Johnson Space Center

Microorganisms have accompanied humans on every spaceflight mission. Thus, any changes in microbial responses to spaceflight could have tremendous implications on our future success in space exploration. Despite this importance, our knowledge of the mechanisms behind these responses is only beginning to be understood. Prior to 1990, over 100 spaceflight experiments had provided insight into novel microbial responses to culture during spaceflight, such as alterations in growth characteristics and cell function [Dickson, 1991] [Taylor, 1974]. Still, many of these studies were limited by the technology of the time and access to spaceflight. Within the past 20 years, advances in analytical capabilities and increased availability aboard spaceflight experimental platforms have provided findings to transform our understanding from observational phenomenology to defined, mechanistic responses of organisms to their environment.

In 1992, Carlo Bruschi was the Principal Investigator of the flight experiment, Microgravitational Effects on Chromosome Behavior (YEAST) [Bruschi and Esposito, 1995] [STS-42 / IML-1, p. 232]. Using a carefully designed combination of mutant strains of Saccharomyces cerevisiae, this experiment was an early trendsetter in spaceflight experimentation by focusing on the genetic mechanism(s) behind the responses of microorganisms to spaceflight culture. The findings from YEAST provided insight into the genetic mechanisms affecting sporulation, recombination, and radiation repair of S. cerevisiae when cultured during spaceflight and encouraged the larger discussion of how microorganisms adapt to this unique environment. In 2004, the use of genetically modified S. cerevisiae for spaceflight research continued with YEAST GAP 1 [Progress 13P / YeastGap1, p. 234], as a mixture of isogenic mutant strains were evaluated to determine the impact of genetic deletions on their growth during spaceflight. While the samples were unfortunately lost in New Orleans during Hurricane Katrina, the experiment demonstrated the rapid advance and potential of new technological approaches.

The use of new advances in technology was highlighted in 2006 in a breakthrough experiment, Effect of Spaceflight on Microbial Gene Expression and Virulence (MICROBE), led by Cheryl Nickerson from the Biodesign Institute at Arizona State University [STS-115 / 12A, p. 235]. MICROBE was designed to investigate the virulence, gene expression, and morphological changes of Salmonella enterica typhimurium, Pseudomonas aeruginosa, and Candida albicans when cultured during spaceflight. In the first paper from this experiment, the investigators found that the disease-causing potential (virulence) of S. typhimurium cultured during spaceflight was increased compared to identically grown cultures on Earth [Wilson et al., 2007]. Interestingly, the gene expression profile from the S. typhimurium spaceflight culture was differentially regulated in key ways that were not associated with increased virulence during its culture on Earth. This molecular-genetic data led to the identification of a master molecular regulator, Hfq, that not only regulates the response of S. typhimurium, but was also found to uniquely regulate spaceflight-induced virulence characteristics of the P. aeruginosa flown in this experiment [Crabbe et al., 2011] These findings were important, as they were the first to (a) identify a molecular mechanism conserved across microbial species in response to spaceflight culture, and (b) clearly connect the spaceflight-induced microbial responses with potential crew health risk. This experiment was quickly followed by a similar experiment by Dr. Nickerson in 2008 in which the increased virulence response was reproduced and an association of the response with the ion concentration in the growth medium was identified [Wilson et al., 2008] [STS-123 / MDRV, p. 237].

The importance of alterations in virulence and virulence characteristics was also investigated by David Niesel from the University of Tex-

as Medical School at Galveston in 2007. Operationally designated as Streptococcus pneumoniae Expression of Genes in Space (SPEGIS), this experiment investigated changes in Streptococcus pneumoniae, a clinically relevant opportunistic pathogen, in response to culture during spaceflight [STS-118 / SPEGIS, p. 236]. In agreement with the results observed using S. typhimurium, S. pneumoniae demonstrated changes in gene expression and virulence characteristics. As SPEGIS demonstrated alterations in gram-positive organisms in response to spaceflight, these results indicated that the changes in virulence characteristics may apply to a wide variety of microorganisms, including many that are commonly associated with crewmembers during a mission.

A fundamental characteristic of many microorganisms that influences their virulence and interaction with their environment is their propensity to attach to surfaces and develop complex communities called biofilms. On Earth, once microorganisms form these biofilms, they can take on new characteristics, which can have important impacts on human health and the successful performance of engineering systems [Costerton et al., 1995]. In an effort to begin understanding if and how microbial biofilms form in the spaceflight environment, Barry Pyle at Montana State University investigated the formation of biofilms on STS-81 using Burkholderia cepacia, a common contaminant of spacecraft systems, such as in the potable water system of the Space Shuttle [Koenig et al., 1995] [STS-81 / Biorack 2, p. 233]. His team confirmed the ability of microorganisms to form biofilms during spaceflight and confirmed their ability to alter their characteristics once attached to surfaces.

In the future, microorganisms have the potential to provide tremendous benefits during human exploration of space, such as biological processing systems and probiotics for crew health; however, they could also pose a hazard to the crew as allergens or agents of infectious disease. Thus, any changes in their characteristics during spaceflight could have a tremendous impact on the mission's success. Interestingly, for the general public on Earth, the knowledge from understanding unique spaceflight responses has the potential to be beneficially translated into novel vaccines, therapeutics, and industrial applications. As such, the avid interest of the scientific and commercial communities in these novel findings is not surprising, especially with the tremendous potential of recent and future spaceflight experiments. On STS-131, Cheryl Nickerson recently challenged mammalian cells with S. typhimurium during a spaceflight experiment to better understand the unique mechanism(s) behind the changes in S. typhimurium virulence when the pathogen encounters its host during flight [STS-131 / STL Immune, p. 242]. Likewise, David Niesel has continued virulence investigations of S. pneumoniae with flight experiments aboard STS-123 [STS-123 / MDRV, p. 238] and STS-129 [STS-129 / SPEGIS-2, p. 241]. Building on the findings of Barry Pyle, [STS-81 / Biorack 2, p. 233] as well as Drs. Nickerson and Niesel, new investigations have been performed by Cynthia Collins from Rensselaer Polytechnic Institute using P. aeruginosa and Staphylococcus aureus, two opportunistic microorganisms of concern during human exploration of space. Her studies on STS-132 [STS-132 / Micro-2, p. 243] and STS-135 [STS-135 / Micro-2A, p. 245] were designed to perform a more thorough investigation of biofilms and lead us toward effective methods to prevent biofilm formation. Taken together, these studies are bridging the gap between fundamental and applied research to build our knowledge of how microorganisms interact with their host and other environments both in space and on Earth.

List of referenced flight experiments:
STS-42 / IML-1, C.V. Bruschi, Cell Division, Mitotic Recombination and Onset of Meiosis by Yeast Cells during Space Flight (YEAST)

STS-81 / Biorack 2, B. Pyle, Bacterial Growth on Surfaces in Microgravity and on Earth

STS-115 / 12A, C. Nickerson, Effect of Spaceflight on Microbial Gene Expression and Virulence (Micrbobe)

STS-118 / SPEGIS, D. Niesel, Streptococcus Pneumoniae Expression of Genes in Space

STS-123 / MDRV(a), C. Nickerson, Effect of Spaceflight on Microbial Gene Expression and Virulence (Microbe)

STS-123 / MDRV(b), D. Niesel, Streptococcus Pneumoniae Expression of Genes in Space (SPEGIS)

STS-129 / SPEGIS-2, D. Niesel, Streptococcus Pneumoniae Expression of Genes in Space (SPEGIS) Raeflight

STS-131 / STL Immune, C. Nickerson, RNA Binding Proteins as Evolutionarily Conserved Cellular Spaceflight Response Mechanisms–Ground/Flight

STS-132 / Micro-2, C. Collins, Gravitational Effects on Biofilm Formation During Spaceflight (Micro-2)

STS-135 / Micro-2A, C. Collins, Gravitational Effects on Biofilm Formation During Spaceflight (Micro-2A)

Progress 13P / YeastGap1, C. Nickerson, Effect of Spaceflight on Microbial Gene Expression in Saccharomyces cerevisiae (Yeast GAP 1)

Literature references cited
Bruschi, C.V.; and Esposito, M.S.: BIORACK on Spacelab IML-1. C. Mattock (Ed.), European Space Agency SP-1162, Noordwijk, The Netherlands, 1995, pp. 83–93.

Costerton, J.W.; Lewandowski, Z.; Caldwell, D.E.; Korber, D.R.; and Lappin-Scott, H.M.: Microbial Biofilms, Annual Review of Microbiol¬ogy, vol. 49, 1995, p. 711.

Crabbe, A.; Schurr, M.J.; Monsieurs, P.; Morici, L.; Schurr, J.; Wilson, J.W.; Ott, C.M.; Tsaprailis, G.; Pierson, D.L.; Stefanyshyn-Piper, H.; and Nickerson, C.A.: Transcriptional and Proteomic Responses of Pseudomonas aeruginosa PAO1 to Spaceflight Conditions Involve Hfq Regulation and Reveal a Role for Oxygen. Ap¬plied Environmental Microbiology, vol. 77, Feb. 2011, p. 1221.

Dickson, K.J.: Missing Summary of Biological Spaceflight Experiments With Cells. Am. Soc. for Gravitational and Space Biology Bulletin, vol. 4, 1991, p. 151.

Koenig, D.W.; Mishra, S.K.; and Pierson, D.L.: Removal of Burkholde¬ria cepacia Biofilms With Oxidants. Biofouling, vol. 9, 1995, p. 51.

Taylor, G.R.: Space Microbiology. Annual Review of Microbiolgy, vol. 28, 1974, p. 121.

Wilson, J.W.: The Effect of Spaceflight on Bone and Cartilage Cell Differentiation. Fifth International Conference on the Chemistry and Biology of Mineralized Tissues, Kohler, Wisconsin, PLoS ONE, vol. 3, 2008, p. e3923.

Wilson, J.W.; Ott, C.M.; Quick, L.; Davis, R.; Höner zu Bentrup, K.; Crabbé, A.; Richter, E.; Sarker, S.; Barrila, J.; Porwollik, S.; Cheng, P.; McClelland, M.; Tsaprailis, G.; Radabaugh, T.; Hunt, A.; Shah, M.; Nel¬man-Gonzalez, M.; Hing, S.; Parra, M.; Dumars, P.; Nor¬wood, K.; Bober, R.; Devich, J.; Ruggles, A.; Cde¬Baca, A.; Narayan, S.; Benjamin, J.; Goulart, C.; Rupert, M.; Catella, L.; Schurr, M.J.; Buchanan, K.; Morici, L.; McCracken, J.; Porter, M.D.; Pierson, D.L.; Smith, S.M.; Mergeay, M.; Leys, N.; Stefanyshyn-Piper, H.M.; Gorie, D.; and Nickerson, C.A.: Media Ion Composition Controls Regula¬tory and Virulence Response of Salmonella in Spaceflight. Proc. of the National Academy of Sciences, USA, Sept. 27, 2007.

Launch Date 1/22/1992 **Landing Date** 1/30/1992

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Cell Division, Mitotic Recombination and Onset of Meiosis by Yeast Cells during Spaceflight (YEAST)

Science Discipline
Microbiology

Investigator	Institute
C.V. Bruschi	East Carolina University

Co-Investigator(s)	Institute
Esposito, M.S.	Lawrence Berkeley Laboratory

Research Subject(s)
Saccharomyces cerevisiae (Yeast)

Ground-Based Controls
Delayed Synchronous Control

Key Flight Hardware
Biorack, Passive Thermal Conditioning Unit (PTCU)

Selected Publications
Bruschi, C.V. and Esposito, M.S.: Cell Division, Mitotic Recombination and Onset of Meiosis by Diploid Yeast Cells During Space Flight. In: BIORACK on Spacelab IML-1, C. Mattock, ed. Noordwijk, The Netherlands, European Space Agency SP-1162, 1995, pp. 83–93.

Bruschi, C.V. and Esposito, M.S.: Diploid Yeast Cells Yield Homozygous Spontaneous Mutations. Current Genetics, vol. 23, nos. 5–6, May–June 1993, pp. 430–434.

Objectives/Hypothesis
The principal objectives of the flight experiment were to determine the effects of the spaceflight environment on cell yield, survival, and ability to undergo meiosis, and to monitor mitotic chromosome segregation and recombination in the spaceflight environment. Saccharomyces cerevisiae, a model unicellular eukaryote was used in the experiment. Two types of yeast cultures were flown: mitotic cell cultures in which yeast cell populations are established by budding and mitosis, and meiotic cell cultures in which yeast cells undergo meiosis and acospore formation. The mitotic cell cultures are microbial analogs of human somatic mitotic cell division, while the meiotic cell cultures are microbial analogs of human meiosis and gamete formation.

Approach or Method
Two yeast diploid hybrids were prepared. STS42-1 is a Rec+, Rad+, Spo+ strain capacle of mitosis or meiosis at both 22° or 36°. STS42-2 is a temperature conditional strain that exhibits Rec+, Rad+, Spo+ at 22°, but Rec-, Rad-, and Spo- at 36°, and grows mitotically at either temperature. The Rec-, Rad-, Spo- phenotype is imposed by the homozygous rec1-1 mutation. Both diploid hybrids incorporate genetic markers that were used to detect mitotic gene conversion, mitotic intergenic recombination, choromosomal loss or nondisjunction, as well as the onset of meiosis in cells at late stationary phase. Two cultures of each strain were incubated in flight under four conditions: in microgravity at 22°, in a 1-g centrifuge at 22°, in microgravity at 36°, and in a 1-g centrifuge at 36°. The ground cultures were incubated under similar conditions, static at 22°, in a 1-g centrifuge at 22°, static at 36°, and in a 1-g centrifuge at 36°. All cell cultures were analyzed with respect to: 1) the total number of cells and buds produced during growth, 2) the percent survival of the cells following plating on nutrient synthetic complete growth medium, 3) frequencies of the cells having phenotypes indicative of mitotic gene conversion, intergenic recombination, and/or mitotic chromosome segregation anomilies, and 4) presence of cells that entered meiosis during the stationary phase following cessation of mitotic cell division.

Results
The analysis of the cultures demonstrates that yeast populations can be propagated in the spaceflight environment and that genetic systems designed to monitor chromosome behavior can be used to study the genetic impacts of spacefight. There was no marked enhancement or reduction in total cell yield due to microgravity conditions. The incubation temperature appears to be the principle factor in the total cell yield; cell densities of cultures incubated at 22° were greater by a factor of 2 or less than the densities of those incubated at 36°. The average survival of the STS42-1 flight culture cells ranged from 51% to 75%, with the 22° cultures being the highest. The average survival rate of the STS42-2 flight cell cultures ranged from 24% to 72% with the 22° cultures being the highest. The most striking discovery was the higher than expected recovery of Rec- intergenic mitotic recombinants from the 36° flight cultures. Rec- is recombinate deficient at 36° in ground controls. STS42-2 also preserved its Rec- phenotype during flight at 36° with respect to resistent segregants due to gene conversion, events that result in mitotic segregants and failure to initiate meiosis. One hypothesis for this behavior is a difference in the nature of the lesion that initiates mitotic recombination in flight as opposed to ground.

STS-81 / Biorack 2

Launch Date 1/12/1997 **Landing Date** 1/22/1997

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Bacterial Growth on Surfaces in Microgravity and on Earth

Science Discipline
Microbiology

Investigator	Institute
B. Pyle	Montana State University

Co-Investigator(s)	Institute
McFeters, G.A.	Montana State University

Research Subject(s)
Burkholderia cepacia (Bacterium)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Biorack, Passive Thermal Conditioning Unit (PTCU), Ambient Temperature Recorder (ATM)

Selected Publications
Pyle, B.H.; Broadaway, S.C.; and McFeters. G.A.: Burkholderia Cepacia Biofilm Growth and Disinfection in Microgravity. 31st International Conference on Environmental Systems, Orlando, Fla. SAE Technical Paper Series 2001-12-2128. Society of Automotive Engineers, Warrendale, Penn., July, 2001.

Pyle, B.H.; McFeters, G.A.; Broadaway, S.C.; Johnsrud, C.K.; Storga, R.T.; and Borkowski, J.: Bacterial Growth on Surfaces and in Suspensions. In Biorack on Spacehab. Biological Experiments on Shuttle to Mir Missions 03, 05, and 06. European Space Agency SP-1222, 1999.

Objectives/Hypothesis
In the context of human life support in spaceflight, there is clearly a need for the highest possible bacterial water quality to limit the risks of infections in human occupants and minimize water systems deterioration. Biofouling bacteria are among the most common organisms isolated from Space Shuttle water systems. This experiment hoped to determine the effects of spaceflight and microgravity on the formation of biofilms by bacteria.

Approach or Method
Approaches have been developed on Earth that are useful for investigating biofilms in the spacecraft environment. Biofilm coupons were inoculated in culture chambers and either fixed in space or returned live for postflight analyses.

Results
The results indicate that biofilms were formed both in all environmental conditions; microgravity, 1-G centrifuge in microgravity, 1-G centrifugation on Earth, and Earth's gravity. Bacterial growth was enhanced in microgravity, particularly in iodine solution and the associated biofilm. Data from the flight suggests that biofilms grew about as well, or slightly more rapidly, in microgravity compared to the spaceflight centrifuge 1-G and Earth-based controls. Further interpretation of the results will be completed at a later date.

Progress 13P / YeastGAP1

Launch Date 1/29/2004

Landing Date 8/9/2005

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Effect of Spaceflight on Microbial Gene Expression in *Saccharomyces cerevisiae* (Yeast GAP 1)

Science Discipline
Microbiology

Investigator / Institute
C. Nickerson — Tulane University

Co-Investigator(s) / Institute
Hammond, T.G. — Tulane University Medical Center

Research Subject(s)
Saccharomyces cerevisiae (Yeast)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Group Activation Pack (GAP)

Selected Publications
None

Objectives/Hypothesis
The objective of this experiment was to identify the *S. cerevisiae* genes that provide a selective advantage or disadvantage for cell survival in the space environment. The genes that convey a survival advantage and those that determine a survival disadvantage in response to spaceflight were identified by whole genome microarray-mediated fitness expression profiling of diploid homozygous and heterozygous *S. cerevisiae* cultures. The results will be compared to those obtained when the same experiments are performed, with the identical microbes cultured under ground-based conditions of modeled microgravity.

Approach or Method
A mixture of molecularly engineered isogenic yeast strains that differ only in a single gene locus were utilized. Each gene had been replaced with an identifying Òbar codeÓ and the mixture contained a deletion strain for every gene in the yeast genome. The yeast deletion strain mixture was dried down and spotted on filter paper preflight. Experiment was activated on orbit with introduction of yeast extract, peptone, dextrose (YPD) media. The cultures grew at ambient temperature under the selective pressure of microgravity for 60–72 hours and terminated by introduction of the fixative RNALater II. Postflight, the Deoxyribonucleic Acid (DNA) was extracted from the cultures, and the bar codes amplified by Polymerase Chain Reaction (PCR). The resulting product was annealed to a gene microarray chip comprising spots for the complementary sequence of each barcode.

Results
Experiment was lost due to Hurricane Katrina. New experiment, Micro-4, was launched on STS-135 in July 2011.

Launch Date 9/9/2006 **Landing Date** 9/21/2006

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Effect of Spaceflight on Microbial Gene Expression and Virulence (Microbe)

Science Discipline
Microbiology

Investigator	Institute
C. Nickerson	Arizona State University

Co-Investigator(s)	Institute
Hammond, T.G.	Tulane University Medical Center
Schurr, M.	Tulane University Medical Center
Buchanan, K.	Tulane University Medical Center

Research Subject(s)
Salmonella typhimurium, Pseudomonas aeruginosa (Bacterium)
Candida albicans (Fungus)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Fluid Processing Apparatus (FPAs), Group Activation Packs (GAPs)

Selected Publications
Wilson, J.W.; Ott, C.M.; Hoener zu Bentrup, K.; Ramamurthy, R.; Quick, L.; Porwollik, S.; Cheng, P.; McClelland, M.; Tsaprailise, G.; Radabaugh, T.; Hunt, A.; Fernandez, D.; Richter, E.; Shah, M.; Kilcoyne, M.; Joshi, L.; Nelman-Gonzalez, M.; Hing, S.; Parra, M.; Dumars, P.; Norwood, K.; Bober, R.; Devich, J.; Ruggles, A.; Goulart, C.; Rupert, M.; Stodieck, L.; Stafford, P.; Catella, L.; Schurr, M.J.; Buchanan, K.; Morici, L.; McCracken, J.; Allen, P.; Baker-Coleman, C.; Hammond, T.; Vogel, J.; Nelson, R.; Pierson, D.L.; Stefanyshyn-Piper, H.M.; and Nickerson, C.A.: Space Flight Alters Bacterial Gene Expression and Virulence and Reveals a Role for Global Regulator Hfq. Proceedings of the National Academy of Sciences of the United States of America, vol. 104, no. 41, 2007, pp. 16299–16304.

Objectives/Hypothesis
The objective of this experiment was to determine the effects of spaceflight, and nutritional status, on gene expression and virulence of three microbrial pathogens: *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Candida albicans*.

Approach or Method
Three Group Activation Packs (GAPs) were assigned to each of four microbial projects: (1) *S. typhimurium* in rich media, (2) *S. typhimurium* in minimal media, (3) *P. aeruginosa* in rich media, and (4) *C. albicans* in rich media. The thermal and radiation profiles of the flight samples were collected by two temperature loggers and two radiation area monitors (RAMs), respectively. Synchronous ground controls (12 GAPs) were loaded at the same time and in the same configuration as the flight samples. Asynchronous ground controls, using the recorded temperature flight data, were performed at the PI lab postflight utilizing a Rotating Wall Vessel (RWV). Fluid Processing Apparatus (FPAs) were loaded with flight reagents (terminators and growth media) and static cultures and installed into the GAPs at approximately launch minus 5 (L-5) days. The loaded GAPs were handed over for a late load into the orbiter to ensure that the healthiest static cultures in stasis medium were utilized for the spaceflight experiment.

Results
Data showed that *Salmonella typhimurium* grown in rich lysogeny broth (LB) media in spaceflight displayed increased virulence, biofilm-like formation, and global alterations in global gene expression, as compared to synchronous ground controls. It was hypothesized that media ion concentrations could be manipulated to prevent/turn off the enhanced Salmonella virulence imparted during spaceflight (tested on STS-123). A total of 167 genes in the flight S. typhimurium were expressed differently to those in the ground controls. Sixty-four of these genes are involved in the expression of Hfq (a protein that binds to messenger ribonucleic acid (mRNA)) suggesting that Hfq is involved in regulating some of the Salmonella responses to spaceflight.

Launch Date 8/8/2007 **Landing Date** 8/21/2007

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Streptococcus pneumoniae Expression of Genes in Space (SPEGIS)

Science Discipline
Microbiology

Investigator / Institute
D. Niesel — University of Texas Medical Branch (UTMB)

Co-Investigator(s) / Institute
None

Research Subject(s)
Streptococcus pneumoniae (Bacteria)

Ground-Based Controls
Asynchronous Control (3 cannisters, 9 vials) Incubated in Ground Merlin Unit at KSC

Key Flight Hardware
SPEGIS Canister

Selected Publications
Allen, C.A.; Galindo, C.; Williams, N.; Pandya, U.; Chopra, A.; and Niesel, D.: Global Transcriptoral Analysis of *Streptococcus Pneumoniae* in Response to Low-Shear Modeled Microgravity. Gravitational and Space Biology, vol. 19, no. 2, Aug. 2006, pp. 143–44.

Allen, C.A.; Galindo, C.L.; Pandya, U.; Watson, D.A.; Chopra, A.K.; and Niesel, D.W.: Transcriptional Profiles of *Streptococcus Pneumoniae* Grown Under Different Conditions of Normal Gravitation. Acta Astronaut, vol. 60, nos. 4–7, Feb.–Apr. 2007, pp. 433–444.

Objectives/Hypothesis
Streptococcus pneumoniae Expression of Gene in Space (SPEGIS) examined the behavior and growth of bacteria in microgravity and investigated the effects of the space environment on the gene expression, protein production, and virulence of the opportunistic pathogenic bacteria, *Streptococcus pneumoniae* ATCC 6304. The data collected provides insight on these types of opportunistic bacterial infections that may occur during long-duration space missions, the risks to crew members, and possible future mitigation strategies for these infections.

Approach or Method
Vials containing bacterial cultures were loaded into the SPEGIS canister Assembly. A total of three canisters were flown on the space shuttle. Each canister contained three 8-ml polypropylene vials. The vials were inserted into vial jackets to improve contact and enhance thermal transfer. The compression pad eliminated space between the vials and canister lid. The SPEGIS payload was launched while stored at +4°C in the Microgravity Environment Research Locker/INcubator (MERLIN). After the orbiter docked with the ISS, the canisters were transferred to the Minus Eighty Laboratory Freezer for ISS (MELFI) for +2°C cold stowage. For the on-orbit incubation growth phase, the canisters were transferred back to MERLIN and then incubated at +37.5°C. After incubation, the canisters were transferred back to MELFI for –95°C cryopreservation. The samples were returned to Earth while stored in the Double Coldbag with ICEPACs at –32°C, which maintain the integrity of the frozen samples for postflight analysis.

Results
Flight and ground control bacterial cultures both reached stationary phase as confirmed by optical density. Viable cell recovery as quantitated by serial dilution and plating were 5 logs lower than expected possibly due to freezing methods used. Protein levels were consistent with late log phase or early stationary phase. A small punctate bacterial colony variation was twice as prevalent in flight samples as observed in ground samples. Activity of enzymes associated with adherence and host-cell binding was increased in flight samples. Neuraminidase activity, measured fluorometrically using the substrate 2'-(4-methyl-umbelliferyl)-d-n-acetylneuraminic acid, was 1.4 times higher in flight than in ground control samples. Beta-galactosidaase activity in flight samples was 1.3–1.4 times higher in supernatants and directly assayed cultures, and twice as high in resuspended pellets. Activity of pneumolysin, a pore-forming toxin associated with cellular and tissue damage, was 3 times higher in flight cultures, 2.3 times higher in flight supernatants, but unchanged in resuspended pellets. Proteome differences were assessed by 2-dimensional gel electrophoresis that revealed the 13 proteins that were up-regulated, while 4 were down-regulated in flight samples. Global transcriptional analysis by NA microarray revealed several differentially expressed genes from diverse functionally groups, including 32 virulence-associated genes. Principle Component Analysis also revealed a clear difference in expression pattern between flight and ground samples, suggesting bacteria adapt to the space environment by expressing a unique set of genes.

Launch Date 3/11/2008 **Landing Date** 3/26/2008

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Effect of Spaceflight on Microbial Gene Expression and Virulence (Microbe)

Science Discipline
Microbiology

Investigator	Institute
C. Nickerson	Arizona State University

Co-Investigator(s)	Institute
Hammond, T.G.	Tulane University Medical Center
Schurr, M.	Tulane University Medical Center
Pierson, D.	NASA Johnson Space Center (JSC)

Research Subject(s)
Salmonella typhimurium, Pseudomonas aeruginosa (Bacterium)
Candida albicans (Fungus)

Ground-Based Controls
Synchronous Control, Asynchronous Control

Key Flight Hardware
Fluid Processing Apparatus (FPAs), Group Activation Packs (GAPs)

Selected Publications
Wilson, J.W.; Ott, C.M.; Quick, L.; Davis, R.; Höner zu Bentrup, K.; Crabbé, A.; Richter, E.; Sarker, S.; Barrila, J.; Porwollik, S.; Cheng, P.; McClelland, M.; Tsaprailis, G.; Radabaugh, T.; Hunt, A.; Shah, M.; Nelman-Gonzalez, M.; Hing, S.; Parra, M.; Dumars, P.; Norwood, K.; Bober, R.; Devich, J.; Ruggles, A.; CdeBaca, A.; Narayan, S.; Benjamin, J.; Goulart, C.; Rupert, M.; Catella, L.; Schurr, M.J.; Buchanan, K.; Morici, L.; McCracken, J.; Porter, M.D.; Pierson, D.L.; Smith, S.M.; Mergeay, M.; Leys, N.; Stefanyshyn-Piper, H.M,; Gorie, D.; and Nickerson, C.A.: Media Ion Composition Controls Regulatory and Virulence Response of Salmonella in Spaceflight. PLoS ONE, vol. 3, no. 12, 2008, p. e3923.

Objectives/Hypothesis
Determine the effect of spaceflight on the virulence potential of the pathogenic microorganisms: *S. typhimurium*, *P. aeruginosa*, and *C. albicans*, immediately following their return from spaceflight
Determine the effect of spaceflight on cellular morphology of the pathogenic microorganisms: *S. typhimurium*, *P. aeruginosa*, and *C. albicans*, immediately following their return from spaceflight.
Compare *S. typhimurium* cultures grown in nutrient limited media to those grown in nutrient rich media to determine the potential effects of nutritional status on changes in gene expression, morphology, and virulence properties during spaceflight.

Approach or Method
Three GAPs were used for each of four projects: *S. typhimurium* in rich media; *S. typhimurium* in minimal media; *P. aeruginosa* in rich media; *C. albicans* in rich media. Temperature loggers and radiation area monitors (RAMs) were used to collect the thermal profile and radiation profile experienced by the flight samples, respectively. Synchronous ground controls (12 Group Activation Packs (GAPs)) were loaded at the same time as the flight. Asynchronous ground controls were performed at the PI lab postflight utilizing the Rotating Wall Vessel and the temperature data recorded from the flight experiment temperature logging devices. The Fluid Processing Apparatus (FPAs) (flight and synchronous) were loaded with the flight reagents (terminators and growth media) and static cultures and installed into the GAPs at approximately launch minus 5 (L-5) days. On-orbit, the cultures were activated with growth media on Flight Day 10 and allowed to grow for 24 ±2 hours. The experiment was terminated with addition of either RNA Later II, for postflight microarray studies, or fresh media for postflight LD50 studies. Postflight, microarray gene expression was performed on the fixed samples. A mouse (465 BALB/c female) LD-50 study was also performed at Kennedy Space Center (KSC) to assess changes in virulence potential.

Results
Confirmed the effects of microgravity observed in the STS-115 Microbe experiments and homed in on the importance of the growth medium for gene expression and virulence of the microbe *Samonella* during spaceflight. A large number of Hfq-regulated genes were differentially expressed (167). Hfq is known to regulate one-third of the 167 differentially expressed genes. Hfq may serve to globally modify bacterial responses to microgravity.

STS-123 / MDRV

Launch Date 3/11/2008 **Landing Date** 3/26/2008

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Streptococcus pneumoniae Expression of Genes in Space (SPEGIS)

Science Discipline
Microbiology

Investigator	Institute
D. Niesel	University of Texas Medical Branch (UTMB)

Co-Investigator(s)	Institute
None	

Research Subject(s)
Streptococcus pneumoniae (Bacteria)

Ground-Based Controls
Synchronous Control (activated 1/2 hour after flight samples)

Key Flight Hardware
Fluid Processing Apparatus (FPAs), Group Activation Packs (GAPs)

Selected Publications
Allen, C.A.; Galindo, C.; Williams, N.; Pandya, U.; Chopra, A.; and Niesel, D.: Global Transcriptoral Analysis of *Streptococcus Pneumoniae* in Response to Low-Shear Modeled Microgravity. Gravitational and Space Biology, vol. 19, no. 2, Aug. 2006, pp. 143–144.

Allen, C.A.; Galindo, C.L.; Pandya, U.; Watson, D.A.; Chopra, A.K.; and Niesel, D.W.: Transcriptional Profiles of *Streptococcus Pneumoniae* Grown Under Different Conditions of Normal Gravitation. Acta Astronaut, vol. 60, nos. 4–7, Feb.–Apr. 2007, pp. 433–444.

Objectives/Hypothesis
The objective of this experiment was to investigate the alteration of virulence of *Streptococcus pneumoniae* ATCC 6304 after growth in spaceflight compared to cultures grown on Earth. This bacterium was investigated on a previous flight (STS-118, SPEGIS I) for alterations in gene and protein expression and is an opportunistic respiratory pathogen. It has been isolated previously from a crew member's nasopharynx preflight indicating that this normally commensal bacterium could become a risk during flight.

Approach or Method
Streptococcus pneumoniae ATCC 6304 was cultured in spaceflight or in parallel ground controls using Group Activation Pack (GAP) hardware. Microbial Drug Resistance and Virulence (MDRV) flew on STS-123. The flight Fluid Processing Apparatus (FPAs) in Group Activation Pack 8 (GAP8) were activated on MET day 11. The ground-control (GC) samples were activated one hour later. FPAs were received ~2.5 hours after landing. The culture media from each sample was collected and aliquots diluted for serial dilution and plate counting on Blood Agar plates. There was minimal growth in the flight samples. The optical density at 600 nm of flight cultures with high initial loading was similar with the ground control with low loading, which showed a slightly higher value. Because of similarity of optical density, which is indicative of bacterial cell numbers, the flight high-load and ground-control low pools were selected for the postflight mouse virulence study.

Results
There was an obvious and clear difference in mouse virulence between the flight and ground-control cultures in terms of LD50 (16-fold lower for the flight-grown cultures). However, the LD50 of these suboptimal cultures were much higher (FLT 35-fold; GC 566-fold) than that observed for strain ATCC 6304 under optimal culture conditions (37C). The differences between flight and ground cultures may reflect virulence differences arising from changes in bacterial gene expression or altered physiology/metabolism in the space environment. Alternatively, the differences observed could arise from the differential growth temperature between the flight and ground controls. This seems unlikely as the ground cultures were at a higher temperature closer to the growth optimal where maximal virulence is observed. However, this remains to be fully established with additional flight experimentation and ground controls.

Launch Date 3/11/2008

Landing Date 3/26/2008

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Bacterial Physiology and Virulence on Earth and in Microgravity

Science Discipline
Microbiology

Investigator / Institute
B. Pyle — Montana State University

Co-Investigator(s) / Institute
None

Research Subject(s)
Pseudomonas aeruginosa (Bacterium)

Ground-Based Controls
Synchronous Control (30 minute delayed)

Key Flight Hardware
Fluid Processing Apparatus (FPAs), Group Activation Packs (GAPs)

Selected Publications
Broadaway, S.; Goins, T.; Crandell, C.; Richards, C.; Patel, M.; and Pyle, B.: Spaceflight Effects on Virulence of *Pseudomonas Aeruginosa*. Proceedings of the Symposium Life in Space for Life on Earth, June 22–27, 2008, Angers, France. European Space Agency SP-663, Dec. 2008.

Objectives/Hypothesis
The focus of this research was to characterize the effects of microgravity on cell proliferation and production of Exotoxin A (ETA) in both *P. aeruginosa* PA01 and PA103. *P. aeruginosa* PA01 is a stock strain that has historically been used for virulence and other studies; it is known to have a low level of ETA production, while strain PA103 produces much higher amounts of ETA under similar conditions.

Approach or Method
Both *P. aeruginosa* PA01 and PA103 were cultured in defined medium (SDM1) that limits the production of Exotoxin A. The experiment was contained in Fluid Processing Apparatus (FPA) tubes that were placed into Group Activation Packs (GAPs); each GAP held eight FPAs. The *pseudomonas* exotoxin experiment occupied two GAPs, including four replicates of each of the Time O (T0) cultures. The first chamber of each FPA tube contained Modified Simple Defined Medium 2 (MSDM2), a growth medium that stimulates ETA production; the second chamber contained the culture suspension; and the third chamber contained a fixative (MSDM2 containing 0.4% formalin). The experiment was activated on-orbit (10 days after launch and 14 days after preparation) by introducing the culture suspension into the growth medium (TO). Ground controls were activated 30 minutes later. After 51.5 hours (ca. 2 days, T2D) incubating at ambient temperature, the experiment was terminated by adding fixative. Postflight, the cells were counted and ETA measured by enzyme-linked immunosorbent assay (ELISA).

Results
Both ETA and cell count results at the start of incubation (T0) were similar for flight and ground cultures. Results after 51.5 hours incubation (2 days) indicate that PA103 produced, as expected, more ETA than PA01 in both flight samples and ground controls. However, cell count numbers for both cultures at 2 days, either after ground or flight incubation, were all very close to 109 cells/ml. This shows that on-orbit incubation did not affect growth of these cultures within 2 days at ambient temperatures. PA103 produced about 10% more ETA in flight samples, both at T0 and after 2 days incubation, while ETA results for PA01 were about the same for flight vs. ground conditions. However, these results are probably not statistically significant (yet to be determined) because of the variability of results after incubation.

STS-123 / MDRV

Launch Date 3/11/2008 **Landing Date** 3/26/2008

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Drug Resistance and Virulence (MDRV): *Saccharomyces cerevisiae* Gene Expression and Susceptibility to Voriconazole During Spaceflight

Science Discipline
Microbiology

Investigator	Institute
M. McGinnis	University of Texas Medical Branch (UTMB)

Co-Investigator(s)	Institute
None	

Research Subject(s)
Saccharomyces cerevisiae (Yeast)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Fluid Processing Apparatus (FPAs), Group Activation Packs (GAPs)

Selected Publications
Parra, M.; Ly, D.; Ricco, A.J.; McGinnis, M.R.; and Niesel, D.: The PharmaSat Nanosatellite Platform for Life Science Experimentation: Effects of Space Flight on Antifungal Activity Against *Saccharomyces Cerevisiae*. American Society for Gravitational and Space Biology Bulletin, vol. 23, no. 1, 2009, p. 30.

Objectives/Hypothesis
The objective was to investigate the alteration in antifungal drug response of *Saccharomyces cerevisiae* after growth in spaceflight compared to growth on Earth. 1. Evaluate the single drug dilution of voriconazole selected to be flown on PharmaSat. 2. Investigate genomic expression as influenced by voriconazole during spaceflight. *Saccharomyces cerevisiae* has been investigated for susceptibility to voriconazole and alterations in gene expression using the High Aspect Ratio Vessel (HARV) bioreactor and Gene Chip Yeast Genome S98 Arrays in the laboratory. Modeled microgravity data indicate that *S. cerevisiae* has an increased resistance to voriconazole and that gene expression involving the ergosterol biosynthetic pathway is altered. GAP experiments allow confirmation of these modeled microgravity data. Susceptibility data during the spaceflight validates the concentration of voriconazole selected to be used in the PharmaSat experiment planned for 2008.

Approach or Method
Yeast was grown under various concentrations of the antifungal compound.

Results
Microgravity and modeled microgravity data suggest that resistance to azoles is increased, which can have an important effect upon management of fungal infections during space habitation.

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Streptococcus pneumoniae Expression of Genes in Space (SPEGIS) Reflight

Science Discipline
Microbiology

Investigator	Institute
D. Niesel	University of Texas Medical Branch (UTMB)

Co-Investigator(s)	Institute
None	

Research Subject(s)
Streptococcus pneumoniae (Bacteria)

Ground-Based Controls
Ground Control

Key Flight Hardware
Fluid Processing Apparatus (FPAs), Group Activation Packs (GAPs)

Selected Publications
Allen, C.A.; Galindo, C.; Williams, N.; Pandya, U.; Chopra, A.; and Niesel, D.: Global Transcriptoral Analysis of *Streptococcus Pneumoniae* in Response to Low-Shear Modeled Microgravity. Gravitational and Space Biology Bulletin, vol. 19, no. 2, Aug. 2006, pp. 143–144.

Allen, C.A.; Galindo, C.L.; Pandya, U.; Watson, D.A.; Chopra, A.K.; and Niesel, D.W.: Transcriptional Profiles of *Streptococcus Pneumoniae* Grown Under Different Conditions of Normal Gravitation. Acta Astronaut, vol. 60, nos. 4–7, Feb.–Apr. 2007, pp. 433–444.

Objectives/Hypothesis
Streptococcus pneumoniae Expression of Gene in Space (SPEGIS) examined the behavior and growth of bacteria in microgravity and investigated the effects of the space environment on the gene expression, protein production, and virulence of the bacteria *Streptococcus pneumoniae*. The data collected also provides insight on opportunistic bacterial infections that may occur during long-duration space missions and the risks to crew members. This was a continuation to the MDRV-SPEGIS study in which the PI discovered a profound increase in the virulence of *Streptococcus pneumoniae* grown during flight and injected into mice postflight. Of particular interest was identification of *S. pneumoniae* virulence factors that might account for the higher virulence seen in mice infected with space-grown bacteria.

Approach or Method
The experimental approach for this flight utilized the Group Activation Pack (GAP,) as used in the MDRV experiment. The GAPs held Fluid Processing Apparatus (FPAs). FPAs are glass tubes with fitted rubber septa that make compartments that hold the bacterial samples and sample processing reagents. The cultures were activated for growth when the astronauts activated the GAP, which pushed the rubber septa together in the FPAs, mixing the different components of the cultures (media, inoculum, and then processing reagents).

Results
A similarity of flight samples, regardless of which shuttle and which culturing method were used, was observed. Genes that were identified as differential on average were also reproducible across shuttle flights. In total, 213 individual genes were identified as significantly altered as a result of growth in space.

Collectively, these results provide strong evidence that microgravity environments increase the virulence of this opportunistic pathogen. Understanding virulence enhancement after bacterial growth in the space environment is critical to the long-term goal to devise countermeasures to protect space travelers/residents from infectious diseases during long-term flight or space habitation.

STS-131 STL Immune

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
RNA Binding Proteins as Evolutionarily Conserved Cellular Spaceflight Response Mechanisms—Ground/Flight

Science Discipline
Microbiology

Investigator	Institute
C. Nickerson	Arizona State University

Co-Investigator(s)	Institute

Research Subject(s)
HT-29 Human Intestinal Epithelial Cell Line, A549 Human Lung Epithelial Cell Line, *Salmonella typhimurium* x3339

Ground-Based Controls
Synchronous Control, Asynchronous Control

Key Flight Hardware
Cell Culture Module (CCM)

Selected Publications
Crabbe, A.; Sarker, S.F.; Van Houdt, R.; Ott, C.M.; Leys, N.; Cornelis, P.; and Nickerson, C.A.: Alveolar Epithelium Protects Macrophages From Quorum Sensing-Induced Cytotoxicity in a Three-Dimensional Co-Culture Model. Cellular Microbiology, vol. 13, no. 3, Mar. 2011, pp. 469–481.

Objectives/Hypothesis
The objectives of this flight experiment were directly related to and complemented ground-based work; the goal of which was to identify universally conserved molecular responses to ground-based spaceflight analogue culture across a variety of bacterial and mammalian cell types. The research focus for the flight portion was to investigate the effects of the space flight environment on the host-to-pathogen interaction by conducting the first-ever in-flight infection of human cells with a pathogenic bacteria.

The specific objectives were: a) determine the evolutionarily conserved role for space flight-responsive ribonucleic acid (RNA) binding proteins in human intestinal and lung epithelial cells before and after infection; b) determine the effect of space flight on select innate immune defense mechanisms and stress responses of human epithelial cells before and after infection; and, c) determine the effect of space flight on cellular differentiation and morphology of human intestinal epithelial cells before and after infection.

Approach or Method
Sample analysis is currently being finalized for alterations in protein expression (via global proteomic response profiling/iTRAQ), cellular differentiation, immune function (inflammatory response mediators), and stress responses associated with space flight in the presence and absence of infection. All flight sample comparisons were made to identical synchronous ground controls housed in duplicate Cell Culture Module (CCM) hardware as that used for flight.

Experimental conditions:
1) Twelve bioreactors: 10 with HT-29 cells, 2 with A549 cells.
2) Cells were adherent to the hollow fibers.
3) Five bioreactors were injected with bacteria in flight on mission Day 11.
4) Eight bioreactors were injected with RNALater 2, and four bioreactors were injected with 4% paraformaldehyde, in flight on mission Day 11.
5) All 12 bioreactors were sampled for conditioned medium.
6) Continuous medium circulation at 37°C with 5% CO2.

Results
Sample analysis is finalized for global alterations in both transcriptomic and proteomic profiling, cellular differentiation, immune function (inflammatory response mediators), and stress responses associated with space flight in the presence and absence of infection. All flight sample comparisons were made to identical synchronous ground controls housed in duplicate CCM hardware as that used for flight. Through iTRAQ analysis, we have identified 86 total unique human proteins (95% confidence, 1% FDR) that were expressed by the HT-29 intestinal epithelial cells cultured on orbit either before or after infection with Salmonella typhimurium. These proteins belonged to a range of different functional classes and categories, including those important for metabolism, apoptosis, cell communication, immune function, and others. Of the 89 proteins identified, a unique subset of these were found to be differentially regulated in response to space flight, and showed differences in response to infection between flight and ground cultures. Collectively, we found intriguing differences in how microgravity uniquely impacted the space flight infected intestinal cells as compared to the ground controls. We have also finalized global proteomic response profiling for the A549 lung epithelial cells as well. Moreover, immunohistochemical profiling has revealed differences in both distribution and expression patterns of targeted host cell proteins and alterations in cellular cytoarchitecture.

Launch Date 5/14/2010 **Landing Date** 5/26/2010

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Gravitational Effects on Biofilm Formation During Spaceflight (Micro-2)

Science Discipline
Cell and Molecular Biology

Investigator / Institute
C. Collins — Rensselaer Polytechnic Institute

Co-Investigator(s) / Institute
None

Research Subject(s)
Pseudomonas aeruginosa, Staphylococcus aureus (Bacterium)

Ground-Based Controls
Ground control conducted in parallel at Kennedy Space Center

Key Flight Hardware
Fluid Processing Apparatus (FPAs), Group Activation Packs (GAPs), Commercial Generic Bioprocessing Apparatus (CGBA)

Selected Publications
Kim, W.S.; Tengra, F.K.; Young, Z.; Shong, J.; Pangule, R.; Plawsky, J. L.; Dordick, J.S.; and Collins, C.H.: Gravitational Effects on Biofilm Formation by Pseudomonas Aeruginosa. 26th Annual Meeting of the American Society for Gravitational and Space Biology Program and Abstracts, National Harbor, Maryland, Nov. 2010, p. 25.

Kim, W.S.; Tengra, F.K.; Young, Z.; Shong, J.; Pangule, R.; Plawsky, J. L.; Dordick, J.S.; and Collins, C.H.: Gravitational Effects on Biofilm Formation by Pseudomonas Aeruginosa. Biofilms-IV International Conference Proceedings, Sept. 2010, p. 89.

Objectives/Hypothesis
This experiment was designed to further the understanding of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms. The specific objectives were: compare biofilms formed on mixed cellulose ester membranes in normal gravity and during spaceflight, and examine the influence of mutations affecting motility on biofilm formation and structure; assess the effects of newly developed, non-leaching, nanotechnology-based, antimicrobial surfaces on biofilm formation during spaceflight and assess their potential to reduce the impact of biolfims in future spacecraft designs; and identify changes in gene expression related to biofilm formation and virulence that occur when cells are cultured during spaceflight.

Approach or Method
This experiment utilized Bioserve's Group Activation Packs (GAPs) and Commercial Generic Bioprocessing Apparatus (CGBA), a flight-certified incubator capable of holding 16 GAPs and controlling the temperature between 4ºC and 37ºC. Each GAP holds eight Fluid Processing Apparatus (FPA) inserts, composed of a glass barrel divided into three chambers that are separated from one another by rubber septa. Each FPA contained growth medium with membranes in chamber A, a microbial culture suspended in stasis medium in chamber B, and a termination reagent, or more growth media, in chamber C. The culture growth was initiated in flight by a crew member. After the growth period (48 ±2 hours), a crew member terminated the experiment.

Results
Results demonstrated that biofilm formation by both of these model organisms, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, can occur during spaceflight and demonstrated that these organisms respond differently to the spaceflight environment. Direct measurements of biomass showed *P. aeruginosa* PA14 formed 3- to 5-fold more biofilm during spaceflight compared to normal gravity. In addition, quantitative analysis of confocal laser scanning microscopy (CLSM) images showed increased biofilm biomass ($p < 0.001$) and increased biofilm thickness ($p < 0.001$) in samples grown during spaceflight. A clear effect of phosphate on biofilm biomass was not observed in any biofilm samples. Spaceflight was also observed to increase planktonic biomass by approximately 10-fold, but only in the presence of 5-mM phosphate.

Conclusions: Direct biomass measurement and image analysis demonstrated that biofilm formation by *P. aeruginosa* PA14 increased during spaceflight. Phosphate concentration did not appear to affect the amount of biofilm formed during spaceflight or in normal gravity. However, both phosphate and spaceflight were observed to affect planktonic biomass.

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: Bone Physiology

Title of Study
Genotypic and Phenotypic Changes in Yeast Related to Selective Growth Pressures Unique to Microgravity (Micro-4)

Science Discipline
Microbiology

Investigator	Institute
T.G. Hammond	Durham Veterans Affairs Medical Center, Durham, NC

Co-Investigator(s)	Institute
Stodieck, L.	University of Colorado, Bioserve Space Technologies

Research Subject(s)
Yeast deletion series

Ground-Based Controls
Synchronous Control

Key Flight Hardware
OptiCell Processing Modules, Plate Habitats, Commercial Generic Biosprocessing Apparatus (CGBA)

Selected Publications
Hammond, T: Genotypic and Phenotypic Changes in Yeast Related to Selective Growth Pressures. 28th Annual Meeting of the American Society for Gravitational and Space Research Program and Abstracts, Nov. 2012, New Orleans, Louisiana, p. 64.

Nislow, C.; Lee, A.Y.; Allen, P.L.; Giaever, G.; Smith, A.; Gebbia, M.; Stodieck, L.; Hammond, J.S.; Birdsall, H.H.; and Hammond, T.G.: Genes required for survival in microgravity revealed by genome-wide yeast deletion collections cultured during spaceflight. Biomed Res Int., 2015, Article ID 976458, in press.

Objectives/Hypothesis
The Micro-4 study investigated how yeast cells adapt to the unique aspects of the space environment by using the yeast deletion series; a collection of yeast strains where every gene has been individually knocked out. In this manner, the selective growth of every strain in the yeast deletion series can be assayed. The goal of the study was to understand the different responses and physical effects of microgravity on yeast cells by examining which specific deletion strains best survive. Direct assessment of selective pressures on cell populations through generations using the yeast deletion series is a critical experiment to directly address risks to biological integrity and life-based support systems for long-term occupation of space. Results from this study allow researchers to gain a global perspective on the genes that play a role in survival under microgravity conditions and allow for a more thoNeurophysiologyrough understanding of the effects of microgravity on a model organism.

Approach or Method
Eight OptiCell Processing Modules and four Plate Habitats were loaded with liquid and solid cultures of the yeast deletion series. Cultures were stored in cold stowage using a Commercial Generic Biosprocessing Apparatus (CGBA) until after launch. Once in orbit, half of the cultures were activated by a temperature shift by transferring to a second CGBA. Liquid cultures were subcultured twice during their growth to ensure sufficient generations. Growth was terminated by a temperature shift via transfer back to the cold CGBA.

Results
Scanning electron microscopy (10,000X) demonstrated differences in shape, surface texture and budding pattern when grown in YPD in Opticells as suspension cultures. Static ground controls had egg to barrel shaped cells, with single pole budding, and an undulated surface. Rotating wall vessel grown yeast were round to barrel shaped, with multiple, bi-polar budding, and greatly undulated surface. Space flown cultured yeast were round to egg shaped, with multiple budding > ground Identification of genetic - environmental interactions in the flown and grown samples versus flown-not grown controls. In this analysis the normalized sizes of each of 4 replicates for each single mutant deletion strain was compared to the flown, not-grown samples, that were grown upon their return.

Pathway analysis: The 50 strains with the greatest space-related fitness defects were imported into the meta-analytical tool, GeneMANIA. GeneMANIA comprises a series of algorithms that search large, publicly available biological datasets to find related genes. We then prepared a visual representation of the output from this analysis where each gene is a node in the network, and the links between each node represents one type of interaction between these 50 genes. This preliminary analysis reveals several clusters of functionally interconnected genes, including clusters of genes involved in DNA repair and metabolism, transcription, chromatin remodeling, mitochondrial function, polarity, ribosome assembly, and stress.

Yeast deletion series are a powerful tool to study pathways mediating evolution fitness for life. These studies have allowed us to define the genes which convey survival advantage and disadvantage during space flight, the pathways which the genes populate, and the control mechanisms which determine the changes.

Physiological/Science Discipline: MICROBIAL GROWTH AND VIRULENCE

Title of Study
Gravitational Effects on Biofilm Formation During Spaceflight (Micro-2A)

Science Discipline
Cell and Molecular Biology

Investigator / Institute
C. Collins — Rensselaer Polytechnic Institute

Co-Investigator(s) / Institute
None

Research Subject(s)
Pseudomonas aeruginosa, Staphylococcus aureus (Bacterium)

Ground-Based Controls
Ground control conducted in parallel at Kennedy Space Center

Key Flight Hardware
Fluid Processing Apparatus (FPAs), Group Activation Packs (GAPs), Commercial Generic Bioprocessing Apparatus (CGBA)

Selected Publications
Kim, W.S.; Tengra, F.K.; Young, Z.; Shong, J.; Pangule, R.; Plawsky, J. L.; Dordick, J.S.; and Collins, C.H.: Gravitational Effects on Biofilm Formation by Pseudomonas Aeruginosa. 26th Annual Meeting of the American Society for Gravitational and Space Biology Program and Abstracts, National Harbor, Maryland, Nov. 2010, p. 25.

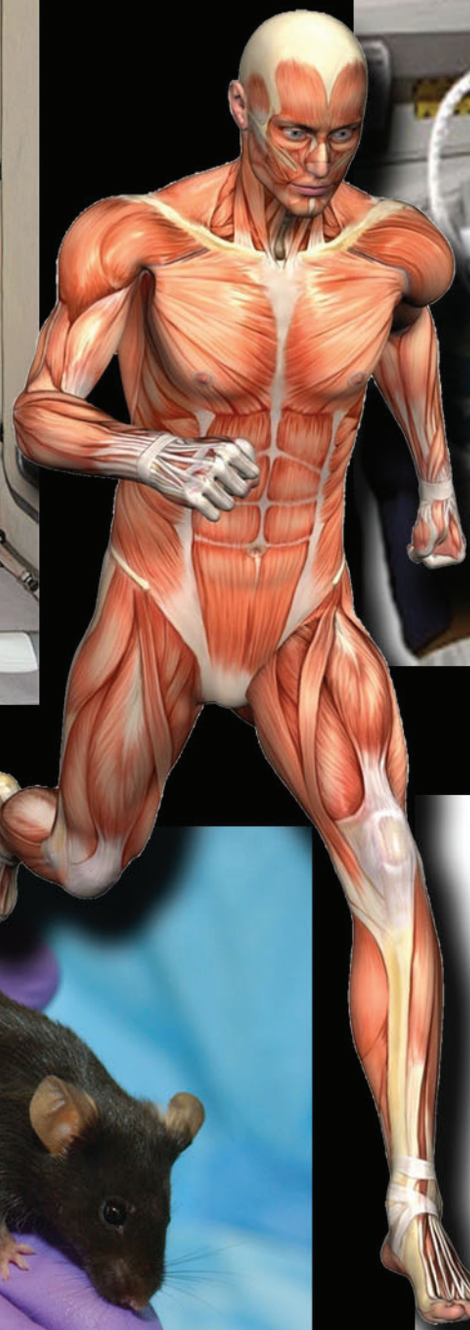
Kim, W.S.; Tengra, F.K.; Young, Z.; Shong, J.; Pangule, R.; Plawsky, J. L.; Dordick, J.S.; and Collins, C.H.: Gravitational Effects on Biofilm Formation by Pseudomonas Aeruginosa. Biofilms-IV International Conference Proceedings, National Harbor, Maryland, Sept. 2010, p. 89.

Objectives/Hypothesis
Micro-2A is a follow-on flight to Micro-2 that flew on STS-132. There is an urgent need to understand the effects of microgravity on the growth, cellular physiology, and cell-cell interactions in microbial biofilms. This information can then be used to curtail harmful activities of microbial consortia thriving as biofilms on the International Space Station and for the long-term success of human space exploration. The Micro-2A experiment studied how gravity alters microbial biofilm formation with the goal of developing new strategies to reduce their impact on the operation of spacecrafts and the health of their crews.

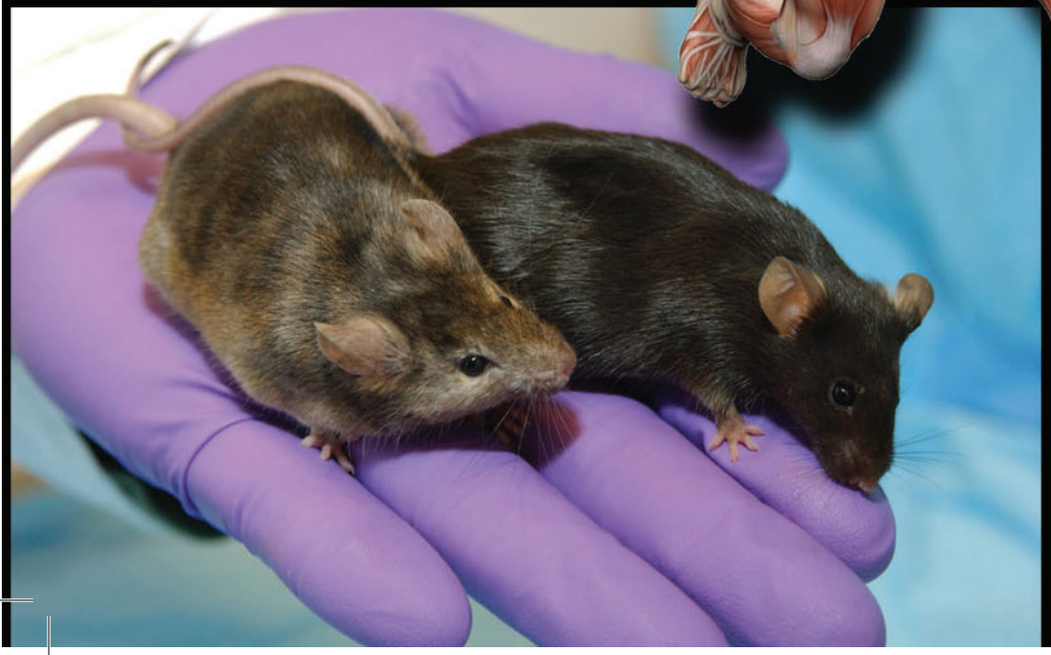
Approach or Method
The experiment compared microbial biofilms formed on membranes in normal gravity and during spaceflight, and examined the influence of mutations affecting bacterial motility on biofilm formation and structure. The experiment assessed the effects of newly developed, non-leaching, nanotechnology-based antimicrobial surfaces on biofilm formation during spaceflight and assessed their potential to reduce the impact of biofilms in future spacecraft designs. The experiment identified changes in gene expression related to biofilm formation and virulence that occur when cells are cultured during spaceflight.

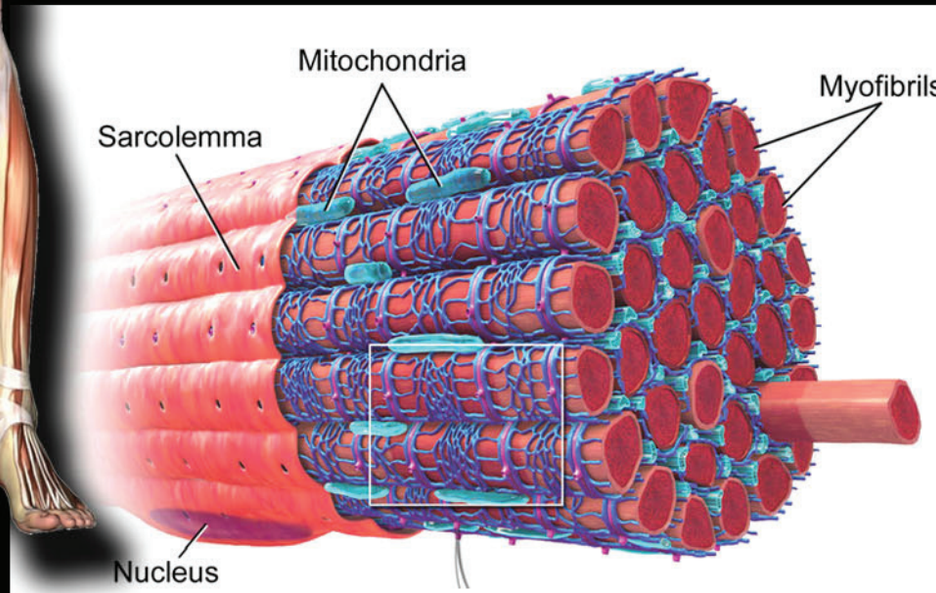
Results
Biofilm formation is significantly increased during spaceflight. Further, it was found that biofilms formed during spaceflight exhibit a novel structure that has not been previously observed and that would be unlikely to form under normal gravity conditions. Recent experiments included *P. aeruginosa* motility mutants and replicates with varied nutrient availability. It was observed that changes in motility, carbon source, and phosphate availability all modulate biofilm formation and the extent to which *P. aeruginosa* responds to spaceflight. Results from these experiments provide insight into the mechanisms underlying the observed differences between biofilms formed in normal gravity and spaceflight.

Muscle Physiology



Muscle Physiology





Muscle Physiology Introduction

Danny A. Riley, Ph.D., Medical College of Wisconsin

During the period 1965–2011, NASA Ames Research Center conducted short-duration (days) spaceflight experiments in Cosmos biosatellite free flyers and Space Shuttle spacecraft for animal studies of skeletal muscle physiology. The space environment generates microgravity (free-fall weightlessness), a unique research tool not available on Earth. These animal studies have provided valuable insights into preventing undesirable adaptation (deconditioning) of muscles in microgravity [Riley, 1996] [STS-40 / SLS-1, p. 276]. The NASA approach to animal flight studies is uniquely multidisciplinary and engages multiple investigators. This powerful holistic approach addresses impacts on multiple organ systems in the same animal. The research literature on astronauts' muscles shows that changes in the muscle physiology of space-flown rodents model the human condition with high fidelity. Knowledge contributed by these structural, physiological, and biochemical studies of muscles in animal models has translated to improved health of astronauts' muscles by influencing the flight surgeon's prescription for muscle maintenance to emphasize aerobic treadmill, resistance exercise with full range of motion.

The spaceflight studies have revealed five major components of skeletal muscle that must be managed to maintain muscle health: contractile strength, shortening velocity, endurance/fatigue resistance, structural strength, and muscle fiber length. The following synopsis describes the advances in understanding how each component adapts. The challenge is to provide stimuli to prevent adaptation to microgravity and retain the capacity to function in gravity-loaded environments.

Contractile strength: Flight studies have revealed that the capacity to generate absolute and specific force declines precipitously in rodent muscles during spaceflights of 4–16 days [Mounier et al., 1997] [STS-40 / SLS-1, p. 277]. Thus, early intervention is required. The declines result from the loss of contractile proteins due to reduced mRNA and protein synthesis, increased degradation, muscle fiber shrinkage in diameter, reduced force per fiber diameter, and decreased myofilament packing density [STS-58 / SLS-2 p. 286] [STS-58 / SLS-2, p. 287]. The antigravity slow muscles atrophy more than the non-antigravity fast muscles[1].Within muscles, the fast fibers atrophy the same or less than slow fibers. Atrophy occurred in bioartificial muscles, suggesting microgravity unloads the cytoskeleton of cultured organoids. Many rodent studies show body weight decreases that implicate nutritional deficits in muscle loss. Spaceflight studies have improved the understanding of atrophy and provided insights into novel targets for exercise, and nutritional and pharmacologic interventions to prevent muscle weakness.

Velocity of shortening: In most studies, the velocity of muscle fiber shortening increased following spaceflight [STS-40 / SLS-1, p. 275]. The major determinant of speed is the myosin heavy chain (MHC) rate of ATPase hydrolysis. Slow type I MHC and slow myosin light chains were preferentially lost [Haddad, 1993] [STS-40 / SLS-1, p. 278] [Vandenburgh, 1999] [STS-66 / NIH.C2, p. 290]. Fast MHC (IIX) increased, and hybrid fibers with slow and fast MHCs were more common [Ciaozzo, 1996] [STS-58 / SLS-2, p. 283]. Speeding up of slow fibers without detectable fast MHC occurred in fibers where thick and thin myofilament packing density was reduced. Similar changes have been reported for astronauts. Weight-bearing activity is not efficiently performed with fast fibers, or fibers with fast velocity, because more ATP per

[1] Most muscles contain a mixture of fast- and slow-twitch muscle fibers. In fast muscles, the fast-twitch fibers are in the majority, and in slow muscles, slow-twitch fibers predominate. Fast fibers contract (shorten) more rapidly than slow fibers because fast fibers contain fast, heavy chain myosin that hydrolyzes ATP 3 to 4 times that of slow myosin predominating in slow fibers. Slow fibers are fatigue-resistant because ATP is generated by oxidative metabolism, whereas fast fibers rely on anaerobic glycolysis from stored glycogen and fatigue more readily.

tension generated is consumed than in slowly contracting fibers. Exercise and pharmacologic techniques for preventing the slow-to-fast conversion are needed to preserve normal muscle function.

Endurance/fatigue resistance: A key component of endurance is the ability to generate ATP oxidatively for contractile activity. During spaceflight atrophy, mitochondrial oxidative enzymes (e.g., SDH) [STS-58 / SLS-2, p. 288] remained close to normal levels because contractile proteins were lost more rapidly. However, biochemical analysis revealed that energy production shifts from oxidative to glycolytic as evidenced by reduced palmitate oxidation, a fall in cytochrome C mRNA, and increases in glycolytic enzymes. This metabolic shift predominated in antigravity muscles. These changes in rodent muscles mirrored those in humans after spaceflight and chronic bed rest. In order to combat muscle fatigue when resuming gravity-loaded movements, the animal studies have pointed to exercise and energy-deriving enzyme pathways targets for preserving oxidative capacity.

Structural strength: Morphological analysis of rodent muscles taken after postflight exposures of many hours to 2 days following spaceflight suggested that microgravity caused muscle edema, muscle fiber atrophy, fiber segmental necrosis, neuromuscular junction denervation, and microvessel disruption [STS-58 / SLS-2, p. 286]. Subsequent in-flight muscle acquisition in the Spacelab [Riley, 1996] [STS-58 / SLS-2, p. 284] (the SLS missions—Spacelab Life Sciences: a series of flights dedicated to the study of life sciences in space using the orbiting scientific laboratory, Spacelab) revealed that atrophy occurred in flight, and the severe tissue disruption was due to progressive damage during reloading of debilitated muscles. Unloading caused structural weakening of muscle fibers; sarcomere filaments broke and the myotendinous junction had reduced muscle fiber area contact with the tendon. Astronauts avoid weight-bearing activities that result in muscle tearing and painful, delayed onset muscle soreness, and resistance exercises have been introduced in flight to prevent structural weakening. While loading stress caused muscle structural failure, the absence of stress in microgravity prevented normal repair of muscle and skin wounds. Astronauts report anecdotally that skin wounds heal poorly in space. Injury repair recapitulates normal muscle development. Rat pups developing in space exhibited delayed connective partitioning of muscles, reduced muscle growth, and slowed maturation of motor nerve endings. These findings illustrate the importance of gravity-loading stimuli for maintaining structural integrity, normal development, and tissue repair.

Muscle fiber length: The postures and patterns of muscle use adopted by astronauts and animals in space lead to muscle shortening. Astronauts adopt a fetal-like posture when floating in microgravity and ambulate with the upper limbs, relegating the lower limbs to perching with a foot-drop plantarflexion posture. Rodents and monkeys in space changed postures and altered muscle recruitment. Rats transitioned to a bipedal forelimb locomotion with the hind feet trailing plantarflexed [Riley et al., 1996] [STS-58 / SLS-2, p. 284]. In hindlimb-suspended unloaded rats, the soleus muscle shortened by approximately 20 percent, which caused a drop in force output when returned to weight bearing because the shorter muscle worked off the plateau of the length/force curve. For the Rhesus monkey and rats in space, soleus electromyographic activity decreased and that of the gastrocnemius increased, suggesting new patterns of muscle recruitment were acquired. These patterns, inappropriate for ambulation on Earth, persisted postflight. Exercise protocols used on the International Space Station (ISS) have recently been modified to maintain normal muscle length. The principles gleaned from the length and muscle use studies are being translated to physical medicine and rehabilitation procedures for patients, including athletes, with conditions involving muscu-

loskeletal trauma, joint replacement, aging sarcopenia, spinal cord injury, and stroke, as well as persons with developmental defects like cerebral palsy and muscle dystrophies.

Lessons for future spaceflight studies: Longer-term spaceflight studies are necessary to ascertain the endpoints of muscle deconditioning in microgravity. Endpoints are important when assessing the effectiveness of countermeasures. For a number of flight studies, the science return was small because of technical problems. The technical issues can be fixed, and flight of the improved experiments should be conducted to increase the science return. In many investigations, control groups such as hindlimb suspension unloading (ground experiments) have provided stand-alone publishable data. Additionally, science return has been maximized by conducting the Biospecimen Sharing Program (BSP) that dispersed tissues not required by the primary investigators. While the ISS is an essential platform for long-term exposure and in-flight tissue acquisition, free flyers with enhanced on-orbit monitoring and measurement capabilities can contribute significantly to the continuation of research enabling long-term human spaceflight.

List of referenced flight experiments:

STS-40 / SLS-1, J.F.Y. Hoh, Skeletal Myosin Isoenzymes in Rats Exposed to Zero Gravity

STS-40 / SLS-1, K.M. Baldwin, Effects of Zero-Gravity Exposure on Biochemical and Metabolic Properties of Skeletal Muscle

STS-40 / SLS-1, D.A. Riley, Electron Microscopy, Light Microscopy, and Protease Activity of Rat Hindlimb Muscles

STS-40 / SLS-1, V.S. Oganov, Contractile Properties of Skeletal Muscles

STS-58 / SLS-2, D.A. Riley, Electron Microscopy, Light Microscopy, and Protease Activity of Rat Hindlimb Muscles

STS-58 / SLS-2, J.-F. Marini, Morphological and Functional Adaptations of Muscle Fibers Muscle-Tendon and Nerve-Muscle Junctions to Spaceflights

STS-58 / SLS-2, K.M. Baldwin, Effects of Zero-Gravity Exposure on Biochemical and Metabolic Properties of Skeletal Muscle

STS-58 / SLS-2, T. Yoshioka, Effects of Spaceflight on Enzyme Activities and Ultrastructure of Fast-Type Skeletal Muscles of Rats

STS-58 / SLS-2, Y. Mounier, Single Fiber Muscle Function

STS-58 / SLS-2, Y. Ohira, Effects of Spaceflight on Beta-Adrenaceptors in Rat Hindlimb Muscles

STS-66 / NIH.C2, H.H. Vandenburgh, The Effect of Space Travel on Skeletal Myofibers

Literature references:

Ciaozzo, V.J.; Baldwin, K.M.; Haddad, F.; Baker, M.J.; Herrick, R.E.; and Prietto, N.: Microgravity-Induced Transformations of Myosin Isoforms and Contractile Properties of Skeletal Muscle. Journal of Applied Physiology, vol. 81, no. 1, July 1996, pp. 123–132.

Haddad, F.; Herrick, R.E.; Adams, G.R.; and Baldwin, K.M.: Myosin Heavy Chain Expression in Rodent Skeletal Muscle: Effects of Exposure to Zero Gravity. J. Applied Physiology, vol. 75, no. 6, 1993, pp. 2471–2477.

Mounier, Y.; Picquet, F.; and Stevens, L.: Postnatal Muscle Development in Unloading Conditions. International J. Sports Medicine, vol. 18, supl. 4, Oct. 1997, pp. S298–S299.

Riley, D.A.; Ellis, S.; Slocum, G.R.; Sedlak, F.R.; Bain, L.W.; Krippendorf, B.B.; Lehman, C.T.; Macias, M.Y.; Thompson, J.L.; Vijayan, K.; and De Bruin, J.A.: In-Flight and Postflight Changes in Skeletal Muscles of SLS-1 and SLS-2 Spaceflown Rats. J. Applied Physiology, vol. 81, no. 1, 1996, pp. 133–144.

Vandenburgh, H.; Chromiak, J.; Shansky, J.; Del Tatto, M.; and Lemaire, J.: Space Travel Directly Induces Skeletal Muscle Atrophy. FASEB J., vol. 13, no. 9, June 1999, pp. 1031–1038.

Cosmos 936 / Bion 4

Launch Date 8/13/1977

Landing Date 8/22/1977

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Spaceflight Effects on Muscle Fibers

Science Discipline
Muscle Physiology

Investigator	Institute
K. Castleman	Jet Propulsion Laboratory

Co-Investigator(s)	Institute
Chui, L.A.	USC School of Medicine
Van Der Meulen, J.P.	USC School of Medicine

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Van Der Meulen, J.P. et al.: Computer Assisted Quantitative Analysis of Muscle Biopsy: Preliminary Observations. Neurology, vol. 27, no. 4, 1977, p. 355.

Castleman, K.R.; Chui, L.A.; Martin, T.P.; and Edgerton, V.R.: Quantitative Muscle Biopsy Analysis. Proceedings of the SPIE, vol. 89, 1976, p. 119.

Objectives/Hypothesis
Whether a muscle fiber employs an oxidative or glycolytic energy mechanism is not immutably fixed, but can be influenced by external factors. Because spaceflight drastically alters the stimulus patterns to which skeletal muscle is exposed, it is relevant to investigate the changes that take place, not only in the muscle fiber size, but also in the energy mechanism. Rather than sampling each muscle only at a few positions along its length, this experiment sought a quantitative total ascertainment of fiber size, number, and type.

Approach or Method
Muscle fiber size and type distribution were studied in the extensor digitorum longus (EDL) muscle of space-flown rats and controls, using histochemical preparation techniques and computer image analysis to quantify the spaceflight-induced changes in muscle fiber size, number, and energy metabolism. The computer program produces a scatter plot showing how the fibers are distributed in diameter and optical density.

Results
Average fiber diameter was largest in the vivarium control animals and smallest in the flight animals. Flight muscles appeared to be shorter than those of other groups. If this length difference is not an artifact of dissection, it could be the result of chronic extension of the foot and/or toes during spaceflight. Fiber number showed no significant difference. The “slow” fiber percentage was quite variable, and no statistically significant fiber type conversion was noted. There were no major cytoarchitectural changes, and necrotic changes and “moth eaten” fibers were not seen. The grouped grand mean fiber diameters show 17% and 7% reductions for the flight and synchronous groups, respectively, when compared to the vivarium group, strongly supporting the contention that hypogravity aggravates the atrophic effects of hypokinesis. While reduced activity may be the major cause of fiber atrophy in spaceflight, other factors may contribute. For example, the stress of negotiating the microgravity environment could produce an ACTH-cortisol response, a possible contributor to reduced fiber size.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Automatic Analysis of Muscle Fibers from Rats Subjected to Spaceflight

Science Discipline
Muscle Physiology

Investigator	Institute
K. Castleman	Jet Propulsion Laboratory

Co-Investigator(s)	Institute
Chui, L.A.	University of Southern California
Van Der Meulen, J.P.	University of Southern California

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages, Cosmos 1129 Russian Hardware Suite

Selected Publications
Chui, L.A. and Castleman, K.R.: Morphometric Analysis of Rat Muscle Fibers Following Spaceflight and Hypogravity. Physiologist, supl., vol. 23, 1980, pp. S76–S78.

Castleman, K.R.; Chui, L.A.; and Van Der Meulen, M.D.: Automatic Analysis of Muscle Fibers From Rats Subjected to Spaceflight. Final Reports of U.S. Rat Experiments Flown on the Soviet Satellite Cosmos 1129. M.R. Heinrich and K.A. Souza, eds., NASA TM-81289, 1981, pp. 267–278.

Objectives/Hypothesis
Spaceflight and microgravity are known to produce systemic and metabolic changes in animals and humans. Even though the effects of weightlessness in various organ systems are well described, the pathophysiological mechanism is largely unknown. In this experiment, muscle size and fiber type distribution were studied in the plantaris and gastrocnemius muscles.

Approach or Method
Muscle histochemistry of flight and control animals were analyzed in the Medical Image Analysis Facility at the Jet Propulsion Laboratory, which includes a microscope-mounted television camera capable of converting the specimen image into numerical form for computer processing. The computer program isolates the individual fibers, and measures the area, perimeter, and average optical density of each. A scatter plot is then produced showing how the fibers are distributed in diameter and optical density. Individual and mean fiber area were also printed with the program.

Results
Both fast-twitch and slow-twitch fibers showed a significant reduction in fiber area. With only two exceptions, the proportion of slow fibers was reduced by spaceflight. The ratio of slow-fiber area to fast-fiber area was lower in the flight groups, indicating that slow fibers suffer size loss more than do fast fibers. These results appear to give a snapshot of how muscle physiology adapts to the spaceflight environment. Hypogravity produces an insufficient loading mechanism, leading to hypokinesia and hypodynamia. This, in turn, produces trophic changes, particularly in antigravity muscles (or slow-twitch oxidative fibers), decreased protein metabolism, negative nitrogen balance, etc., producing muscle atrophy as the final result. Slow fibers, important in maintaining posture against gravity, are of little use in space, and their size, and even proportion, were reduced by the adaptation process. Fast fibers also suffered a disuse atrophy, but to a lesser extent since they are used for locomotion.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Morphological and Biochemical Investigations of Microgravity-Induced Nerve and Muscle Breakdown: I. Investigation of Nerve and Muscle Breakdown During Spaceflight

Science Discipline

Muscle Physiology

Investigator	Institute
D.A. Riley	Medical College of Wisconsin

Co-Investigator(s)	Institute
Ellis, S.	San Jose State University
Ilyina-Kakueva, E.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Wistar rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications

Riley, D.A.; Ilyina-Kakueva, E.; Ellis, S.; Bain, J.L.; Slocum, G.R.; and Sedlak, F.R.: Skeletal Muscle Fiber, Nerve, and Blood Vessel Breakdown in Space-Flown Rats. FASEB Journal, vol. 4, no. 1, 1990, pp. 84–91.

Riley, D.A.: Effects of Microgravity on Rat Muscle. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 45–49.

Objectives/Hypothesis

Extended human exposure to microgravity produces progressive skeletal muscle weakness. The mechanism of the loss must be understood in order to develop rational countermeasures. While simple atrophy should be reversible by exercise, restoration of pathological changes depends upon complex processes of regeneration. This study attempted to further the understanding of the effects of spaceflight on muscle weakness.

Approach or Method

Rats were sacrificed two days after landing. Methods included light and electron microscopy examination of the adductor longus (AL), the extensor digitorum longus (EDL), the soleus, and plantaris muscles. Fast- and slow-twitch types were classified using histochemical staining properties. Ubiquitin conjugates were localized by immunostaining.

Results

Damage was confined to the AL and soleus muscles. The midbelly region of the AL had more segmental necrosis and edema than the ends. Macrophages and neutrophils were the major mononucleated cells infiltrating and phagocytosing the cellular debris. Increased ubiquitination of disrupted myofibrils may have promoted myofilament degradation. Overall, mitochondria content and succinate dehydrogenase activity (SDH) activity were normal, except for a decrease in the subsarcolemmal region. The myofibril ATPase activity shifted toward the fast type in the flight AL muscles as compared to controls. About 17% of the flight AL end plates exhibited total or partial denervation. Initial signs of muscle and nerve fiber regeneration were detected. Myoblast-like cells were present in the segmental necrotic lesions cleared of cell debris, and there was a direct correlation between the distributions of hypertrophied satellite cells and segmental necrosis.

Launch Date 9/29/1987 **Landing Date** 10/11/1987

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Morphological and Biochemical Investigations of Microgravity-Induced Nerve and Muscle Breakdown: II. Biochemical Analysis of EDL and PLT Muscles

Science Discipline
Muscle Physiology

Investigator	Institute
S. Ellis	San Jose State University

Co-Investigator(s)	Institute
Riley, D.A.	Medical College of Wisconsin
Ilyina-Kakueva, E.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Ellis, S. et al.: Morphological and Biochemical Investigations of Microgravity-Induced Nerve and Muscle Breakdown: II. Biochemical Analysis of EDL and PLT muscles. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 259–261.

Objectives/Hypothesis
Carbonic anhydrase III (CA III) is present in highest concentrations in slow oxidative muscle fibers whereas fast fibers have very low concentrations, and the levels of the enzyme can be affected by a number of physiological perturbations. Although the plantaris (PLT) and the extensor digitorum longus (EDL) are quite gravity insensitive, it was of interest to determine the influence of spaceflight on levels of CA III. Conversely, parvalbumin (PVA) is highest in concentration in the fast-twitch muscle and functions as a relaxing factor facilitating the sequestration of calcium in the sarcoplasmic reticulum. In view of reports that unloading slows the 1/2 relaxation time, it was also of interest to measure possible concentration decreases of this protein.

Approach or Method
Measurements were made of two enzymes, the lysosomal tripeptidyl aminopeptidase (TAP) and CA III, and also the calcium binding protein, PVA, in PLT and EDL muscles.

Results
The PLT muscle in flight rats showed a 30% decrease in TAP; the EDL did not show a decrease in flight activity. There was no difference in CA III content of these muscles, except for the PLT of the basal group. The PVA concentrations of the PLT were not significantly different in any of the groups. In the case of EDL, only the synchronous group showed a significant reduction in PVA, whereas the basal, vivarium and flight groups did not differ. Spaceflight showed significant perturbation only in the TAP concentration of the PLT; the concentrations of the CA III and the PVA were unaffected in either of the two muscles. Simulation of freezer failure holding the frozen muscle showed that a thawed condition for 24 hours using fresh muscles did not result in a reduction in TAP or CA III activities, whereas PVA was reduced by 24% in the EDL and unchanged in the PLT.

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Effects of Zero Gravity on Myofibril Protein Content and Isomyosin Distribution in Rodent Skeletal Muscle

Science Discipline

Muscle Physiology

Investigator	Institute
K.M. Baldwin	University of California, Irvine

Co-Investigator(s)	Institute
Herrick, R.	University of California, Irvine
Oganov, V.S.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Wistar rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications

Thomason, D.B.; Herrick, R.E.; and Baldwin, K.M.: Activity Influences on Soleus Muscle Myosin During Rodent Hindlimb Suspension. Journal of Applied Physiology, vol. 63, 1987, pp. 138–144.

Baldwin, K.M.; Herrick, R.E.; Ilyina-Kakueva, E.; and Oganov, V.S.: Effects of Zero Gravity on Myofibril Content and Isomyosin Distribution in Rodent Skeletal Muscle. FASEB Journal, vol. 4, no. 1, 1990, pp. 79–83.

Thomason, D.B.; Herrick, R.E.; Surdyka, D.; and Baldwin, K.M.: Time Course of Soleus Muscle Myosin During Hindlimb Suspension and Recovery. Journal of Applied Physiology, vol. 63, 1987, pp. 130–137.

Objectives/Hypothesis

The purpose of this experiment was to investigate the effects of 12 days of microgravity exposure on the enzymatic properties, protein content, and isomyosin distribution of the myofibril fraction of the slow-twitch vastus intermedius (VI) and the fast-twitch vastus lateralis (VL) muscles of adult male rats. The study tested the general hypothesis that zero gravity would induce: 1) a preferential loss of slow myosin and a corresponding increase in myofibril ATPase activity in the VI, and 2) minimal changes in the VL.

Approach or Method

Myofibrils extracted from the VI and the VL were analyzed using electrophoresis and a soft laser scanning densitometer. Myofibril ATPase specific activity was determined at a free calcium concentration with the use of a buffer system. Both the flight and ground-control groups were examined for muscle mass, myofibril protein content and ATPase specific activity, and estimates of absolute and relative isomyosin content.

Results

The results were largely in support of the hypothesis. Compared to the two control groups, VI weight was lower by 23% ($p < 0.10$); whereas no such reduction was observed for the VL muscle. No evidence of loss of the fast isomyosins was apparent for either muscle following spaceflight. Myofibril ATPase activity of the VI was increased in the flight group compared to the controls, which is consistent with the observation of preferential slow-myosin degradation. These data suggest that muscles containing a high percent of slow-twitch muscle fibers undergo greater degrees of myofibril protein degradation than do muscles containing predominantly fast-twitch fibers in response to a relatively short period of microgravity exposure, and the primary target appears to be the slow-myosin molecule. This observation is consistent with previous findings on the soleus muscle of hindlimb suspended rats, further suggesting that the absence of ground support activity is an important factor in inducing the atrophy response.

Launch Date 9/29/1987 **Landing Date** 10/11/1987

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Actin mRNA and Cytochrome-C mRNA Concentrations in the Triceps Brachia Muscle of Rats

Science Discipline
Muscle physiology

Investigator	Institute
F.W. Booth	University of Texas Medical School, Houston

Co-Investigator(s)	Institute
Morrison, P.R.	University of Texas Medical School
Thomason, D.B.	University of Texas Medical School
Oganov, V.S.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Morrison, P.R.; Muller, G.W.; and Booth, F.W.: Actin Synthesis Rate and mRNA Level Increase During Early Recovery of Atrophied Muscle. American Journal of Physiology, vol. 253, 1987, pp. C205–C209.

Morrison, P.R.; Montgomery, J.A.; Wong, T.S.; and Booth, F.W.: Cytochrome c Protein-Synthesis Rates and mRNA Contents During Atrophy and Recovery in Skeletal Muscle. Biochemistry Journal, vol. 241, no. 1, 1987, pp. 257–263.

Objectives/Hypothesis
Some skeletal muscles atrophy as a result of weightlessness and as a result of hindlimb suspension. The content of protein is determined by the rate of protein synthesis and degradation. Any decrease in protein synthesis could be caused by decreases in messenger ribonucleic acid (mRNA) concentrations. In suspended rat hindlimbs, an increased protein degradation and a decreased protein synthesis were observed, as well as decreases in the concentration and content of alpha-actin mRNA and cytochrome-C mRNA. From these findings, it was hypothesized that the same pattern could be observed in the triceps brachia muscle of space-flown rats.

Approach or Method
Relevant determinations included muscle wet weight, RNA concentration, RNA content, alpha-actin mRNA concentration, and cytochrome-C mRNA concentration. Variance was determined by analysis of variance (ANOVA).

Results
The triceps brachia was not atrophied after 42 hours of recovery from the 12.5-day flight. Both of these factors (lack of atrophy and delayed recovery time) likely contributed to the lack of change in RNA content and alpha-actin mRNA concentration per unit of RNA. Rapid recovery of alpha-actin mRNA concentrations has been noted in atrophied muscle recovering from 7 days of hindlimb immobilization. It is also possible to speculate that the triceps brachia was recruited frequently in space, as the rat attempted to hold on to a position in the cage or move between two points, and that this prevented atrophy. The failure to observe a significant decrease in cytochrome-C mRNA was likely related either to the absence of atrophy or to a speculated absence of a decline in the electromyographic activity of the triceps brachia muscle. It is unlikely that the recovery period was the explanation for this, because cytochrome-C mRNA did not recover for the first 2 days after 7 days of limb immobilization.

Launch Date 9/29/1987 **Landing Date** 10/11/1987

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effect of Microgravity on: I. Metabolic Enzymes of Individual Muscle Fibers

Science Discipline
Muscle physiology

Investigator	Institute
O.H. Lowry	Washington University School of Medicine, St. Louis

Co-Investigator(s)	Institute
McDougall, Jr., D.	Washington University, St. Louis
Carter, J.	Washington University, St. Louis
Krasnov, I.B.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Lowry, O.; McDougal, D., Jr.; Nemeth, P.M.; Chi, M.M.-Y.; Pusateri, M.; Carter, J.; Manchester, J.; Norris, B.; and Krasnov, I.: Effect of Microgravity On: I. Metabolic Enzymes of Individual Muscle Fibers. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 393–411.

Manchester, J.K.; Chi, M.M.; Norris, B.; Ferrier, B.; Krasnov, I.; Nemeth, P.M.; McDougal, D.B., Jr.; and Lowry, O.H.: Effect of Microgravity on Metabolic Enzymes on Individual Muscle Fibers. FASEB Journal, vol. 4, no. 1, 1990, pp. 55–63.

Objectives/Hypothesis
The individual fibers of any muscle vary greatly in enzyme composition, a fact that is obscured when enzyme levels of a whole muscle are measured. The purpose of this study was, therefore, to assess the changes due to weightlessness on the enzyme patterns composed by the individual fibers within the muscles of rats subjected to spaceflight.

Approach or Method
Studies were made on 64 soleus (slow-twitch) and 164 tibialis anterior (fast-twitch) fibers from two synchronous and two flight animals. Each fiber was analyzed in duplicate for two to eight different enzymes, and the size (μg/mm) determined, involving more than 2,300 quantitative measurements. Because each assay required only 0.1 to 0.2 μl of extract (equivalent to 10–20 ng of dry fiber), a single 5-μl extract was sufficient for duplicate assays on a large number of different enzymes.

Results
The average size (weight per unit length) was about 35% lower in flight than in synchronous muscles of both types (fast and slow twitch). In the soleus muscle, the only conclusive enzyme change was in hexokinase, which increased an average of 137% on the dry weight basis. In the tibialis anterior (TA) muscles, hexokinase increased about the same percentage as in the soleus, but in addition all the enzymes of oxidative metabolism were increased about 60%. The glycolytic, glycogenolytic enzymes in TA, in contrast to the soleus muscles, were all somewhat lower (12%–25%) in the flight muscles. In contrast, on a fiber length basis, it is apparent that although hexokinase increased in absolute terms, the increase was no more than 50%, and that six of the enzymes decreased by 10% to 40%. Similarly in the TA muscle, whereas in absolute terms (fiber length basis) oxidative enzymes were almost unchanged, hexokinase increased, but only by 25%, and phosphorylase and the glycolytic enzymes decreased about 50%.

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Metabolic and Morphologic Properties of Muscle Fibers After Spaceflight

Science Discipline

Muscle Physiology

Investigator	Institute
V.R. Edgerton	University of California, Los Angeles

Co-Investigator(s)	Institute
Miu, B.	University of California, Los Angeles
Marini, J.F.	Unite de Recherches Neurobiologique
Oganov, V.S.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Miu, B.; Martin, T.P.; Roy, R.R.; Oganov, V.; Ilyina-Kakueva, E.; Marini, J.F.; Leger, J.J.; Bodine-Fowler, S.C.; and Edgerton, V.R.: Metabolic and Morphologic Properties of Single Muscle Fibers in the Rat After Spaceflight. FASEB Journal, vol. 4, no. 1, 1990, pp. 64–72.

Hauschka, E.O.; Roy, R.R.; and Edgerton, V.R.: Size and Metabolic Properties of Single Muscle Fibers in Rat Soleus After Hindlimb Suspension. Journal of Applied Physiology, vol. 62, no. 6, 1987, pp. 2338–2347.

Objectives/Hypothesis

It is apparent that a variety of biochemical and physiological properties of the rat skeleton are altered following 5–22 days of exposure to microgravity. Because these studies have been based primarily on the analysis of whole muscle properties, and given the potential difference in the response of muscle fibers differing in alkaline adenosine triphosphatase (ATPase) type and size, the purposes of this study were: 1) to define the size and metabolic responses of single fibers to spaceflight, and 2) to determine the specificity of these responses to the muscle and the myosin type and size of its fibers.

Approach or Method

The left soleus (SOL) and the medial gastrocnemius muscles were examined for ATPase fiber density, succinate dehydrogenase activity (SDH), and alpha-glycerol-phosphate dehydrogenase activity (GPD). Frozen sections of SOL were also reacted to antibodies for slow and fast myosin. Fascicles of fibers free of tissue artifact and considered visually to be representative of the tissue section were chosen for analyses. A computer-assisted image analysis system was used to quantify the reaction product based on the rate of optical density for each fiber.

Results

In the SOL more fibers stained darkly with the ATPase stain in the flight than control rats. In conjunction, it appears the same fibers maintained or increased their GDP activity while maintaining their SDH activity. As a result, a greater percentage of fibers in these muscles could be categorized as fast oxidative-glycolytic. The GDP activity data suggest that some flight muscles may have an elevated capacity to utilize carbohydrate derived from carbon sources. Also, in the present data the degree of atrophy in flight muscles depended more on the muscle and the region of the muscle than on fiber type as defined by ATPase staining or the immunohistochemical properties. This differential response among muscles and muscle regions is similar to responses to hindlimb suspension. The present study demonstrates that the general capability of skeletal muscles to maintain proteins decreases rapidly in response to spaceflight.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Biochemical and Histochemical Observations of Vastus Medialis

Science Discipline
Muscle Physiology

Investigator	Institute
X. Musacchia	University of Louisville

Co-Investigator(s)	Institute
Steffen, J.M.	University of Louisville
Fell, R.D.	University of Louisville
Oganov, V.S.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Musacchia, X.J.; Steffen, J.M.; and Fell, R.: Biochemical and Histochemical Observations of Vastus Medialis From Rats Flown in Cosmos 1887. Physiologist, supl., vol. 32, no. 1, 1989, pp. S21–S22.

Steffen, J.M. and Musacchia, X.J.: Effect of Hypokinesia and Hypodynamia on Protein, RNA and DNA in Rat Hindlimb Muscles. American Journal of Physiology, vol. 247, 1984, pp. R728–R732.

Objectives/Hypothesis
The principal objectives of this study were to ascertain if the vastus medialis (VM) responded to microgravity exposure. Three approaches were used: 1) a histochemical evaluation of cellular morphology (fibers and capillaries); 2) an assessment of biochemical composition (protein, RNA and DNA concentrations), and 3) an estimation of metabolic activities and capacities (oxidative and glycolytic metabolism).

Approach or Method
Frozen muscle sections were stained for ATPase activity; muscle fibers and capillaries were differentiated. Fiber area and density measurements were made, and capillary distribution was assessed. The remaining samples were lyophilized, weighed, and powdered. Aliquots were used for protein; RNA and DNA concentration determinations; and for lactate dehydrogenase (LDH), citrate synthase (CS) activities, and lipoprotein lipase (LPL) activities.

Results
Although some of the morphological parameters suggest a small degree of atrophy in the VM, the biochemical analysis (protein, RNA, and DNA) suggest that these may be minimal and functionally nonsignificant. The relatively similar CS and LDH activities of VM from flight and various control groups, as well as the lack of difference between flight and synchronous rats, suggest that there is little or no effect on the oxidative or glycolytic function of this muscle. Because the VM is chiefly a mixed fast-twitch muscle, these metabolic indices of energy production are relatively unchanged. The results of this study are in agreement with previous observations of another type II fast-twitch muscle, the extensor digitorum longus, from Spacelab-3 rats, which did not respond markedly to weightlessness and whole body suspension.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Skeletal Muscle Atrophy in Response to 14 Days of Weightlessness

Science Discipline
Muscle Physiology

Investigator	Institute
X. Musacchia	University of Louisville

Co-Investigator(s)	Institute
Steffen, J.M.	University of Louisville
Fell, R.D.	University of Louisville
Oganov, V.S.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Musacchia, X.J.; Steffen, J.M.; Fell, R.D.; Dombrowski, M.J.; Oganov, V.W.; and Ilyina-Kakueva, E.I.: Skeletal Muscle Atrophy in Response to 14 Days of Weightlessness: Vestus Medialis. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S44–S50.

Musacchia, X.J.: An Assessment of Suspension Systems: Models That Reproduce Responses to Weightlessness. Physiologist, supl., vol. 35, no. 1, 1992, pp. S92–S95.

Objectives/Hypothesis
Muscles of the rat hindlimb have been used to demonstrate the effects of unloading in weightlessness and in models developed to mimic the response seen during exposure to microgravity. The principal objectives of this study were to ascertain how the vastus medialis (VM) responded to 14 days of microgravity and if hindlimb unloading (tail-suspension) was comparable to microgravity.

Approach or Method
Three experimental approaches were used: a histochemical evaluation of microscopic morphology, including fibers and capillaries; an assessment of biochemical composition, including protein, DNA and RNA concentrations; and an estimation of metabolic capacity. From each muscle a piece was taken from the belly for histochemical analysis. Cross sections were stained for myosin ATPase under different pH conditions and used for distinguishing fiber types and capillaries. Fiber cross-sectional area and cell density measurements were made and capillary density was assessed using an image analysis system. A second portion was utilized for protein, RNA, and DNA determinations; both contents and concentrations were determined. Lactate dehydrogenase and citrate synthase activities were measured on lyophilized samples.

Results
Flight animals displayed losses in fiber area and increases in fiber density. Data show that the type II fibers in both the mixed and unmixed portions of the VM were affected by the spaceflight, particularly in those reduced cross-sectional areas in type I fibers of the mixed portion, which averaged between 20–30%. There was a similar atrophy in flight and tail-suspended slow-twitch fibers of the mixed VM portion, suggesting a good correlation between ground and flight protocols. The view that significant changes can occur even in the predominately type II muscles was evidenced by increased fiber densities in flight animals. These results suggest that even non-load-bearing muscles, such as the VM, show measurable responses to weightless flight. Metabolic studies indicated small reductions in this fast-twitch muscle, with a tendency for increased anaerobic capacity (flight and hindlimb unloading).

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effect of Zero Gravity on Myosin Isoform Expression in Rodent Skeletal Muscle

Science Discipline
Muscle Physiology

Investigator	Institute
K.M. Baldwin	University of California, Irvine

Co-Investigator(s)	Institute
Kerrick, R.E.	University of California, Irvine
Ilyina-Kakueva, E.	Institute of Biomedical Problems
Oganov, V.S.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Thomason, D.B.; Morrison, P.R.; Oganov, V.; Ilyina-Kakueva, E.; Booth, F.W.; and Baldwin, K.M.: Altered Actin and Myosin Expression in Muscle During Exposure to Microgravity. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S90–S93.

Objectives/Hypothesis
The purpose of this study was to extend previous findings on the effects of spaceflight on the biochemical properties of rodent fast-twitch and slow-twitch knee extensor muscles. A primary focus was to ascertain the effect on whole homogenate protein, myofibril protein yields, myofibril ATPase, isomyosin distribution patterns, and alpha-glycerolphosphate dehydrogenase activity in vastus intermedius (VI), vastus lateralis (VL), and vastus medialis (VM) muscles.

Approach or Method
Aliquots of homogenized muscles were processed for total protein analysis. Myofibril ATPase specific activity was determined as a free calcium concentration by use of a buffer, expressed as nanomoles of inorganic phosphate released per milligram of myofibril protein per minute. Gel bands of the native myosins separated by pyrophosphate electrophoresis were analyzed densitometrically by directly scanning at 630 nm using a Zenith Soft Laser Densitometer. Isomyosins were fully characterized in terms of their heavy-chain and light-chain composition.

Results
Surprisingly, the muscle mass of the VI did not undergo atrophy in either the flight or tail-suspended groups; however, there was evidence that myofibril yields in the VI were reduced in these same animals. This suggests that degradation of the myofibril machinery in slow-twitch muscle is an early event in the adaptation to zero gravity. Other parameters were not altered in any of the muscles, with the exception that suspension did induce isomyosin shifts in the VI. With regard to the VI, these findings are not fully consistent with Cosmos 1887 observations, suggesting, in part, that there may have been some difficulty in removing the VI from the quadriceps complex.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Messenger RNA Levels in Skeletal and Smooth Muscles: I. mRNA Decrease in Skeletal Muscle During Spaceflight

Science Discipline

Muscle Physiology

Investigator	Institute
F.W. Booth	University of Texas Medical School

Co-Investigator(s)	Institute
Thomason, D.B.	University of Texas Medical School
Morrison, P.R.	University of Texas Medical School
Oganov, V.S.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Thomason, D.B.; Morrison, P.R.; Oganov, V.; Ilyina-Kakueva, E.; Booth, F.W.; and Baldwin, K.M.: Altered Actin and Myosin Expression in Muscle During Exposure to Microgravity. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S90–S93.

Objectives/Hypothesis

The sequence of chemical steps linking non-weight-bearing to the loss of muscle protein remains unknown. Using a model of non-weight-bearing (tail-suspension) to mimic the effect of microgravity on skeletal muscle, decreases in the messenger ribonucleic acids (mRNAs) for skeletal alpha-actin and cytochrome-C have been observed in skeletal muscle. The purpose of this study was to determine if similar events occur in skeletal muscle of rats during spaceflight.

Approach or Method

RNA was extracted from skeletal muscle by the LiCl-urea method. Hybridizations were performed with a 32P-labeled rat skeletal alpha actin probe consisting of 560 bases of the coding region and with a 32P-labeled 960-base fragment of the rat somatic cytochrome-C gene. The concentration of the mRNA was determined by RNA dot blots. Following autoradiography, laser beam densitometry and scintillation counting were done to estimate 32P bound to the mRNA. Analysis of covariance was used to determine significance among the slopes for intensity and for counts per RNA quantity.

Results

Directional changes in skeletal alpha-actin mRNA in skeletal muscle during spaceflight were in the same pattern as those previously reported in the non-weight-bearing model at 1 g. Decreases of 25% and 36% were found in the vastus intermedius and lateral gastrocnemius muscles, respectively. Cytochrome-C mRNA decreased 36% in the vastus intermedius muscle. No decrease in cytochrome-C mRNA was found in the lateral gastrocnemius muscle in flight and one of the two tail-suspension studies or in the vastus intermediate during tail suspension studies. Thus, as opposed to responses of skeletal alpha-actin mRNA to non-weight-bearing, cytochrome-c mRNA changes were not directionally consistent.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Messenger RNA Levels in Skeletal and Smooth Muscles: II. mRNA Levels in Smooth Muscle

Science Discipline

Muscle Physiology

Investigator	Institute
N. Weisbrodt	University of Texas Medical School

Co-Investigator(s)	Institute
Booth, F.W.	University of Texas Medical School
Lai, M.	University of Texas Medical School
Thomason, D.B.	University of Texas Medical School

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Weisbrodt, N.W.; Booth, F.W.; Lai, M.; and Thompson, D.B.: mRNA Levels in Skeletal and Smooth Muscles: II. mRNA Levels in Smooth Muscle. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 1, J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-108802, 1994, pp. 363–371.

Objectives/Hypothesis

Spaceflight effects on the structure and function of the gastrointestinal tract have received little study. Although previous investigations suggest that the net function of the gut is unaltered by gravitation changes, more detailed studies are warranted. Data from a newly developed intestinal bypass model suggest that if there are any changes in the gut associated with spaceflight, they may be expressed as early changes in gene expression in the intestinal smooth muscle. The purpose of this experiment was to define the effects of spaceflight on the weight and protein content, and on the abundance of actin messenger ribonucleic acid (mRNA) in the intestinal smooth muscle of rats flown on Cosmos 2044.

Approach or Method

Intestinal smooth muscle samples were analyzed for changes in weight, protein content, RNA, and mRNA levels for actin. Segments of mid-small intestine were removed, opened lengthwise, rinsed, and frozen. After storage at -80°C, muscles were thawed at 4°C and lengths measured. The longitudinal layer of smooth muscle was removed, weighted, and processed. For analysis of mRNA actin, a special riboprobe was developed, containing a cDNA insert that codes for a portion of the 3' untranslated region, all of the coding region, and a portion of the 5' untranslated region of the mRNA that, in turn, codes for the synthesis of smooth muscle actin. While the method used to isolate total RNA yields a relatively pure product, recovery is incomplete and variable. For this reason, the amount of RNA recovered spectrophotometrically was determined and presented as a comparative estimate of the total RNA contained in each segment of longitudinal muscle. Wet weight, total protein, and total RNA were normalized to the length of each segment.

Results

Wet weight and protein content were less in tissue taken from the flight animals compared to vivarium controls. However, no differences were detected between tissues taken from flight, synchronous, and tail-suspended animals. Detected differences may be explained on the basis of food consumption or weight gain. Total RNA differed among tissues from all groups, with decreasing amounts identified among vivarium, tail-suspended, synchronous, and flight groups, respectively. Size fraction of the RNA demonstrated significant degradation. Analysis of northern blots failed to show any hybridization to the riboprobe for smooth muscle actin, more than likely due to the degradation. Although not conclusive, the RNA data suggest that there may be an influence of spaceflight on intestinal smooth muscle gene expression. That the lowest RNA levels were found in flight animals may indicate the influence of diet and/or spaceflight; however, because hormonal and other changes also occur during flight, the effect, if any, could be secondary.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effects of Microgravity in the Adductor Longus Muscle and Receptors in the Forebrain of Rats

Science Discipline
Muscle Physiology
Neurophysiology

Investigator	Institute
N.G. Daunton	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
D'Amelio, F.	San Jose State University Foundation
Ilyina-Kakueva, E.	Institute of Biomedical Problems
Harris, E.	San Jose State University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
D'Amelio, F. and Daunton, N.G.: Effects of Spaceflight in the Adductor Longus Muscle of Rats Flown in the Soviet Biosatellite Cosmos 2044: A Study Employing Neural Cell Adhesion Molecule (N-CAM) Immunocytochemistry and Conventional Morphology Techniques. Journal of Neuropathology and Experimental Neurology, vol. 51, no. 4, 1992, pp. 415–431.

Objectives/Hypothesis
Although the effects of microgravity upon muscle tissue are far from being understood, previous studies have shown that "slow" muscle, mostly composed of type I fibers (e.g., soleus, adductor longus), carry the burden of the changes. This study placed emphasis on some particular responses to weightlessness observed in the adductor longus muscle of rats, namely: 1) muscle fiber injury, 2) regenerative phenomena, and 3) alterations of the neuromuscular junctions.

Approach or Method
The motor endplate region was identified by acethylcholinesterase activity under the stereomicroscope and dissected; silver-gold ultrathin sections were stained with uranyl acetate-lead citrate, mounted in copper grids (100 mesh), and observed with an electron microscope. For light microscopy observations, fixed adductor samples were stained with hematoxylin and eosin. For N-CAM immunocyto-chemistry, sections (25 μm) were mounted, rinsed, and incubated with anti-N-CAM rabbit antibodies.

Results
N-CAM immunoreactivity was seen on the myofiber surface, satellite cells, and in regenerating myofibers reminiscent of myotubes. Light microscopy revealed myofiber atrophy, contraction bands, and segmental necrosis accompanied by cellular infiltrates of macrophages, leukocytes, and mononuclear cells. The principal electron microscopic changes of the neuromuscular junctions consisted of a decrease or absence of synaptic vesicles, degeneration of axon terminals, increased numbers of microtubules, vacant axonal spaces, and axonal sprouting. These results indicate that major alterations such as myofibrillar disruption and necrosis, muscle regeneration, and denervation and synaptic remodeling at the level of the neuromuscular junction may take place during spaceflight.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Rodent Tissue Repair: I. Skin Repair Studies

Science Discipline
Muscle Physiology

Investigator	Institute
A.C. Vailas	University of Wisconsin

Co-Investigator(s)	Institute
Durnova, G.	Institute of Biomedical Problems
Kaplansky, A.S.	Institute of Biomedical Problems
Vanderby, R.	University of Wisconsin

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Vailas, A.C.; Vanderby, R., Jr.; Martinez, D.A.; Ashman, R.B.; Ulm, M.J.; Grindeland, R.E.; Durnova, G.N.; and Kaplansky, A.: Adaptations of Young Adult Rat Cortical Bone to 14 Days of Spaceflight. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. 4S–9S.

Objectives/Hypothesis
The aim of this experiment was to determine the effects of microgravity on the healing and repair of skin connective tissue and skeletal muscle. Two days preflight, skin and lateral head of the medial gastrocnemius, medial soleus, and fibula of hindlimbs from five of the flight rats were cut and wounds sutured after surgery.

Approach or Method
Flight samples were compared histologically and immunocytochemically to similarly treated tail-suspended, synchronous, and vivarium control animals. All protein markers were isolated from rat sources and antibodies prepared and tested for cross reactivity with other molecules. Non-lesion skin was prepared for measurements of DNA content, collagen content by hydroxyproline, and uronic acid content by an estimation of ground substance.

Results
Skin repair studies were somewhat problematic for the following reasons: 1) it was very difficult to locate the wound and many of the lesions were not the same dimensions, and 2) thawing and fixation of frozen tissue caused problems with immunocytochemical staining for better resolution with light microscopy image processing. Significant qualitative differences were not detected for the wound markers collagen type III, hematotoxyline and eosin, and macrophage factor XIII. Other results indicated there was a nonsignificant increase (10%) in flight animal skin DNA concentration; however, data expressed as a ratio of DNA/collagen estimates of the cell or nuclear density that supports a given quantity of collagen showed a dramatic increase in the flight group (33%). This means flight conditions may have slowed down collagen secretion and/or increased cell proliferation in adult rat skin.

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Rodent Tissue Repair: II. Changes in Muscle Serine Proteases, Serpins, and Matrix Molecules

Science Discipline
Muscle Physiology

Investigator	Institute
B. Festoff	University of Kansas Medical Center

Co-Investigator(s)	Institute
Ilyina-Kakueva, E.	Institute of Biomedical Problems
Rayford, R.A.	Veterans Affairs Medical Center
Reddy, B.R.	Veterans Affairs Medical Center

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Festoff, B.W; Ilyina-Kakueva, E.I.; Rayford, A.R.; Burkovskaya, T.E.; Reddy, B.R.; and Rao, J.S.: Connective Tissue Studies: II. Changes in Muscle Serine Proteases, Serpins, and Matrix Molecules. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 2, J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-108802, 1994, pp. 239–254.

Objectives/Hypothesis
In microgravity, type I muscle fibers atrophy and lose predominance, especially in slow-twitch muscles. No increase in mononuclear cells has been observed, as is the case with simple denervation, where both type I and II fibers atrophy, without infiltration of cells but with clear satellite cell proliferation. However, degradation of the extracellular matrix (ECM) takes place after denervation and, if reinnervation is encouraged, functional recovery to near control levels may be achieved. No information is available concerning the ECM milieu, the activation of serine proteases, their efficacy in degrading ECM, and the production of locally derived natural protease inhibitors (serpins) in effecting surface proteolytic control. This study examined the activation of these enzymes in microgravity and their response to muscle injury, both in space and on the ground.

Approach or Method
Gastrocnemius muscle was crushed by clamping down a hemostat for 30 seconds. Rat cells were grown in microcarrier cultures as a source of purified Protease Nexin I (PNI), and affinity chromatography was used to obtain it. To determine if differences in previously injured gastrocnemius muscles could be detected, nitrocellulose or immobilon-bound samples were sequentially probed with antibodies to plasminogen activator (PA) and two serpins (PAI and PAI-I). PA activity was also determined by amidolytic assay; serpin activity by several synthetic chromogenic assays specific for the target protease. Immunologically stained slides were viewed under epifluorescence with a microscope. Protein determination was estimated using staining.

Results
Although PNI increased in the flight group, levels were less than in the tail-suspended group, as compared to vivarium and synchronous controls. An increase in moles of active PAI-1 occurred after muscle crush, and in all cases PAI-1 was increased compared with basal extracts. Enzyme-linked immunosorbent assays for both serpins indicated that crush injury itself increased the amount of active plus complexed serpin, just significantly greater in injured flight muscles. In adult innervated muscle, urkinase-like PA (uPA) activity was barely detectable, uPA messenger ribonucleic acid (mRNA) expression was second only to the kidney, suggesting that uPA message in kidney is in “ready reserve” awaiting some type of injury for rapid activation. This supports the theory that neutral, extracellular proteases are essential to degrade components of the old basement membrane/ECM in order to allow for a basement membrane to scaffold so that orderly regeneration can take place. Likewise, from the results it may be speculated that some interference with this “ready reserve” of uPA mRNA after injury characterizes zero gravity.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Rodent Tissue Repair: III. Skeletal Muscle

Science Discipline
Muscle Physiology

Investigator	Institute
W. Stauber	West Virginia University

Co-Investigator(s)	Institute
Fritz, V.K.	West Virginia University
Ilyina-Kakueva, E.	Institute of Biomedical Problems
Kaplansky, A.S.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Stauber, W.T.; Fritz, V.K.; and Dahlmann, B.: Extracellular Matrix Changes Following a Blunt Trauma to Rat Skeletal Muscles. Experimental Molecular Pathology, vol. 52, no. 1, 1990, pp. 69–86.

Stauber, W.T.; Fritz, V.K.; Burkovskaya, T.E.; and Ilyina-Kakueva, E.I.: Effect of Injury on Mast Cells of Rat Gastrocnemius Muscle with Respect to Gravitational Exposure. Experimental Molecular Pathology, vol. 59, no. 2, 1993, pp. 87–94.

Objectives/Hypothesis
Myofiber injury repair was studied in the rat gastrocnemius following a crush injury to the lower leg prior to flight in order to understand if the regenerative responses of muscles are altered by the lack of gravitational forces during spaceflight. Skeletal muscle atrophy in actual or simulated weightlessness appears to be due to a combination of local and systemic factors—muscle tension and exogenous growth factors. If gravitational forces are necessary for optimal connective tissue organization and muscle repair, then muscle injuries in microgravity might require a special type of rehabilitation to prevent muscle fibrosis and/or movement dysfunction.

Approach or Method
Histochemical and immunohistochemical techniques were used to examine myofiber, vascular, and connective tissue for alterations. Muscle tissues were sectioned at –20°C in a cryostat at a thickness of 4 μm. Macrophages were localized using an alpha-naphthyl acetate esterase kit. Blood vessels and capillaries in the muscle sections were visualized using a histochemical procedure for the localization of dipeptidyl peptidase IV employing fast blue staining. Localizations of proteinases, proteins, and proteoglycans were performed by indirect immunohistochemical techniques using fluorescein-labeled second antibodies.

Results
In general, the repair process was somewhat similar in all muscle samples with regard to the extracellular matrix organization and myofiber regeneration. Small and large myofibers were present within a newly organized extracellular matrix indicative of myogenesis and muscle regeneration. In the tail-suspended animals, a more complete repair was observed with no enlarged area of non-muscle cell of matrix material visible. In contrast, flight muscle samples were less well differentiated with more macrophages and blood vessels in the repair region, but small myofibers and proteoglycans were, nevertheless, in their usual configuration. Myofiber repair did vary in muscles from different groups, but for the most part resulted in functional muscle tissue. However, an increase in the vascularity of the repair site and in the number of macrophages in flight muscle might suggest the development of granulation tissue at the repair site of the flight animals, or that muscle regeneration was slowed down due to a variation in growth factors such as growth hormone and insulin.

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Metabolic and Morphologic Properties of Muscle Fibers and Motor Neurons After Spaceflight: I. Muscle Fibers

Science Discipline
Muscle Physiology
Neurophysiology

Investigator	Institute
V.R. Edgerton	University of California, Los Angeles

Co-Investigator(s)	Institute
Ohira, Y.	University of California, Los Angeles
Roy, R.R.	University of California, Los Angeles
Jiang, B.	University of California, Los Angeles

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Ohira, Y.; Jiang, B.; Roy, R.R.; Oganov, V.; Ilyina-Kakueva, E.; Marini, J.F.; and Edgerton, V.R.: Rat Soleus Muscle Fiber Responses to 14 Days of Spaceflight and Hindlimb Suspension. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S51–S57.

Jiang, B.; Ohira, Y.; Roy, R.R.; Nguyen, Q.; Ilyina-Kakueva, E.I.; Oganov, V.; and Edgerton, V.R.: Adaptation of Fibers in Fast-Twitch Muscles of Rats to Spaceflight and Hindlimb Suspension. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S58–S65.

Objectives/Hypothesis
This study addressed several issues raised by Cosmos 1887. These include the difference in atrophy suggested by wet muscle and fiber size data (40%), and the adaptability of selected metabolic enzymes to spaceflight. Also, a question not addressed in previous studies of tail-suspension or spaceflight was whether myosin ATPase activities of single fibers change in parallel with the changes in expression of fast myosin.

Approach or Method
Serial sections (10 μm) were cut. Qualitative staining of ATPase was performed at pH 8.7; quantitative staining for myofibrillar ATPase activity at pH 8.6. Triplicate sections were stained at 37°C with ATP in the substrate medium, and two sections were stained without ATP. The difference in optical density between the with and without substrate was used to calculate activity (optical density/min.). Additional serial sections were stained with monoclonal antibody against fast or slow myosin heavy chain. Fibers were identified as having reacted positively for the slow, fast, or both antibodies.

Results
Significant atrophy was found in both dark and light myosin ATPase fibers following spaceflight and tail suspension. In suspension, dark and light (Type II and Type I) were 40% and 38% smaller than controls, respectively, while flight fibers were 28% and 38% smaller. The distribution of fibers that were positive to a monoclonal antibody for fast myosin heavy chain increased from 9.6% in controls to 24.1% after spaceflight; however, the percent responding positively to slow myosin antibody did not change. The ATPase activity in light ATPase fibers was less in flight than control muscle. Those fibers that stained intermediately with ATPase had intermediate quantitative ATPase, SDH, and GPD enzyme activities suggesting that these fibers were in a transitional state.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Morphohistochemical, Immunocytochemical, and Biochemical Investigation of Microgravity Induced Nerve and Muscle Breakdown: I. Muscle Histology

Science Discipline

Muscle Physiology

Investigator	Institute
D.A. Riley	Medical College of Wisconsin

Co-Investigator(s)	Institute
Ilyina-Kakueva, E.	Institute of Biomedical Problems
Oganov, V.S.	Institute of Biomedical Problems
Ellis, S.	San Jose State University

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Riley, D.A.; Ellis, S.; Giometti, C.S.; Hoh, J.F.; Ilyina-Kakueva, E.I.; Oganov, V.S.; Slocum, G.R.; Bain, J.L.; and Sedlak, F.R.: Muscle Sarcomere Lesions and Thrombosis After Spaceflight and Suspension Unloading. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S33–S43.

Riley, D.A.; Slocum, G.R.; Bain, J.L.; Sedlak, F.R.; Sowa, T.E.; and Mellender, J.W.: Rat Hindlimb Unloading: Soleus Histochemistry, Ultrastructure, and Electromyography. Journal of Applied Physiology, vol. 69, 1990, pp. 58–66.

Objectives/Hypothesis

Extended exposure of humans to spaceflight produces a progressive loss of muscle strength. Rats orbited in longer Cosmos missions manifested more severe atrophy and greater tissue necrosis than those on shorter missions, suggesting the degenerative processes were progressive. The purpose of this study was to examine the hindlimb muscle from flight rats sacrificed as close to landing as possible so that changes induced by spaceflight and early readaptation to weight bearing could be distinguished from the changes that resulted from the 2-day postflight period during Cosmos 1887.

Approach or Method

Rats were sacrificed 8 to 11 hours after landing. The adductor longus (AL), extensor digitorum longus (EDL), and plantaris muscles were cut into sections and stained appropriately for light and electron microscopy. Qualitative atrophic changes in muscle fibers, nerves, neuromuscular junctions, microcirculatory vessels, interstitial tissue, and the myotendious junctions were assessed. Estimates of interstitial edema for the AL and EDL muscles were made by quantifying the percentage area of the muscle fibers and non-muscle fiber connective tissues within stained 0.5-μm sections using computer programs. Indirect immunofluorescence was utilized to localize antibodies against ubiquitin conjugates, complement IgG, fibrinogen, immune cell types, red blood cells, platelets, and fast and slow myosins.

Results

This data reconfirms that AL muscle fibers atrophy during spaceflight and tail-suspension. In the flight AL, absolute mitochondrial content decreased, but the relatively greater breakdown of myofibrillar proteins maintained mitochondrial concentration near normal. At the ultrastructural level, a 53% decrease in subsarcolemmal mitochondria concentration was detected in the flight AL muscles compared to vivarium animals. The flight muscles exhibited more eccentric, contraction-like lesions than did the suspended AL, and the high reentry g-forces appear to explain this difference. Muscle atrophy appears to increase the tendency to form eccentric contraction-like lesions following reloading; this may reflect weakening of the muscle fiber cytoskeleton and extracellular matrix. Microcirculation is also compromised by spaceflight such that there is increased formation of thrombi in the postcapillary venules and capillaries.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Morphohistochemical, Immunocytochemical, and Biochemical Investigation of Microgravity Induced Nerve and Muscle Breakdown: II. Muscle Biochemistry

Science Discipline

Muscle Physiology

Investigator	Institute
S. Ellis	San Jose State University

Co-Investigator(s)	Institute
Riley, D.A.	San Jose State University
Giometti, C.S.	Argonne National Laboratory
Oganov, V.S.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Ellis, S.; Riley, D.A.; and Giometti, C.S.: Morphological, Histochemical, Immunocytochemical, and Biochemical Investigation of Microgravity Induced Nerve and Muscle Breakdown: II. Muscle Studies. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 1, J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-108802, 1994, pp. 327–337.

Objectives/Hypothesis

Earlier studies on microgravity effects by means of hindlimb unloading showed marked changes in relative concentrations of several proteins in rat soleus muscles. The Cosmos 2044 mission offered an opportunity to determine if qualitatively similar changes in protein pattern occurred in skeletal muscle after exposure to microgravity.

Approach or Method

Two types of analyses were performed on the muscle samples. The first was a two-dimensional gel electrophoretic resolution of the proteins extracted with 9 M urea from sections of the adductor longus (AL) muscles. The second was an analysis of the plantaris and EDL for three protease activities using synthetic peptide derivatives: lysosomal tripeptidyl aminopeptidase, cytosolic multicatalytic protease, and cytosolic activity for free calpain protease activity.

Results

The electrophoretic analyses of the AL muscle showed a strong similarity in the pattern of specific protein changes in the atrophying flight and tail-suspended muscle, except the degree of change was more intense in the flight muscle. The flight muscle showed a reduction in the light chains 1s and 1sa, and 2s, and the appearance of fast muscle chains 1f, 2f, and 3f, suggesting that a conversion of fiber types was initiated by hypogravity. The flight EDL showed a 19% increase of tripeptide peptidyl hydrolase activity, whereas the flight plantaris remained unchanged. Results suggest that tail-suspension does not entirely mimic the muscle atrophy induced by microgravity.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Functional Neuromuscular Adaptation to Spaceflight

Science Discipline
Muscle Physiology
Neurophysiology

Investigator	Institute
V.R. Edgerton	University of California, Los Angeles

Co-Investigator(s)	Institute
Bodine-Fowler, S.C.	University of California, Los Angeles
Roy, R.R.	University of California, Los Angeles
Hodgson, J.A.	University of California, Los Angeles

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Laboratory Control

Key Flight Hardware
Cosmos Primate-BIOS

Selected Publications
Hodgson, J.A.; Bodine-Fowler, S.C.; Roy, R.R.; de Leon, R.D.; de Guzman, C.P.; Koslovskaya, I.; Sirota, M.; and Edgerton, V.R.: Changes in Recruitment of Rhesus Soleus and Gastrocnemius Muscles Following a 14 Day Spaceflight. Physiologist, vol. 34, no. 1, 1991, pp. S102–S103.

Bodine-Fowler, S.C.; Roy, R.R.; Rudolph, W.; Hague, N.; Kozlovskaya, I.B.; and Edgerton, V.R.: Spaceflight and Growth Effects on Muscle Fibers in the Rhesus Monkey. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S82–S89.

Objectives/Hypothesis
This study was designed to determine the effects of the absence of weight support on flexor and extensor muscles of the hindlimb. These effects were assessed morphologically and biochemically from muscle biopsies taken from a slow extensor: the soleus; a fast extensor: the medial gastrocnemius (MG); and a fast flexor: the tibialis anterior (TA). A second objective was to determine the relative importance of activity (by electromyogram (EMG)) and force (by joint torque) on the adaptation of muscle.

Approach or Method
Pre- and postflight EMGs were analyzed using amplitude histograms, scatterplots, and joint probability density distributions of rectified, smoothed EMGs. Of the two flight animals, only one provided a complete data set from implanted soleus, MG, and TA muscles. Muscle biopsies of 8–14 mg were taken from two independent sites with a Bergstrom needle. For each biopsy mean fiber cross-sectional area and succinic dehydrogenase (SDH) activity were measured. Joint probability distribution was generated on a logarithmic scale using three consecutive trials and totaling 20,000 data points.

Results
Activity of the TA muscles appeared unchanged after the flight, while the amplitude of soleus EMG was reduced, and MG amplitude was elevated, possibly to compensate for the soleus. Two weeks postflight, MG amplitude had declined to normal values but recovery of soleus was incomplete. Joint probability distribution showed a similar correlation between soleus and MG. This redistribution may be caused by a reduction of activity in the vestibulospinal pathways that normally excites motorneurons innervating the slow motor units of extensor muscles. Mean cross-sectional area of MG and soleus fibers were increased postflight, while TA fibers were significantly smaller. Mean SDH activity was not significantly different in soleus, but decreased in postflight biopsies of the MG and TA.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Biomechanical, Biochemical, and Morphological Alterations of Muscle and Dense Fibrous Connective Tissues After 14 Days Spaceflight: I. Connective Tissue Studies

Science Discipline
Muscle Physiology

Investigator	Institute
A.C. Vailas	University of Wisconsin

Co-Investigator(s)	Institute
Durnova, G.	Institute of Biomedical Problems
Kaplansky, A.S.	Institute of Biomedical Problems
Vanderby, R.	University of Wisconsin

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Vailas, A.C.; Vanderby, R. Jr.; Martinez, D.A.; Ashman, R.B.; Ulm, M.J.; Grindeland, R.E.; Durnova, G.N.; and Kaplansky, A.: Adaptations of Young Adult Rat Cortical Bone to 14 Days of Spaceflight. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. 4S–9S.

Objectives/Hypothesis
The objectives of this experiment were to characterize the structural and material properties of cortical and trabecular bone samples, and to correlate the biochemical properties of these tissues to the type and quality of structural proteins. This study examined tendon and connective tissue components in muscle for alterations following a spaceflight of a short duration in a skeletally mature rat.

Approach or Method
Connective tissue studies were conducted in the Achilles and patellar tendons and humeri. The parameters examined included humeri stiffness, flexural rigidity, and failure load; cortical lengths, cross-sectional areas, densities, and moments of inertia. It should be noted that a number of unique methods were employed such as: 1) ultrasonics for humerus elastic properties, 2) lysylpyridnoline collagen cross-link analysis in cortical bone, and 3) skin biochemical markers using enzyme digestion.

Results
The study demonstrates that the spaceflight induced no significant changes for all the parameters studied. However, the results regarding skin biochemical properties showed a significant increase in DNA/mg collagen ratio. Specifically, data indicated that spaceflight induced an increase in the amount of nuclear material that supports a given quantity of collagen (structural protein). In some cases, the tail-suspended group induced changes in tissues (as compared to the controls) that would suggest in some ways that the model does not mimic all aspects of spaceflight as an effecter of connective tissue.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Biomechanical, Biochemical, and Morphological Alterations of Muscle and Dense Fibrous Connective Tissues: II. Composition of the Invertebral Disk

Science Discipline

Muscle Physiology

Investigator	Institute
A. Pedrini-Mille	University of Iowa

Co-Investigator(s)	Institute
Maynard, J.A.	University of Iowa
Durnova, G.	Institute of Biomedical Problems
Krasnov, I.B.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Pedrini-Mille, A.; Maynard, J.A.; Durnova, G.N.; Kaplansky, A.S.; Pedrini, V.A.; Chung, C.B.; and Fedler-Troester, J.: Effects of Microgravity on the Composition of the Intervertebral Disc. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. 26S–32S.

Objectives/Hypothesis

The intervertebral disc is formed by three structures: the nucleus pulposus (NP), the annulus fibrosis (AF), and the cartilaginous endplates (EP). This study was designed to determine the effects of weightlessness on the size, water content, and composition of the lumbar annuli intervertebral disc in space-flown rats. Changes in the composition of the AF may render it unable to withstand swelling pressures of the NP and/or the stress and strain associated with movement under load, making the return to normal gravitational fields potentially hazardous.

Approach or Method

Discs for light microscopy were fixed, cut into halves along the anterior-posterior axis, and stained, with one-half embedded flat and the other at 90° to obtain both horizontal and sagittal sections of the disc. For electron microscopy each disc was trimmed while immersed in glutaraldehyde so as to divide each disc into anterior, transitional, and posterior components, with the transitional area representing a small segment on the anterior one-half of the AF immediately adjacent the NP. Longitudinal sections were photographed at 20,000 and 60,000x to determine collagen fibril-proteoglycan relationships. For biochemical analysis minced samples were mixed with various aliquots.

Results

Data indicate that resulting from weightlessness: 1) there was significant reduction in annuli weight that was attributed to an actual loss of tissue components and not to water loss; 2) the annular matrix became proportionally more collagenous, but without the abnormal changes in the relative proportions of Type I or II collagen or number of pyridinoline crosslinks; 3) the collagen proteoglycan ratio in flight animals was significantly greater than controls; and 4) when annuli were immersed in water or saline solutions for 2 hours, proteoglycans leached out of annuli, suggesting the presence of abnormal or smaller proteoglycans.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Effect of Microgravity on: I. Metabolic Enzymes in Type I, IIA, and IIB Muscle Fibers

Science Discipline

Muscle Physiology

Investigator	Institute
O.H. Lowry	Washington University School of Medicine, St. Louis

Co-Investigator(s)	Institute
Krasnov, I.B.	Institute of Biomedical Problems
Ilyina-Kakueva, E.	Institute of Biomedical Problems
Nemeth, P.M.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Chi, M. M.-Y.; Choksi, R.; Nemeth, P.; Krasnov, I.; Ilyina-Kakueva, E.; Manchester, J.K.; and Lowry, O.H.: Effects of Microgravity and Tail-Suspension on Enzymes of Individual Soleus and Tibialis Anterior Fibers. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S66–S73.

Objectives/Hypothesis

Individual fibers of any given muscle vary widely in enzyme composition, a fact obscured when enzyme levels of the whole muscle are measured. Therefore, this study was to assess the effects of microgravity and hindlimb unloading on the enzyme patterns within a slow-twitch muscle (soleus) and a fast-twitch muscle (tibialis anterior, TA).

Approach or Method

Samples were prepared for assays two different ways. In one method, samples of 0.5 μg were added to 5 μl of special detergent-containing medium known to preserve without loss all but a few of the enzymes of interest. In an alternate method, thinner sections (16 μm) were stained for myosin ATPase to type the individual fibers, while thicker slices (32 μm) were dissected from individual fibers identified as to type by the adjacent stained sections. Altogether, over 2,200 individual enzyme measurements were made.

Results

Average fiber size was much smaller for both flight and tail-suspended muscles ($P < 0.01$) than the synchronous samples. Pyruvate kinase, glycerol-3-phosphate dehydrogenase, and hexokinase activities were higher in both flight and tail-suspended soleus muscles, while 3-ketoacid CoA transferase was decreased. In contrast, there were only two statistically significant differences ($P < 0.05$) between TA enzyme activities of synchronous fibers and those of either flight or tail-suspended TA fibers: Hexokinase activity of one tail-suspended sample averaged 57% higher than the average for the higher synchronous fiber set; thiolase activity of one flight sample was 27% lower than the average for the lower synchronous sample.

STS-41 / PSE.01

Launch Date 10/6/1990 **Landing Date** 10/10/1990

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Physiological Systems Experiment

Science Discipline
Muscle Physiology

Investigator	Institute
M. Cronin	Genentech, Inc.

Co-Investigator(s)	Institute
Tidball, J. and Quan, D. Grindeland, R.E.	University of California, Los Angeles NASA Ames Research Center (ARC)
Hancock, W.; Schwall, R.; Clark, R.; Battersby, J.	Genentech, Inc.
Mastro, A.M.; Hymer, W. C.; Nash P.	Pennsylvania State University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Flight Simulated, Control Flight Simulated Protein-Treated Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications

Nash, P. and Mastro, A.: Variable Lymphocyte Responses in Rats after Spaceflight. Experimental Cell Research, vol. 202, 1992, pp. 125–131.

Cronin, M.; Battersby, J.; Hancock, W.; Schwall, R.; and Clark, R.: Delivery of Recombinant Human Growth Hormone to Rats During Exposure to Microgravity on NASA Space Shuttle Discovery. Physiologist, supl., vol. 35, no. 1, 1992, pp. S51–S52.

Tidball, J.G. and Quan, D.M.: Reduction in Myotendinous Junction Surface Area of Rats Subjected to 4-Day Spaceflight. Journal of Applied Physiology, vol. 73, no. 1, 1992, pp. 59–64.

Objectives/Hypothesis
The objective of this experiment was to test the hypothesis that a growth-hormone (GH) deficiency occurs during spaceflight and that this deficiency contributes to the bone loss and decreased tissue function observed following microgravity exposure. It was expected that replacement with recombinant GH in flight, in combination with adequate nutrition and exercise, would halt the process of bone and tissue degeneration. As the first commercially sponsored life science payload, the experiment was to investigate whether biological changes caused by microgravity were similar enough to Earth-based medical conditions to facilitate pharmacological evaluation of potential new therapies. Other objectives included evaluation of muscle and immune function, which are known to be affected/regulated by GH.

Approach or Method
Static measurements were made of long bone length, diaphyseal cross-sectional surface area, medullary areas, and cortical sectional areas in femora and humeri. Dynamic measurements were made of the periosteal surface of femora and humeri. The soleus muscles were weighed, and cross-sectional surface area was determined. Surface area of the myotendinous junctions (MTJs) was also measured. Immunocompetence of T-lymphocytes from the lymph nodes and spleen was determined.

Results
Neither short-term spaceflight nor rhGH treatment altered long bone length, diaphyseal cross-sectional surface area, medullary areas, and cortical sectional areas in femora and humeri. In both flight and ground-control animals, rhGH treatment significantly increased bone formation and mineral apposition rates at the periosteal surface of femora and humeri. However, because 4 days of spaceflight was not enough to inhibit periosteal bone formation, the effect of rhGH treatment on spaceflight-induced osteopenia could not be determined. Muscle weight, muscle fiber cross-sectional area, and MTJ surface area were significantly reduced by 4 days in space, comparable to reductions seen after 7–14 days of spaceflight. Administration of rhGH did not reduce the muscle atrophy observed. This is consistent with the hypothesis that another hormone is mediating the response to muscle unloading. T-lymphocyte responsiveness in the lymph nodes was significantly depressed compared to ground controls. There appeared to be a tissue-specific immune response to microgravity exposure.

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Skeletal Myosin Isoenzymes in Rats Exposed to Zero Gravity

Science Discipline
Muscle Physiology
Regulatory Physiology

Investigator / Institute
J.F.Y. Hoh — University of Sydney

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control, Mission Length Control

Key Flight Hardware
Research Animal Holding Facility (RAHF), Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
This study was to determine how microgravity affects muscle fiber type and muscle isomyosin composition. It was postulated that under zero gravity some slow fibers would convert to fast. Because stimuli to the slow-twitch antigravity muscles should be greatly reduced in low gravity, the concentration of myosin isoenzymes in these fibers should also be changed.

Approach or Method
Muscles were analyzed using monoclonal antibodies against myosin heavy chains. Antibodies specific to different myosin-heavy-chain types were used to identify fast and slow fibers. Mab 5-4D was specific to the slow type I fibers, and mab 5-2B was specific for type IIa and type IIx.

Results
Soleus muscles of the flight animals showed a marked increased in the proportion of fibers expressing fast type II isomyosin. Muscle fibers tended to change from slow to fast, however the change was not as dramatic as observed in tail-suspension studies. Slow fibers were more atrophied than fast fibers. It is likely that the conversion from slow- to fast-twitch fibers was not complete by the end of the flight. Changes in fiber type distribution were not detected in the extensor.

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Electron Microscopy, Light Microscopy, and Protease Activity of Rat Hindlimb Muscles

Science Discipline
Muscle Physiology

Investigator	Institute
D.A. Riley	Medical College of Wisconsin

Co-Investigator(s)	Institute
Ellis, S.	San Jose State University
Haas, A.L.	Medical College of Wisconsin

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)
Animal Enclosure Module (AEM)

Selected Publications
Riley, D.A.; Ellis, S.; Slocum, G.R.; Sedlack, 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. Journal of Applied Physiology, vol. 81, 1996, pp. 133–144.

Riley, D.A.; Thompson, J.L.; Krippendorf, B.B.; and Slocum, G.R.: Review of Spaceflight and Hindlimb Suspension Unloading Induced Sarcomere Damage and Repair. Basic and Applied Myology, vol. 5, 1995, pp. 135–141.

Objectives/Hypothesis
This spaceflight study examined the degree of atrophy for muscles used primarily to oppose gravity with those not weight bearing and investigated the cellular and chemical basis for atrophy. Another objective was to characterize the degeneration of neuromuscular junctions. Understanding how structural and chemical changes in muscle are induced by the stress of launch, low gravity, reentry, and readaptation to Earth's gravity will help define how several factors contribute to muscle weakening, and effective countermeasures can be developed to overcome atrophy during spaceflight.

Approach or Method
A total of 1,490 muscles were analyzed by light and electron microscopy for evidence of shrinkage or death of muscle cells, breakdown of muscle fibers, or degeneration of motor nerves. The chemical basis for atrophy was investigated by immunostaining for ubiquitin proteins that catalyze the breakdown of proteins. Cross-sectional areas of muscle fiber types in the slow and mixed (fast and slow) fiber regions of the adductor longus and the central portion of soleus were measured by computer-assisted digitizing morphometry of fibers in myofibrillar ATPase reacted sections.

Results
Spaceflight induced significant atrophy (fiber shrinkage) and increased expression of fast muscle characteristics (fast myosin) in the slow fibers. The slowly adapting myosin change most likely occurred in flight. Adductor longus muscles showed increased susceptibility to pathological damage upon resumption of weight bearing activity at 1 G. Postflight damage included thrombosis of the microcirculation, interstitial and cellular edema, muscle fiber fragmentation, sarcomere disruptions, activation of phagocytic cells, and elevated ubiquitin conjugation suggestive of increased protein breakdown. Accelerated aging-like involution of neuromuscular junctions was significantly more prominent in rats housed in flight cages in flight and during Delayed Flight Profile Test (DFPT), indicating caging-induced effects. The soleus also atrophied but showed less pathology than the adductor longus, which appeared related to greater resumption of loaded contractile activity postflight by the adductor longus.

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Contractile Properties of Skeletal Muscles

Science Discipline
Muscle Physiology

Investigator	Institute
V.S. Oganov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Murasko, L.M.	Institute of Biomedical Problems
Kabitskaya, O.E.	Institute of Biomedical Problems
Riley, D.A.	Medical College of Wisconsin
Edgerton, V.R.	University of California, Los Angeles

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Oganov, V.S.; Riley, D.; Edgerton, R.; Murashko, L.M.; and Kabitskaya, O.E.: Contractility Properties of Skeletal Muscles. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, pp. 35–36.

Objectives/Hypothesis
In this study the zero-gravity effect upon contractile properties of skeletal muscles was studied in rats after a 9-day flight (R+0) and a 9-day postflight readaptation period. A comparative analysis of the effect of microgravity of varying duration on the contractile properties of skeletal muscles of different functional profiles (slow and fast) was performed.

Approach or Method
Using glycerated myofibers, the following contractile properties were measured: maximum isometric strain; velocity of contraction; velocity of semi-relaxation; work capacity; time of maximum contraction development; time of semi-relaxation; and diameter of myofibers treated with ATP+Ca2+.

Results
The results obtained demonstrated that the greatest changes occurred in the weight-bearing soleus and included a decrease of diameter of the muscle fibers, and decreases of isometric tension and contraction velocity. There was a trend towards increase of contractile force in the fast locomotor muscle, the extensor digitorium longus (EDL), and in both heads of the gastrocnemius. A decrease of velocity of contraction and semi-relaxation was also seen in the EDL. During the readaptation period, R+9, these parameters demonstrated a trend towards normalization. These results confirm the in-flight dependency of the contractile characteristics of the muscles from their functional profile.

Launch Date 6/5/1991

Landing Date 6/14/1991

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effects of Zero-Gravity Exposure on Biochemical and Metabolic Properties of Skeletal Muscle

Science Discipline
Muscle Physiology

Investigator	Institute
K.M. Baldwin	University of California, Irvine

Co-Investigator(s)	Institute
None	

Research Subject(s)
*Rattus norvegicus (*Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF), Animal Enclosure Module (AEM)

Selected Publications
Haddad, F.; Herrick, R.E.; Adams, G.R.; and Baldwin, K.M.: Myosin Heavy Chain Expression in Rodent Skeletal Muscle: Effects of Exposure to Zero Gravity. Journal of Applied Physiology, vol. 75, no. 6, 1993, pp. 2471–2477.

Baldwin, K.; Herrick, R.E.; and McCue, S.: Substrate Oxidation Capacity in Rodent Skeletal Muscle: Effects of Exposure to Zero Gravity. Journal of Applied Physiology, vol. 75, no. 6, Dec. 1993, pp. 2466–2470.

Objectives/Hypothesis
Exposure to microgravity causes mechanical unloading of skeletal muscles. It is this unloading that is thought to play a major role in producing a loss in muscle mass and other phenotypic alterations. Given the lack of data regarding the influence of spaceflight in muscle function, it is important that these earlier observations be confirmed and that the effects of longer spaceflight missions be studied. The second area of investigation that requires examination is related to the transcriptional, translational and post-translational regulation of myosin isoform expression. The influence of microgravity on myosin heavy chain (MHC) and messenger ribonucleic acid (mRNA) isoform expression remains relatively unexplored. This study examined the effects of microgravity on the contracrile properties of the soleus, an antogravity skeletal muscle; and the MHC protein and mRNA isoform content of the solius, vastus intermedius, plantais, and tibialis anterior muscles.

Approach or Method
The relative and total content of isomyosin protein expression was determined by gel electrophoresis for type I, type IIa-IIx, and type IIb isoforms. Total RNA was isolated from muscles for slot-blot analysis, and mRNA was determined for type I, type II, and type IIb isoforms. Oxidative rates of palminate and pyruvate were determined from measurements of 14CO2 production. Oxidative enzymes were measured, and mitochondrial and cytoplasmic isoforms were identified by alcohol inactivation.

Results
Findings demonstrate a reduced expression of the two slow myosin heavy chain and an increased expression of the two fastest myosin heavy chain isoforms. Coupled with muscle atrophy, this tends to reduce the effective muscle mass to support antigravity function and locomotor activity. There also appears to be a selective reduction in the capacity of the muscle to produce long-chain fatty acids, which may impair endurance during spaceflight. A decrease in palminate oxidizing capacity was observed in the flight animals, but no decrease was found in pyruvate oxidizing capacity in fast-twitch muscles.

STS-48 / PARE.01

Launch Date 9/12/1991

Landing Date 9/18/1991

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Physiological and Anatomical Rodent Experiment 1

Science Discipline
Muscle Physiology

Investigator	Institute
M.E. Tischler	University of Arizona

Co-Investigator(s)	Institute
None	

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Controls, Tail-Suspension Control

Key Flight Hardware
Ambient Temperature Recorder (ATR)

Selected Publications
Tischler, M.E.; Henriksen, E.J.; Munoz, K.A.; Stump, C.S.; Woodman, C.R.; and Kirby, C.R.: Spaceflight on STS-48 and Earth-Based Unweighting Produce Similar Effects on Skeletal Muscle of Young Rats. Journal of Applied Physiology, vol. 74, no. 5, 1993, pp. 2161–2165.

Henriksen, E.J., Tischler, M.E., Woodman, C.R., Munoz, K.A., Stump, C.S., and Kirby, C.R.: Elevated Interstitial Fluid Volume in Soleus Muscles Unweighted by Spaceflight or Suspension. Journal of Applied Physiology, vol. 75, 1993, pp. 1650–1653.

Objectives/Hypothesis
Previous to PARE 01, the only studies comparing unweighting through microgravity and unweighting through hindlimb suspension were performed on adult rats. These studies have shown that posterior hindlimb muscles, the function of which depend on opposing the pull of gravity, are the most responsive to unweighting. Another effect found in unweighting studies has been the increased response of insulin to glucose transport. This experiment was designed to test the validity of using tail suspension methods as models for studying microgravity unweighting effects on developing rats. Several muscle parameters were compared between rats subjected to spaceflight and rats subjected to hindlimb suspension. A main objective was to determine whether microgravity causes an increase in glucose uptake in the presence or absence of insulin.

Approach or Method
Flight rats and ground-control rats were 26 days old at the beginning of the experiment. Rats were flown for 5.4 days, and muscles were removed within 3 hours of landing. Body weights and muscle weights were measured for flight and control rats. Protein analysis for total protein content and concentration was performed on the soleus, gastrocnemius, plantaris, tibialis anterior, and extensor digitorum. The soleus and extensor digitorium were used for glucose uptake studies. The extensor digitorium served as a control to the soleus because it is not affected by loading or systemic effects. The uptake of 2-deoxyglucose was measured in the presence and absence of insulin.

Results
Despite similar food and water consumption, flight rats gained more weight than the control rats. Comparisons of muscle sizes normalized to body mass showed that flight and suspension reduced muscle mass of the soleus by 38% and 33% respectively. Smaller differences were seen in masses of plantaris and gastrocnemius muscles, and no differences were seen in anterior muscles. Protein content of the soleus muscles from flight rats was reduced by 20% and was characterized by a lower concentration. Protein content from the soleus muscles of suspended rats was reduced by 23%. In the absence of insulin, there was no difference in the rate of uptake of 2-deoxyglucose in the soleus or the extensor digitorium. However, in the presence of insulin, the uptake of 2-deoxyglucose was significantly higher in flight and suspended rats than in control rats. This increase may be due to an increase in insulin binding capacity due to unloading effects.

Launch Date 12/29/1992 **Landing Date** 1/10/1993

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Morphologic and Metabolic Properties of Single Muscle Fibers in Hindlimb Muscles of the Rhesus

Science Discipline
Muscle Physiology

Investigator	Institute
S.C. Bodine-Fowler	University of California, San Diego

Co-Investigator(s)	Institute
Pierotti, D.J.	University of California, San Diego School of Medicine
Edgerton, V.R.	University of California, Los Angeles

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
Bodine-Fowler, S.C.; Pierotti, D.J.; and Talmadge, R.J.: Functional and Cellular Adaptation to Weightlessness in Primates. Journal of Gravitational Physiology, vol. 2, no. 1, 1995, pp. P43–P46.

Bodine-Fowler, S.: Adaptive Response of Slow and Fast Skeletal Muscle in the Monkey to Spaceflight (Final Report). NASA contractor report 202120, 1996, p. 11.

Bodine-Fowler, S.C. and Pierotti, D.J.: Effect of Spaceflight on Muscle Fibers in the Rhesus Monkey [abstract]. American Society of Gravitational Space Biology Bulletin, vol. 7, no. 1, Oct. 1993, p. 96.

Objectives/Hypothesis
Previous studies on rats have shown that within 7 days of muscle unloading, there is considerable muscle atrophy and a small increase in the percentage of muscle fibers that express fast myosin isoforms mainly within slow muscles. These responses seem to be dependent on the function of the muscle and of its original myosin composition. Two questions have arisen from these studies: 1) what are the physiological signals that trigger these changes, and 2) how do these responses in rats compare to other animals? The purpose of this study was to further define the effects of spaceflight on selected morphology and metabolic properties of single muscle fibers from selected extensor and flexor muscles of the Rhesus monkey.

Approach or Method
Muscle biopsies were taken from two independent sites (one was taken 90–98 days prior to flight, the other was taken 2–5 days after flight) in the soleus (Sol), medial gastrocnemius (MG), tibialis anterior (TA), and the vastus lateralis (VL) muscles. Fiber cross-sectional area and succinate dehydrogenase (SDH) activity were determined for individual fibers (50–80 fibers) in a 10-micrometer cross section. Fibers were classified as type I (slow), type IIa (fast), or type IIb (fast) based on monoclonal antibodies specific for myosin heavy chains. To access for differences in fiber cross-sectional area after flight, a sample of 200–500 fibers were measured from tissue cross section stained with an antibody specific for laminen, a protein in the basal lamina surrounding the muscle fiber.

Results
The TA muscle showed significant atrophy in both flight monkeys (flight monkeys were numbered 151 and 906). In contrast, the Sol and MG of 906 showed a significant increase in size after flight, whereas in 151, the muscles showed atrophy. In the Sol of 151 there was a decrease in SDH of all fiber types, however the decrease in size was limited to type IIa and hybrid fibers. In contrast, the Sol of 906 exhibited an increase in SDH activity for all fiber types and a size increase for fast and hybrid fibers. The MG of 151 showed a decrease in size and SDH activity in all fiber types. The MG of 906 showed an increase in size and a decrease in SDH activity. The TA of 151 had a decrease in SDH activity in the type I and type IIa fibers and a decrease in size of the type IIb fibers. The TA of 906 had a decrease in SDH activity and size for all fiber types.

Launch Date 1/13/1993 **Landing Date** 1/19/1993

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effects of Zero Gravity on Biochemical and Metabolic Properties of Skeletal Muscle Fiber Types

Science Discipline
Muscle Physiology

Investigator	Institute
K.M. Baldwin	University of California, Irivine

Co-Investigator(s)	Institute
Caiozzo, V.	University of California, Irvine

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications

Caiozzo V.J.; Baker M.J.; Herrick R.E.; Ming, T.; and Baldwin, K.M.: Effect of Spaceflight on Skeletal Muscle: Mechanical Properties and Myosin Isoform Content of a Slow Muscle. Journal of Applied Physiology, vol. 76, no. 4, Apr. 1994, pp. 1764–1773.

Caiozzo, V.J.; Haddad, F.; Baker, M.J.; and Baldwin, K.M.: Functional and Cellular Adaptations of Rodent Skeletal Muscle to Weightlessness. Journal of Gravitational Physiology, vol. 2, no. 1, 1995, pp. P39–P42.

Objectives/Hypothesis
Microgravity has a dramatic effect on skeletal muscle during spaceflight. The types and quantities of contractile proteins of skeletal muscle fibers play an important role in determining the muscle function. The objective of this study was to determine the effect of spaceflight on myosin composition of the soleus muscle through the investigation of mechanical properties of skeletal muscle determined by the types and quantity of myosin, the myosin heavy chain (MHC) protein isoform composition, and the MHC messenger ribonucleic acid (mRNA) isoform content.

Approach or Method
The soleus muscle was taken from rats after 6 days of spaceflight. Maximal isometric tension (P_o) was measured and normalized to the physiological cross section area of the muscle. Maximal shortening velocity was determined and expressed as mm/s and muscle length/s. The percentage of slow type I and fast type IIA MHC protein isoforms were determined for the soleus muscle, as well as the corresponding mRNA content for each MHC fiber type protein isoform.

Results
A 5% reduction in maximal isometric tension was found in the soleus muscle, expressed in N/cm^2. In contrast to the decreased tension force, maximum shortening velocity increased 15% in the soleus muscle. It is speculated that to compensate for decreased isometric tension, maximum shortening velocity increases so the muscle can be stimulated at higher frequencies. Little change was seen in the percentage of slow Type I and fast Type IIA MHC protein isoforms. Fast Type IIX MHC protein isoform content increased by 10% of the total MHC protein isoform content. This increase may account for the increase in maximum shortening velocity. The mRNA content for fast Type IIX MHC isoform was also significantly increased. This is consistent with the increase in the fast Type IIX MHC protein isoforms. Based on this and other information, it appears muscle atrophy in microgravity is isoform specific.

Launch Date 1/13/1993 **Landing Date** 1/19/1993

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effect of Spaceflight on Oxidative and Antioxidant Enzyme Activity in Rat Diaphragm and Intercostal Muscles

Science Discipline
Muscle Physiology
Regulatory Physiology

Investigator	Institute
B. Girten	Houghten Pharmaceuticals

Co-Investigator(s)	Institute
Tuttle, R.	Houghten Pharmaceuticals
Lee, M.	Houghten Pharmaceuticals

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM), Ambient Temperature Recorder (ATM)

Selected Publications
Lee, M.D.; Tuttle, R.; and Girten B.: Effect of Spaceflight on Oxidative and Antioxidant Enzyme Activity in Rat Diaphragm and Intercostal Muscles. Journal of Gravitational Physiology, vol. 2, no. 1, 1995, pp. 68–69.

Objectives/Hypothesis
Previous studies show oxidative marker enzymes respond to microgravity differently in different muscle fibers. In hindlimb suspension studies, oxidative enzyme levels have been shown to both increase and decrease in different experiments. The purpose of this experiment was to examine the effects of microgravity on oxidative and antioxidative enzyme levels in respiratory muscles.

Approach or Method
Diaphragm and intercostal muscles were taken from rats after 7 days of spaceflight. A portion of each tissue sample was homogenized, and enzyme analysis was performed on the supernatent. Citrate sythase activity was measured using spectrophotometry. Superoxide dismutase levles were measured to indicate antioxidant activity. Lipid peroxidation was determined through levels of malondialdehyde and 4-hydroxyalkenal, by-products of lipid peroxidation.

Results
An increase in citrate synthase activity (an oxidative activity) was seen in diaphragm muscles but not in intercostal muscles. A significant decrease was seen in lipid peroxidation activity of the diaphragm. Lipid peroxidation of the intercostals was not significantly affected. Antioxidant activity remained unchanged in the diaphragm. These results are inconsistent with previous hindlimb unloading studies in which citrate synthase activity decreased and peroxidation products increased. This may be due to the fact that respiratory muscles do not experience unloading as a result of spaceflight, instead diaphragm muscles remain passively tense and tonic activity of intercostal muscles increases.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effects of Zero-Gravity Exposure on Biochemical and Metabolic Properties of Skeletal Muscle

Science Discipline
Muscle Physiology

Investigator	Institute
K.M. Baldwin	University of California, Irvine

Co-Investigator(s)	Institute
Ciaiozzo, V.	University of California, Irvine

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Caiozzo, V.J.; Haddad, F.; Baker, M.J.; and Baldwin, K.M.: Functional and Cellular Adaptations of Rodent Skeletal Muscle to Weightlessness. Journal of Gravitational Physiology, vol. 2, no. 1, 1995, pp. 39–42.

Caiozzo, V.J.; Baldwin, K.M.; Haddad, F.; Baker, M.J.; Herrick, R.E.; and Prietto, N.: Microgravity-Induced Transformations of Myosin Isoforms and Contractile Properties of Skeletal Muscle. Journal of Applied Physiology, vol. 81, no. 1, July 1996, pp. 123–132.

Objectives/Hypothesis
Exposure to microgravity causes mechanical unloading of skeletal muscles. It is this unloading that is thought to play a major role in producing a loss in muscle mass and other phenotypic alterations. Given the paucity of data regarding the influence of spaceflight in muscle function, it is important that these earlier observations be confirmed and that the effects of longer spaceflight missions be studied. The second area of investigation that requires examination is the transcriptional, translational, and post-translational regulation of myosin isoform expression. The influence of microgravity on myosin heavy chain (MHC) and messenger ribonucleic acid (mRNA) isoform expression remains relatively unexplored. This study examined the effects of microgravity on the contracrile properties of the soleus, an antogravity skeletal muscle; the MHC protein and mRNA isoform content of the solius, vastus intermedius, plantais, and tibialis anterior muscles.

Approach or Method
Approximately 4 hours after landing, the hindlimb musculature of the first flight animal was isolated and in situ contractile measurements were made on the soleus muscle. Upon completion, the left and right soleus, vastus intermedius, plantaris, and tibialis anterior muscles were removed and weighed. Samples from these muscles were used in the following procedures. Immunaohistochemical analyses were performed, determining the presence of slow and fast MHC isoforms using two different monoclonal antibodies. Isolation and purification of myofibrils were performed. MHC isoforms were separated electrophoretically. Total cellular RNA was isolated from skeletal muscle using the RNAzol method. Northern blots were run, dried, and used for subsequent hybridization. Four synthetic oligonucleotides, complementary to the 3’ nontranslated sequences of rat skeletal muscle, were used as a probe for MHCisoform mRNA.

Results
Microgravity had the greatest effect on muscle fiber composition in the soleus muscle, with a reduction in slow muscle fibers and an increase in hybrid fibers. There were significant decreases in slow type I protein isoforms and increases in fast type IIX MHC protein isoforms of the soleus and the vastus intermedius muscles. Consistent with this data was an increase in the type IIX MHC mRNA isoform. In contrast, the plantaris and tibialis anterior muscles showed increases in fast type IIB MHC mRNA isoforms without a corresponding increase in the protein content. The force-velocity relationships of the flight soleus muscle had a significant reduction in maximal isometric tension and a corresponding increase in maximal shortening velocity.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Electron Microscopy, Light Microscopy, and Protease Activity of Rat Hindlimb Muscles

Science Discipline
Muscle Physiology

Investigator	Institute
D.A. Riley	University of Wisconsin

Co-Investigator(s)	Institute
Ellis, S.	San Jose State University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications

Riley, D.A.; Thompson, J.L.; Krippendorf, B.B.; and Slocum, G.R.: Review of Spaceflight and Hindlimb Suspension Unloading Induced Sarcomere Damage and Repair. Basic and Applied Myology, vol. 5, 1995, pp. 135–141.

Riley, D.A.; Ellis, S.; Slocum, G.R.; Sedlak, F.R.; Bain, L.W.; Krippendorf, B.B.; Lehman, C.T.; Macias, M.Y.; Thompson, J.L.; Vijayan, K.; and De Bruin, J.A.: In-Flight and Postflight Changes in Skeletal Muscles of SLS-1 and SLS-2 Spaceflown Rats. Journal of Applied Physiology, vol. 81, no. 1, 1996, pp. 133–144.

Objectives/Hypothesis
Prior to SLS-2, all subject dissections were performed postflight. These rats were exposed to landing stress and gravity reloading on the skeletal muscles, making it difficult to distinguish microgravity adaptation from other factors. SLS-2 marked the first in-flight dissections of experiment subjects, allowing specimens to be studied without postflight effects. This study examined the histochemistry and protease activity of the adductor longus and soleus muscle of a rat exposed to microgravity. The long-term objective of this study was to define the cellular and molecular basis for spaceflight-induced neuromuscular deterioration, and to assist development of countermeasures to ensure the health, safety, and performance of humans who work in space.

Approach or Method
Adductor longus, extensor digitorum longus, and soleus muscles were used for tissue processing and analysis. The occurrence of aberrant myofibers was determined in hematoxylin and eosin-stained sections of muscles. Myofiber cross-sectional areas and nonmyofiber areas were measured by computerized digitizing morphometry of myofibers in hematoxilin and eosin stained crystsat sections and toluidine blue-stained Epon semithin sections. Electron microscopy was performed on the aforementioned muscles in addition to the respiratory diaphragm muscles. Eccentric contraction-like sarcomere lesions were defined as two or more hyperstretched sarcomeres with pale A bands and wavy extracted Z lines. The percentages of myofibers with these sarcomere lesions were counted. Groups were subjected to 2 minutes of videotaping of voluntary movements against a calibration grid square matrix in an open cage to permit quantitation of walking speed.

Results
In microgravity, rats adopted bipedal forelimblocomotion with the hindlimbs relegated to grasping activities. On landing day, body posture was abnormally low and walking was stilted at a rate one-third of normal. The AL and soleus muscles exhibited decreased myofiber areas that did not recover 14 days postflight. Doubling of the nonmyofiber area indicated interstitial edema in AL muscles 2.3 hours postflight. Solei did not manifest edema postflight, and neither muscle showed edema in flight. Sarcomere eccentric contraction-like lesions were detected in 2.6% of AL fibers 4.5 hours postflight, but were absent earlier postflight and in flight. At 9 days postflight, these lesions were repaired but regenerating AL myofibers were present suggesting myofiber necrosis occurred 1–2 days postflight. These studies demonstrate that muscle atrophy occurs in microgravity, whereas interstitial edema and sarcomere lesions are postflight phenomena.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Single Fiber Muscle Function

Science Discipline
Muscle Physiology

Investigator	Institute
Y. Mounier	Universite de Lille

Co-Investigator(s)	Institute
Stevens, L.	Universite de Lille
Cordonnier, C.	Universite de Lille
Picquet, F.	Universite de Lille

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Stevens, L.; Picquet, F.; Catinot, M.P.; and Mounier, Y.: Differential Adaptation to Weightlessness of Functional and Structural Characteristics of Rat Hindlimb Muscles. Journal of Gravitational Physiology, vol. 3, no. 2, Sept. 1996, pp. 54–57.

Mounier, Y.; Picquet, F.; and Stevens, L: Postnatal Muscle Development in Unloading Conditions. International Journal of Sports Medicine, supl. 4, vol. 18, Oct. 1997, pp. S298–S299.

Objectives/Hypothesis
Previous studies examining the soleus (SOL) and extensor digitorum longus (EDL) muscles of the rat hindlimb indicate that muscles exposed to microgravity undergo atrophy and a change of fiber composition from slow- to fast-twitch types. These changes can vary depending upon the muscle participation in antigravitational activity (that is, posture) and on the functional profile of the muscle (slow- or fast-twitch). In order to corroborate these earlier findings, this study examined the functional (activated tension characteristics) and structural (myofibril composition) changes caused by microgravity in the tibialis anterior (TA), vastus intermedius (VI), as well as the soleus (SOL), and extensor digitorum (EDL) muscles of the rat hindlimb.

Approach or Method
Fiber bundles were removed, isolated, and skinned from selected rat hindlimb muscles (SOL, VI, TA, EDL). In order to quantify the atrophy of the fiber, cross-sectional area (CSA), and maximal tension were measured. Then, each fiber underwent force measurements to establish tension/pCa and tension/pSr and to determine the functional properties of the muscle. Additional calculations and measurements were performed in order to determine Ca and Sr affinity. After the completion of these physiological measurements, the muscle fibers underwent analysis with sodium dodecyl sulphate polyacrimide gel electrophoresis (SDS-PAGE) in order to determine myosin heavy and light chain composition. The results were analyzed using a two-way analysis of variance (ANOVA).

Results
Electrophoretic analysis revealed that SOL fibers exposed to microgravity showed a significant increase in the proportion of fast fibers when compared to control fibers. In the mixed VI muscle, there was a progressive rearrangement between the different fiber types within the slow and fast populations without changes in the proportion (50/50) of each population. The transformation concerned the coexpression of slow and fast myosins with an increase in the proportion of fast isoforms. Decreases in CSA and maximal force appeared in SOL and were more marked for the slow fibers. VI exhibited only losses in force, while no change in CSA or force was detected in TA and EDL muscles. Another important effect of weightlessness concerned the Ca2+ activation characteristics of the fast transformed fibers, which showed a decrease in Ca affinity and an increase in the cooperativity of the different proteins of the thin filament.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Morphological and Functional Adaptations of Muscle Fibers Muscle-Tendon and Nerve-Muscle Junctions to Spaceflights

Science Discipline
Muscle Physiology

Investigator	Institute
J.F. Marini	Université de Nice; Centre National Recherche Scientifique

Co-Investigator(s)	Institute
Carnino, A.	Centre National Recherche Scientifique, Marseille
Zamora, A.J.	Centre National Recherche Scientifique; INSERM Marseille

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Zamora, A.J.; Carnino, A.; Roffino, S.; and Marini, J.F.: Respective Effects of Hindlimb Suspension, Confinement and Spaceflight on Myotendinous Junction Ultrastructure. Acta Astronaut, vol. 36, nos. 8–12, Oct.–Dec. 1995, pp. 693–706.

Objectives/Hypothesis
Most of the generated myofilament contractile forces are transmitted from the skeletal muscle fibers to the tendon collagen fibers, across the plasma membrane, via the myotendinous junction (MTJ). The structure of this specialized region at the endings of the muscle fibers depends on the mechanical constraints imposed on muscle. Previous studies have shown ultrastructural MTJ modifications caused by an increase or a decrease in muscle-loading. This study compared the respective effects on MTJ ultrastructure of 8, 18, and 29 days of hindlimb suspension and 14 days of microgravity exposure or 14 days of confinement.

Approach or Method
Ground simulation of microgravity was obtained by suspending animals by their tails, making the hindlimbs neither active nor weight bearing. After different hindlimb suspension (HS) durations, the region of the distal MTJ was removed from both the soleus and plantaris muscles in the HS controls, confinement controls, and spaceflight animals. All muscles were fixed in a glutaraldehyde solution, then post-fixed, dehydrated, and embedded in epoxy resin. Ultrathin sections were cut, stained with uranyle acetate and lead citrate, and examined using an electron microscope.

Results
The first morphological modifications in the hindlimb suspension group were seen after 18 days on the soleus muscle, an antigravity postural muscle. Twenty-nine days of hindlimb suspension showed profound morphological and cytoarchitectural modifications and degenerative changes. The animals that experienced 14 days of microgravity showed greater morphological and cytoarchitectural modifications than did the 18-day hindlimb suspension group. The muscle fibers' endings presented longer and thinner finger-like processes than controls. Numerous caveolae and subplasmalemmal vacuoles evidenced the intense membrane remodelling at MTJ. An histomorphometric quantification showed a 60% increase in the length of this interface between muscle fiber and tendon after the 14-day spaceflight. The morphological modifications of the plantaris MTJ were found to be much less profound than those observed in the soleus MTJ, under all experimental conditions. These qualitative and quantitative studies suggest that the mechanical charge imposed on muscle plays an important role in the structural organization of the MTJ.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effects of Spaceflight on Beta-Adrenaceptors in Rat Hindlimb Muscles

Science Discipline
Muscle Physiology

Investigator	Institute
Y. Ohira	National Institute of Fitness and Sports

Co-Investigator(s)	Institute
Yasui, W.	National Institute of Fitness and Sports
Kariya, F.	National Institute of Fitness and Sports
Tanaka, T.	National Institute of Fitness and Sports

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Ohira, Y.; Yasui, F.K.; Hinds, W.E.; Kariya, F.; Tanaka, T.; Kitajima, I.; Maruyama, I.; Nagaoka, S.; and Sekiguchi, C.: Spaceflight Effects on Beta-Adrenoceptor and Metabolic Properties in Rat Plantaris. Journal of Applied Physiology, vol. 81, no. 1, July 1996, pp. 152–155.

Ohira, Y. and Saito, K: Responses of Beta-Adrenoceptor in Rat Soleus to Phosphorous Compound Levels and/or Unloading. American Journal of Physiology, vol. 266, no. 5:1, May 1994, pp. C1257–C1262.

Objectives/Hypothesis
Gravitational unloading has been observed to cause changes in the slow-twitch muscle fibers of the antigravity soleus muscle. The density of ß-adrenoceptors (ß-AR) is greater in slow-twitch red muscle fibers than fast-twitch white muscle fibers. The density of ß-ARs in the rat soleus decreases in response to gravitational unloading, which has lead to the hypothesis that the same metabolic adaptation would occur in the rat plantaris muscle.

Approach or Method
Plantaris muscles were taken from the right limb of rats approximately 5 hours after 14 days of spaceflight and were cut into 20-μm consecutive cross sections. Quantitative autoradiographic analysis was performed determining the maximum binding capacity (Bmax) and the dislocation constant of b-1 and b-2 ARs. Qualitative histochemical analysis was performed after staining for myosin adenosine triphosphatase. Fibers were categorized as slow, intermediate, or fast. The activities of ß-hydroxyacyl CoA dehydrogenase (HAD) and succinate dehydrogenase (SDH) were measured spectrophotometrically.

Results
The Bmax of ß-AR was significantly lower after flight and did not normalize after 9 days of recovery. The dissociation constant remained unchanged, suggesting the changes in Bmax were caused by a change in the number of receptors. SDH activity was approximately 24% subnormal but normalized after 9 days of recovery. No significant responses were seen in HAD activity or in fiber-type percentages of flight animals. The decrease in Bmax seems to be associated with a decrease in the inner membrane enzymes of the mitochondria rather than with the matrix enzyme HAD.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effects of Spaceflight on Enzyme Activities and Ultrastructure of Fast-Type Skeletal Muscles of Rats

Science Discipline
Muscle Physiology

Investigator	Institute
T. Yoshioka	St. Marianna University School of Medicine

Co-Investigator(s)	Institute
Yamashita, K.	St. Marianna University School of Medicine
Tanaka, O.	St. Marianna University School of Medicine
Uchida, H.	St. Marianna University School of Medicine

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Macias, M.Y.; Lehman, C.T.; Sanger, J.R.; and Riley, D.A.: Myelinated Sensory and Alpha Motor Axon Regeneration in Peripheral Nerve Neuromas. Muscle Nerve, vol. 21, no. 12, Dec. 1998, pp. 1748–1758.

Riley, D.A.: Review of Primary Spaceflight-Induced and Secondary Reloading-Induced Changes in Slow Antigravity Muscles of Rats. Advances in Space Research, vol. 21, nos. 8/9, 1998, pp. 1073–1075.

Objectives/Hypothesis
There is little information available about the characteristics of fast muscles exposed to space microgravity. Calcium release from the sarcoplasmic reticulum (SR) is an important step in the excitation-contraction (E-C) coupling of skeletal muscle. It is generally accepted that transverse (T)-tubule is identified as a signal pathway from sarcolemma to the SR. However, there is no observation regarding the ultrastructure of these architectures in skeletal muscles exposed to actual microgravity. This study was designed to investigate structural changes in the myofilaments, the T-tubules, and the SR, as well as changes in the volume fraction of mitochondria and the activities of oxidative and glycolytic enzymes in fast-type skeletal muscles after spaceflight.

Approach or Method
The skeletal muscles examined in this study were the tibialis anterior, the plantaris, the extensor digitorum longus (EDL), the medial gastrocnemius, and the lateral gastrocnemius sampled from the right limb. These muscles are generally classified as fast-type muscles. Several small sample blocks were dissected from the muscles and were fixed. These blocks were stained by modified Golgi's staining. The fraction was analyzed by point counting and a digitizer from electron micrographs of transverse sections for each muscle. A portion of each muscle (except the EDL) was homogenized, and the supernatants were collected for biochemical analyses. The succinate dehydrogenase (SDH) and phosphofructokinase (PFK) activities were determined at 20°C.

Results
Activity of SDH in medial gastrocnemius muscles of rats was significantly increased following 2-week spaceflight ($p < 0.05$). That of PFK in plantaris muscles was lowered after flight ($p < 0.05$). Overall activities of both enzymes were effectively maintained during flight. No structural alterations in the mitochondria and other organelles were observed in response to spaceflight. However, a myofilament disordering and central nucleus were often seen in the fast muscle during recovery after landing, but not immediately after landing. These observations indicated that spaceflight increases susceptibility to sarcomere damage and metabolic activity in a specific muscle during reloading.

Launch Date 11/3/1994 **Landing Date** 11/14/1994

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effects of Spaceflight on Muscles and Nerves

Science Discipline
Muscle Physiology

Investigator	Institute
K.I. Clark	University of Michigan

Co-Investigator(s)	Institute
Barald, K.F.	University of Michigan
Feldman, E.L.	University of Michigan
Sullivan, K.A.	University of Michigan

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control

Key Flight Hardware
Ambient Temperature Recorder,(ATR), Animal Enclosure Module (AEM)

Selected Publications
Clark, K.I.: Effects of Spaceflight on Development of Neuromuscular Systems. American Society for Gravitational and Space Biology Bulletin, no. 9, 1995, p. 173.

Brown, D.; Clark, K.I.; Pellis, N.R.; and Goodwin, T.: Characterization of Skeletal Muscle Atrophy Induced in Simulated Microgravity Culture Systems. In Vitro Cellular and Developmental Biology, vol. 31, no. 3, 1995, p. 2.

Objectives/Hypothesis
The purpose of this experiment was to determine the influence of gravity on anatomical, cellular, and molecular aspects of neuromuscular development. Specific aims were to: 1) assess the effects of microgravity on the formation of individual muscles following cleavage by comparing muscles that developed in microgravity to those that developed under the influence of the normal gravity on earth; 2) ascertain the effects of microgravity on the placement of the large nerves that course through muscles leading to innervation; and 3) use isoform-specific DNA probes to study levels of myosin heavy chain (MHC) fibers, myosin light chain (MLC) fibers and actin from experimental (microgravity) and control (earth-bound) embryos to ascertain whether gravity plays a role in the molecular development of contractile proteins.

Approach or Method
The muscle and the nerves of the thigh were delineated using immunohistochemistry antibody staining of fixed and frozen cross-sections of the entire thigh. Tissues were double-labeled with MF20, an anti-myosin antibody which binds to embryonic, fetal, and adult skeletal muscle cells, and TUJ-1, an antibody against a neuron-specific tubulin isoform. Slides were used to compare muscle size, placement of divisions among muscles, and locations of major nerve branches between control and experiment thighs. In situ hybridization with riboprobes provided a sensitive method for examining the localization (cellular distribution) of transcripts. Serial sections of tissues were cut and hybridized with anti-sense riboprobes generated to be non-isotopic. Probes were generated to be complementary to the messenger ribonucleic acid (mRNA) of MHC slow, MLC 1f, MLC 2f, and gamma-actin. Sections were compared to those from embryos developed in normal gravity.

Results
From the data of this experiment, it was discovered that the general shape of the thigh muscle was similar between the flight and control animals. However, in the flight subjects there were sections within muscles that appeared to be separated from the rest of the muscles. Results also showed a lack of connective tissue formation in the space between the muscles in the flight group. Three distinct proteins of the muscle responded to microgravity. The fetuses of the flight group displayed less alpha-skeletal actin RNA than the control. Alpha-skeletal actin RNA appeared in similar levels in the pups of both flight and control groups. MLC RNA and MHC were found in a greater amount in the fetuses of the flight group than the control. However, the amount of MHC decreased from fetuses to pups in flight animals while the amount of MHC increased from the fetuses to pups in control animals.

STS-66 / NIH.C2

Launch Date 11/3/1994

Landing Date 11/14/1994

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
The Effect of Space Travel on Skeletal Myofibers

Science Discipline
Muscle Physiology

Investigator / Institute
H.H. Vandenburgh — Miriam Hospital/Brown University School of Medicine

Co-Investigator(s) / Institute
None

Research Subject(s)
Gallus gallus (White leghorn chicken) skeletal muscle organoids

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Space Tissue Loss unit-A (STL)

Selected Publications
Vandenburgh, H.; Chromiak, J.; Shansky, J.; Del Tatto, M.; and Lemaire, J.: Space Travel Directly Induces Skeletal Muscle Atrophy. FASEB Journal, vol. 13, no. 9, June 1999, pp. 1031–1038.

Objectives/Hypothesis
Previous studies indicate that muscle atrophy in space can result from a wide variety of local and systemic factors. Studies at the molecular level utilizing tissue cultures have shown a wide variety of interactions between muscle tension and exogenous growth factors. However, tissue culture studies have the disadvantage that the skeletal fibers used are neonatal in morphology and isoform expression. Utilizing tissue engineering techniques, bioartificial muscle (BAM) tissues were formed to simulate the adult myofiber. This experiment attempted to determine whether spaceflight induces damage and/or atrophy in these engineered myofibers.

Approach or Method
Bioartificial muscles (BAMs) were engineered from embryonic avian muscle cells. Samples were collected during flight. Postflight, BAM cell cultures were analyzed with a variety of biochemical assays. Glucose metabolism and lactate production were assayed. Cultures were also analyzed for total noncollagenous protein content and total DNA content. Fibronectin and Myosin Heavy Chain (MHC) content were analyzed with gel electrophoresis. Protein turnover rates were assessed by [3H] phenylalanine incorporation and [14C] phenylalanine release from pre-labeled proteins. Morphometric measurements were also made of the cell cultures. Cells stained with hematoxylin and eoisin or through immunolabeling of tropomyosin were used to measure mean myofiber diameter, length, and surface area.

Results
Flight culture and ground cultures had similar total collagenous protein and DNA content. Flight BAMs and control BAMs exhibited similar rates of cellular metabolism that increased linearly throughout the flight. As assessed with [14C] phenylalanine, the rate of total muscle degradation was not significantly different for the two groups. However, protein synthesis rates were decreased (79 percent) in flight BAMs on day 9. After return to Earth, protein synthesis rates in the flight BAMs rapidly elevated to ground control levels. MHC levels increased 42 percent in ground control over the course of the experiment while MHC levels in flight BAMs only increased 21 percent. In contrast, fibronectin levels decreased by similar amounts in both ground and flight cultures over the course of the experiment. Mean myofiber size was significantly (10 percent) decreased in flight BAMs when compared to ground controls.

Launch Date 7/13/1995 **Landing Date** 7/22/1995

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Histological Effects of Microgravity on Rat Body Wall Musculature

Science Discipline
Muscle Physiology

Investigator	Institute
R. Wassersug	Dalhousie University

Co-Investigator(s)	Institute
Fejtek, M.	Dalhousie University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control, Vivarium Control, Nonlaparotomized, Control, Laparotomized Control

Key Flight Hardware
Ambient Temperature Recorder (ATR), Animal Enclosure Module (AEM)

Selected Publications
Fejtek, M. and Wassersug, R.J.: Effects of Laparotomy, Cage Type, Gestation Period and Spaceflight on Abdominal Muscles of Pregnant Rodents. Journal of Experimental Zoology, vol. 284, no. 3, Aug. 1, 1999, pp. 252–264.

Fejtek, M. and Wassersug, R.J.: Survey of Studies on How Spaceflight Affects Rodent Skeletal Muscle. Advances in Space Biology and Medicine, vol. 7, 1999, pp. 1–30.

Objectives/Hypothesis
The purpose of this study was to examine the effects of four variables on histological properties of three muscles from the body wall. The muscles examined were the rectus abdoninus (RA), transverse abdominis (TA), and external oblique (EO). These muscles function in both locomotion and in raising intra-abdominal pressure to expel material, including pups at birth, from the body cavity. The variables examined were: 1) pregnancy, 2) animal caging, 3) the effect of a midline laparotomy performed early in gestation to determine fetus numbers, and 4) exposure to spaceflight.

Approach or Method
One group of flight rats underwent cesarean section immediately following flight after which muscles were removed and frozen for shipment. The same procedure was performed on the second group 3 hours postflight. Serial cross sections were taken from frozen muscles of the specimens. Fiber shape, size, and fascicle density were determined by image analysis. Samples were incubated with monoclonal antibodies specific for slow myosin heavy chain (MHC) and fast MHC. Primary antigen-antibody complexes were detected using IgA for slow MHC and IgG for fast MHC. Oxidative enzyme markers succinate dehydrogenase and alpha-glycerophosphate dehydrogenase were used to determine enzyme levels. Changes in enzyme levels were correlated with fiber type.

Results
All four variables listed in the objectives had effects specific to both individual muscles and to experimental conditions. The TA and RA muscles showed signs of stretching with increased gestation. EO, a rotator of the torso, hytrophied in rats housed in complex 3-dimensional group cages as compared to control rats housed singly in flat-bottom cages. The TA and EO muscles, whose contractions would pull on the suture line, showed signs of atrophy in laparotomized animals. The hypertrophy of the EO, an unexpected result, was consistent with the behavior of rats in orbit. In microgravity, the rats did not float freely but instead crawled over the cage walls and each other. This behavior requires active rotation of the torso, indicating weightlessness does not necessarily involve muscle inactivity or unloading. Changes in these muscles did not compromise the ability of the rats to give birth.

STS-77 / NIH.C7

Launch Date 5/19/1996 **Landing Date** 5/29/1996

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effect of Space Travel on Skeletal Myofibers

Science Discipline
Muscle Physiology

Investigator / Institute
H.H. Vandenburgh — Miriam Hospital/Brown University

Co-Investigator(s) / Institute
None

Research Subject(s)
Gallus gallus (White leghorn chicken) skeletal muscle organoids

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Space Tissue Loss-A (STL-A) Module

Selected Publications
Vandenburgh, H.; Chromiak, J.; Shansky, J.; Del Tatto, M.; and Lemaire, J.: Space Travel Directly Induces Skeletal Muscle Atrophy. FASEB Journal, vol. 13, no. 9, June 1999, pp. 1031–1038.

Borselli, C.; Storrie, H.; Benesch-Lee, F.; Shvartsman, D.; Cezar, C.; Lichtman, J.W.; Vandenburgh, H.H.; and Mooney, D.J.: Functional Muscle Regeneration With Combined Delivery of Angiogenesis and Myogenesis Factors. Proceedings of the National Academy of Sciences USA, vol. 107, no. 8, Feb. 23, 2010, pp. 3287–3292.

Objectives/Hypothesis
The physiological mechanisms that lead to a loss of skeletal muscle mass during spaceflight are not understood. This study utilized engineered 3-dimensional skeletal muscle tissues composed of several thousand aligned post-mitotic myofibers. These bioartificial muscles (BAMs) were used to study the effects of microgravity upon skeletal muscle tissues.

Approach or Method
Pre-launch experimental procedures were identical to NIH.C5 (STS-72). On STS-77, 24 BAMs were flown on the Shuttle and another 24 were used as ground controls. In flight, lactate production and glucose metabolism were measured by removing culture medium samples (1–3 ml), storing them in the Space Tissue Loss (STL) Module, and analyzing the aliquots with a YSI Glucose/Lactate Model 2000 analyzer. On the last day of flight, selected bioreactors were infused with 50% (w/v) trichloroacetic acid (TCA) to a concentration of 10% (w/v) and left for 12–24 hours. Postflight, the BAMs were removed from their culture wells and sonicated in 1 ml of sucrose buffer. DNA and total noncollagenous protein content were then determined. [3H] phenylalanine (Phe) was used to determine protein synthesis rates both during and postflight. Quantitative polyacrylamide gels (PAGEs) were used to determine muscle contractile protein myosin heavy chain (MHC), fibronectin, and beta-1 collagen synthesis rates. BAMs were fixed postflight, embedded in Epon, thin sectioned, stained, and randomly selected cross sections were analyzed to determine cross-sectional area.

Results
The tissue-cultured myofibers' reaction to spaceflight was analogous to that of humans and animals in space. These results suggest that BAMs may be used to test possible countermeasures to the effects of microgravity. However, caution must be taken when comparing neonatal-like avian muscle cells with adult mammalian muscle cells. Alterations in protein synthesis rates in microgravity could possibly be due to the coupling of muscle cells tension and protein synthesis via the cytoskeleton. The effects of spaceflight on the BAMs were most likely caused by alterations in the cytoskeletal/sarcomeric network of fibrils, although it is likely that there are numerous secondary effects. Further analysis suggests that anabolic factors such as GH or IGF-1 could be used to counteract muscle atrophy in space. Overall, a combination of exercise and delivery of anabolic factors might be the most effective countermeasure to muscle wasting in microgravity.

Bion 11

Launch Date	Landing Date
12/24/1996	1/7/1997

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Structural and Metabolic Plasticity of Leg Muscle

Science Discipline
Muscle Physiology

Investigator	Institute
B.S. Shenkman	Institute of Biomedical Problems
S.C. Bodine-Fowler	University of California, La Jolla

Co-Investigator(s)	Institute
Vailas, A.C.	University of Wisconsin, Madison
Grindeland, R.E.	NASA Ames Research Center (ARC)
Edgerton, V.R.	University of California, Los Angeles

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications

Shenkman, B.S.; Belozerova, I.N.; Lee, P.; and Nemirovskaya, T.L.: Structural and Metabolic Chracteristics of Rhesus Monkey M. Soleus. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S39-S42.

Belozerova, I.N. , Nemirovskaya, T.L.; and Shenkman, B.S: Structural and Metabolic Profile of Rhesus Monkey m. vastus lateralis after Spaceflight. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, p. S55-S58.

Mazin, M.G.; Kiselyova, E.V.; Nemirovskaya, T.L.; and Shenkman, B. S.; Ultrastructure of Skeletal Muscles of Rhesus Monkeys after Spaceflight. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S59-S62.

Objectives/Hypothesis
Changes in the structure and metabolism of the skeletal muscles of the leg have been observed in both humans and animals after space flight. This experiment examined the effect of microgravity and diminished motor activity (restraint) on the structure and metabolism of the m. vastus lateralis and m. soleus muscles of the rhesus monkey.

Approach or Method
Muscle biopsies were taken from flight control monkeys approximately 100 days before launch and at R +1. Samples were frozen and cut into serial cross-sections 10 μm thick and stained for succinate dehydrogenase (both muscles) or myofibrillar ATPase (m. soleus only). Cross-sectional areas (CSA) of type I and type II fibers were measured from each biopsy. Morphometric measurements were made in a minimum of 50 fibers of each type. In the m. vastus lateralis, total protein, mitochondrial respiration, mitochondrial creatine kinase, oxygen uptake, and mitochondrial respiration were measured. In the m. soleus, capillary/fiber ratio, capillary density, and total protein were measured.

Results
In the m. vastus lateralis postflight, both monkeys showed a significant decrease in the CSA of type I fibers. One of the monkeys also showed a significant decrease in the CSA of type II fibers. In R+17 capsule controls, significant decrease in the CSA of type I fibers was seen in only one monkey, and no change was seen in the CSA of type II fibers. In the vivarium controls, CSA of both fiber types increased. Total protein content remained essentially unchanged in the three groups of monkeys. Maximum oxygen uptake was lower in the flight animals than in the controls. SDH activity in the flight and capsule controls was unchanged in type I fibers, and lower in type II fibers. In the m. soleus, flight monkeys showed a significant decrease in the CSA of type I fibers. One of the monkeys also showed a significant decrease in the CSA of type II fibers. The R+17 control monkeys showed mixed effects. In vivarium controls, three monkeys showed an increase in the CSA of type I fibers and one monkey showed a decrease. The CSA of type II fibers diminished in all vivarium animals. Total protein in type I fibers decreased in both flight animals and the CSA of type II fibers decreased in one flight animal. In the vivarium controls, total protein remained essentially unchanged. The number of capillaries per fiber decreased in the m. soleus of both flight monkeys, but the capillary density varied differentially.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Morphological and Functional Adaptations of Muscle Fibers Myotendonous Junction to Hypokinesia and Hypogravity

Science Discipline
Muscle Physiology

Investigator / Institute
J.F. Marini — University of Aix-Marseille II

Co-Investigator(s) / Institute
None

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Carnino, A.; Roffino, S.; Chopard, A.; and Marini, J.F.: Effects of a 14-day Spaceflight on Soleus Myotendinous Junction Ultrastructure in the Rhesus Monkey. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S65-S68.

Chopard, A.; Leclerc, L.; Pons, F.; Leger, J.J.; and Marini, J-F.: Effects of 14-day Spaceflight on Myosin Heavy Chain Expression in Biceps and Triceps Muscles of the Rhesus Monkey. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S47-S49.

Objectives/Hypothesis
Previous studies have shown that both space flight and simulated microgravity (hindlimb suspension) induce ultrastructural changes in the myotendinous junction (MTJ) of the soleus in the rat. A chronic change in muscle use and/or loading leads to a structural remodeling of the MTJ. Previously, only one histological study has examined the muscle-tendon interface in non-human primates. This experiment will examine the effects of space flight on the soleus MTJ ultrastructure in the rhesus monkey.

Approach or Method
Biopsies were taken from the distal MTJ region in the soleus muscles of flight and vivarium control monkeys after the 14-day space flight. Since the MTJ morphology would not significantly differ after a 14-day period, no growth control biopsies were used. Biopsies were fixed in a graded series of glutaraldehyde solution in 0.4 M cacodylate buffer at pH 7.4, then postfixed in a 2% osmium tetroxide solution. The fixed samples were dehydrated, then embedded in epoxy resin and cut into longitudinal ultra-thin sections (70 nm) through the muscle-tendon interface. The sections were mounted on copper grids and stained with uranyl and lead citrate for electron microscopy.

Results
Structural remodeling occurred at the MTJs after a 14-day space flight, in a similar manner as observed in rat soleus MTJs during the SLS-2 mission. Soleus MTJs of the flight monkeys appeared more shredded than vivarium control MTJs, due to deeper and larger invaginations of the plasma membrane. As observed in rats, the larger, deeper invaginations of the sarcolemma seemed to increase the myotendinous interface. Signs of membrane and basal lamina remodeling were also observed. Granular and vesicular globules observed at the MTJs are likely due to muscle fiber degenerative material. An alteration of contractile material and myofilament anchoring structures was suggested by the presence of numerous vacuoles and caveolae along the lateral sides of the muscle fiber processes. Unlike in the rat soleus MTJ, the contractile apparatus in the rhesus monkey appeared to be less affected by a 14-day space flight. The only misalignment observed was that of adjacent Z disks in the parajunctional region. The structural remodeling described appears to be influenced both by weightlessness and constrained movement during flight.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study

Single Muscle Function and EMG Analysis Associated with Microgravity Conditions

Science Discipline

Muscle physiology

Investigator	Institute
Y. Mounier	Universite des Sciences et Technologies de Lille
B.S. Shenkman	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Desplanches, D.	UER de Grange Blanche - Lyon
Falempin, M.	Universite des Sciences et Technologies de Lille

Research Subject(s)

Macaca mulatta (Rhesus monkey)

Ground-Based Controls

Vivarium Control, Asynchronous Control

Key Flight Hardware

Bion 11 Hardware Suite

Selected Publications

Mounier, Y.; Stevens, L.; Shenkman, B.S.; Kischel, P.; Lenfant, A.M.; Montel, V.; Catinot, M.P.; Toursel, T.; and Picquet, F.: Effect of Spaceflight on Single Fiber Function of Triceps and Biceps Muscles in Rhesus Monkeys. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S51-S52.

Kischel, P.; Stevens, L.; Montel, V.; Picquet, F.; and Mounier, Y.: Plasticity of Monkey Triceps Muscle Fibers in Microgravity Conditions. Journal of Applied Physiology, vol. 90, no. 5, May 2001, pp. 1825-1832.

Objectives/Hypothesis

Atrophy of some lower limb muscles, especially the antigravity muscles, has been observed in animals exposed to microgravity. Few studies have been done on the effects of microgravity on the upper limbs. Primates appear to be a good model for examining muscle contractile and biochemical properties, and making EMG recordings. This experiment will examine the effects of microgravity on the slow-type muscle of the triceps and the fast-type muscle of the biceps.

Approach or Method

Two sets of muscle biopsies were performed on each group of monkeys: flight, 1 G simulation control, and vivarium control (used to simulate growth effects). Biopsies were taken from the biceps and the deep portion of the triceps medialis. Baseline biopsies were taken approximately five months before in-flight (control) or postflight biopsies. Fibers were EGTA-skinned and examined for structural and functional properties. Single fiber types were analyzed by SDS-PAGE for their myosin heavy chain (MHC) composition, and identified as either slow, fast, slow hybrid, or fast hybrid. After being structurally identified, each fiber was studied for its functional Ca activation characteristics (T/pCa curves).

Results

In the baseline biopsies, no significant differences between the monkeys were seen in the maximal tension P0 (kN/m2) and the T/pCa relationship within each fiber type. Significant differences were observed in the T/pCa parameters between slow and fast fiber groups. The biceps muscle was found to be composed exclusively of fast fibers. In the vivarium growth control monkeys, no growth effect was observed in the maximal tension P0 (kN/m2) and the T/pCa relationship. In the confinement controls, the populations of hybrid and fast fibers were increased after confinement conditions. In the flight monkeys, the proportion of fast and hybrid fibers in the triceps increased in comparison to controls. The slow fibers showed signs of atrophy. Postflight, the P0 (kN/m2) was diminished in the slow triceps fibers, and the T/pCa relationships were shifted towards lower pCa values for the slow fibers indicating a decrease in Ca-affinity. The T/pCA curves of the biceps showed no change. Both microgravity and confinement were involved in the changes in muscle properties, and atrophy but microgravity had a specific effect on the cooperativity between the proteins of the thin filament. EMG activity of the biceps and triceps muscles during goal-directed arm movements were studied before during and after 14 days of space flight and flight simulation at normal gravity. The EMG activity was also recorded during treadmill locomotion before and after space flight. Mean EMG was significantly decrease during the flight comparatively to the pre- and postflight values, which were very similar. After space flight, quadrupedal locomotion was modified and abnormal steps were numerous. The integrated area of triceps bursts was increased for the stance phase during locomotion.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effect of Weightlessness on Single Muscle Fiber Function in Rhesus Monkeys

Science Discipline
Muscle Physiology

Investigator	Institute
D. Desplanches	UER de Grange Blanche
R.H. Fitts	Marquette University

Co-Investigator(s)	Institute
None	

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Fitts, R.H.; Romatowski, J.G.; De La Cruz, L.; Widrick, J.; and Desplanches, D.: Effect of Spaceflight on the Maximal Shortening Velocity, Morphology, and Enzyme Profile of Fast- and Slow-twitch Skeletal Muscle Fibers in Rhesus Monkeys. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S37-S38.

Fitts, R.H.; Desplanches, D.; Romatowski, J.G.; and Widrick, J.J. Spaceflight Effects on Single Skeletal Muscle Fiber Function in the Rhesus Monkey. American Journal of Physiology, vol. 279, no. 5, Nov 2000, pp. R1546-R1557.

Objectives/Hypothesis
This experiment sought to determine the extent to which the isotonic contractile properties of the slow- and fast-twitch fiber types of the soleus and gastrocnemius muscles are altered by a 14-day space flight. Elevated velocity in the antigravity slow type I fibers, and the extent to which weightlessness alters glycolytic and oxidative enzyme capacity of individual slow- and fast-twitch fibers, were also examined.

Approach or Method
Soleus and gastrocnemius muscles of the flight and control animals were biopsied approximately 4 months preflight. Flight animals were biopsied 1 and 2 days postflight. Two flight simulation controls were biopsied after 14 days restraint. Small cross-sections were cut from the bottom of the samples and used for determination of myofibrillar and mitochondrial density. The remaining portions were divided longitudinally into three sections, skinned, and separated into single fibers. Single fibers were used to measure peak force (P0), the force-velocity relationship, the pCa-force relationship, maximal unloaded shortening velocity (V0), and peak rate of tension redevelopment (ktr). Fibers were then solubilized and used to determine the myosin heavy and light chain isozyme profiles. Remaining single fibers were freeze-dried and assayed for biochemical content by enzymatic cycling.

Results
Growth had no significant effects on the diameter or peak force of slow type I fibers. In contrast, fiber diameter and peak force declined in both flight and flight simulation animals. Relative force decreased only in flight animals. A larger flight-induced decline in fiber size and peak force was observed in the gastrocnemius than the soleus, but could be explained by muscle atrophy alone, as relative force stayed the same. Post-flight, type II fibers in the gastrocnemius showed a 28% decline in relative force, but no significant change in fiber diameter or peak force. Maximal shortening velocity increased during weightlessness, which could be explained by growth. Postflight, soleus type I fibers showed a shifted pCa-force relationship. However, changes in Ca2+ sensitivity were attributed to growth, as the growth controls showed the same effects. Myofibrillar volume density of the soleus fibers was unchanged by flight. No significant effect of space flight was seen on any of the glycolytic enzymes, in either the slow- or fast-twitch fiber types. The primary effects of space flight were type I fiber atrophy and reduced peak force and power.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Neural Thyroid Interaction on Skeletal Isomyosin Expression in Zero-G

Science Discipline
Muscle Physiology
Neurophysiology

Investigator	Institute
K.M. Baldwin	University of California, Irvine

Co-Investigator(s)	Institute
Takeda, S.	National Institute of Neuroscience

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Adams, G. R.; Haddad, F.; McCue, S.A.; Bodell, P.W.; Zeng, M.; Qin, L.; Qin, A.X.; and Baldwin, K.M.: The Effects of Space Flight on Rat Hindlimb Development II: Expression of Myosin Heavy Chain Isoforms. Journal of Applied Physiology, vol. 88, no. 3, Mar. 2000, pp. 904–916.

Adams, G.R.; McCue, S.A.; Bodell, P.W.; Zeng, M.; and Baldwin, K. M.: The Effects of Space Flight on Rat Hindlimb Development I: Muscle Mass and IGF-1 Expression. Journal of Applied Physiology, vol. 88, no. 3, Mar. 2000, pp. 894–903.

Objectives/Hypothesis
This experiment examined the interactive roles of gravity, innervation, and thyroid hormone (T3) in the developmental programming of myosin heavy chain (MHC) isoform expression in neonatal rodent antigravity and locomotor skeletal muscle. The central hypothesis tested was that gravity exerts a profound influence on the development and maintenance of slow (Type I) MHC expression in antigravity and locomotor muscle. An additional objective was to determine whether 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.

Approach or Method
Flight and ground control rodents were divided into two subgroups: normal (euthyroid) and thyroid deficient (TD). At recovery, key muscles were removed to study MHC isoform expression at both the messenger ribonucleic acid (mRNA) and protein level of analysis using electrophoretic, immuno-histochemical, and in situ hybridization technology. Due to the loss of neonates in flight, tissue samples could not be obtained from animals allowed to recover for 30 days postflight, as originally planned.

Results
Body weights of both the normal (euthyroid) and TD flight rats were significantly lower than the counterpart ground-control groups. Data suggest that lack of nutrition cannot account for the lack of weight gain. Absolute muscle weights were also significantly reduced in all flight groups relative to age-matched ground controls. Both body weight and muscle weight were lower in the TD groups than in the euthyroid groups. In the anti-gravity slow-twitch soleus muscle, spaceflight caused a greater relative atrophy response than in their fast-twitch counterparts. When soleus data was normalized for body mass, it was shown that the relative muscle mass of the soleus did not increase as a result of spaceflight; it remained essentially the same as that seen at 7 days of age. Spaceflight also blunted slow MHC gene expression in the developing soleus muscle and created a profile typically seen in most fast muscles. In contrast, in the TD animals, expression of the Type I MHC was essentially augmented along with retention of small amounts of both the embryonic and neonatal MHCs in both the flight-based and ground-based groups relative to what is typically seen in euthyroid animals during normal development in 1 G. Non-weight-bearing leg muscles such as the tibialis anterior appeared to be the least affected by spaceflight.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
The Effects of Microgravity on Neuromuscular Development

Science Discipline
Muscle Physiology, Neurophysiology, Developmental Biology

Investigator / Institute
D.A. Riley — Medical College of Wisconsin

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Nuclear Yellow (non-radioactive), Vivarium Control, Simulated Flight Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Homick, J.L.; Delaney, P.; and Rodda, K.: Overview of the Neurolab Spacelab Mission. Acta Astronautica, vol. 42, nos. 1–8, Jan.–Apr. 1998, pp. 69–87.

Campbell, M.R.; Williams, D.R.; Buckey, J.C. Jr.; and Kirkpatrick, A.W.: Animal Surgery During Spaceflight on the Neurolab Shuttle Mission. Aviation, Space, and Environmental Medicine, vol. 76, no. 6, June 2005, pp. 589–593.

Objectives/Hypothesis
Previous research has indicated that a critical period of weight bearing may exist for the development of the motor system in animals. This experiment tested the hypothesis that gravity-associated weight bearing is required postnatally for normal neuromuscular development of motor neurons, neuromuscular junctions, and muscle fiber types of the antigravity soleus muscle, but not for that of the extensor digitorum longus (EDL), a non-weight-bearing muscle.

Approach or Method
Muscle and spinal cord tissues of rats were processed and analyzed to evaluate muscle fiber type differentiation, cytoplasm/nucleus ratios, neuromuscular junction development, and spinal motor properties. A ground-control group consisting of hindlimb-suspended pups with a schedule of 4 hours unloaded and 2 hours returned to the dam for nursing, was repeated 24 hours a day for 9 days. Litter mates of the hindlimb-suspension control were removed from the dam on the same schedule as the unloaded rats and singly housed to provide isolation controls.

Results
Exposure to spaceflight resulted in microgravity-induced unloading as well as reduced neonate-dam and neonate-neonate interactions. The in-flight retardation of neonate body weight gain was recovered 1 month postflight. Fewer large soleus fibers postflight suggested stunted growth for some fibers. Higher cytoplasm/nuclear ratios indicated a persistent deficit in soleus myoblast function. Differentiation from embryonic to slow fiber type was transiently retarded and enhanced toward fast type. Spaceflight temporarily increased the susceptibility of developing soleus fibers to reloading damage. Elimination of multiple innervation was completed during spaceflight. The pattern of terminal branching of motor nerve endings was less complex, implying delayed maturation. Spaceflight retarded the growth of spinal motor neurons. Down-regulation of mitochondrial cytochrome oxidase activity and gene expression in lumbar spinal motor neurons indicated lower oxidative capacity in the flight rats. Lower levels of choline acetyltransferase and Cat-301 proteoglycan indicated delayed motor neuron maturation, regarding neurotransmitter synthesis and extracellular matrix organization. Overall, the results from the neonates emphasize the importance of weight-bearing exercise for the normal development of the infant neuromuscular system.

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effects of Microgravity on Postnatal Motor Development

Science Discipline
Muscle Physiology, Neurophysiology, Developmental Biology

Investigator / Institute
K. Walton — New York University Medical Center

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
García-Ovejero, D.; Trejo, J.L.; Ciriza, I.; Walton, K.D.; and García-Segura, L.M.: Space Flight Affects Magnocellular Supraoptic Neurons of Young Prepuberal Rats: Transient and Permanent Effects. Brain Research, Developmental Brain Research, vol. 130, no. 2, Oct. 24, 2001, pp. 191–205.

DeFelipe, J.; Arellano, J.I.; Merchán-Pérez, A.; González-Albo, M.C.; Walton, K.; and Llinás, R.: Spaceflight Induces Changes in the Synaptic Circuitry of the Postnatal Developing Neocortex. Cerebral Cortex, vol. 12, no. 8, Aug. 2002, pp. 883–891.

Objectives/Hypothesis
This experiment examined the adaptability of the motor nervous system to environmental demands. The hypothesis tested in this experiment was: 1) a normal gravitational field is essential for the normal postnatal development of the motor system; 2) elimination of weight-bearing leads to profound changes in motor system organization; 3) changes in motor function are most marked when animals are exposed to microgravity during sensitive periods of development; and 4) functional changes persist into adulthood when animals are exposed during critical periods of motor development.

Approach or Method
Rat pup ages at flight allowed exposure to microgravity during both the sensitive period (P8–P24) and critical period (P14–P30) of neuromotor development. In-flight experiments evaluated locomotion, complex motor skills, and vestibular reflexes with the use of an Animal Walking Apparatus (AWA). Animals were videotaped in flight using two cameras simultaneously while they progressed along rods of varying diameters, along a 1/2-inch mesh surface, on a foam surface, and during tests of surface (or contact) righting. Postflight, animals underwent numerous noninvasive behavioral tests to test their motor skills including swimming, walking, startle reflex, and surface righting.

Results
Three general observations were made over the course of the experiment: (1) The exact set of movements used to achieve a goal, i.e., motor tactics, is influenced by the physical environment during development. This was found in both swimming and walking. (2) The age of the animals influenced the magnitude and duration of the effect. (3) The length of time an animal spends in an altered environment determines if the effects will be transient or long lasting. Locomotion in the microgravity environment was dominated by the forelimbs; when the hindlimbs were used, overstepping was observed. In addition, poor interlimb coordination was observed in these animals. Anatomical studies found that the arborization of the dendritic tree of cervial motorneurons is less rich in flight animals on the day of landing. Studies in the cerebral cortex have found differences on the day of landing that persist for at least 3 months postflight. A preliminary analysis indicates that an Earth-normal gravitational field is needed for the normal postnatal development of motor function.

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Alterations in Skeletal Muscle Gene Expression in Relation to the Muscle

Science Discipline
Muscle Physiology

Investigator / Institute
N. Gonzales-Cadavid — Charles R Drew University

Co-Investigator(s) / Institute
Bhasin, S. — Charles R Drew University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Lalani, R.; Bhasin, S.; Byhower, F.; Tarnuzzer, R.; Grant, M.; Shen, R.; Asa, S.; Ezzat, S.; and Gonzalez-Cadavid, N.F.: Myostatin and Insulin-Like Growth Factor-I and -II Expression in the Muscle of Rats Exposed to the Microgravity Environment of the NeuroLab Space Shuttle Flight. Journal of Endocrinology, vol. 167, no. 3, Dec. 2000, pp. 417–428.

Taylor, W.: Alteration of Gene Expression Profiles in Skeletal Muscle of Rats Exposed to Microgravity During a Spaceflight. Journal of Gravitational Physiology, vol. 9, 2002, pp. 61–70.

Objectives/Hypothesis
This experiment investigated whether muscle loss associated with spaceflight is accompanied by increased levels of myostatin and a reduction in insulin like growth factor IGF-I and -II levels in the muscle, and whether these changes correlate with an increase in muscle proteolysis and apoptosis.

Approach or Method
Rats were divided upon return to Earth into two groups, and sacrificed either 1 day later (R1) or after 13 days of acclimatization (R13). Ground-based control rats were maintained for the same periods in either vivarium (R3 and R15, respectively), or flight-simulated cages (R5 and R17, respectively). Ribonucleic acid (RNA) and protein were isolated from the tibialis anterior, biceps femoris, quadriceps, and gastrocnemius muscles. Myostatin, IGF-I, IGF-II and proteasome 2c mRNA concentrations were determined by reverse transcription/polymerase chain reaction (PCR); myostatin and ubiquitin mRNA were also measured by Northern blot analysis; myostatin protein was estimated by immunohistochemistry; the apoptotic index and the release of 3 methylhistidine were determined respectively by the Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) assay and by high-performance liquid chromatography (HPLC).

Results
Muscle weights were 19–24% lower in the R1 rats compared with the control R3 and R5 rats, but were not significantly different after the recovery period. The myostatin/b-actin mRNA ratios were higher in the muscles of the R1 rats compared with the control R5 rats: 5-fold higher in tibialis, 3-fold in biceps, 1.9-fold in quadriceps, and 2.2-fold in gastrocnemius. These values also normalized upon acclimatization. Western blotting showed that myostatin immuno-staining was increased in muscle sections from R1 rats, compared with control R3 rats, and normalized upon acclimatization. In contrast, IGF-II mRNA concentrations in the muscles from R1 rats were 64–89% lower than those in R3 animals. With exception of the gastrocnemius, IGF-II was also decreased in R5 animals and normalized upon acclimatization. The intramuscular IGF-I mRNA levels were not significantly different between the flight rats and the controls. No increase was found in the proteolysis markers 3-methyl histidine, ubiquitin mRNA, and proteasome 2C mRNA. In conclusion, the loss of skeletal muscle mass that occurs during spaceflight is associated with increased myostatin mRNA and protein levels in the skeletal muscle, and a decrease in IGF-II mRNA levels. These alterations are normalized upon restoration of normal gravity and caging conditions.

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Effects of Microgravity on the Body Wall Muscles in Rodents

Science Discipline
Muscle Physiology

Investigator	Institute
R. Wassersug	Dalhousie University

Co-Investigator(s)	Institute
Fejtek, M.	Dalhousie University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Fejtek, M. and Wassersug, R.: Effects of Spaceflight and Cage Design on Abdominal Muscles of Male Rodents. Journal of Experimental Zoology, vol. 289, no. 5, Apr. 15, 2001, pp. 330–334.

Objectives/Hypothesis
Numerous studies on rodent skeletal muscles exposed to orbital spaceflight have documented histological changes associated with muscle deconditioning. This experiment examined the effects of a 16-day spaceflight mission on the size of muscle fibers in the rectus abdominis, external oblique, and transversus abdominis muscles of adult male Fisher rats.

Approach or Method
Five male Fischer 344 rats were individually housed in orbit in the Research Animal Holding Facility (RAHF), in contrast to the one previous spaceflight investigation of the same muscles, where the rats were group-housed pregnant females. Ground controls consisted of rats similarly kept in the RAHF and another five rats maintained in standard vivarium cages to determine cage effects. Rats were euthanized approximately 24 hours after Shuttle landing and the cross-sectional area of the muscle fibers was used as a measure of muscle atrophy or hypertrophy.

Results
The transversus, which is presumed to be the primary expiratory muscle and consequently works against internal hydrostatic pressures that are not likely to change much between 1 G and weightlessness, did not change in size. However, both the rectus abdominis (a spinal flexor) and the external oblique (a rotator of the torso), which resist gravity in the 1-G environment, showed significant signs of atrophy after extended exposure to microgravity. The atrophy of the external oblique was diametrically opposite to hypertrophy of the same muscle observed in group-housed rodents previously exposed to spaceflight. Although the two missions differed in several factors, such as the gender of the rats and mission duration, the housing of the animals was believed to be the key factor that accounted for the different responses of the external oblique. Previous research has shown that group-housed rats in spaceflight exhibited seven times more rotations of their torsos than matched ground controls. Thus, unloading of the musculoskeletal system may not be achieved in weightlessness when animals have the freedom to interact with each other. No cage effects between RAHF- and vivarium-housed rats was observed.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Mechanisms Underlying Loss of Proprioception

Science Discipline
Muscle Physiology

Investigator	Institute
F.W. Booth	University of Texas Medical School, Houston

Co-Investigator(s)	Institute
Gordon, S.	University of Texas Medical School, Houston

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Deschenes, M.R.; Britt, A.A.; Gomes, R.R.; Booth, F.W.; and Gordon, S.E.: Recovery of Neuromuscular Junction Morphology Following 16 Days of Spaceflight. Synapse, vol. 42, no. 3, Dec. 1, 2001, pp. 177–184.

Objectives/Hypothesis
Adaptations of skeletal muscle to exposure to 0 G have been well documented. It has previously been established that spaceflight elicits alterations in the morphology of the neuromuscular system that includes expansion of the neuromuscular junction (NMJ) and myofiber atrophy. The purpose of this study was to determine the capacity of the neuromuscular system to recover from spaceflight-induced modifications upon return to normal gravity.

Approach or Method
Soleus muscles were obtained from rats participating in the 16-day Neurolab Space Shuttle mission at 1 day and 14 days after returning to Earth; solei were also taken at the same time points from ground-based control rats. Cytofluorescent techniques, coupled with confocal microscopy, were used to assess NMJ morphology. Histochemistry, in conjunction with phase contrast microscopy, was employed to examine myofiber size and type.

Results
One day post landing, the neuromuscular junction were significantly larger in the spaceflight group compared to control. Muscle fiber atrophy was present. By 14 days post landing, neuromuscular area and muscle fiber size were not different from control, suggesting a robust capacity to recover from spaceflight-induced perturbations upon return to normal gravitation influences.

ICE-FIRST / Expedition 8

Launch Date 4/19/2004 **Landing Date** 4/29/2004

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Caenorhabditis elegans Gene Expression and Muscle Physiology in the Space Environment

Science Discipline
Muscle Physiology

Investigator	Institute
C. Conley	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
None	

Research Subject(s)
Caenorhabditis elegans (Nematode)

Ground-Based Controls
Parallel Sets of Transport and Ground Controls

Key Flight Hardware
Kubik incubator

Selected Publications
Adenle, A.A.; Johnsen, B.; and Szewczyk, N.J.: Review of the Results From the International *C. Elegans* First Experiment (ICE-FIRST). Advances in Space Research, vol. 44, 2009, pp. 210–216.

Objectives/Hypothesis
The objectives of the experiment were to: 1) investigate the effects of the spaceflight environment on the expression of known and novel genes by performing full genome microarray analysis; 2) test the hypotheses that radiation repair genes are up-regulated, and genes involved in muscle specification and contractility are down-regulated; 3) investigate regulation of genes involved in worm 'immune' function and aging; 4) determine if developmental timing is altered by spaceflight using cuticles shed in flight as a metric of larval development; and 5) determine if there are major changes in muscle in response to flight by investigating the localization of Tropomodulin and other contractile proteins of muscle.

Approach or Method
ICE-FIRST was a collaborative effort among four nations: France, the United States, Japan, and Canada. Fifty-three sets of *C. elegans* were prepared and loaded in flight hardware in Toulouse, France 5–7 days prior to launch from the Kennedy Space Center, Florida to the International Space Station (ISS). Parallel sets of transport and ground control animals were also utilized.

Results
The Apoptosis investigation of this experiment showed that checkpoint and physiological apoptosis in germ cells occurred normally in space flown *C. elegans* suggesting that radiation damaged cells should be able to be removed via apoptosis in flight and that *C. elegans* can be employed as a biological accumulating dosimeter. However, no statistically significant mutational changes in spaceflown animals were detected indicating that mutagenic effects of radiation in LEO are small. The expression of myogenic transcription factors was decreased in space-flown worms. Insulin and TGF-beta signaling in flight were altered.

STS-118 / CBTM-2

Launch Date 8/8/2007 **Landing Date** 8/21/2007

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Commercial Biomedical Testing Module-2 (CBTM-2)

Science Discipline
Muscle Physiology

Investigator / Institute
H. Q. Han — Amgen Research
D. Lacey

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (C57/B6 mouse)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Baqai, F.P.; Gridley, D.S.; Slater, J.M.; Luo-Owen, X.; Stodieck, L.S.; Ferguson, V.; Chapes, S.K.; and Pecaut, M.J.: Effects of Spaceflight on Innate Immune Function and Antioxidant Gene Expression. Journal of Applied Physiology, vol. 106, no. 6, Jun. 2009, pp. 1935–1942.

Allen, D.L.; Bandstra, E.R.; Harrison, B.C.; Thorng, S.; Stodieck, L.S.; Kostenuik, P.J.; Morony, S.; Lacey, D.L.; Hammond, T.G.; Leinwand, L.L.; Argraves, W.S.; Bateman, T.A.; and Barth, J.L.: Effects of Spaceflight on Murine Skeletal Muscle Gene Expression. Journal of Applied Physiology, vol. 106, no. 2, Feb. 2009, pp. 582–595.

Objectives/Hypothesis
Commercial Biomedical Test Module-2 (CBTM-2) used a validated mouse model to examine the effectiveness of an experimental therapeutic as a possible countermeasure for muscle atrophy. Combined with exercise, this experimental therapeutic developed by Amgen could one day form the basis for a treatment that will help maintain a high level of physical fitness in future flight crews. Also, through an extensive tissue sharing program, several additional investigations were performed to determine the effects of microgravity on the skeletal, cardiovascular, immune system, liver and kidney function, as well as other physiological systems.

Approach or Method
Nine-week-old female C57/B6 mice were launched on this 13-day flight on the Space shuttle, for a total of 11–12 days in microgravity. Flight mice were treated once with a placebo vehicle or therapeutic agent approximately 24 hours before launch. Ground-control mice were treated similarly but with a 48-hour offset and were housed under the same environmental conditions (temperature, light/dark cycle, humidity, oxygen levels, and carbon dioxide levels) as the flight mice. All mice received the same full access to food and water. Upon return to Earth, bone marrow cells were isolated from the humeri (long bones of the upper limb or forelimb) of the mice and counted. The expression of several molecules (Ly6C, CD11b, CD31 (PECAM-1), Ly6G (Gr-1), F4/80, CD44, and c-Fos) that define the maturation state of cells in the granulocytic lineage on three bone marrow cell subpopulations (R1, R2, and R3) were defined by their size and light-scattering properties. Body weight of the mice was also measured pre- and postflight.

Results
There were no observable characteristic differences between total bone marrow cells isolated from flight and ground-control mice. Nevertheless, there were subpopulation differences observed that suggested neutrophil activation in response to landing. Decreases were noticed in Ly6C, c-Fos, CD44high, and Ly6G. An increase in F4/80 suggested that the cells in the bone marrow R3 subpopulation of the mice flown on Shuttle were more differentiated compared to the ground controls. A loss in body weight was also noticed in the mice that flew in space, which suggest that they were subjected to chronic stress beyond what was endured during landing. Therefore, it is not unreasonable to suggest that there are significant changes in bone marrow phenotype in response to the stress of the spaceflight experience.

STS-133 / BSP

Launch Date 2/24/2011 **Landing Date** 3/9/2011

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
The Response of Articular Cartilage to Microgravity

Science Discipline
Muscle Physiology

Investigator	Institute
J. Fitzgerald	Oregon Health and Science University

Co-Investigator(s)	Institute
None	

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Simulated Flight Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
The objective was to investigate alterations in gene expression in articular cartilage, skeletal muscle, and skin exposed to microgravity.

The data from STS-131 animals demonstrate that in cartilage, muscle, and skin, genes that respond to different types of common stresses such as oxidative stress and genotoxic stress are also upregulated under conditions of microgravity. This is a completely novel finding and may explain some of the tissue-level effects of microgravity reported, such as muscle atrophy.

This experiment examined the hypothesis that one effect of microgravity in these tissues is the activation of stress response genetic networks, and this has effects on cell proliferation and tissue maintenance.

Approach or Method
The molecular data from STS-131 has generated new hypotheses to test. The availability of muscle samples from STS-133 and STS-135 animals was extremely useful for validating and extending current data. The STS-131 samples were assessed for gene expression differences using defined microarrays for Affymetrix. The tissue samples stored in formalin were used to examine microgravity-related changes in protein levels and were guided by the gene expression analyses.

Results
At the time of publication data analysis is still in progress.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Spaceflight's Effects on Vascular Atrophy in the Hindlimbs of Mice

Science Discipline
Muscle Physiology and Vasculature

Investigator / Institute
R.J. Midura — The Cleveland Clinic Foundation

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Carpenter, R.D.; Lang, T.F.; Bloomfield, S.A.; Bloomberg, J.J.; Judex, S.; Keyak, J.H.; Midura, R.J.; Pajevic, P.D.; and Spatz, J.M.: Effects of Long-Duration Spaceflight, Microgravity, and Radiation on the Neuromuscular, Sensorimotor, and Skeletal Systems. Journal of Cosmology, vol. 12, Oct.–Nov. 2010, pp. 3778–3780.

Androjna, C.; McCabe, N.P.; Cavanagh, P.R.; and Midura, R.J.: Effects of Spaceflight and Skeletal Unloading on Bone Fracture Healing. Clinical Reviews in Bone and Mineral Metabolism, vol. 10, no. 2, June 2012, pp. 61–70.

Objectives/Hypothesis
Assess muscle- and bone-associated vasculature volumes in the hindlimbs of mice flown in space (STS -135) and compare them to ground-based mice undergoing weight bearing (WB) or hindlimb unloading (HLU). Hypogravity leads to sarcopenia and a loss of cortical bone mass and bone mineral density (BMD) in the lower hindlimb in rodents. Skeletal muscle and bone tissues contain an intrinsic vascular supply that is recognized as essential for proper maintenance of vital tissue functions. It has been suggested that hypogravity may induce some vascular degeneration within hindlimb tissues thereby contributing to sarcopenia and osteopenia, but this concept has not been extensively investigated. Lower hindlimb muscle- and bone-associated vascular tissue outcomes were evaluated in mice after 12 days of STS-135 spaceflight exposure, and compared to vascular tissue outcomes over an identical time period of synchronous ground-based WB and HLU mice. Vasculature outcomes in a standardized region of the mid-lower hindlimb (including portions of the soleus muscle and tibia/fibula) were assessed using both immunostaining of vascular cell markers in tissue sections along with quantitative polymerase chain reaction (PCR) gene array analyses for genes associated with endothelial cell function from adjacent tissue sections. These vascular outcomes were compared to muscle and bone tissue assessments in the same tissue regions as assessed by histomorphometry.

Approach or Method
Hypogravity leads to sarcopenia and a loss of cortical bone mass and BMD in the lower hind limb in rodents. Skeletal muscle and bone tissues contain an intrinsic vascular supply that is recognized as essential for proper maintenance of vital tissue functions. It has been suggested that hypogravity may induce some vascular degeneration within hind limb tissues thereby contributing to sarcopenia and osteopenia, but this concept has not been extensively investigated. Lower hind limb muscle- and bone-associated vascular tissue outcomes will be evaluated in mice after 12 days of STS-135 space flight exposure, and compared to vascular tissue outcomes over an identical time period of synchronous ground based WB and HLU mice. Vasculature outcomes in a standardized region of the mid-lower hind limb (including portions of the soleus muscle and tibia/fibula) will be assessed using both immunostaining of vascular cell markers in tissue sections along with quantitative PCR gene array analyses for genes associated with endothelial cell function from adjacent tissue sections. These vascular outcomes will be compared to muscle and bone tissue assessments in the same tissue regions as assessed by histomorphometry.

Results
qRT-PCR Analyses: A greater than 1.5-fold increase or decrease in gene expression levels between spaceflight and ground control was chosen as physiologically significant. For "bone" samples, 10 of 84 interrogated genes were altered in spaceflight specimens as compared to ground controls. Gene expression levels for 3 genes (Cxcr5, Ccl2, Il1b) were decreased by ≥ 2-fold and that for 1 gene (Mmp1a) was increased ≥ 2-fold. Gene expression levels for 3 genes (Selplg, Icam1, Vegfa) were decreased by > 1.5-fold and those for 3 genes (Fgf1, Cpb2, Serpine1) were increased by > 1.5-fold; these changes were also statistically significant. For "muscle" samples, 16 of 84 interrogated genes were altered in spaceflight specimens as compared to ground controls. Gene expression levels for 8 genes (Adam17, Bcl2l1, Cdh5, Pf4, Edn2, Mmp9, Selplg, Serpine1) were increased ≥ 2-fold. Gene expression levels for 1 gene (Tek) was decreased by > 1.5-fold and those for 7 genes (Ace, Agtr1a, Bcl2, Cx3cl1, Il6, Mmp2, Ptgis) were increased by > 1.5-fold; these changes were also statistically significant. Selplg and Serpine1 were the only gene expression alterations occurring in both bone and muscle samples; ≥ 2 for muscle and between 1.5 and 2.0 for bone samples, but fold trends were opposite in the two tissue compartments.

Immuno-histomorphometry Analyses: Tissue sections have been immuno-stained and photomicrograph images have been collected. Data analysis is pending upon completion of histomorphometry measurements.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: MUSCLE PHYSIOLOGY

Title of Study
Skeletal Muscle Regeneration

Science Discipline
Muscle Physiology

Investigator / Institute
E.R. Barton — University of Pennsylvania

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

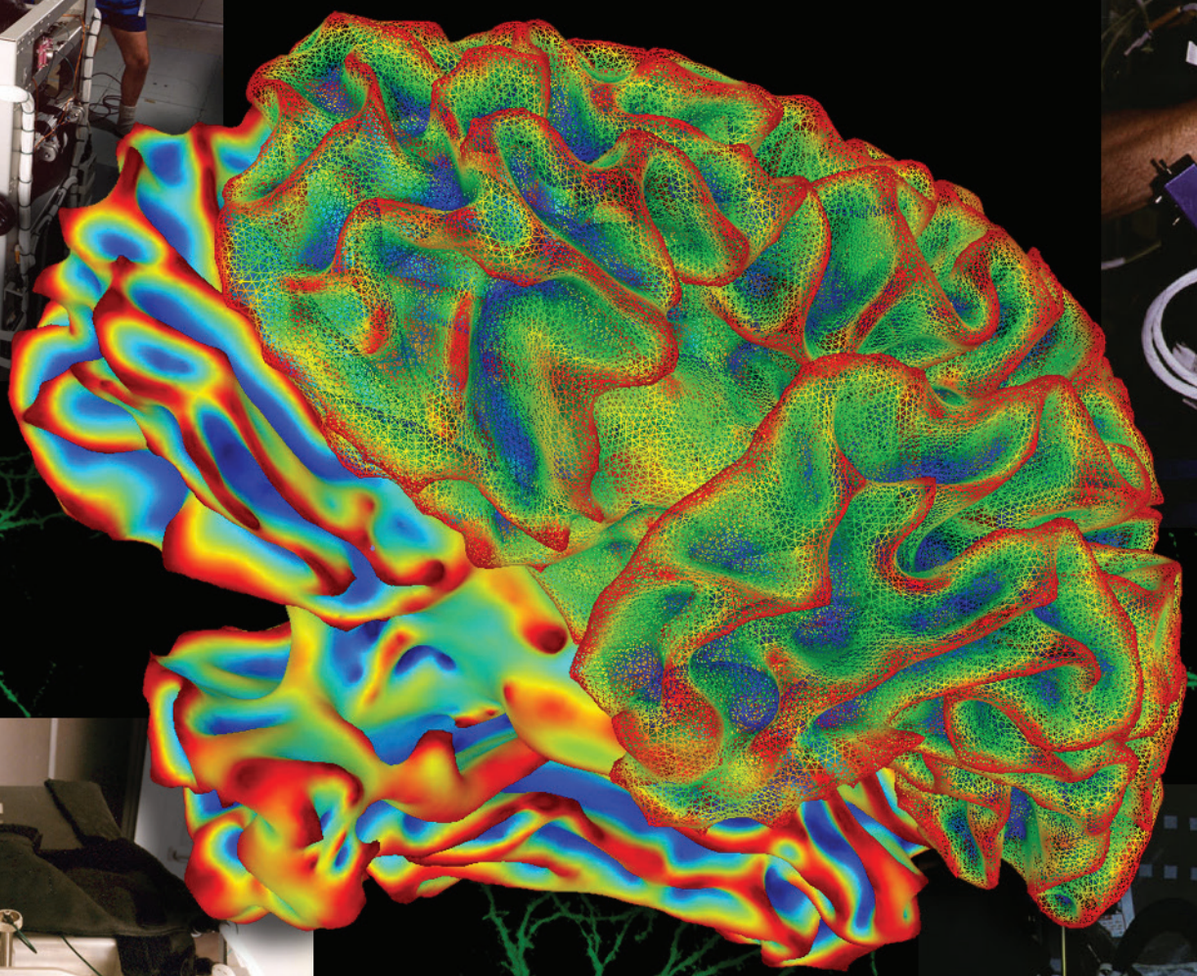
Selected Publications
None

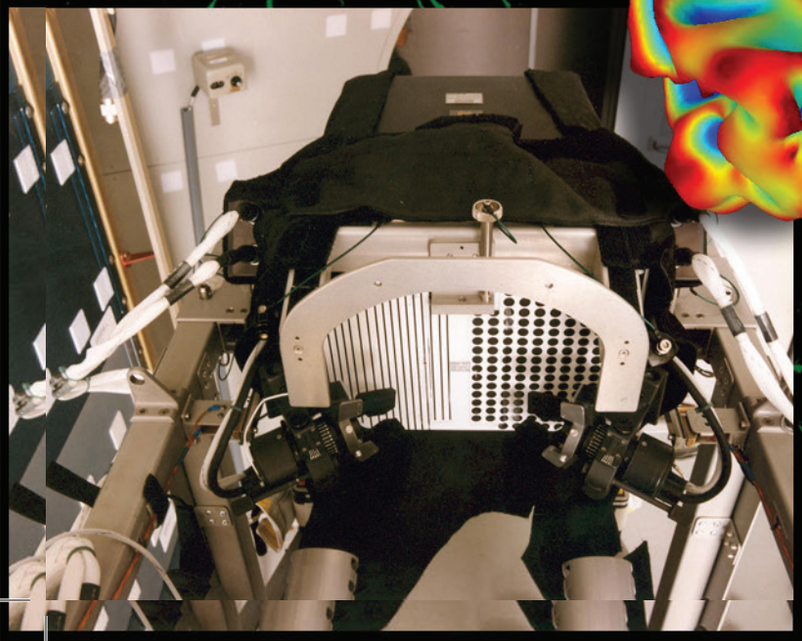
Objectives/Hypothesis
The goals of this project were to understand the contribution of a protein complex in muscle cell membranes to sensing load. While much has been achieved in previous shuttle missions with mouse samples, the sarcoglycan complex involvement has not been studied. From related projects, clear differences between the masseters and tibialis anterior (TA) muscles in terms of loading signals and expression differences have been reported. This experiment examined the hypothesis that the loading of masseter muscles comes in part from normal chewing activity, and so the mouse masseters may be spared from atrophy. It was further hypothesized that load (or lack of it) affects the post-translational state of the sarcoglycan complex, which included both phosphorylation of intracellular tyrosines as well as the glycosylation of the complex. These studies established a mechanistic understanding of the mechanical responses through each membrane complex, and provided the groundwork for developing novel strategies to prevent muscle atrophy in space.

Approach or Method
Muscles were harvested from the mice available from STS-135. The experimental animals included baseline (pre-flight) controls, ground controls, and those that were subjected to spaceflight and microgravity. We obtained TA, extensor digitorum longus (EDL), and masseter muscles from these mice. For the masseters, EDL, and TA muscles, the muscles from one side of the mouse were weighed and rapidly frozen for biochemical measurements. The muscles from the contralateral side were fixed in 4% paraformaldehyde, processed in 10% sucrose, then frozen in melting isopentane for morphological measurements. Note: 1) that EDL and TA muscle weight were obtained prior to receipt of the samples; and 2) that masseters from all mice of each group (N = 15) were obtained, whereas EDL and TA muscles from N = 7 mice of each group were obtained. Comparisons within groups utilized paired t-tests; comparisons across groups utilized 1-way analysis of variance (ANOVA) followed by Tukey post-hoc analysis.

Results
All muscle weights for the masseters have been obtained, but the results for other muscles are pending. Note that a subset of masseters (N = 8) was from animals treated with a compound that may affect results; they will be removed from the study once the list of treated animals is received. The mean body weight of flight animals was ~7% lower than the mean body weight of the baseline animals ($P < 0.05$), but there was no other significant difference between conditions. For masseter mass, the absolute muscle mass for flight animals was significantly lower than ground animals (a 9% decrease), and the masseter muscles from the baseline animals was significantly lower than both other groups. Note that the most important comparison for this study is between flight and ground animals, where the only difference was microgravity. Thus, from the standpoint of absolute muscle mass, the hypothesis that masseters were spared from atrophy must be rejected. Because the animals also lost body weight during weightlessness, it was determined if the loss of masseter muscle mass was proportional to total body weight in the animals. No statistically significant difference in normalized masseter mass between flight and ground animals was found, suggesting that the initial hypothesis is upheld. Additional testing will occur upon receipt of the list of treated animals that should be removed from the study. In addition, the changes in mass in the masseter will be compared to those in the limb muscles to determine if the susceptibility of muscles to microgravity is uniform across different muscle groups.

Neurophysiology





Neurophysiology Introduction

Richard Boyle, Ph.D., NASA Ames Research Center

Of all the environmental factors that an animal has been exposed to in the course of its evolution, only gravity has stayed constant. Predation, climate, vegetation, and terrestrial or aquatic habitation, for example, have changed, but the intensity and direction of gravity have not. The ability of an organism to detect gravity and to live under a gravitational load is critical for its survival. Even rudimentary ciliated protozoa display positive or negative geotaxis. Fossil evidence shows that the elaborate sensory structures used to sense the acceleration forces are remarkably conserved among vertebrates. Although less is available in the fossil record on invertebrate neurosensory structures, most, if not all, invertebrate species can orient their bodies' axis with respect to gravity.

Over 50 years before the advent of the space age, neuroscientists in laboratories and clinics throughout the world were identifying the neural mechanisms underlying spatial orientation: self-movement detected by transducers in the inner ear organs and the receptors associated with muscles and joints, and motion perception of an external visual object and whole field from retinal receptors in the eye. The arrival of aviation offered a new, more complex laboratory where man and machine interacted in a rapidly changing environment. This environment proved dangerous to many less experienced pilots when an Earth stationary visual reference, such as the horizon, was lost, leading the craft into a graveyard spiral. To avoid this deadly entrapment, the pilot must override the state of equilibrium sensed by the inner ear indicating a level flight path and rely entirely on the craft's instrumentation. The pervasiveness of gravity on Earth provided the nervous system of the diverse animal species a common reference about which to optimize sensory transduction mechanisms and sensory perception. This reference was now completely violated by the speed at which the individual and visual world were moving, and by the rapidly changing orientation of the gravity vector with respect to the individual in the aircraft. Thus, we entered the space age in the early 1960s with our eyes wide open when Yuri Gagarin journeyed into "outer space" in his Vostok spacecraft.

With the advent of spaceflight it is possible to address fundamental questions on the biological principle(s) of how the nervous system in animals, from humans to invertebrates, responds and adapts to weightlessness [STS-40 / SLS-1, p. 321]. A key question is, despite the constancy of gravity, does the nervous system adapt to novel transitions in gravity states, even for transitions of brief duration measured in days [STS-40 / SLS-1, p. 324]? Another key question is, because of the elemental nature of, say, gravi-reception, do animals respond similarly to altered gravity states and adapt to the new, and in the case of postflight, the original, environment? This chapter illustrates the attempts made by neuroscientists to address these questions designed to identify how the nervous system responds to the space environment. This is, of course, not an easy task given the limitations of conducting fundamental animal research on unmanned orbital missions starting with Cosmos 1887 / Bion 8 in 1987 and ending with the manned STS-135 mission in 2011. During this period of investigation, the rodent was the primary animal model, but other models such as monkey, fish, mollusks, birds, and insects were also used to explore a wide range of neural mechanisms. Scientists from across the globe participated in these pioneering efforts.

Two key publications provide a thorough description of selected experiments, insights into experimental design, and offer detailed interpretations of the scientific findings. The first publication is by Bernie Cohen and colleagues. It focuses on the neurovestibular and oculomotor systems, and summarizes the key findings [Cohen

et al.,2005]: "From the 1970s, the Russian Space Agency made orbital space flight available to INTERCOSMOS scientists from many countries for animal experimentation, primarily on rats. Between 1983 and 1995, monkeys and rats flew for approximately 1–2 week periods on a Vostok Space Capsule in experiments supported by the Russian Space Agency and the National Aeronautics and Space Administration (NASA). Rodents and invertebrates also flew on NASA Space Shuttle missions, particularly on the Neurolab Mission (STS-90) in 1998. The purpose of this chapter is to provide a summary of these experiments." Pertinent to the present chapter of Ames Research Center's participation in space studies, Section 1 of Cohen et al. examines the cellular responses to altered gravitational environments in adult animals, and Section 3 highlights the NASA-Russian monkey experiments on the angular and linear vestibulo-ocular reflex [Cosmos 2229 / Bion 10, p. 331] [Cosmos 2044 / Bion 9, p. 317]. For the interested reader, Section 2 gives an excellent summary of the in-flight studies in the Russian Cosmos Project that focused on the effects of microgravity on the angular vestibulo-ocular reflex and gaze control, and on single-unit neural activity in the vestibular nuclei and cerebellar flocculus of monkeys.

The second publication is particularly important and provides an in-depth analysis of the orbital STS-90, a mission dedicated solely to the study of the nervous system response to spaceflight, launched during the Decade of the Brain—the mission called Neurolab [Buckey and Homick, 2003]: "On April 17, 1998, the Neurolab Spacelab Mission lifted off from Kennedy Space Center. On board were 26 experiments dedicated to studying the effects of weightlessness on the brain and nervous system." Six experiments were designed to study how spaceflight affects balance in humans, rats, and fish [STS-90 / Neurolab, p. 350]. How spaceflight influences sensory integration of different senses and navigation was studied in three human psychophysics studies and in one rat neurophysiology experiment. A major effort in this mission was made to determine if the presence of gravity is a prerequisite for normal neural development, with seven studies using the rodent model [STS-90 / Neurolab, p. 351] [STS-90 / Neurolab, p. 299], one investigation applying a comparative approach with several invertebrate species [STS-90 / Neurolab, p. 352] and one vertebrate (fish) species [STS-90 / Neurolab, p. 360], and one fly neural development study. The loss of the gravity vector has a clear impact on blood pressure control mechanisms, and this was studied in four human studies. Lastly, the light and dark cycles during orbital missions roughly approximate a 90-minute day, and thus sleep and circadian rhythms are affected, and three experiments (two human and one rat), [STS-90 / Neurolab, p. 392] were dedicated to this problem.

Despite our best efforts we have suggestive, but nevertheless, marginal data on the effects of microgravity, and even limited data on the long-term effects of hypergravity in ground-based studies, on neural development and function. We do know that relatively short-term exposure to weightlessness dramatically alters vestibular sensation. As the astronaut begins the mission, the inner ear mechanisms are functioning normally. Within the first days in space and for some time upon return following a relatively short exposure to weightlessness, the astronaut experiences difficulties in orientation and stability not unlikely symptoms of a vestibular patient. The underlying conflict is likely due to an adaptive, temporary change in synaptic strength, and in time the astronaut recovers to normal function. However, this is not the case for the patient who is in it for the "long haul" and must learn new adaptive strategies to manage even simple behaviors. The impact of long-term absence of gravity on the animal's nervous system is unknown and will likely involve complex adaptive mechanisms. Without sufficient countermeasures, some of these compensatory

mechanisms might lead to structural changes and modifications of interconnectivity between neuronal populations, and thus prove maladaptive to the organism when reintroduced into a gravity environment. A wealth of clinical and experimental data is known on the long-term consequences of inner ear damage on motor control function along the neuraxis, and we need to design new translational studies to understand the scope and extent of the neural compensatory mechanisms. The behavior and plasticity of synaptic organization and neural function will have important relevance to crew health and performance during exploration of space, and we have a formidable challenge ahead of us.

List of referenced flight experiments:

Cosmos 2044 / Bion 9, B. Cohen, Adaptation of Optokinetic Nystagmus to Microgravity

Cosmos 2229 / Bion 10, B. Cohen, Reduction of Ocular Counter-Rolling by Adaptation to Space

STS-40 / SLS-1, L.N. Dyachkova, Ultrastructure of the Brain Cortex

STS-40 / SLS-1, M.D. Ross, Effects of Space Travel on Mammalian Gravity Receptors

STS-90 / Neurolab, C.A. Fuller, CNS Control of Rhythms and Homeostasis During Space Flight

STS-90 / Neurolab, E. Horn, Development of an Insect Gravity Sensory System in Space

STS-90 / Neurolab, G. Holstein, Anatomical Studies of Central Vestibular Adaptation

STS-90 / Neurolab, K. Walton, Effects of Microgravity on Postnatal Motor Development

STS-90 / Neurolab, M.L. Wiederhold, Development of Vestibular Organs in Microgravity

STS-90 / Neurolab, S. Highstein, Chronic Recording of Otolith Nerves in Microgravity (Toadfish)

Literature references cited:

Buckey, J.C., Jr. and Homick, J.L. (Eds.): The Neurolab Spacelab Mission: Neuroscience Research in Space. NASA SP-2003-535, 2003.

Cohen, B.; Yakushin, S.B.; Holstein, G.R.; Dai, M.; Tomko, D.L.; Badakva, A.M.; and Kozlovskaya, I.B.: Vestibular Experiments in Space. Advances in Space Biology and Medicine, vol. 10, 2005, pp. 105–164.

Biosatellite III

Launch Date 6/28/1969

Landing Date 7/7/1696

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Sleep/Wake Activity Patterns of a Pig-Tailed Monkey During Nine Days of Weightlessness

Science Discipline
Neurophysiology

Investigator	Institute
W.R. Adey	University of California, Los Angeles

Co-Investigator(s)	Institute
Durham, R.	University of California, Los Angeles
Hoshizaki, T.	University of California, Los Angeles

Research Subject(s)
Macaca nemestrina (Pig-Tailed Monkey)

Ground-Based Controls
Laboratory (Flight Backup Subjects); Flight Simulated (to 30 days)

Key Flight Hardware
Primate Life Support System; Primate Physiological Sensors

Selected Publications

Hahn, P.M.: Circadian Rhythms of the Macaca nemestrina Monkey in Biosatellite III. BIOSPEX: Biological Space Experiments, NASA TM -58217, 1979, p. 109.

Hahn, P.M.; Hoshizaki, T.; and Adey, W.R.: Circadian Rhythms of the Macaca nemestrina Monkey in Biosatellite III. Aerospace Medicine, vol. 42, 1971, pp. 295-304.

Hoshizaki, T.; Hahn, P.M.;, and Adey, W.R.: Circadian Rhythms and Sleep/Wake Activity in the Biosatellite Monkey. Physiologist, vol. 16, 1973, pp. 202-208.

Objectives/Hypothesis
The rhythmicity of activity levels, metabolism, excretion rates, thermoregulation, and cardiovascular measures persist in terrestrial laboratory conditions where environmental factors such as light, temperature, and humidity are kept in con- stant and unvarying conditions. It is believed that if these circadian processes become arrhythmic or desynchronized a deterioration of the organism can result. As the possibility of desynchronosis of the circadian rhythm and its consequences in the space environment is of great concern, this experiment was designed to study the effect of weightlessness on circadian rhythms.

Approach or Method
A variety of parameters measured inflight were analyzed and compared to simi- larly maintained ground-control subjects in order to determine if desynchronosis occurred. Telemetry included implanted sensors for EEG, EMG, ECG, and respiration, vascular catheters to monitor venous and arterial pressures, temperature sensors in the brain, and general environmental parameters. Computer programs and plotting techniques were used to estimate periodicity. Due to the rapid changes in parameters recorded during the last thirty hours, only 7.5 cycles of 24-hour rhythms were used in analysis from the 8.8-day flight. Day averaging was the most common method: data obtained during the flight were interpolated to fixed 1.5-hour intervals; an average for a four-day period was obtained; and deviations were plotted to give the parameter a cyclic representation. Time displacement of two such tracings was an indication of an altered circadian rhythm.

Results
All physiological sensors functioned well throughout the flight, and the subject displayed a define desynchronosis in some physiological processes. The pCO2, brain and body temperatures and heart rate were well correlated and indicated a rhythm of greater than 25 hours; however arterial blood pressure remained at 24 hours. Such internal desynchronization of temperature, cardiac, and respiratory cycles from the blood pressure and the external desynchronization from the imposed 24-hour daily routine may have been detrimental to the well-being of the flight subject. The derangement of the cardiovascular system suggested as a concomitant of space flight, and the desynchronization found in the flight subject, may well have acted together to bring about its rapid deterioration. There was no evidence of this desynchronosis in any ground controls, including Biosatellite simulations lasting up to thirty days. This suggests the existence of a gravity dependent mechanism in the control of circadian rhythm.

Biosatellite III

Launch Date 6/28/1969 **Landing Date** 7/7/1696

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Digital Computer Analysis of Neurophysiological Data from Biosatellite III

Science Discipline
Neurophysiology

Investigator	Institute
W.R. Adey	University of California, Los Angeles

Co-Investigator(s)	Institute
Walter, D.O.	University of California, Los Angeles
Berkhout, J.I.	University of California, Los Angeles
Buchness, E.	University of California, Los Angeles
Kram, E.	University of California, Los Angeles
Rovner, L.	University of California, Los Angeles

Research Subject(s)
Macaca nemestrina (Pig-Tailed Monkey)

Ground-Based Controls
Laboratory (Flight Backup Subjects)

Key Flight Hardware
Primate Life Support System; Primate Physiological Sensors

Selected Publications

Walter, D.O.; Berkhout, J.I.; Buchness, R.; Kram, E.; Rovner, I.; and Adey, W.R.: Digital Computer Analysis of Neurophysiological Data from Biosatellite III. Aerospace Medicine, vol. 42, 1971, pp. 314-321.

Walter, D.O.: Digital Computer Analysis of Neurophysiological Data from Biosatellite III. Aerospace Medicine, vol. 42, 1971, pp. 314-321.

Adey, W.R. and Hahn, P.M.: Introduction: Biosatellite III Results. Aerospace Medicine, vol. 42, 1971, pp. 273-280.

Objectives/Hypothesis
Two goals were formulated for computer analysis of Biosatellite III data: 1) a short term analysis to assist in animal monitoring and mission abort decisions and 2) a long-term analysis to support the general physiological studies, including circadian rhythm studies. Down-linked data for short-term analysis were available from telemetry captures at prime receiving stations in Quito, Ecuador; Lima, Peru; Santiago, Chile; and Fort Myers, Florida. Data for long-term analysis were available from the prime stations and many others following flight.

Approach or Method
Spectra and coherences were presented principally in the form of contour maps which compress much data into brief compass. Transient changes in the animal's responsive states, circadian rhythms in neuro-electric parameters, and the general course of EEG are represented in one, highly compressed set of maps. Short term analysis and transmission of output graphs to Mission Control was initiated within seven hours of the data's generation in space. A composite map of EEG spectral intensity contours from insertion of the animal into the capsule to de-orbit was obtained by plotting contours across 180 data-capture epochs occurring at irregular intervals approximately 1.5 hours apart. In this mapping, the left parietal cortex was representative of the four cortical channels, and the left amygdala was representative of the six deep bipolar leads. A total of 46,270 seconds of "long- term" data was processed in ten-second epochs, for these and other maps.

Results
Launch was a mildly traumatic event for the animal, intensity contours show alterations during the two hours immediately following launch, then return to stable, prelaunch levels. Visible spectral peaks on the left parietal channel on days two, three, four, and five suggest that the animal was aroused during per-formance tasks, although the actual performance was quite low. The animal appears to have had a functionally intact cortex until flight day six, and to have had a functional cortical impairment on flight days seven and eight. This was compatible with a minimal response to alterations of light versus dark and with maintenance of normal subcortical electrical activity. The animal became grossly pathological and unresponsive on flight day nine, when the mission was terminated. Considerable fluctuations in spectral intensity persisted within certain frequency bands. This pathological state resembled, but was not identical with, a state of acute hypothermia under anesthesia. Death occurred eight hours after recovery, the acute cause being ventricular fibrillation.

Biosatellite III

Launch Date 6/28/1969

Landing Date 7/7/1696

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Sleep and Wake States in Biosatellite III Monkey: Visual and Computer Analyses of Telemetered Electroencephalographic Data

Science Discipline
Neurophysiology

Investigator	Institute
W.R. Adey	University of California, Los Angeles

Co-Investigator(s)	Institute
Hanley, J.	University of California, Los Angeles

Research Subject(s)
Macaca nemestrina (Pig-Tailed Monkey)

Ground-Based Controls
Laboratory (Flight Backup Subjects)

Key Flight Hardware
Primate Life Support System; Primate Physiological Sensors

Selected Publications
Hanley, J. and Adey, W.R.: Sleep and Wake States in the Biosatellite III Monkey: Visual and Computer Analysis of Telemetered Electroencephalographic Data from Earth Orbital Flight. Aerospace Medicine, vol. 42, 1971, pp. 304-313.

Hoshizaki, T.; Durham, R.; and Adey, W.R.: Sleep-Wake Activity Patterns of a Macaca nemestrina Monkey During Nine Days of Weightlessness. Aerospace Medicine, vol. 42, 1971, pp. 288-295.

Objectives/Hypothesis
It is well established that the different sleep states are necessary in sufficient quantity for the continuance of physiological and psychological well-being. This circadian rhythm, as well as others, can be perturbed by a variety of factors such as unfamiliar surroundings, selective deprivation, rapid travel across time zones, etc. This study was to investigate the effects of the weightless environment on the sleep and wake states in system of the nonhuman primate.

Approach or Method
Ten EEG, two EOG, and two EMG channels were among the 33 channels of physiological data monitored on Biosatellite III. EEG electrodes were stereotaxically positioned bilaterally in the parietal and visual cortex, the hippocampus, and the amygdala. EOG leads were placed at the right and left outer canthi, and EMG sensors were implanted in posterior cervical and lower scapular sites. Data were telemetered to Earth-based tracking stations at the rate of 22.4 kilobits per second, collected every 97 minutes, and length of capture varied with elevation of spacecraft above the horizon, typically five to seven minutes. The monkey was acclimated to twelve-hour day/twelve-hour night cycle in the capsule.

Results
The sleep of the Biosatellite III primate in orbital flight was abnormal in amount and bizarre in its distribution. It was characterized by rapid transitions in sleep state, brevity of state, and unusual transitions from one state to another. The monkey never achieved its normal terrestrial cycle, and the remarkable fragmentation of consciousness required meticulous and virtually microscopic scoring of EEG records. A dramatic reduction was noted in REM and stage four sleep. Eye movements, normally only seen in REM, were observed during stage two and stage four sleep. The changes began concurrently with the onset of the weightlessness and were not secondary to altered fluid balance or body temperature. There was a complex response to the independent variable of weightlessness, with a sudden decline on day eight attributable to fluid loss and redistribution of blood in the thorax consequent to zero gravity state. The observed fragmentation of consciousness appeared similar to the sleep of medical patients with high cervical cord transections.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Study of Muscarinic and GABA (Benzodiazepine) Receptors in the Sensory-Motor Cortex, Hippocampus, and Spinal Cord

Science Discipline
Neurophysiology

Investigator	Institute
N.G. Daunton	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
D'Amelio, F.	NASA Ames Research Center (ARC)
Krasnov, I.B.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Daunton, N.G.; D'Amelio, F.; and Krasnov, I.: Study of Muscarinic and GABA (Benzodiazepine) Receptors in the Sensory-Motor Cortex, Hippocampus, and Spinal Cord. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 365–370.

Objectives/Hypothesis
In this experiment, frontal lobe samples from brains of space-flown rats were processed for the study of muscarinic (cholinergic) and GABA (benzodiazepine) receptors, and for immunocytochemical localization of the neurotransmitter gamma-aminobutyric acid (GABA) and glial fibrillary acidic protein (GFAP). The aim was to explore the feasibility of investigating neurotransmitters and their receptors in spaceflight experiments.

Approach or Method
For receptor binding studies, slides with 20-micrometers frontal lobe sections were incubated with 3H-ligand, washed, and later placed in an x-ray cassette. Tritium sensitive film was placed over the slides and later developed. For GABA and GFAP immunocytochemistry, slide-mounted tissue sections were fixated with appropriate solutions and rinsed in cold phosphate buffer saline.

Results
Although radioactive labeling of both muscarinic cholinergic and GABA receptors proved to be successful with the techniques employed, distinct receptor localization of individual laminae of the frontal neocortex was not possible because the sampling area was different in the various groups of animals. In spite of efforts made for proper orientation and regional identification of laminae, it was found that a densitometric (quantitation of autoradiograms) analysis of tissue did not contribute to the final interpretation of the effects of weightlessness on these receptors. As to the immunocytochemical studies, the use of both markers, GFAP and GABA antiserum, confirmed the suitability of the techniques for frozen material. Similar problems to those encountered in the receptor studies prevented an adequate interpretation of the effects of microgravity exposure on the localization and distribution of GABA and GFAP.

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study

Effect of Microgravity on: II. Metabolic Enzymes of Hippocampus and Spinal Cord

Science Discipline

Neurophysiology

Investigator	Institute
O.H. Lowry	Washington University School of Medicine, St. Louis

Co-Investigator(s)	Institute
McDougall, Jr., D.	Washington University, St. Louis
Carter, J.	Washington University, St. Louis
Krasnov, I.B.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Wistar rat)

Ground-Based Controls

Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications

Lowry, O.; McDougal, D., Jr.; Nemeth, P.M.; Chi, M.M.-Y.; Pusateri, M.; Carter, J.; Manchester, J.; Norris, B.; and Krasnov, I..: Effect of Microgravity On: II. Metabolic Enzymes of Hippocampus and Spinal Cord. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 413–418.

Objectives/Hypothesis

The question of possible enzyme changes due to exposure to microgravity is much more complicated in the case of the central nervous system than it is for skeletal muscle. The brain is enormously complex. Valid comparisons must be made between exactly the same regions of control and flight brains, otherwise natural differences will confuse the issue. This study compared flight and synchronous enzymes from the hippocampus and spinal cord with regards to specific regions.

Approach or Method

Nine different enzymes in six regions of the hippocampus, and four and five enzymes in five regions of the spinal cord (a total of almost 500 quantitative measurements) were measured in two vivarium and two flight animals. Whenever possible, the assays for a number of enzymes were in duplicate with aliquots from an extract of a single, relatively large tissue sample. The spinal cord data was limited to one (synchronous) control and one flight animal.

Results

The six enzymes of the hippocampus were in most cases remarkably similar in flight and control (vivarium) brains. ß-hydroxyacyl CoA dehydrogenase was 35% lower in the molecular layer of CA1 of the flight brain; however, the other two enzymes of oxidative metabolism in this region were within 10% of the control. The glutamate decarboxylase activities were quite variable, nevertheless it probably should not be ignored that levels for the flight samples from all of the hippocampal regions were on the average 30% higher than controls. For glutaminase, in CA1 average values for the three regions assayed were 25% to 59% higher in flight than control tissue. For the spinal cord, the higher aspartate aminotransferase values for the pyramidal tract and outer dorsal horn, and lower glutaminase levels in the dorsal column and pyramidal tract of the flight animal, would be worth further investigation.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Adaptation of Optokinetic Nystagmus to Microgravity

Science Discipline
Neurophysiology

Investigator	Institute
B. Cohen	Mount Sinai School of Medicine

Co-Investigator(s)	Institute
Kozlovskaya, I.B.	Institute of Biomedical Problems
Raphan, T.	Brooklyn College
Solomon, D.	Mount Sinai School of Medicine

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Laboratory Control

Key Flight Hardware
Cosmos Primate-BIOS

Selected Publications
Cohen, B.; Kozlovskaya, I.; Raphan, T.; Solomon, D.; Helwig, D.; Cohen, N.; Sirota, M.; and Yakushin, S.: Vestibulo-ocular Reflex of Rhesus Monkeys After Spaceflight. Journal of Applied Physiology, supl., vol. 73, no. 2, Aug. 1992, pp. S121–S131.

Objectives/Hypothesis
The goal of these experiments was to study the effect of adaptation to microgravity on the various components of the vestibulo-ocular reflex (VOR) of two space-flown Rhesus monkeys. Horizontal, vertical, and roll eye movements were recorded in these and six other monkeys implanted with scleral search coils.

Approach or Method
Pre- and postflight experiments were performed on juvenile (3–4 kg) monkeys. Head restraint rings were chronically fixed to each animals' head and two scleral search coils were implanted on one eye. Eye movements were induced by rotating the animals or the visual surround in a three-axis vestibular and optokinetic stimulator. Animals were rotated around a vertical axis to determine the gain of the horizontal, vertical, and roll VOR; they were subjected to off-vertical axis rotation (OVAR) to determine steady state gains and effects of gravity on modulations in eye position and eye velocity. Animals were also tested for cross coupling of horizontal to pitch and roll optokinetic after-nystagmus (OKAN) and for tilt dumping of post-rotatory nystagmus.

Results
The gain of horizontal VOR was close to unity when animals were tested 15 and 18 hours after flight. VOR gain values were similar to those registered before the flight. If the gain of the horizontal VOR changes in microgravity, it must revert to normal soon after flight. Steady state velocities of nystagmus induced by OVAR were unchanged by adaptation to microgravity, and the phase of modulations was similar before and after flight. However, modulations in horizontal eye velocity were on average about 50% larger for angles of tilt of the axis of rotation between 50° and 90° after flight. The difference in both animals was similar and significant. One animal lost its ability to tilt-dump its nystagmus. This loss persisted several days after return and implies an alteration in spatial orientation of velocity storage after flight. Results are consistent with the postulate that adaptation to microgravity causes alterations in the way that otolith information is processed in the central nervous system.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study

Metabolic and Morphologic Properties of Muscle Fibers and Motor Neurons After Spaceflight: II. Ventral Horn Cell Responses to Spaceflight and Suspension

Science Discipline

Neurophysiology

Investigator	Institute
B. Jiang	University of California, Los Angeles

Co-Investigator(s)	Institute
Roy, R.R.	University of California, Los Angeles
Ilyina-Kakueva, E.	Institute of Biomedical Problems
Krasnov, I.B.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Jiang, B.; Roy, R.R.; Polyakov, I.V.; Krasnov, I.B.; and Edgerton, V.R.: Ventral Horn Cell Responses to Spaceflight and Hindlimb Suspension. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S107–S111.

Objectives/Hypothesis

Spaceflight or hindlimb (tail) suspension result in a loss of mass and in alteration of the metabolic and contractile protein profiles of skeletal muscles towards that resembling faster muscles. Given the influence of motorneurons on muscle properties, ventral horn cells of the lumbosacral enlargement of the spinal cord were studied to determine whether similar adaptations were present in those cells.

Approach or Method

Spinal cords were quick-frozen and the succinate dehydrogenase (SDH) activity and cross-sectional area (CSA) of the soma of ventral horn cells were measured using a computer enlargement processing system. The optical density (OD) for SDH activity was determined after 8 minutes of incubation in a reaction medium which gave a steady-state enzymatic reaction. Soma sizes were determined in cells having a visible nucleus.

Results

Although there were no significant differences in mean CSA and SDH activity, the population distributions of both variables shifted significantly. In flight rats, there was a shift toward smaller cells. Compared to control, the population of SDH activities shifted to higher activities in flight samples, while the distribution shifted toward lower activities in suspended animals. When considering the interactive effects within individual cells, there was a higher percentage of small cells having high SDH activities in the flight compared to control or suspended animals. These contrasting effects of spaceflight and suspension suggest that the changes observed in ventral horn cells were due to factors other than simply the absence of weight support.

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Studies of Vestibular Primary Afferents in Normal, Hyper- and Hypogravity

Science Discipline
Neurophysiology

Investigator	Institute
M.J. Correia	University of Texas Medical School

Co-Investigator(s)	Institute
Kozlovskaya, I.B.	Institute of Biomedical Problems
Sirota, M.G.	Institute of Biomedical Problems
Perachio, A.A.	University of Texas Medical School

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Laboratory Control

Key Flight Hardware
Cosmos Primate-BIOS

Selected Publications
Correia, M.J.; Perachio, A.A.; Dickman, J.D.; Kozlovskaya, I.B.; Sirota, M.G.; Yakushin, S.B; and Beloozerova, I.N.: Changes in Monkey Horizontal Semicircular Canal Afferent Responses After Spaceflight. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S112–S120.

Correia, M.J. et al.: Vestibular Primary Afferent and Vestibulo-ocular Changes in Rhesus Monkey Following 14 Days of Microgravity. Society for Neuroscience - Abstracts, vol. 16, 1990, p. 735.

Objectives/Hypothesis
This study was conducted to determine if the task of making numerous coordinated active horizontal eye and head movements during spaceflight modified the response of vestibular primary afferents to controlled passive rotations. Because a multi-axis rotator could not be placed on the spacecraft, it was assumed that if these afferents could be tested immediately postflight, information could be obtained about the dynamic properties of the horizontal semicircular canals before they recalibrated to the terrestrial gravitational environment.

Approach or Method
Head restraint rings were chronically fixed to animals' heads; corneo-retinal potential electrodes for recording eye movements were chronically secured in the bone near the outer canthi above and below one eye. Of the five monkeys operated on, two were flown while the remaining three served as ground controls. Single unit recordings were made, using tungsten microelectrodes, from semi-circular canal and otolith primary afferents. During all recording sessions animals were awake; during eye movement tests animals were aroused with auditory cues and tested using step, sinusoidal, and sum of sinusoidal rotations about an Earth vertical axis and axes other than Earth vertical.

Results
The responses from the horizontal canal afferents suggest that the vestibular end-organ remains normal during and following activities related to the flight; however, it appears that the gain and neural adaptation increase. Also, the "DC bias" of horizontal nystagmus during horizontal axis constant velocity rotation was greater for the two flight monkeys when they were tested postflight day one (R+1) as compared to day 9 (R+9), and horizontal nystagmus during step rotation about an Earth vertical axis, with horizontal canals in the plane of rotation, produced roughly the same results at R+1 as R+9. But when the head was pitched down 45° on R+1, the nystagmus slow phase velocity was greater and the duration was doubled. This suggests that this otolith-mediated response to changing linear acceleration, and the response involving the interaction of the horizontal and vertical semicircular canals and otoliths, did not completely recalibrate immediately after the flight (R+1).

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study

Effect of Microgravity on: II. Metabolic Enzymes, Neurotransmitter Amino Acids, and Neurotransmitter Associated Enzymes in Selected Regions of the Central Nervous System

Science Discipline

Neurophysiology

Investigator	Institute
O.H. Lowry	Washington University School of Medicine, St. Louis

Co-Investigator(s)	Institute
Krasnov, I.B.	Institute of Biomedical Problems
Ilyina-Kakueva, E.	Institute of Biomedical Problems
Nemeth, P.M.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Lowry, O.H.; Krasnov, I.; Ilyina-Kakueva, E.I.; Nemeth, P.M.; McDougal, D.B., Jr.; Choksi, R.; Carter, J.G.; Chi, M.M.Y.; Manchester, J.K.; and Pusateri, M.E.: Part II: The Distribution of Selected Enzymes and Amino Acids in the Hippocampal Formation. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 2, J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM -108802, 1994, pp. 137–154.

Objectives/Hypothesis

Six key metabolic enzymes plus glutaminase and glutamate decarboxylase (GAD), as well as glutamate, aspartate, and GABA (gamma-aminobutyric acid), were measured in 11 regions of the hippocampal formation of synchronous, flight, and tail-suspended rats.

Approach or Method

All of the assays were performed with samples dissected from 20-μm freeze-dried coronal microtome sections; the size of utilized sample and preparation for assay varied with the substance to be measured. All of the methods employed were based on the conversion of NAD+ to NADPH, NADH to NAD+, or NADPH to NADP+. The amount of pyridine product formed varied from about 1x10-12 mole in the aspartate assays to about 200x10-12 mole in the pyruvate kinase assays. It was therefore necessary in all cases to increase the sensitivity 1,000 to 10,000-fold by enzymatic cycling.

Results

Glucose-6-P dehydrogenase and especially aspartate aminotransferase varied least among the different regions. Glutaminase, associated with the role of glutamate as a transmitter, showed a wider range of activities than any of the six purely metabolic enzymes; moreover, the distribution did not resemble any of these. Both glutamate and aspartate varied over about a 30% range among the 12 subdivisions of the hippocampal formation analyzed. GABA data were somewhat more consistent than GAD data, with relatively high levels for both found in the fascia dentate and the pyramidal cell and molecular layers. While major differences were observed in the normal distribution patterns of each enzyme and amino acid, no substantive effects of either microgravity or tail suspension on these patterns were clearly demonstrated.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Effects of Space Travel on Mammalian Gravity Receptors

Science Discipline
Neurophysiology

Investigator / Institute
M.D. Ross — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF), Animal Enclosure Module (AEM)

Selected Publications
Ross, M.D.: Morphological Changes in Rat Vestibular System Following Weightlessness. Proceedings of the Barany Society, Symposium on Space Research, Prague, Czech Republic, 1992. Journal of Vestibular Research, vol. 3, no. 3, 1993, pp. 241–251.

Ross, M.D.: Synaptic Plasticity in Utricular Maculas of Rats Exposed to Microgravity. American Society for Gravitational and Space Biology Bulletin, vol. 6, no. 1, 1992, p. 100.

Objectives/Hypothesis
Previous research indicates that vestibular gravity sensors (maculas) are functionally specialized structures. There are two main interacting circuits: type I macular sensory hair cells are part of the highly channeled (or direct) circuit; type II macular hair cells sense, distribute, and modify information flowing through the system as part of the distributed modifying (or local) circuit. Based on their participation in local circuits, it was predicted that type II cells would show more synaptic changes in an altered gravitational environment than type I cells.

Approach or Method
Synapses were recorded from four sets of 50 serial thin sections from right maculas. Two consecutive series of 50 sections were obtained from the macula of one animal in each group to determine whether results would vary between the two sites. Only those synapses with an electron opaque central ribbon and a halo of vesicles were counted. Photographic mosaics were made of every seventh section for locating and numbering cell profiles accurately, and to ensure that no synapses were counted twice. More than 6,000 synapses in over 1,000 utricular macular hair cells were analyzed.

Results
Increased numbers of hair cells in R+0 flight animals support the thesis that the local circuit is more dynamic and would exhibit more change. Spaceflight appears to retune vestibular gravity sensors so that they can function in microgravity. The observed increments in pairs and groups of synaptic ribbons may increase the efficacy of the synaptic site in microgravity (i.e., the addition of synaptic ribbons at a site increases the probability of release of transmitter substance, making the hairs more sensitive). If postflight stress was a factor, it acted selectively on neural elements of the local circuitry, which did not differ at R+ML. This is in contrast to findings at R+0, when both receptor hair cell types were affected.

Launch Date 6/5/1991

Landing Date 6/14/1991

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Primary Perceptive Structure of the Brain: Morphology and Histochemistry

Science Discipline
Neurophysiology

Investigator	Institute
I.B. Krasnov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Daunton, N.G.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Sergutina, A.V.; Gershtein, L.M.; D'Amelio, F.D.; Donton, N.; and Krasnov, I.B.: Some Cytochemical Features of the Motor System of the Rat Brain After Spaceflight. Bulletin of Experimental Biology and Medicine, vol. 119, no. 3, Mar. 1995, pp. 288–290.

Krasnov, I.B.; Fidelina, O.V.; Gorbatiuk, O.S.; and Vikhreva, O.V.: Repeated Exposure in Hypergravity: Morphology of Locus Coeruleus, Hypothalamic Paraventricular Nucleus and Vagal Nerve Dorsal Nucleus in Rats. Aviakosm Ekolog Med, vol. 34, no. 3, 2000, pp. 21–27.

Objectives/Hypothesis
Electron microscopic examinations of the nodulus cortex of the cerebellum of Cosmos rats demonstrated ultrastructural changes that suggested that the vestibular flow to the cortex decreased in microgravity and drastically increased after return to the Earth's gravity. This study was designed to evaluate the effects of spaceflight on glutamic acid metabolism enzymes and ultrastructure of the nodulus of the cerebellar vermis, the medulla oblongata, and pons varolii.

Approach or Method
Vermis and right hemisphere of the cerebellum, left side of medulla oblongata, and pons varolii were collected. Quantitative histochemical analysis of glutaminase and glutamate-asparte transaminase was performed on the cortical layers isolated from freeze-dried sections of the nodulus, and in fragments isolated from freeze-dried sections of the vestibular nuclei medialis and lateralis. Nervous and glial elements of the nodular cortex and nucleus gracilis were examined by electron microscopy.

Results
Analysis of fragments of the granular layer of the cortex of the nodulus and medial vestibular nucleus, and fragments of lateral vestibular nucleus, demonstrated that, after 9 days of spaceflight, the activity of glutaminase is decreased at the endings of primary vestibular fibers. However, this was not statistically significant. In animals sacrificed 9 days postflight, glutaminase activity in the structures studied did not differ from controls.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Neuronal Morphology

Science Discipline
Neurophysiology

Investigator	Institute
T.A. Leontovich	Institute for Brain Research

Co-Investigator(s)	Institute
Belichenko, P.V.	Institute of Brain Research
Makhanov, M.A.	Institute of Brain Research
Fedorov, A.A.	Institute of Brain Research
Lowry, O.H.	Washington University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Leontovich, T.A.; Lowry, O.; Belichenko, P.V.; Fedorov, A.A.; and Makhanov, M.A.: Morphology of Neurons. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, pp. 34–35.

Objectives/Hypothesis
Previous spaceflight studies have demonstrated consistent changes in morphometric parameters of the geometry and orientation of dendrites of neurons of medulla oblongata of rats. This experiment was to assess the effects of spaceflight on the geometry and orientation of dendrites of command neurons of the giganto-cellular reticular nucleus, and neurons of the superior and median vestibular nuclei.

Approach or Method
Tissues collected included medulla oblongata and pons varolii, right side (one sample). Semi-automatic morphometric analysis of the geometry and orientation of dendrites of nerve cells after impregnation was conducted according to Golgi. By means of a digitizer, a graphic drawing of the neurons was obtained.

Results
Morphometric investigation of dendrite geometry of giant multipolar neurons of nucleus reticularis giganto-cellularis of medulla oblongata did not reveal significant differences between flown and ground-based control animals. However, significant differences in the number and mean branching of dendrites between R+0 and R+9 rats suggested structural rearrangement of the dendrite tree of neurons that developed during and after flight. Comparison of these findings, along with the data obtained during similar studies in Cosmos-1667, Cosmos-1887, and Cosmos-2044 flights, helped identify time course variations of the dendrite tree of gigantic multipolar neurons of the reticular formation at different stages of animal adaptation to microgravity.

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Ultrastructure of the Brain Cortex

Science Discipline
Neurophysiology

Investigator	Institute
L.N. Dyachkova	Severtsev Institute of Evolutionary Morphology and Ecology of Animals

Co-Investigator(s)	Institute
Lowry, O.H.	Washington University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Dyachkova, L.N.; and Lowry, O.H.: Cerebral Cortex Ultrastructure. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, p. 35.

Objectives/Hypothesis
Earlier examinations of the ultrastructure of the cerebral cortex on Cosmos has suggested that the system of interneuronal contacts of the neocortex showed the highest level of adaptive changes in microgravity. This experiment was to assess the effects of spaceflight on the ultrastructure of nervous, glial, and vascular elements of the brain cortex.

Approach or Method
Fragments of the motor, somatosensory, visual, and olfactory cortex were collected, and a layer-by-layer electron microscope examination of nervous, glial, and vascular elements of the brain cortex was conducted. While embedding in araldite, brain sections were oriented in such a way as to have all cortical layers sectioned in frontal ultrathin sections.

Results
Results demonstrated changes in neuronal and glial cells, which pointed to an active restructuring in the cortical connections of the flight rats. Motor and sensomotory ultrastructure at R+0 suggested that synapses and stellate cells were in an excitation state, which was associated with an increased afferent flow to the cortex during the 2–3 hours after recovery. Examinations at R+9 indicated both an enhanced afferent flow, as well as an increased functional activity of large pyramidal neurons of the V layer. Changes in the visual cortex of the flight rats were similar to those in the somatosensory cortex, but less significant. Changes of the olfactory cortex suggested a slight decrease of the afferent flow and an increase of the functional activity of neurons postflight.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Cytochemistry of Neurons

Science Discipline
Neurophysiology

Investigator	Institute
L.M. Gershtein	Institute for Brain Research

Co-Investigator(s)	Institute
Sergutina, A.V.	Institute for Brain Research
Mehler, W.R.	NASA Ames Research Center (ARC)
Daunton, N.G.	NASA Ames Research Center (ARC)
D'Amelio, F.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Gershtein, L.M.; Daunton, N.; Mehler, W.; D'Amelio, F.; and Sergutina, A.V.: Cytochemistry of Neurons. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, p. 35.

Objectives/Hypothesis
This experiment was to assess the effects of spaceflight on enzymes involved in neurotransmitter and energy metabolism in neurons of the motor and somatosensory cortex, and the head of the caudate nucleus of the brain.

Approach or Method
The left hemisphere of the brain was collected. Activities of acetyl cholinesterase, succinate dehydrogenase, and glucose-6-phosphate dehydrogenase were detected in the frontal sections of the brain by histochemical staining. Subsequent measurement of enzyme activities in neuronal elements of the motor and somatosensory cortex, and the head of the caudate nucleus, was conducted by densitometry methods.

Results
The study suggests that microgravity exposure results in a decreased monoamine oxidase activity in fibrillar structures of the fifth layer of the somatosensory cortex and the head of the caudate nucleus, as well as a decreased acetyl cholinesterase in the bodies of neurons in the head of the caudate nucleus, which may be interpreted as a sign of: 1) a decrease, during microgravity, of the modulating influence of brain monoaminergic structures upon the somatosensory cortex and the head of the caudate nucleus; and/or 2) a decrease, during microgravity, of the inhibitory influence of the neurons of the caudate nucleus upon the globus pallidus, n. ruber, substantia nigra, and other brain structures.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Spinal Cord and Dorsal Root Ganglion Morphology and Histochemistry

Science Discipline
Neurophysiology

Investigator	Institute
I.B. Krasnov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Drobyshev, V.I.	Voronezh Medical Institute
Polyakov, I.V.	Voronezh Medical Institute
Edgerton, V.R.	University of California, Los Angeles
Lowry, O.H.	Washington University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Fidelina, O.V.; Corbatyuk, O.S.; Krasnov, I.B.; and Akmayev, I.G.: Reactions of the Hypothalamic Paraventricular Nucleus and Vagus Nerve Dorsalis Nucleus in the Rats Exposed to Changed Gravitational Environment [in Russian]. Problemy Endokrinologii, vol. 41, no. 6, 1995, pp. 35–38.

Sergutina, A.V.; Gershtein, L.M.; D'Amelio, F.D.; Donton, N.; and Krasnov, I.B.: Some Cytochemical Features of the Motor System of the Rat Brain After Spaceflight. Bulletin of Experimental Biology and Medicine, vol. 119, no. 3, Mar. 1995, pp. 288–290.

Objectives/Hypothesis
Examinations of motorneurons of the anterior horns of the lumbar and cervical enlargements of the spinal cord of rats have revealed changes suggesting a lowered activity of the nerve cells after a 14- to 22-day exposure to weightlessness. This experiment was performed to evaluate the effect of spaceflight factors on the nervous, glial, and vascular elements of the cervical and lumbar enlargements of spinal cord and dorsal root ganglion.

Approach or Method
The upper half of the cervical enlargement and the lower half of the lumbar enlargement of spinal cord and dorsal root ganglion were removed and fixated. Parameters measured included the activities of cytochrome oxidase, acetyl cholinesterase, and alkaline phosphatase; the volume of the neuronal body and nucleus; and the neuron-glial index. Studies were performed on animals sacrificed at recovery (R+0), and 9 days postflight (R+9).

Results
No changes were observed at R+0 or R+9 in the enzyme activity of the anterior horns of the spinal cord at the C2-C4 level, while a lowered cytochromoxidase activity was observed in the motorneurons of the anterior horns of the spinal cord at the L1-L2 level. The latter fact suggests the development of a motorneurons hypofunction in the lumber enlargement as a result of the spaceflight. A recovery of cytochromoxidase activity in the motorneurons of the lumber enlargement at R+9 demonstrates the reversibility of the observed changes and a recovery of functional motorneurons activity during the readaptation period. An increased number of active capillaries in the anterior horns of the lumber enlargement at R+9 probably reflects an increased transport of active metabolites through the capillaries of the anterior horns, suggesting the development of a compensatory process directed at activation of metabolism in the spinal cord during the readaptation period.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Histochemistry of Hypothalamus

Science Discipline
Neurophysiology

Investigator	Institute
I.B. Krasnov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Grindeland, R.E.	NASA Ames Research Center (ARC)
Sawchenko, P.E.	Salk Institute
Vale, W.	Salk Institute

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Sergutina, A.V.; Gershtein, L.M.; D'Amelio, F.D.; Donton, N.; and Krasnov, I.B.: Some Cytochemical Features of the Motor System of the Rat Brain After Spaceflight. Bulletin of Experimental Biology and Medicine, vol. 119, no. 3, Mar. 1995, pp. 288–290.

Krasnov, I.B.; Fidelina, O.V.; Gorbatiuk, O.S.; and Vikhreva, O.V.; Repeated Exposure in Hypergravity: Morphology of Locus Coeruleus, Hypothalamic Paraventricular Nucleus and Vagal Nerve Dorsal Nucleus in Rats. Aviakosm Ekolog Med, vol. 34, no. 3, 2000, pp. 21–27.

Objectives/Hypothesis
Previous analysis of the anterior hypothalamus and the anterior lobe of the pituitary gland of rats flown on Cosmos biosatellites and Spacelab-3 revealed inhibition of the synthesis and excretion of growth hormone, and the system controlling these processes. This experiment was to assess the effects of spaceflight on gamma-aminobutyric acid (GABA) and other enzymes in the system of the hypothalamus.

Approach or Method
The portion of the brain containing the thalamus and hypothalamus oriented in the anterior-posterior direction was dissected. Single tissue fragments, 0.2 to 1.0 μg in mass, were separated by micro-instruments from lyophilized sections (20 μm in thickness) of the arcuate nucleus and medial eminence hypothalamus. Quantitative histochemical analysis of glutamate decarboxylase and glutaminase, as well as determinations of the content of lipids and the defatted dry substance, were performed.

Results
After spaceflight, glutaminase activity in the arcuate nucleus was decreased by 22.7%, and glutaminase activity in the medial eminence was decreased by 30.4%. The ratios of lipids and defatted dry substance in both structures remained unchanged. Because data indicated a high sensitivity of somatoliberan-containing neurons of the arcuate nucleus to glutamate, the possible participation of glutamate in the regulation of growth hormone secretion has been suggested.

STS-40 / SLS-1

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Morphology of Neurons of the Brain Cortex

Science Discipline
Neurophysiology

Investigator	Institute
T.A. Leontovich	Institute of Brain Research

Co-Investigator(s)	Institute
Makhanov, M.A.	Institute of Brain Research
Fedorov, A.A.	Institute of Brain Research
Belichenko, P.V.	Institute of Brain Research
Lowry, O.H.	Washington University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Leontovich, T.A.; Lowry, O.; Makhanov, M.A.; Belichenko, P.V.; and Fedorov, A.A.: Morphology of Neurons of the Cerebral Cortex. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, pp. 36–37.

Objectives/Hypothesis
Morphological evaluation of neurons of the visual cortex of the brain of animals exposed to spaceflight may help gain an insight into mechanisms underlying adaptive responses of the visual organ to microgravity. This experiment was to assess spaceflight effects on dendrite geometry and orientation, as well as the number of dendrite processes of neurons, in the visual and somatosensory cortex.

Approach or Method
The somatosensory (right side) and visual cortex (left side) were collected. Semiautomatic morphometric analysis of the geometry and orientation of dendrites of nerve cells after impregnation was conducted according to Golgi. Pyramidal neurons of the third layer of the visual cortex were outlined from histological preparations at a magnification of x400. Altogether, 49 neurons were outlined. By means of a digitizer, a graphic drawing of the neurons was obtained.

Results
There was a significant increase in the body size of pyramidal neurons of the flight animals. The findings show an increase in the length of apical dendrites located in the upper layers of the visual cortex, among the pyramidal neurons of the third layer. Examinations show a well-developed apical system and participation in the establishment of associative connections between various cortical compartments. This process may have been induced by the need for an additional afferent input and can act as a foundation for new connections between the visual cortex and other cortical compartments in microgravity. An enlargement of the profile size of the body of pyramidal neurons of the third layer, also at R+0, can be viewed as another indication of the restructuring of the dendrite system of these neurons in microgravity.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Catecholamines, Vasopressin, Atrial Natriuretic Factor (ANF), and ANF Receptors in the Rat Brain

Science Discipline
Neurophysiology

Investigator	Institute
C. Gharib	Centre National Recherche Scientifique

Co-Investigator(s)	Institute
Gabrion, J.B.	Centre National Recherche Scientifique
Pequignot, J.-M.	Centre National Recherche Scientifique

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)
Animal Enclosure Module (AEM)

Selected Publications
Herbute, S.; Oliver, J.; Davet, J.; Viso, M.; Ballard, R.; Gharib, C.; and Gabrion, J.: ANP Binding Sites Are Increased in Choroid Plexus of SLS-1 Rats After 9 Days of Spaceflight. Aviation, Space and Environmental Medicine, vol. 65, 1993, pp. 134–138.

Fareh, J.; Cottet-Emard, J.-M.; Pequignot, J.-M.; Jahns, G.; Meylor, J,; Viso, M.: Norepinephrine Content in Discrete Brain Areas and Neurohypophysial Vasopressin in Rats After a 9-Day Spaceflight. Aviation, Space and Environmental Medicine, vol. 64, 1993, pp. 507–511.

Objectives/Hypothesis
Changes in blood volume during spaceflight have been related to modifications of fluid regulating hormones. This study was to evaluate the effect of spaceflight on the neurological basis of endocrine regulating factors. Catecholamines, vasopressin, atrial natriuretic factor (ANF) and ANF receptors were studied in the rat brain.

Approach or Method
Brain stem noradrenergic cell groups (A1, A2, A5, and A6) were removed and supernatants were assayed for norepinephrine, and central tissues were analyzed by liquid chromatography and electrical detection. Vasopressin content in the hypothalamus and hypophysis was determined by radioimmunoassay. ANF binding sites were studied with 125I-rANP and autoradiography.

Results
After flight, vasopressin was decreased in the hypothalamus and increased in the postier pituitary. Norepiniphrine was unchanged in the A2 and A5 groups. Norepiniphrine content was decreased in the locus coeruleus (A6), but showed no change at R+9, indicating a stress reaction associated with landing. This stress effect may mask microgravity effects. Results also indicated an increase in binding sites in the choroid plexus after flight and a decreased affinity of meningial ANF receptors. These changes suggest that ANF may be involved in fluid electrolyte imbalances in the brain occurring during flight.

Cosmos 2229 / Bion 10

Launch Date 12/29/1992

Landing Date 1/10/1993

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Adaptation to Microgravity of Oculomotor Reflexes

Science Discipline
Neurophysiology

Investigator	Institute
D.L. Tomko	NASA Ames Research Center

Co-Investigator(s)	Institute
Kozlovskaya, I.B.	Institute for Biomedical Problems
Paige, G.D.	University of Rochester Medical School
Badakva, A.M.	Institute for Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
Tomko, D.L.; Kozlovskaya, I.B.; Paige, G.D.; and Badakva, A.M.: Adaptation to Microgravity of Oculomotor Reflexes (AMOR): Otolith-Ocular Reflexes. Final Reports of the U.S. Experiments Flown on the Russian Biosatellite Cosmos 2229. J.P. Connolly, M.G. Skidmore, and D.A. Helwig, eds., NASA TM-110439, Apr. 1997, pp. 293–351.

Tomko, D.L. and Paige, G.D.: Linear Vestibuloocular Reflex During Motion Along Axes Between Nasooccipital and Interaural. Annals of the New York Academy of Sciences, vol. 656, 1992, pp. 233–241.

Objectives/Hypothesis
The objective of these experiments was to study the linear vestibulo-ocular reflexes (LVORs) during gravity receptor stimulation (linear acceleration) before and after spaceflight. The LVOR is likely to change during exposure to microgravity, because it is primarily controlled by the gravity-sensing otoliths. These experiments will characterize the re-adaptation of otolith reflexes to Earth's gravity after exposure to microgravity.

Approach or Method
Pre- and postflight response characteristics were measured during passive head movements at two stimulus frequencies, 1.5 and 5.0 Hz, in darkness (LVOR), and during viewing of a head-fixed (visual suppression-VSLVOR) or an Earth-fixed (visual linear-VLVOR) visual scene. Motion was delivered along the inter-aural (IA), naso-occipital (NO), and dorso-ventral (DV) head axes, as well as along intermediate oblique ones. Angular VORs were recorded during sinusoidal yaw, pitch, or roll motion delivered manually with Earth-fixed visual targets (VVOR) during yaw and pitch, and with animal-fixed visual targets during roll. Data acquisition and analysis were done on PC-based programs. Response gain, response phase, gaze position, and vergence state were calculated. The gain and phase of differentiated, de-saccadded eye position recordings were calculated using Fourier analysis.

Results
LVORs compensatory for head displacement were recorded during IA, DV, NO, and intermediate axis motion. All responses were affected by visual target distance. NO responses were also affected by gaze direction. AVORs during yaw and pitch had roughly compensatory gains, while torsional gains of between 0.4 and 0.7 were recorded. Both flight monkeys had lower AVOR gain in response to pitch and yaw head movements immediately postflight. During IA and DV head motion at 5 Hz, subject M906 had larger reductions in the slope of the function relating LVOR sensitivity to vergence that did not recover by R+39 hours. Subject M151 displayed similar responses under the same conditions. During NO head motion, pre- and postflight responses for subject M151 were similar to one another, while responses of subject M906 were smaller and more variable postflight.

Launch Date 12/29/1992 **Landing Date** 1/10/1993

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Reduction of Ocular Counter-Rolling by Adaptation to Space

Science Discipline
Neurophysiology

Investigator	Institute
B. Cohen	Mount Sinai School of Medicine

Co-Investigator(s)	Institute
Diai, M.	Mount Sinai School of Medicine
McGarvie, L.	Mount Sinai School of Medicine
Kozlovskaya, I.B.	Institute of Biomedical Problems
Sirota, M.G.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
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. Experimental Brain Research, vol. 102, no. 1, 1994, pp. 45–56.

Dai, M.; Cohen, B.; and Raphan, T.: Ocular Vergence Induced by Off-Vertical Axis Rotation (OVAR) Before and After Spaceflight. Neuroscience Abstract, vol. 21, 1995, p. 137.

Objectives/Hypothesis
Although the average head angular movements in space does not change, stimulation of semicircular canals with pitch and torsional head movement at high frequency may be reduced due to a lack of locomotional forces. Consistent with this, little change has been found in the angular horizontal vestibulo-ocular reflex (VOR). On the other hand, the otolith organ, with its dependence on gravity, should undergo changes when in microgravity. A reinterpretation of otolith input has been proposed in which a linear force sensed by the otolith is interpreted as translational. The purpose of this experiment was to examine changes in the VOR and the hypothesis that there is a shift in the yaw axis orientation vector of velocity storage from a gravitational frame of reference to a body frame of reference.

Approach or Method
Horizontal and vertical eye movements were measured using a magnetic scleral search coil implanted in the frontal plane. Ocular torsion (roll) about the optic axis was recorded with a magnetic scleral search coil implanted on the top of one eye. Ocular counter rolling (OCR) was studied using static tilts of 90 degrees and off-vertical axis rotation (OVAR). Roll VOR was measured during steps of velocity about a naso-occipital axis with the monkey prone, and during sinusoidal oscillation with the animal upright. Spatial orientation of velocity storage was examined using a optokinetc nystagmus (OKN) and optokinetic after nystagmus.

Results
Static and dynamic OCR was dramatically reduced by about 70% in both monkeys after spaceflight, with no apparent recovery in the magnitude of torsion over 11 days of testing. This was not seen in ground-control monkeys. Roll VOR was also decreased. These data indicate long-lasting depression of torsional or roll eye movement after adaptation to microgravity. Before flight, yaw axis orientation vectors of velocity storage were closely aligned to the spatial vertical axis. After flight, there was a significant shift of the yaw axis toward the body axis. The major finding of these experiments is that the torsional otolith-ocular reflex, induced by head tilt with regard to gravity, was substantially reduced in the two flight monkeys after adaptation to space, and that the reduction in OCR persisted for a prolonged period after reentry.

Cosmos 2229 / Bion 10

Launch Date 12/29/1992 **Landing Date** 1/10/1993

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Functional Neuromuscular Adaptation to Spaceflight

Science Discipline
Muscle Physiology

Investigator	Institute
V.R. Edgerton	Department of Physiological Science University of California, Los Angeles

Co-Investigator(s)	Institute
Roy, R.R.	University of California, Los Angeles
Hodgson, J.A.	University of California, Los Angeles

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
Edgerton, V.R. and Roy, R.R.: Regulation of Skeletal Muscle Fiber Size, Shape and Function. Journal of Biomechanics, vol. 24, supl. 1, 1991, pp. 123–133.

Edgerton, V.R. and Roy, R.R.: Neuromuscular Adaptation to Actual and Simulated Weightlessness. Advances in Space Biology and Medicine, vol. 4, 1994, pp. 33–67.

Objectives/Hypothesis
Experiment objectives were to determine the effects of the absence of weight support on flexor (tibialis anterior) and extensor (soleus, medial gastrocnemius, and vastus lateralis) muscles of the leg. The study also focused on the relative importance of activity (as measured by intramuscular electromyography) and force (as measured by a tendon force transducer) on the adaptation of muscle to microgravity.

Approach or Method
Activity of different parts of motor control systems and peripheral motor mechanisms were studied in flight, as well as pre- and postflight, during active performance of motor tasks. A Tendon Force Sensor was surgically implanted into each flight subject on the distal tendon of the medial gastrocnemius of the left leg. Electromyography (EMG) electrodes were implanted in the soleus, medial gastrocnemius, tibialis anterior, and vastus lateralis muscles of the left leg. Muscle biopsies were also taken from these four muscles in the right leg. Preflight histograms for soleus and medial gastrocnemius activity were taken during 24 hours of cage activity for one of the flight monkeys and three control animals. Pre- and postflight chair trials were done for the force recordings. In-flight data for the Tendon Force Sensor was generated by a monkey using its left leg to perform a sinusoidal lever movement against a changing torque. EMG activity was recorded for all four implanted muscles during flight.

Results
One of the flight animals had a defective force transducer, which was not connected during flight. The transducer in the other animal drifted to such a degree that it was outside the measurable range during flight. However, postflight chair trials showed that they were still functional and that the transducer life was sufficient for this type of experiment. The monkeys performed the motor tasks poorly during flight, and the EMG signals were clipped due to the amplifiers being set at high gains. Some data analysis was accomplished despite the clipped signals. The flight EMG recording suggests that significant changes in muscle control may occur in spaceflight.

Launch Date 12/29/1992 **Landing Date** 1/10/1993

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Studies of Vestibular Neurons in Normal, Hyper-, and Hypogravity

Science Discipline
Neurophysiology

Investigator	Institute
M.J. Correia	University of Texas Medical Branch at Galveston

Co-Investigator(s)	Institute
Dickman, J.D.	University of Mississippi Medical School
Perachio, A.A.	University of Texas Medical School
Kozlovskaya, I.B.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
Correia, M.J.; Ricci, A.J.; and Rennie, K.J.: Filtering Properties of Vestibular Hair Cells: An Update. Annals of the New York Academy of Sciences, vol. 781, June 19, 1996, pp. 138–149.

Correia, M.J. and Lang, D.G.: An Electrophysiological Comparison of Solitary Type I and Type II Vestibular Hair Cells. Neuroscience Letters, vol. 116, nos. 1–2, Aug. 14, 1990, pp. 106–111.

Correia, M.J.: Filtering Properties of Hair Cells. Annals of the New York Academy of Sciences, vol. 656, May 22, 1992, pp. 46–57.

Objectives/Hypothesis
Two types of neurons were studied in this experiment; horizontal (lateral) semicircular canal afferents and type I or type II vestibular nuclei neurons found in the medial vestibular nucleus. The purpose of this study was to gain an understanding of neural adaptation of the semicircular canals to microgravity and to compare results to the previous study performed on Cosmos 2044.

Approach or Method
Monkeys were implanted with electrodes to monitor neuronal activity. Recordings were made during pre- and postflight studies from 118 semicircular canal afferents and 27 vestibular nucleus neurons from 7 rhesus monkeys. Five of these monkeys were ground controls and 2 were flight subjects. One hundred thirty-seven pulse rotation protocols were executed. Usable data were obtained from 127 horizontal afferents concerning spontaneous discharge. Rotation protocols for the semicircular canals included tests of spontaneous discharge, pulse rotation test, sum of sines test, and sinewave test. Rotation protocols for the vestibular nuclei neurons included spontaneous sinusoidal discharge test, oscillation at 0.2, 0.5 and 1.0 hertz, pulse constant velocity of 60 degrees per second, and a sum of sines stimulus covering the bandwidth from 0.02 hertz to 1.0 hertz.

Results
The mean spontaneous rate varied from 128 spikes per second during preflight tests to 92 spikes per second during postflight tests, for a change of 28%. The best filtered neural adaption operator (k) and the gain of pulse response were decreased during postflight when compared to preflight. This contrasts with data obtained from Cosmos 2044. The best filtered gain and and k values for the sum of sines were slightly elevated postflight. For periodic stimuli (pulse and sine waves) no change was found in gain and neural adaptation postflight. This is different from results found in 2044 but may be attributable to differences in experimental procedures.

Launch Date 1/13/1993 **Landing Date** 1/19/1993

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Effects of Spaceflight Stress on Proopiomelanocortin, Proenkephalin A, and Tachykinin Neurpepetidergic Systems in the Rat Posterior Pituitary

Science Discipline
Muscle Physiology

Investigator / Institute
D.M. Desidero — University of Tennessee, Memphis

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Zhu, X. and Desiderio, D.M.: Effects of Space Flight Stress on Proopiomelanocortin, Proenkephalin A, and Tachykinin Neuropeptidergic Systems in the Rat Posterior Pituitary. Life Sciences, vol. 55, no. 5, 1994, pp. 347–350.

Objectives/Hypothesis
Stress is known to cause an increase in the secretion of beta endorphins and methionine enkephalins into the blood stream. Both of these substances have a sedative effect or inhibit stimulation. Substance P is also an important neuropeptide in the stress process due to its ability to normalize stress disorders. The objective of this study was to examine the levels of these peptides in response to the unique stresses of spaceflight.

Approach or Method
Flight rats experienced 5 days of spaceflight including launch and landing stresses. The posterior pituitary was dissected from the rats 5–12 hours after landing. Total protein content was determined using micro-colorimetry. Radioimmunoassays were used to determine beta-endorphin-like immunoreactivity (BE-li), methionine enkephalin-like immunoreactivity (ME-li), and substance P-like immunoreactivity. These results were compared to the same measurements taken from ground-control rats.

Results
Statistical analysis showed levels of ME-li and SP-li in the flight rats to be significantly lower than levels in control rats. There were no differences found in BE-li levels of flight and control rats. This indicates that proenkephalin A and tachykinin neuropeptidergic systems respond to the stresses of spaceflight (as indicated by lower levels of ME-li and SP-li), whereas the proopiomelancortin neuropeptidergic system does not (as indicated by the lack of change in BE-li levels).

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Effects of Space Travel on Mammalian Gravity Receptors

Science Discipline
Neurophysiology

Investigator / Institute
M.D. Ross — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Ross, M.D.: Mammalian Vestibular Macular Synaptic Plasticity: Results From SLS-2 Spaceflight. ARO Midwinter Meeting, Feb. 5–9, 1995.

Objectives/Hypothesis
Results of an analysis of the number, type, and distribution of synapses in rats flown on the SLS-1 mission and ground control rats demonstrated that mammalian gravity sensors retain the property of neural plasticity into the adult stage. This experiment intended to replicate and expand the findings of the SLS-1 experiment with the following objectives: 1) to determine the acute effects of spaceflight on the ultrastructure of otoconia and neuroepithelium in vestibular organs by inflight tissue fixation for later microscopy; 2) to determine the chronic and/or progressive effects of spaceflight on the integrity of the vestibular organs by studying these organs immediately postflight and after a postflight recovery period; and 3) to determine the feasibility of in-flight tissue dissection.

Approach or Method
Synapses were analyzed from 100 serial sections taken from maculas of flight and ground control animals. Maculas were oriented so that sections were obtained from the posterior portion. Collection of sections, 150 nm thick, began ~64 μm into the tissue. Samples were taken from several areas across the macula to learn whether differences in synaptic count occur from site to site. Tissues were fixed and examined ultrastructurally with a transmission electron microscopy (TEM) microscope. Synapses were photographed and counted in blocks of 100 serial sections, using mosaics of every fifth or seventh section to locate the synapses to numbered cells. Ultrastructural and statistical analysis of variance (ANOVA) followed by Scheffe's S procedure for post-hoc comparison were carried out.

Results
The maculas of two rats dissected in flight (IF), two 13-day controls (IFC), and two R+0 rats have been studied. Synapses were counted in 100 serial sections. The difference between synaptic means of IF and control rat Type I cells was statistically significant ($p < 0.02$). The difference between synaptic means of Type II cells in IF and control rats was also significant ($p < 0.0001$). There was close correspondence between: 1) low counts at initial sampling of controls in both flight experiments (SLS-1, R0, 6.0 ± 4.5, $n = 180$; SLS-2, F13, 5.4 ± 3.9, $n = 120$); and 2) mean values for flown rats at R+0 in both flights (9.3 ± 6.8, $n = 142$ for SLS-1 and 8.8 ± 6.6, $n = 94$ for SLS-2). These preliminary results replicate the main findings from the SLS-1 experiment.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Ultrastructure and Histochemistry of Vestibular Structures and Vegitative Nuclei of the Brain

Science Discipline
Neurophysiology

Investigator	Institute
I.B. Krasnov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Grindeland, R.E.	NASA Ames Research Center (ARC)
Sawchenko, P.E.	Salk Institute
Vale, W.	Salk Institute

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Krasnov, I.B.; Fidelina, O.V.; Gorbatiuk, O.S.; and Vikhreva, O.V.; Repeated Exposure in Hypergravity: Morphology of Locus Coeruleus, Hypothalamic Paraventricular Nucleus and Vagal Verve Dorsal Nucleus in Rats. Aviakosm Ekolog Med, vol. 34, no. 3, 2000, pp. 21–27.

Fidelina, O.V.; Corbatyuk, O.S.; Krasnov, I.B.; and Akmayev, I.G.: Reactions of the Hypothalamic Paraventricular Nucleus and Vagus Nerve Dorsalis Nucleus in the Rats Exposed to Changed Gravitational Environment (in Russian). Problemy Endokrinologii, vol. 41, no. 6, 1995, pp. 35–38.

Objectives/Hypothesis
Morphological and physiological investigations of animals have demonstrated that the cerebellar vermis receives proprioceptive signals from hindlimbs and produces a regulatory effect on antigravitational muscles. Morphological examinations of the nervous system of Cosmos and SLS-1 rats have revealed structural changes in spinal ganglia, somatosensory cortex, and spinal cord, which pointed to a reduced amount of extero- and proprioceptive signals entering the brain and a reduced activity of spinal motoneurons. Another change is a decreased tone of antigravitational muscles. In this context, it is important to study the anterior vermis, one of the structures regulating antigravitational muscles, of animals flown in space with the purpose of better understanding the mechanism of adaptation of antigravitational muscles to microgravity. In this study quantitative histochemical and morphometric examinations of the upper central lobe of the vermis were studied.

Approach or Method
The upper central lobe of the vermis of rats were examined. Cytochrome oxidase was determined histochemically. In parallel sections the nucleolus and neuronal soma were stained according to the modified method of Howell and Black. The dorsal central lobe was identified using a rat brain atlas. Cytochrome oxidase activity in the Purkinje cell cytoplasm, molecular layer, neuropile, and granular layer glomerules of the dorsal central lobe was measured densitometrically at 450 mm by means of a discrete method. Optical density of the cytoplasm was measured in 3–5 sites of 50 Purkinje cells of each animal. Fifty optical density measurements were taken in the molecular layer, neuropile, and granular layer glomerules. The nucleolar and neuronal cross sections were measured in 40 stained Purkinje cell sections by means of an image analyzer. The resulting data were processed according to the Student-Fisher test.

Results
The results of quantitative histochemistry of flight rats sacrificed 4–5 hours after spaceflight showed a trend toward a higher cytochrome oxidase activity in Purkinji cells, molecular layer, and granular layer glomerules. This trend was most significant in the molecular layer with an increase of 10%. Cytochrome oxidase activity measured histochemically indicates dorsal central lobe function during flight. There were no changes observed in the size of the Purkinje cell nucleolus and cell body taken from the dorsal central lobe of flight rats. This suggests there were no changes in the synthesis rate of Purkinje cells, and their activity was equal to that of vivarium controls. Therefore, these Purkije cells maintained their capability to inhibit neurons of the dorsal caudal compartment of the lateral vestibular compartment during spaceflight. Observations give evidence that the amount of afferent signals reaching the dorsal central lobe in microgravity is adequate to maintain its activity at a a level comparable to that on the ground.

Launch Date 10/18/1993

Landing Date 11/1/1993

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Morphology of End and Intermediate Brain

Science Discipline
Neurophysiology

Investigator	Institute
I.B. Krasnov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Dyachkova, L.N.	Russian Academy of Sciences

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Krasnov, I.B.; Fidelina, O.V.; Gorbatiuk, O.S.; and Vikhreva, O.V.: Repeated Exposure in Hypergravity: Morphology of Locus Coeruleus, Hypothalamic Paraventricular Nucleus and Vagal Nerve Dorsal Nucleus in Rats. Aviakosm Ekolog Med, vol. 34, no. 3, 2000, pp. 21–27.

Sergutina, A.V.; Gershtein, L.M.; D'Amelio, F.D.; Donton, N.; and Krasnov, I.B.: Some Cytochemical Features of the Motor System of the Rat Brain After Spaceflight. Bulletin of Experimental Biology and Medicine, vol. 119, no. 3, Mar. 1995, pp. 288–290.

Objectives/Hypothesis
One goal of spaceflight is to determine the neuronal mechanisms by which an organism adapts to microgravity in spaceflight. One structure that has shown changes in previous spaceflights is the cerebral cortex. However, in all previous studies, subjects had been exposed to reentry stresses, increasing the proprioceptive impulses to the brain. SLS-2 offered the first opportunity to examine the somatosensory and visual cortex of rats exposed to microgravity with out reentry effects.

Approach or Method
Three groups of rats were used; one group was decapitated on flight day 13, the others were decapitated 5 hours postflight and 14 days postflight. Brains were removed from skulls no more than 3 minutes after decapitation and sectioned in half along the midline. Sections were fixed in glutaraldehyde in 0.1M cacodylate buffer, pH 7.3 at 4 C. After fixation, cortical fragments were cut into 0.3- to 0.5-mm-wide strips, dehydrated in ethanol of increasing concentrations and in acetone. They were then embedded in araldite oriented in such a way that all cortical sections were cut when making ultrathin frontal sections. Electron microscopy was performed on these sections by Russian specialists. Ultrathin sections of 3–5 blocks of each cortical area was examined with a JEM electron microscope.

Results
Electron microscopic examinations of the somatosensory cortex of rats decapitated in flight revealed ultrastructural changes in the II-IV layers, which pointed to a lower number of signals entering the cortex in microgravity. This was seen as an emergence of presynaptic axonal terminals with a low electron density of the matrix and an insignificant content of synaptic vessicles, termed "light" axonal terminals. The study of the ultrastructure of the somatosensory cortex of rats decapitated 14 days after recovery indicates that microgravity-induced changes are reversible, but not complete after 14 days.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Ultrastructural Changes in Choroid Plexus of Rats Maintained in Microgravity During a Spaceflight

Science Discipline
Neurophysiology

Investigator	Institute
J.B. Gabrion	Universite de Montpiler

Co-Investigator(s)	Institute
Gharib, C.	Université de Lyon
Herbuté, S.	Universite de Montpiler

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Asynchronous Control, Hindlimb Suspension Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Gabrion J. et al.: Choroidal Adaptation to Real Microgravity (Mission SLS-2) or Simulated (Rat Hindlimb-Suspended Model): Effects on the Cellular Structures and on the Natriuretic Peptide Receptors (in French). In: Four Years of Scientific Research in Space, CNES/COSPAR, J. Seylaz, ed., 1994, pp. 319–322.

Gabrion, J.; Herbute, S.; Oliver, J.; Maurel, D.; Davet, J.; Clavel, B.; Gharib, C.; Fareh, J.; Fagette, S.; and Nguyen, B.: Choroidal Responses in Microgravity. (SLS-1, SLS-2 and Hindlimb-Suspension Experiments). Acta Astronautica, vol. 36, 1995, pp. 439–448.

Objectives/Hypothesis
The aim of this experiment was to evaluate the effects of microgravity on fine structure and protein organization of choroidal cells after a spaceflight. The functional consequences of spaceflight and hindlimb suspension were evaluated by immunocytochemistry and by molecular biology by detecting changes in the expression of cytoskeletal and membrane proteins in choroidal cells, which produce cerebrospinal fluid. Moreover, qualitative changes in the biosynthesis and storage of natriuretic peptides (using electron microscopy and immunocytochemistry) in hypothalamus and heart were evaluated under microgravity conditions.

Approach or Method
Choroid plexuses from five brains dissected and fixed in flight and three others dissected 5–8 hours after landing were carefully isolated, fixed, and embedded in LX-112 epoxy resin. Those from two other animals dissected 5–8 hours after landing were fixed in the same conditions in 3% paraformaldehyde in phosphate buffered saline (PBS). Hypothalamus and the remaining brain with brainstem from these same brains were removed and directly frozen on dry ice before storage at –80°C until sectioning. Whole brains from two animals in each group were fixed, washed, and dehydrated before embedding in Paraplast. Four other whole brains were directly frozen on dry ice and stored at –80°C. Hearts were similarly processed for electron microscopy and immunocytochemistry. Atrial and ventricle samples were frozen for radioimmunoassay and Northern blot.

Results
Ultrastructural observations of choroidal cells (dissected in flight or 5–8 hours postflight) from adult rats showed a loss of cell polarity (altered kinocilia, loss of microvilli, decreases in apical ezrin) and a reduced choroidal secretion (accumulation of apical vesicles, loss of apical membrane and cytoplasmic molecules, involved in water and ionic transports such as aquaporin 1, Na/K ATPase, carbonic anhydrase II). Similar effects were observed in hindlimb-suspended rats, whereas control rats displayed typical choroidal features. Those results suggested that after 2 weeks in weightlessness choroidal functions were altered, indicating a reduction in the secretory processes. As cerebrospinal fluid (CSF) is mainly produced by these cells, it was concluded that spaceflight and head-down suspension probably induce a reduced CSF production. Analyses of natriuretic peptides in hypothalamus and heart are still in progress.

STS-65 / IML-2

Launch Date 7/8/1994 **Landing Date** 7/23/1994

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Early Development of a Gravity-Receptor Organ in Microgravity

Science Discipline
Neurophysiology

Investigator / Institute
M.L. Wiederhold — University of Texas

Co-Investigator(s) / Institute
None

Research Subject(s)
Cynops pyrrhogaster (Newt)

Ground-Based Controls
Vivarium Fertilized Egg Control

Key Flight Hardware
Aquatic Animal Experiment Unit (AAEU)

Selected Publications

Wiederhold, M.L.; Yamashita, M.; Larsen, K.; and Asashima, M.: Formation of Otoconia in the Japanese Red-Bellied Newt, Cynops Pyrrhogaster. Advance Space Research, vol. 14, no. 8, 1994, pp. 327–330.

Steyger, P.S. and Wiederhold, M.L.: The Morphogenic Features of Otoconia During Larval Development of Cynops Pyrrhogaster, the Japanese Red-Bellied Newt. Hearing Research, vol. 84, 1995, pp. 61–71.

Wiederhold, M.L. and Sharma, J.S.: Development of the Statocyst in Aplysia Californica I. Observations on Statoconial Development. Hearing Research, vol. 49, nos. 1–3, Nov. 1990, pp. 63–78.

Objectives/Hypothesis
The objective of this study was to examine the effects of microgravity on the inner ear of juvenile developing newts. These organs contain sensory hair cells covered by a layer of dense stones called otoconia.

Approach or Method
Pre-mated adult female newts and fertilized eggs were flown. Newts were induced to produce viable eggs through injections of human chorionic gonadotropin (HCG). Two of the adult newts did not survive the flight, one perishing on flight day 5 and the other on flight day 9. After recovery, flight and ground larvae were fixed on days R+0, R+3, and R+5. An X-ray micro-focus system was used to study the development of the otoliths.

Results
Preliminary analysis of these data indicate that the saccular otolith was significantly larger in the flight, on all three days of fixation. However the growth rate did not differ significantly between the flight and ground control groups. Otoconia from spaceflown larvae also appear more susceptible to speculation and in a few cases appear to contain only longitudinal filaments, indicating their alteration in their assembly.

STS-66 / NIH.R1

Launch Date 11/3/1994

Landing Date 11/14/1994

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Development of Sensory Receptors in Skeletal Muscle

Science Discipline
Neurophysiology

Investigator / Institute
M.E. DeSantis — University of Idaho

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM), Temperature Recording System-Modification 1 (ATR-4), Circadian Periodicity Experiment (CPE) Package

Selected Publications
DeSantis, M.; Helmick, C.; and Wong, A.: Proprioceptors in Skeletal Muscle of Rats That Developed in Utero Aboard the Space Shuttle Atlantis. Journal of Idaho Academy of Science, vol. 32, no. 1, 1996, p. 13.

Wong, A.M. and DeSantis, M.: Rat Gestation During Space Flight: Outcomes for Dams and Their Offspring Born Upon Return to Earth. Integrative Physiological and Behavioral Science, vol. 32, no. 4, 1997, pp. 322–342.

Objectives/Hypothesis
The objective of this experiment was to learn whether exposure to the microgravity conditions of spaceflight during the latter half of gestation affects the development of encapsulated sensory receptors in skeletal muscle among the offspring after they have been returned to Earth to be born.

Approach or Method
The number, size, and intramuscular distribution of muscle spindles and tendon organs in flexor and extensor muscle were compared. The ultrastructure of muscle spindles (and tendon organs) in rats undergoing gestation in microgravity conditions were compared with receptors in synchronous control rats, paying particular attention to the size and number of intrafusal fibers, and to the integrity and relative size and locations of motor and sensory nerve terminals. The hindlimb walking patterns during the initial 2 months of life for rats that underwent gestation in microgravity conditions were compared with those of synchronous controls.

Results
Data from the experiment showed the presence of muscle spindles in all flight and control soleus muscles. However, no significant differences were found between the experimental and flight subjects when all the measures for muscle spindles were examined. In a separate analysis, the vestibular nucleus was examined. Although there were no statistically significant differences between the composite volume of synchronous control, vivarium control, and flight subjects, it was observed that female rats had significantly smaller vestibular nuclear complex volume than male rats. Thus, gender seemed to be involved in the size of the adult vestibular nuclear complex. It was concluded that exposure to microgravity during the latter half of gestation does not preclude the existence of normal proprioceptive structure in newborn animals or in those that have grown to adulthood on Earth.

Launch Date 11/3/1994 **Landing Date** 11/14/1994

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Effects of Weightlessness on Vestibular Development

Science Discipline
Neurophysiology

Investigator	Institute
B. Fritzsch	Creighton University

Co-Investigator(s)	Institute
Bruce, L.L.	Creighton University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM), Temperature Recording System-Modification 1 (ATR-4)

Selected Publications

Fritzsch, B. and Bruce, L.L.: Utricular and Saccular Projections of Fetal Rats Raised in Normal and Microgravity. American Society for Gravity and Space Biology Bulletin, vol. 9, 1995, pp. 97.

Bruce, L.L. and Fritzsch, B.: The Development of Vestibular Connections in Rat Embryos in Microgravity. Journal of Gravitational Physiology, vol. 4, no. 2, July 1997, pp. 59–62.

Nichols, D.H.; Kingsley, J.D.; Bruce, L.L.; and Fritzsch, B.: Incomplete Target Segregation of Facial, Vestibular and Cochlear Efferents in Rodents. Society of Neuroscience Abstracts, vol. 21, 1995, p. 1040.

Objectives/Hypothesis
Previous studies indicate that the presence of gravity appears to be a critical factor in the development of the vestibular system. This study examined the effect of microgravity on the development of peripheral collateralization (receptor to receptor connections) and central projections to the medial vestibular nucleus of both gravistatic (utricle and saccule) and non-gravistatic (posterior vertical canal) afferents.

Approach or Method
The embryos of 10 pregnant rats were subjected to microgravity from gestational day 9 (G9) (before vestibular ganglion neurons contact vestibular nuclei) to G19 (approximately when the vestibular system becomes somewhat functional). Approximately 3 hours after shuttle landing, embryos were anesthetized and fixed. The saccule, utricle, and posterior vertical canal sensory epithelia were surgically revealed and exposed to DiI-soaked filter strips in order to label sensory afferent projections. After labeling, the rat brains were sectioned, mounted, and examined with epifluorescent microscopy for analysis of fiber distribution in the sensory epithelia and the medial vestibular nuclei. To determine synaptic density, suitable sections were further processed with electron microscopy.

Results
Animals subjected to microgravity conditions had slightly greater peripheral efferent collateralization than control animals, indicating that peripheral vestibular branches developed similarly in flight and control embryos. Additionally, labeled axonal projections from the saccule and utricle (gravistatic receptors) into the medial vestibular nucleus of flight animals had comparatively small growth cones and rarely had side branches. When these projections were examined under light microscopy, synaptic boutons were observable in the control animals but not in the flight animals. However, labeled projections from the posterior vertical canal (non-gravistatic receptors) into the medial vestibular nucleus were equally elaborate in both flight and control animals. Furthermore, on these projections, well developed synapses were observable on both flight and control animals. This data suggests that gravity stimulus is important for the proper development of central projections from gravistatic receptors but not important in the proper development of projections from non-gravistatic receptors.

STS-66 / NIH.R1

Launch Date 11/3/1994 **Landing Date** 11/14/1994

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Choroid Plexus, Brain and Heart Natriuretic Peptide Development in Space

Science Discipline
Neurophysiology

Investigator	Institute
J.B. Gabrion	Universite' de Montpellier II

Co-Investigator(s)	Institute
Gharib, C.	Universite Claude-Bernard
Gauquelin, G.	Universite Claude-Bernard
Bouille, C.	Universite de Montpellier
Herbuté, S.	Universite de Montpellier

Research Subject(s)
*Rattus norvegicus (*Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM), Temperature Recording System-Modification 1 (ATR-4)

Selected Publications
Davet, J.; Fagette S.; Mani-Ponset, L.; Bayard, B.; Dumars, P.; Reiss-Bubenheim, D.; Güell, A.; Gharib, C.; and Gabrion J.: Cardiac Atrial Natriuretic Peptide (ANP) in Rat Dams and Fetuses Developed in Space (NIH-R1 and NIH-R2 Experiments). Life Sciences, vol. 64, no. 17, 1999, pp. 1533–1541.

Davet, J.; Clavel, B.; Datas, L.; Mani-Ponset, L.; Maurel, D.; Herbuté, S.; Viso, M.; Hinds, W.; Jarvi, J.; and Gabrion, J.: Choroidal Readaptation to Gravity in Rats After Spaceflight and Head-Down Tilt. Journal of Applied Physiology, vol. 84, no. 1, Jan. 1998, pp. 19–29.

Objectives/Hypothesis
The objectives of this study were: 1) to investigate the choroidal alterations induced by 11 days of development in space in developing rats and pups, and to compare results obtained in adult rats flown during the SLS-2 experiments with the ultrastructural and immunocytochemical observations obtained from dams dissected 2 days after landing from an 11-day spaceflight; and 2) to evaluate qualitative or quantitative changes in storage and biosynthesis of natriuretic peptides in cardiac tissues in rats that developed for 11 days in space and in the dams that delivered 2 days after landing.

Approach or Method
Brains from dams, fetuses, and pups were removed after decapitation. Choroid plexuses were dissected either 3–4 hours or 2 days after landing. Samples were fixed and embedded in LX-112 epoxy resin or in 3% paraformaldehyde in PBS. The hypothalamus and remaining brain with brainstem from the same brains were removed and frozen until sectioning and observation. Whole brains from two additional animals in each group were prepared and embedded in Paraplast. Four other whole brains were frozen and stored at 80°C. Hearts were similarly processed for electron microscopy, immunocytochemistry. Atrial and ventricle samples were frozen to be studied by radioimmunoassay, in situ hybridization and competitive reverse transcription polymerase chain reaction (RT-PCR).

Results
In dams, a strong restoration of the cell polarity and a restored choroidal protein expression was observed. These results suggested that after 2 days of readaptation to Earth's gravity, choroidal functions were restored, indicating that secretory processes were reestablished early after landing. In fetuses and pups, maturation of choroid plexuses appeared slightly delayed, in comparison with fetuses and pups developed on Earth. Results on the expression of natriuretic peptides in heart showed that cardiac atrial natriuretic peptide (ANP) biosynthesis of flight dams was increased by about twice, when compared to synchronous and vivarium control rats. Rat fetuses developed in space displayed an altered maturation of cardiac ANP, whereas the cardiac ANP storage was slightly reduced in both flight and synchronous control groups, compared to vivarium control rats. This suggested that ANP metabolism during development is impacted by both the microgravity environment and the housing conditions.

Launch Date 11/3/1994

Landing Date 11/14/1994

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
An Experiment to Study the Role of Gravity in the Development of the Optic Nerve

Science Discipline
Neurophysiology

Investigator	Institute
J.L. Lambert	Jet Propulsion Laboratory

Co-Investigator(s)	Institute
Borchert, M.S.	University of Southern California

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications

Lambert, J.; and Borchert, M.: Investigation of Optic Nerve Development Using Neuronal Tracing Dyes. SPIE Proceedings, vol. 2137, Jan 22–28, 1994, pp. 49–54.

Brochert, M.; and Lambert, J.: Morphometric Refinement of Retinotectal Connections in the Rat. Investigative Opthalmology and Visual Science, supl., vol. 35, 1994, p. 1771.

Lambert J.: System Would Generate Virtual Heads-up Display. NASA Tech Brief, vol. 18, no. 7, July 1994, pp. 22–23.

Objectives/Hypothesis
At birth, the retinal ganglion cells project fairly accurately to the superior colliculus, the primary visual center of the rat. Previous studies show that visual stimuli is important for the proper refinement of these projections. However, it is uncertain what role other sensory stimuli may have in the development and refinement of these projections. This NIH.R1 mission provided an opportunity to observe the effects of gravity on these connections. Because the superior colliculus receives and integrates both visual and vestibular information, gravity might be expected to influence these retino-tectal connections.

Approach or Method
Pups were exposed to microgravity during gestation, which included nearly the entire period of prenatal development of connections between the retina and the brain. On postnatal days 2, 14, 21, and 47, the rats were examined. Two days prior to examination, Fast Blue dye was microinjected into the right superior colliculus of the pups. After 2 days of recovery, the pups' retinas were dissected and prepared for microscopic analysis. The centroid area, the 2-D Gaussian distribution, and the moment invariants of the cluster of stained cells were determined. The rat brains were then dissected, fixed, and visualized under epifluorescent microscopy. A 3-D rendering of the entire superior colliculus and fluorescent injection was constructed. The percent of the superior colliculus occupied by the injection site was then calculated. The area of the 2-D Gaussian distribution of 80% of the labeled retinal ganglion cells was calculated as a percent of the area of the total retina. The ratio of the percent of retinal area stained to the percent of superior colliculus volume injected represents the expansion of the retinotopic map in the superior colliculus.

Results
In this experiment retinotopic magnification was defined as normalized area stained of retina/normalized volume stained of superior colliculus. The findings showed a normal reduction in retinotopic magnification. There were no statistically significant differences found between the flight and control groups. The results suggested that neuronal connections between the eye and the brain develop normally in weightlessness, thus, gravity does not seem to play a significant role in neuronal connection development in general.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Adaptations of Motor Control in Response to Spaceflight

Science Discipline
Neurophysiology

Investigator	Institute
D.M. Rumbaugh	Georgia State University
V.R. Edgerton	University of California, Los Angeles

Co-Investigator(s)	Institute
Kozlovskaya, I.B.	Institute for Biomedical Problems
Badakva, A.M.	Institute for Biomedical Problems
Roy, R.R.	University of California, Los Angeles
Hodgson, J.A.	University of California, Los Angeles

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications

Hodgson, J.A., et al.: Rhesus Leg Muscle EMG Activity during a Foot Pedal Pressing Task on Bion 11. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, p. S87.

Recktenwald, M.R. et al.: Quadrupedal Locomotion in Rhesus Monkeys after 14 Days of Spaceflight. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, p. S71.

Roy, R.R. et al.: Fiber Size and Myosin Phenotypes of Selected Rhesus Hindlimb Muscles After a 14-day Spaceflight. Journal of Gravitational Physiology, vol. 6, no. 2, Oct 1999, pp. 55-62.

Objectives/Hypothesis
Although past experiments have proven that some muscles weaken and atrophy during space flight, the exact mechanism behind this is unknown. Three hypotheses help explain the phenomenon: 1) Some "normal" level of activity, which is lacking in microgravity, is needed to maintain normal muscle properties; 2) Force generated by a muscle is directly related to muscle fiber size, loss of which occurs in space; and 3) Hormonal deficiencies, such as those seen during microgravity exposure, contribute to a loss in muscle mass. This experiment will help to quantify the response of muscle to space flight.

Approach or Method
Muscle biopsies were taken from the soleus (Sol), medial gastrocnemius (MG), tibialis anterior (TA), and vastus lateralis (VL), before and after a 14-day space flight. Samples were used to determine myosin heavy chain (MHC) composition, fiber type distribution, and fiber size. Single fibers from the soleus muscle were analyzed for cross-sectional area (CSA), myonuclear number/mm, and myonuclear domain.

Results
Percentage of pure type I fibers was decreased in the Sol postflight, in both the flight and the flight simulation groups. The number of hybrid fibers increased in both groups, and the MHC composition changed in a similar direction. In the MG or TA, no consistent changes in MHC, fiber type composition, or size of type II fibers were observed. However, pure type I fibers were smaller postflight in both the MG and the TA. In the single soleus fibers, mean CSAs of type I MHC fibers were smaller postflight. Mean myonuclear number/mm was similar between pre- and postflight in all groups, but the mean myonuclear domains of the type I MHC fibers were decreased postflight. The Sol, a slow antigravity muscle, was affected the most by the 14-day space flight.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Effect of Microgravity on the Mechanisms of Eye, Head and Hand Movement Coordination

Science Discipline
Neurophysiology

Investigator	Institute
I.B. Kozlovskaya	Institute of Biomedical Problems

Co-Investigator(s)	Institute
None	

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Antsiferova, L.I.; Shlyk, G.G.; and Ignatenko, A.V.: Effect of Microgravity on the Implementation of Conditioned Reflex Skills of Rhesus Monkeys. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S95-S98.

Objectives/Hypothesis
The effects of microgravity on human eye-head-hand coordination have been investigated for several years. In early studies on humans during short-term exposure to microgravity, scientists concluded that human response time increased and precision decreased in performing hand movement tasks. Similar studies were carried out later on rhesus monkeys flown on biosatellites. This experiment continues the study on two rhesus monkeys during a 14-day Bion 11 flight.

Approach or Method
Each monkey was required to touch a light stimuli, illuminated subsequently on a panel in front of it, with its left hand. The stimulus consisted of five concentric circles located 20-22 cm directly in front of the monkey, one in the center and the others 20 and 40 angular degrees. After successfully completing a task, the monkey was rewarded with a small quantity of juice, which would increase as more tasks were completed. Two hundred and fifty-six stimuli were presented with each stimulus occurring 2.5 seconds after a successful completion. These activities occurred at set times twice a day during flight.

Results
In comparison to ground experiments, monkey #357 demonstrated a decrease in response time during the entire flight. In fact, response times decreased even more from FD2 to FD7. These results contradicted the conclusions made from previous flights. Monkey #484, on the other hand, demonstrated behavior more consistent with hypotheses; that is, during the first part of the flight, it had a shorter response time for one stimulus, but longer response times for others. These response times lengthened from FD3 to FD6. In terms of precision, #357 had an initial increase for all stimuli from 35% on the ground to 75% in space. This level remained constant until FD3. FD5-6 showed a decrease to 25% for the ±40 degree stimuli and 50-60% for the ±20 degree stimuli. The highest levels of precision were reached on FD7 and FD8, upon which #357 achieved 50-70% for all stimuli. Monkey #484 did not perform as well on the ±40 degree stimuli during FD4-6, but improved greatly in the FDs thereafter to all stimuli. #484 performed particularly well on the ±20 degree stimuli.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Effect of Microgravity on Sympathetic and Parasympathetic Compartments of the Autonomic Nervous System

Science Discipline
Neurophysiology

Investigator	Institute
A.M. Badakva	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Miller, N.E.	Yale University

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Badakva, A.M. and Miller, N.V.: The Effect of Microgravity on the Autonomic Nervous System of Rhesus Monkeys. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S113-S118.

Objectives/Hypothesis
The mechanisms that regulate the autonomic nervous system undergo a pattern of adaptations as they adjust to microgravity. Alterations in gastric myoelectric activity can be used to evaluate changes in vagal and sympathetic effects. Varying R-R intervals allow sympathetic effects and vagal variations to be estimated. The vagal control of the heart can also be evaluated by the respiratory sinus arrhythmia (RSA). This study focused on the adaptation of rhesus monkeys' autonomic nervous system to microgravity and its effect upon cardiac and gastric rhythms.

Approach or Method
Four months prior to launch, flight animals were implanted with electrodes in order to monitor and record their electrocardiogram (EKG), electrogastrogram (EGG), and rheopneumogram (RPG). Physiological data were recorded during flight for a period of five minutes every two hours. EKG signals were interpolated and resampled at 20 Hz. Breathing depth was measured as an rheopneumogram amplitude (Arpg). R-R interval, BR, and Arpg means were calculated.

Results
During space flight, the vagal effects upon both the cardiac and respiratory function varied between the two animals. In animal #357, gastric myoelectric activity was enhanced, which may be associated with the cessation of juice intake on flight day (FD) 11. Early in the flight, #357's RSA increased despite an increase in Arpg, which suggests significantly lower vagal effects on the heart. #484 had a lower level of gastric myoelectric activity when compared to the rest of the flight. RSA levels rose through the flight and then dropped after landing. Further analysis of the results for animal #484 suggests that vagal effects on both the cardiac system and gastric functions were lower at the beginning of the flight. In summary, the vagal effects in #357 increased at the beginning of the flight and then decreased towards the end while #484's decreased through the duration of the flight. Further, the inhibition of vagal effects in animal #484 was accompanied by space motion sickness symptoms.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Partial Oxygen Pressure in the Somatosensory Cortex of Monkeys in Space Flight

Science Discipline
Neurophysiology

Investigator / Institute
V.P. Krotov — Institute of Biomedical Problems

Co-Investigator(s) / Institute
None

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Krotov, V.P. and Nosovsky, A.M.: Oxygen Tension in the Somatosensory Cortex of Rhesus Monkeys in Space. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S125-S127.

Objectives/Hypothesis
During microgravity exposure, body fluids shift towards the head. This fluid shift may affect cerebral circulation, causing venous congestion. Theory has predicted that this may decrease oxygen supply to the brain, causing hypoxemia. Few experiments have actually measured oxygen tension (pO2)—a measurement of regional circulation and oxygen supply—in space. One study performed on the skin of cosmonauts during initial exposure to microgravity showed a decrease in both pO2 and oxygen uptake. In contrast, previous studies of pO2 in the brain of primates during space flight did not show any significant changes in pO2. This experiment will continue the study of pO2 in the primate brain during space flight.

Approach or Method
Platinum electrodes 50 μm in diameter were used to measure pO2 in the somatosensory cortex. The electrodes were covered with polysterol film dissolved in toluene to reduce protein absorption. For both flight and control subjects, electrodes were calibrated in saline solution equilibrated with O2 prior to implantation and immediately after removal. Ten to 14 days before the first measurements were taken, the active electrodes were inserted into previously implanted cannulas in the cortex at a depth of 1-1.5 mm. pO2 was recorded continuously during the first 3 hours of flight, then for 5 minutes every 2 hours for the duration of the flight.

Results
Both monkeys experienced a pO2 in the somatosensory cortex higher than the baseline during the first 30 -55 minutes of flight. This increase was most likely due to the emotional response of the animals. Although hypoxemia does not develop in the somatosensory cortex immediately after launch, there may be a mismatch between the metabolic rate and oxygen supply to the area during the first 1-2 hours in space. During flight, pO2 of the somatosensory cortex of both monkeys was higher than the baseline. This indicates that in microgravity, the regulatory mechanisms in charge of local circulation and metabolism may be changed.

STS-89 / CEBAS

Launch Date 1/22/1998 **Landing Date** 1/31/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Development of Vestibular Organs in Microgravity

Science Discipline
Neurophysiology
Developmental Biology

Investigator	Institute
M.L. Wiederhold	University of Texas Health Science Center

Co-Investigator(s)	Institute
Becker, W.	University of Hamburg
Bluem, V.	Ruhr University Bochum

Research Subject(s)
Biomphalaria glabrata (Snail)
Xiphophorus helleri (swordtail fish)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Closed Equilibrated Biological Aquatic System (CEBAS)

Selected Publications
Wiederhold, M.L.; Harrison, J.L.; Parker, K.; and Nomura, H.: Otoliths Developed in Microgravity. Journal of Gravitational Physiology, vol. 72, no. 2, July 2000, pp. 39–42.

Objectives/Hypothesis
Otoliths, dense calcified masses that respond to gravitational forces, are most likely affected by the change in gravity that accompanies spaceflight. The development of otoliths, as well as their respective synaptic connections, are susceptible to changes due to microgravity. This study utilized two model systems to study otoliths' embryonic and larval stages of development during spaceflight.

Approach or Method
Thirty-five *Biomphalaria glabrata*, freshwater snails, were flown in the closed equilibrated biological aquatic system (CEBAS) on board STS-89. Upon landing, a portion of the specimens were dissected and fixed for light and electron microscopy. These specimens' statoconia were compared with those of specimens grown on Earth. The other specimens were allowed to continue developing in order to study how their statoliths and statoconia respond to a return to gravity. The crawling patterns of 1- to 7-mm-sized snails were videotaped for about 5 days after landing in order to test the development of gravitactic reflex behavior in animals raised in microgravity. Also, about two hundred *Xiphophorus helleri* (swordtail fish) were flown, and the differences in otolith structure between flight and ground-control specimens were analyzed at light-microscopic, electron-microscopic, and atomic-force microscopic levels.

Results
Preliminary analysis showed a significant increase in the total statoconial volume of the snails reared in microgravity in relation to the ground control. When comparing ground and space-raised snails of similar diameter, those reared in microgravity were, on average, 30% larger. However, the increase in statoconial volume in space-reared specimens was due to an increase in the number of statoconia, not an increase in individual size. The cross-sectional area for both ground and flight-reared animals was the same.

STS-89 / CEBAS

Launch Date 1/22/1998 **Landing Date** 1/31/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Brain Pituitary Axis Development in the CEBAS Minimodule

Science Discipline
Neurophysiology

Investigator	Institute
M. Schreibman	Brooklyn College, New York

Co-Investigator(s)	Institute
Magliulo-Cepriano, L.	Brooklyn College, New York

Research Subject(s)
Xiphophorus helleri (Swordtail fish)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Closed Equilibrated Biological Aquatic System (CEBAS)

Selected Publications
Schreibman, M.P.; Magliulo, L.; Bluem, V.; and Paris, F.: (Abstract) Preliminary Report of Brain-Pituitary Axis Development in the CEBAS-Minimodule: Shuttle Flights STS-89 and STS-90. American Society for Gravitational and Space Biology Bulletin, vol. 12, 1998, p. 53.

Objectives/Hypothesis
Using the CEBAS, which could lead to an aquatic controlled ecological life support system (CELSS) to provide plant and animal biomass for nutrition on long-duration flight, this study focused on the reproductive biology of animals in space. Information was gathered on microgravity-induced changes to the brain-pituitary axis and the organs that receive information from the environment. Spaceflight conditions may affect the development and functioning of the neuroendocrine system, which regulates the reproductive system.

Approach or Method
Xiphorous helleri, a freshwater teleost, was used to study the brain-pituitary axis in embryos, neonates, immature, and mature specimens. Two hundred juveniles and four gravid females were flown in the CEBAS. Histology, cytology, immunohistochemistry, morphometry, and in situ histochemistry were used to evaluate the synthesis, storage and release of neurotransmitters, neuroregulatory peptides, neurohormones, pituitary hormones, and the structure of the specimens' cells and organs. The olfactory system and the pineal organ were also studied in a similar fashion. Four living specimens were kept to monitor postflight growth and development.

Results
The distributions of Neuropeptide Y (NPY), Neurotensin (NT), Androgen Receptor (AR), and Dynorphin (DYN) were found to be identical in both flight specimens and the ground-based laboratory module. Intense distribution of Immunoreactiv-Galanin (ir-GAL) in the cell bodies of the nucleolar organizing regions (NOR) was observed in all flight specimens while ir-GAL was absent in the laboratory module. The distribution of FMRF-amide fibers in the brains of the flight specimens appeared sparse and less intense when compared to laboratory controls. There was no significant difference in growth rate and maturity in both flight animals and laboratory control groups.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Chronic Recording of Otolith Nerves in Microgravity

Science Discipline
Neurophysiology

Investigator	Institute
S. Highstein	Washington University

Co-Investigator(s)	Institute
Usui, S.	Toyohashi University of Technology

Research Subject(s)
Opsanus tau (Oyster toadfish)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Vestibular Function Experiment Unit (VFEU)

Selected Publications
Mensinger, A.F.; Anderson, D.J.; Buchko, C.J.; Johnson, M.A.; Martin, D.C.; Tresco, P.A.; Silver, R.B.; and Highstein, S.M.: Chronic Recording of Regenerating VIIIth Nerve Axons With a Sieve Electrode. Journal of Neurophysiology, vol. 83, 2000, pp. 611–615.

Boyle, R.; Mensinger, A.F.; Yoshida, K.; Usui, S.; Intravaia, A.; Tricas, T.; and Highstein, S.M.: Neural Readaptation to Earth's Gravity Following Return From Space. Journal of Neurophysiology, vol. 86, 2001, pp. 2118–2122.

Objectives/Hypothesis
The goals of this study were to record the responses of primary afferents of the otolithic organs to document otolithic organ response dynamics in normal gravity and in microgravity. Studies of these aspects of the vestibular system and its efferent control can add to knowledge of its function and may suggest future therapies for control of Earth-bound motion sickness.

Approach or Method
Experimental difficulties precluded obtaining useful in-flight data as originally planned. However, fish were received within 8 hours of the Shuttle's return to Earth and utricular nerve afferents recorded sequentially for 5 days postflight. For this, anesthetized, paralyzed toadfish underwent a small craniotomy to allow the implantation of glass micro-electrodes (2 M $LiCl_2$) in the nerves innervating the utricle. Records of primary afferents in response to linear acceleration were taken. Position and motion of the fish were documented by linear and rotary potentiometers.

Results
Results from Neurolab were combined with those from STS-95 for a total of four experimental subjects. Control responses were obtained from three fish that did not fly. For flight subjects, the magnitude of response to an applied linear acceleration was on average three times greater than for controls within the first day postflight. By 30 hours postflight, responses had returned to normal and were statistically similar to controls. Directional selectivity appeared unaffected by exposure to microgravity. To examine for possible recording bias, all measured parameters were compared between control and postflight afferents; no statistical difference was found in the range and mean of afferent discharge rate and regularity of discharge between postflight and control fish. Thus, the reduced gravitational vector and linear acceleration in orbit apparently resulted in an up-regulation of the sensitivity of utricular afferents. More tests must be done to determine whether a specific population of afferents demonstrated increased sensitivity or whether this finding is a general feature of all classes of cells. The original plan called for the implementation and perfection of a sieve electrode to allow for continuous recording of afferents during flight. Although experimental difficulties precluded the completion of the entire original plan, the perfection and utilization of the sieve electrode was achieved.

Launch Date 4/17/1998

Landing Date 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Anatomical Studies of Central Vestibular Adaptation

Science Discipline
Neurophysiology

Investigator / Institute
G. Holstein — Mount Sinai School of Medicine

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Holstein, G.R.; Kukielka, E.; Martinelli, G.P.: Anatomical Observations of the Rat Cerebellar Nodulus After 24 hr of Spaceflight. Journal of Gravitational Physiology, vol. 6, no. 1, July 1999, pp. P47–P50.

Cohen, B.; Yakushin, S.B.; Holstein, G.R.; Dai, M.; Tomko, D.L.; Badakva, A.M.; and Kozlovskaya, I.B.: Vestibular Experiments in Space. Advances in Space Biology and Medicine, vol. 10, 2005, pp. 105–164.

Objectives/Hypothesis
Alterations in sensory and motor function occur during weightlessness. The vestibular abnormalities experienced by astronauts include immediate-reflex motor responses, sensations of rotation, nystagmus, dizziness, and vertigo, and space motion sickness. Adaptation to the microgravity environment usually occurs within 1 week. The mechanisms underlying this adaptation process have not been completely elucidated. This experiment studied the neuronal basis for microgravity-induced changes in the vestibular system. The specific objectives were: 1) to assess the quantitative differences in cerebellar synaptic morphology and ultrastructure of flight rats compared to controls; 2) to study the qualitative and/or quantitative differences in excitatory amino acid neurotransmission in the nodulus and flocculus of flight rats compared to controls; and 3) to evaluate the qualitative and/or quantitative differences in GABAergic neurotransmission in the nodulus and flocculus of flight rats compared to controls.

Approach or Method
Brain tissue was taken from both flight and ground-control animals on flight day 2 (FD 2), FD 14, recovery day one (R+1), and R+13. The entire cerebellum was fixed and the tissue processed for stereological analysis, immunocytochemical studies of excitatory amino acid neurotransmission, and immunocytochemical studies of GABAergic Purkinje cell interactions. Thin wafers of cerebellar tissue were cut, embedded in plastic, and examined by light microscopy. The tissue wafers were then traced using a Trisimplex projector. The tracings were used to estimate the volume of the nodulus and the partial volume fraction occupied by the molecular layer of the nodulus for each subject. After volume measurement, selected thin sections were obtained from four wafers from the FD 2 rats and prepared for ultrastructural, quantitative, and post-embedding immunocytochemical studies.

Results
Cytoplasmic and neuropil alterations were observed in sections from the flight animals that were not apparent in the controls. Throughout entire nodular Purkinje cells (including somata, dendrites, thorns, and axon terminal) the smooth cisterns of endoplasmic reticulum were substantially more complex in flight animals than controls. Some enormous mitochondria were found in the Purkinje cell somata of flight animals, and enhanced glial sheathing between Purkinje cells and granule cells was also observed. Neuropil alterations included frequent, sometimes large protrusions of neuronal elements into neighboring profiles, suggesting enhanced fluidity of neuronal shape. Ultrastructural indications of degeneration and synaptic reorganization were also observed in the nodular molecular layer of flight animals. The above morphologic changes were not apparent in control animals. These preliminary results provide ultrastructural evidence for neuronal and synaptic plasticity in the nodulus of adult rats after 24 hours of exposure to spaceflight.

Launch Date 4/17/1998

Landing Date 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Development of an Insect Gravity Sensory System in Space

Science Discipline
Neurophysiology
Developmental Biology

Investigator	Institute
E.R. Horn	University of Ulm

Co-Investigator(s)	Institute
Kamper, G.	University of Ulm
Neubert, J.	DLR, Institute of Aerospace Medicine

Research Subject(s)
Acheta domesticus (cricket) eggs and larvae

Ground-Based Controls
Asynchronous Control, 3-G Hypergravity Control

Key Flight Hardware
BOTany EXperiments (BOTEX) incubator system

Selected Publications
Horn, E.R.: Critical Periods in Vestibular Development or Adaptation of Gravity Sensory Systems to Altered Gravitational Conditions? Archives Italiennes de Biologie, vol. 142, no. 3, May 2004, pp. 155–174.

Horn, E.R.: Crickets in Space: Morphological, Physiological and Behavioral Alterations Induced by Space Flight and Hypergravity. Advances in Space Research, vol. 30, no. 4, 2002, pp. 819–828.

Horn, E.R.: The Development of Gravity Sensory Systems During Periods of Altered Gravity Dependent Sensory Input. Advances in Space Biology and Medicine, vol. 9, 2003, pp. 133–171.

Objectives/Hypothesis
The objective of the CRISP (Crickets in Space) experiment was to examine to what extent genetics or environmental factors affect the development of a neuronal sensory system. The question under investigation was whether irreversible anatomical and physiological changes in sensory, neuronal, and motor systems could be induced by periods of altered environmental conditions during early development. Crickets provide an excellent model system for neurobiology experiments. They possess external gravity receptors located on their abdominal cerci, which can regenerate morphologically and functionally if lost during postembryonal development. Neural information generated from the stimulation of these receptors is transmitted by way of a single interneuron called the position sensitive interneuron (PSI), the activity of which relates to the posture of the animal's body. The CRISP experiment tested the functionality of this neural system in crickets raised without normal gravitational cues.

Approach or Method
Cricket eggs and first-, fourth-, and sixth-stage larvae were flown on Neurolab in the BOTEX incubator. The use of four age groups allowed for comparison of adaptation to microgravity between nervous systems in differing stages of development. Lesions of the gravity sensory system were performed on sixth-stage larvae. A 1-G in-flight control (on the BOTEX reference centrifuge) and a 3-G hypergravity control were performed for the four larval stages. Postflight, cricket roll-induced compensatory head response (rCHR) was videotaped and analyzed for the flight and control groups. Recordings of PSI activity were taken and compared between 1) flight and ground groups, and 2) intact and regenerated cerci.

Results
Eggs hatched and larvae developed peripheral and central gravity-sensing structures in spite of the altered gravitational cues. Larvae that had undergone lesions of the cerci were able to successfully regenerate the organs in microgravity as on the ground. Postflight, larvae demonstrated normal walking behavior. Significant behavioral differences in the rCHR were not observed between lesioned and intact larvae in either the flight or the ground samples. Larvae that hatched in orbit showed a retardation of 1-G re-adaptation. The PSI showed a significant sensitivity to microgravity exposure, contrasting with the behavioral analysis. A sensitivity was also seen in the 3-G hypergravity but only during the period of 1-G readaptation. Overall, the study indicates that the physiology of an identified gravity-sensitive neuron is strongly affected by microgravity exposure while behavior of the larvae remains less or unaffected.

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Neuronal Development Under Conditions of Spaceflight

Science Discipline
Neurophysiology
Developmental Biology

Investigator / Institute
K. Kosik — Bringham Women's Hospital

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Temple, M.D.; Kosik, K.S.; and Steward, O.: Spatial Learning and Memory is Preserved in Rats After Early Development in a Microgravity Environment. Neurobiology of Learning and Memory, vol. 78, no. 2, Sept. 2002, pp. 199–216.

Homick, J.L.; Delaney, P.; and Rodda, K.: Overview of the Neurolab Spacelab Mission. Acta Astronaut, vol. 42, nos. 1–8, Jan.–Apr. 1998, pp. 69–87.

Objectives/Hypothesis
Considerable development of the hippocampus occurs in rats after birth. Therefore, the hippocampus, which is required for spatial learning, is more likely to be affected by microgravity exposure at a young age. This experiment examined the hypothesis that exposure of rat neonates to microgravity results in altered activity that leads to altered development. This study evaluated the cognitive mapping abilities of rats that spent part of their early development in a microgravity environment. Another objective of this experiment was to analyze neuronal morphology and determine the synaptic functional plasticity in the developing nervous system of rat neonates raised in microgravity. Finally, an analysis of the distribution of specific protein components of the nervous system was conducted, with particular emphasis on the cytoskeletal synaptic proteins, in rat neonates raised in microgravity.

Approach or Method
Upon return to Earth, all animals were tested in three different tasks to measure general activity and exploratory activity: the Morris water maze (MWM); a modified version of the radial arm maze (RAM); and an open field apparatus (OFA). Performance and search strategies were evaluated in each of these tasks using an automated tracking system (Poly-Track, San Diego Instruments). The analysis of the hippocampus included a determination of dendritic extents, a quantitative analysis of the synaptic structure and spinal architecture, an analysis of isoform expression of specific cytoskeletal and synaptic proteins, and a determination of the onset of long-term potentiation.

Results
There were remarkably few differences between the flight (FLT) groups and their earth-bound controls in these tasks. FLT animals learned the MWM and RAM as quickly as controls. Evaluation of search patterns suggested subtle differences in patterns of exploration and in the strategies used to solve the tasks, but these differences normalized rapidly. Together, these data suggest that development in an environment without gravity has minimal long-term impact on cognitive mapping. Any differences due to development in microgravity quickly reversed after return to Earth's normal gravity.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Ensemble Neural Coding of Place and Direction in Zero-G

Science Discipline
Neurophysiology

Investigator / Institute
B. McNaughton — University of Arizona

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications

Knierim, J.J.; McNaughton, B.L.; and Poe, G.R.: Three-Dimensional Spatial Selectivity of Hippocampal Neurons During Space Flight. Nature Neuroscience, vol. 3, no. 3, Mar. 2000, pp. 209–210.

Homick, J.L.; Delaney, P.; and Rodda, K.: Overview of the Neurolab Spacelab Mission. Acta Astronaut, vol. 42, nos. 1–8, Jan.–Apr. 1998, pp. 69–87.

Campbell, M.R.; Williams, D.R.; Buckey, J.C. Jr.; and Kirkpatrick, A.W.: Animal Surgery During Spaceflight on the Neurolab Shuttle Mission. Aviation, Space, and Environmental Medicine, vol. 76, no. 6, June 2005, pp. 589–593.

Objectives/Hypothesis
Brain cells in the hippocampus, called place cells, play an important role in the ability of animals to orient themselves within their environment. Another class of neurons associated with spatial orientation, called head direction (HD) cells, are located in the thalamus and limbic cortex. Firing properties of the place cells in the hippocampus and HD cells are tightly coupled. The purpose of this experiment was to study how the hippocampal neural representations of place and direction are affected by 0-G conditions in rats performing tasks requiring navigation in three dimensions.

Approach or Method
Three rats were singly tracked with two position-image-locator cameras synchronized with a data acquisition system as they navigated a special walking apparatus. Animals were implanted with probes that monitored neuronal spikes simultaneously from about 20 or more hippocampal neurons, to determine whether they fired in a stable fashion with respect to the rat's location in 3-dimensional space. Rats were also trained to visit each of four arms on a 2-dimensional cross-shaped track. The animals were tracked as above to determine whether place cells fire in synchronization with the rat's spatial location relative to the track or relative to the reference frame of the work volume of the General Purpose Work Station.

Results
During the first 0-G recording session on flight day (FD) 4, rat 2 did not show strong spatial tuning in any of the cells that fired while on the track. In contrast, this animal had normal, highly specific place fields in cells that fired when tested on a very similar track 4 days before launch. A spatial-tuning index for rat 2 was significantly different between preflight and FD 4 recordings. Rat 1 exhibited a different pattern of abnormal spatial selectivity; almost all the spatially selective firing recorded occurred when the rat moved its head off the track. In contrast to rats 1 and 2, rat 3 displayed normal spatial tuning on FD 4; however, these data came from the animal's second run on the track. Data from the first run were lost due to technical difficulties, so it remains unknown if the animal gained spatial experience during the first trial. Spatial selectivity of rats 1 and 2 improved by FD 9, showing hippocampal adaptation. The main conclusion is that the rat hippocampus is capable of forming a stable code for 3-dimensional space under low-gravity conditions; however, the development of this code may be somewhat slower than under normal gravity conditions.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Reduced Gravity: Effects in the Developing Nervous System

Science Discipline
Neurophysiology
Developmental Biology

Investigator	Institute
R. Nowakowski	University of Medicine & Dentistry of New Jersey

Co-Investigator(s)	Institute
None	

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM), General Purpose Work Station (GPWS)

Selected Publications
Homick, J.L.; Delaney, P.; and Rodda, K.: Overview of the Neurolab Spacelab Mission. Acta Astronautica, vol. 42, no. 1, Jan.–Apr. 1998, pp. 69–87.

Objectives/Hypothesis
The central nervous system (CNS) is the single most complicated organ of the body, and its normal development is the consequence of a complex series of events such as cell proliferation, migration, and differentiation. The objective of these experiments was to determine the short-term and intermediate-term physiological effects of microgravity on the cells of the developing CNS. There were also two secondary goals: to assay cell proliferation in the adult tissue and also to assay cell proliferation in a second brain region at a later stage of development.

Approach or Method
This study used fetal mice to examine the short-term and intermediate-term effects of spaceflight and reduced gravity on the cells of the developing CNS. Timed-pregnant mice were launched at embryonic (E) days E8, E10, and E12. Two markers of cell proliferation, bromodeoxyuridine (BUdR, which is detected immunohisto-chemically), and tritiated thymidine (3H-thymidine, which is detected autoradiographically) were used to track cell proliferation and migration. The markers were administered by intraperitoneal injection to pregnant mice on FD 3 and FD 6.

Results
The pregnancy rates (> 90%) were well within the planned guidelines, and all six of the planned experiments were completed successfully. After 2 or 5 days in the microgravity environment, the following results were obtained: (1) The height of the ventricular zone was greater in the Flight animals than in either control group. (2) Flight animals had a greater number of nuclei per unit of ventricular surface than either control group. (3) Flight animals had a greater number of labeled nuclei per unit of ventricular surface than either control group. (4) In the flight group, the ratio of cells labeled with 3H-TdR to those labeled with BUdR in the proliferative zone in a 300-um extent along the ventricular surface was significantly greater than in either control group. (5) Differences in relative maturity and labeling characteristics suggest that the housing conditions provided by the Animal Enclosure Module (AEM) had an impact on fetal development. Although housing conditions in the AEM appear to have contributed to some of the observed differences, that contribution was insufficient to account for the significant differences observed in the flight group in the number of 3H-TdR-only labeled nuclei OR the total number of cells. These findings need to be corroborated by replication on future spaceflights.

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Effects of Microgravity on Gene Expression in the Brain

Science Discipline
Neurophysiology

Investigator	Institute
O. Pompeiano	University of Pisa, Italy

Co-Investigator(s)	Institute
d'Ascanio, P.	University of Pisa, Italy
Centini, C.	University of Pisa, Italy
Pompeiano, M.	University of Pisa, Italy

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Asynchronous Control, Simulated Flight Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Pompeiano, M.: Short-Term (Fos) and Long-Term (FRA) Protein Expression in Rat Locus Coeruleus Neurons During the Neurolab Mission: Contribution of Altered Gravitational Fields, Stress, and Other Factors. Neuroscience, vol. 115, no. 1, 2002, pp. 111–123.

Pompeiano, M.: Fos-Related Antigens are Involved in the Transcriptional Responses of Locus Coeruleus Neurons to Altered Gravitational Fields in Rats. Acta Oto-Laryngologica, supl., vol. 545, 2001, pp. 127–132.

Objectives/Hypothesis
Immediate early genes (IEGs) are useful indicators of changes in neuronal activity and plasticity. This experiment investigated whether changes in IEG expression, occurring during spaceflight, affect brain structures involved in the control of: a) somatic (postural and motor) functions; b) vegetative (cardiovascular, respiratory, and gastrointestinal) functions; and c) the regulation of the sleep-waking cycle.

Approach or Method
Adjacent sections of the same brain tissues were alternatively stained with either a Fos or a FRA antibody, to mark both short- and long-lived responses to changes in gravity stimulus. These were characterized by hypergravity followed by exposure to μG at flight day (FD) 2 after launch, stabilization to μG at FD 14, hypergravity followed by exposure to 1 G at R+1 after landing, stabilization to 1 G at R+13. Tissue was removed from the fixative, cryoprotected in 30% sucrose solutions in phosphate buffer (PB) at 4°C, frozen, and sectioned into 40-mm sections with a cryostat. Immunocytochemistry was used to detect Fos and FRA proteins in the forebrain and brainstem. The distribution of labeled cells in each brain region was examined and quantified. The number of labeled cells was compared between flight and asynchronous groups.

Results
Variable numbers of Fos- and FRA-positive cells were found during different spaceflight conditions in Medial (MVe) and Spinal Vestibular nuclei (SpVe), which contribute to the vestibulo-ocular and the vestibulo-collic reflex, and in Group F nuclei, which projects to the vestibulocerebellum. An increased level of Fos expression occurred in the noradrenergic Locus Coeruleus nucleus (LC) of flight rats with respect to ground controls, without variations at different time points of the spaceflight. However, the number of FRA-positive cells selectively increased in the LC of flight animals sacrificed at FD 2 and more prominently at R+1. An increase in Fos expression was also observed in the spinal areas of both the Inferior Olive (IO) and the Lateral Reticular nucleus (LRt) at FD 2 and FD 14, i.e., during and after the launch. However, only few labeled cells were observed in these precerebellar nuclei at R+1 and R+13. In addition, the increase in gravity force at R+1 selectively increased the number of FRA-positive cells in the dorsomedial cell column of the IO (which corresponds to the vestibular area of this structure), while no such changes affected the Fos expression.

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Microgravity and Development of Vestibular Circuits

Science Discipline
Neurophysiology
Developmental Biology

Investigator / Institute
J. Raymond — Universite de Montpellier II

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control, Simulated Flight Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Raymond, J.: Developmental Study of Rat Vestibular Neuronal Circuits During a Spaceflight of 17 Days. Journal of Gravitational Physiology, vol. 7, no. 2, pp. P55–P58.

Dememes, D.: Development of the Rat Efferent Vestibular System on the Ground and in Microgravity. Developmental Brain Research, vol. 128, no. 1, May 31, 2001, pp. 35–44.

Homick, J.L.; Delaney, P.; and Rodda, K.: Overview of the Neurolab Spacelab Mission. Acta Astronautica, vol. 42, no. 1, Jan.–Apr. 1998, pp. 69–87.

Objectives/Hypothesis
Exposure of the vertebrate inner ear to microgravity has been shown to result in an alteration of the gravity receptors of the vestibular system as well as vestibular sensory-motor rearrangement. This experiment investigated the vestibular system at the level of the gravity receptors (sensory hair cells), and the primary neurons relaying the sensory signals (the vestibular neurons) and their potential plasticity at the structural and biochemical levels.

Approach or Method
In-flight tissue samples were collected as part of the integrated neonate perfusions and dissections on flight day (FD) 8 and FD 15. Postflight samples were collected 8 hours after landing. Vestibular receptors were dissected out in cold phosphate buffer solution (PBS). For Vibratome sectioning, brainstems, ampullar cristae, and utricular and saccular maculae were separately embedded in 4% agarose in PBS and cut into 50-mm sections with a Vibratome. For cryostat sectioning, the receptors were incubated overnight at 4°C in 30% sucrose in PBS and cut into 14-mm sections. Vibratome sections were prepared for immunofluorescent microscopy by incubation with varied primary and secondary antibodies.

Results
Preliminary observations did not indicate a significant reorganization of the macular sensory organs, or their afferent and efferent connections. Expression of parvalbumin, calretinin, and calbindin were unaffected. Sensory cells and their afferent fiber distribution, as well as the distribution of synaptic proteins, remained unchanged in flight animals. This lack of effect on the organization of the maculae and ganglion may indicate that the peripheral otolithic organs and their afferent networks are less sensitive to environmental changes than the integrative structures of the brain. Another possible explanation is that the critical periods of development for these peripheral organs occur at an earlier age than postnatal day 8. Peripheral nerve processes of the efferent system appeared to develop and mature normally in flight. It is not known whether the lack of a detectable effect of microgravity is due to the parameter analyzed; ultrastructural observations should be done for clarification. Microgravity exposure did impair the development of vestibular neurons and Purkinje cell axonal branching in the vestibular nuclei. However, it is not clear if this implies delayed development or a definitive failure resulting from microgravity exposure during a critical period of development.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Multidisciplinary Studies of Neural Plasticity in Space

Science Discipline
Neurophysiology

Investigator / Institute
M.D. Ross — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Simulated Flight Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications

Homick, J.L.; Delaney, P.; and Rodda, K.: Overview of the Neurolab Spacelab Mission. Acta Astronautica, vol. 42, nos. 1–8, Jan.–Apr. 1998, pp. 69–87.

Campbell, M.R.; Williams, D.R.; Buckey, J.C. Jr.; and Kirkpatrick, A.W.: Animal Surgery During Spaceflight on the Neurolab Shuttle Mission. Aviation, Space, and Environmental Medicine, vol. 76, no. 6, June 2005, pp. 589–593.

Ross, M.D. and Varelas, J.: Synaptic Ribbon Plasticity, Ribbon Size and Potential Regulatory Mechanisms in Utricular and Saccular Maculae. Journal of Vestibular Research, vol. 15, no. 1, 2005, pp. 17–30.

Objectives/Hypothesis
Ribbon synapses in the hair cells of the inner ear transmit information to the brain about linear acceleration forces acting on the body. This experiment was designed to build on previous data collected on Spacelab Life Sciences 1 and 2 (SLS-1 and SLS-2), which showed that the ribbon synapses of gravity-sensor hair cells changed in number, kind, and distribution during spaceflight, a phenomenon known as neural plasticity. The three major points of interest in this study were: 1) whether the ribbon synapses would rapidly increase in number upon initial exposure to weightlessness; 2) whether the changes observed early in flight would remain stable throughout the flight (expecting an initial overshoot as the body compensates for the new environment); and 3) whether different parts of the gravity sensors would show different synaptic counts.

Approach or Method
Flight rats used in this study were part of the integrated adult rat dissections performed on flight day (FD) 2 and FD 14. Inner ear tissues were removed from four rats on FD 2, nine rats on FD 14, six rats 2 days postflight, and six rats 14 days postflight. In addition, tissues were taken from 10 basal controls on FD 2. Labyrinth dissection, microdissection, tissue preparation, and statistical analysis were performed as described in Ross 1993, 1994, 2000, except that the Neurolab study concentrated on the striolar macular area rather than on the posterior border. Only one of the four utricular maculae from FD 2 was in condition for transmission electron microscopy. To compensate for lost data, the saccular maculae were also examined.

Results
Although findings from the utricular maculae must be considered anecdotal due to small sample size, the analysis provided several interesting results. In summary: 1) by FD 2, an upward change in the mean number of synaptic ribbons could already be observed in macular vestibular hair cells; 2) a decline from FD 2 values occurred in both kinds of hair cells by FD 14; 3) on both FD 2 and FD 14, increments in synaptic means were prominent in Type II hair cells and were significant compared to the basal; and 4) mean values of synapses for Type II hair cells of the striolar area were lower than those found previously in SLS-2. These findings support previous evidence that changes in synaptic ribbons of utricular gravity sensor hair cells occur rapidly in weightlessness, and that the changes are more prominent in Type II hair cells. The decline seen in the striolar area by FD 14 is a new observation, suggesting a synaptic overshoot on FD 2. Notably, the number of processes on Type II hair cells of the saccule were less numerous than those observed previously in the utricular macula. Overall, the results provide the first evidence that maculae and macular location (defined by variation in otoconial distribution, hair cell morphology, and vestibular afferent wiring) are related to the range of synaptic changes observed in weightlessness. The differences in saccular versus utricular macular results indicate that the two maculae have differing functional (and integrative) responses to translational and gravitational forces acting on the systems.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Development of the Aortic Baroreflex Under Conditions of Microgravity

Science Discipline
Neurophysiology

Investigator	Institute
T. Shimizu	Fukushima Medical University School of Medicine

Co-Investigator(s)	Institute
Wago, H.	Fukushima Medical University School of Medicine
Okouchi, T.	Fukushima Medical University School of Medicine

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Shimizu, T.: Development of the Aortic Baroreflex in Microgravity. In: The Neurolab Spacelab Mission: Neuroscience Research in Space. J.C. Buckey Jr. and J.L. Homick, eds., NASA Johnson Space Center Press, Houston, Tex., NASA SP-2003-4525, May 12, 2003, pp. 151–159.

Yamasaki, M.; Shimizu, T.; Miyake, M.; Miyamoto, Y.; Katsuda, S.; O-Ishi, H.; Nagayama, T.; Waki, H.; Katahira, K.; Wago, H.; Okouchi, T.; Nagaoka, S.; and Mukai, C.: Effects of Space Flight on the Histological Characteristics of the Aortic Depressor Nerve in the Adult Rat: Electron Microscopic Analysis. Biological Sciences in Space, vol. 18, no. 2, June 2004, pp. 45–51.

Objectives/Hypothesis
This study examined the effects of microgravity on the structural and functional development of the aortic baroreflex. The experiment objectives were to observe and analyze the effect of microgravity mainly on the baroreflex responses and on the fine structure of the aortic nerves.

Approach or Method
Six 25-day-old rat neonates raised from 9 days old for 16 days in space were selected for examination on the day of landing (Recovery + 0 days = R+0), and another six neonates were reserved for experiments on 30 days postflight (R+30). Following anesthesia, blood pressure, heart rate, and aortic nerve activity were recorded, and baroreflex tests were performed. The animal body was perfused with a fixative and tissues were prepared for electron and light microscopy analysis. Reological properties of the aorta extirpated from other shared neonates on R+0 were also observed.

Results
Flight (FLT) pups had significantly lower body weights than ground controls, but there were no significant correlations between body weight and other parameters examined. Differences in blood pressure and heart rate seen at R+0 between the FLT and control groups dissipated by R+30. Each parameter of blood pressure, including mean blood pressure (MBP), systolic blood pressure, and diastolic blood pressure (DBP), was lower in the FLT group than controls at R+0, though there was no significant difference between FLT and control groups at R+30. Blood pressure in the R+30 FLT group was significantly higher than that measured on R+0. Similarly, basal heart rate (HR) at R+0 was higher in the FLT group than in the control groups. On R+30, all groups showed lower HR than at R+0. On R+0, the index of baroreceptor reflex sensitivity (delta HR% / delta MBP%) showed significant differences between the FLT and control groups, with two FLT pups having the lowest value. At R+30, there was no significant index differences between the groups. Afferent sensitivity in the aortic baroreceptor reflex was also decreased in the FLT group on R+0, and again differences were not seen between any groups at R+30. On R+0, the numbers of unmyelinated fibers and the ratio of unmyelinated to all fibers were significantly less in the FLT group than in the controls, and these differences were still observed at R+30. However, myelination of the aortic nerve on both R+0 and R+30 was quite nominal. Aortic wall tension produced by strain was significantly smaller in FLT than in both control groups on R+0. The thickness of the aortic walls in FLT pups was about 70% of those in the other groups; the amount of smooth muscle cells and fine elastin fibers were markedly reduced. Examination and analysis of various organs continues.

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Development of Vestibular Organs in Microgravity

Science Discipline
Neurophysiology
Developmental Biology

Investigator	Institute
M.L. Wiederhold	University of Texas Health Science Center

Co-Investigator(s)	Institute
Becker, W.	University of Hamburg
Bluem, V.	Ruhr University Bochum

Research Subject(s)
Biomphalaria glabrata (Snail)
Xiphophorus helleri (Swordtail fish)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Closed Equilibrated Biological Aquatic System (CEBAS)

Selected Publications
Wiederhold, M.L.; Harrison, J.L.; Parker, K.; and Nomura, H.: Otoliths Developed in Microgravity. Journal of Gravitational Physiology, vol. 72, no. 2, July 2000, pp. 39–42.

Homick, J.L.; Delaney, P.; and Rodda, K.: Overview of the Neurolab Spacelab Mission. Acta Astronautica, vol. 42, nos. 1–8, Jan.–Apr. 1998, pp. 69–87.

Objectives/Hypothesis
Many species sense gravity, and therefore linear acceleration, by way of otoliths (dense calcium carbonate crystals attached to hair cells and associated nerve fibers). This study was designed to test the hypothesis that during development the mass of the otolith is regulated to achieve a desired weight; as such, one would expect to see larger-than-normal otoliths in animals reared in microgravity.

Approach or Method
The statoconia (analogous to otoliths) of the pond snail, *Biomphalaria glabrata*, and the otoliths of the swordtail fish, *Xiphophorus helleri*, were the focus of this study. Thirty-five adult snails, four pre-mated adult female swordtail fish, and 50 to 200 juvenile swordtail fish were flown. The pregnant adult swordtail fish were selected at a stage that they would produce developing fry but not have any hatch by the end of the mission. On landing day, approximately half the juvenile snails were fixed; the other half were fixed 5 days postflight. Adult fish ovaries and juvenile fish were fixed in alcohol on landing day. Fixed snails were removed from their shell and sectioned at 1–2 mm and examined by transmission electron microscopy.

Results
Results were obtained from both the Neurolab flight (16-day) and a similar experiment of 9-days duration flown on STS-89. Total statoconia volume in the snails varied considerably within both the flight and ground-control groups. However, the difference in mean values for the two groups was significant at $p < 0.05$, with the average total volume greater in the flight group. In most of the groups of flight snails the increase in statoconia volume increased as the animals developed another 5 days on Earth. Overall, the flight-reared snails had approximately 50% more statoconia than the ground controls. This indicated that rearing in microgravity causes the supporting cells to produce more statoconia of approximately the same size as in ground-reared animals. Otoliths of the embryonic fish from STS-90 (Neurolab) exhibited a greater growth, with increased body length in the flight-reared animals, compared to ground controls. The embryos from STS-89 were much smaller that those retrieved after STS-90, because this was a shorter mission. In this case, the growth of otoliths of the ground-reared fish embryos was actually larger than that of the flight-reared animals. These results suggest a critical period, in the late embryonic stages, where rearing in microgravity produces larger otoliths.

STS-95 / VFEU

Launch Date 10/29/1998

Landing Date 11/7/1998

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Chronic Recording of Otolith Nerves in Microgravity

Science Discipline
Neurophysiology

Investigator	Institute
S. Highstein	Washington University

Co-Investigator(s)	Institute
Usui, S.	Toyohashi University of Technology

Research Subject(s)
Opsanus tau (Oyster toadfish)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Vestibular Function Experiment Unit (VFEU)

Selected Publications

Mensinger, A.F.; Anderson, D.J.; Buchko, C.J.; Johnson, M.A.; Martin, D.C.; Tresco, P.A.; Silver, R.B.; and Highstein, S.M.: Chronic Recording of Regenerating VIIIth Nerve Axons With a Sieve Electrode. Journal of Neurophysiology, vol. 83, 2000, pp. 611–615.

Boyle, R.; Mensinger, A.F.; Yoshida, K.; Usui, S.; Intravaia, A.; Tricas, T.; and Highstein, S.M.: Neural Readaptation to Earth's Gravity Following Return From Space. Journal of Neurophysiology, vol. 86, 2001, pp. 2118–2122.

Objectives/Hypothesis
The goals of this study were to record the responses of primary afferents of the otolithic organs to document otolithic organ response dynamics in normal gravity and in microgravity. Studies of these aspects of the vestibular system and its efferent control can add to our knowledge of its function and may suggest future therapies for control of Earth-bound motion sickness.

Approach or Method
Experimental difficulties precluded obtaining useful in-flight data as originally planned. However, fish were received within 8 hours of the shuttle's return to Earth and utricular nerve afferents recorded sequentially for 5 days postflight. For this, anesthetized, paralyzed toadfish underwent a small craniotomy to allow the implantation of glass micro-electrodes (2 M LiCl2) in the nerves innervating the utricle. Records of primary afferents in response to linear acceleration were taken. Position and motion of the fish were documented by linear and rotary potentiometers.

Results
Results from STS-95 were combined with those from Neurolab for a total of four experimental subjects. Control responses were obtained from three fish that did not fly. For flight subjects, the magnitude of response to an applied linear acceleration was on average three times greater than for controls within the first day postflight. By 30 hours postflight, responses had returned to normal and were statistically similar to controls. Directional selectivity appeared unaffected by exposure to microgravity. To examine for possible recording bias, all measured parameters were compared between control and postflight afferents; no statistical difference was found in the range and mean of afferent discharge rate and regularity of discharge between postflight and control fish. Thus, the reduced gravitational vector and linear acceleration in orbit apparently resulted in an up-regulation of the sensitivity of utricular afferents. More tests must be done to determine whether a specific population of afferents demonstrated increased sensitivity or whether this finding is a general feature of all classes of cells. The original plan called for the implementation and perfection of a sieve electrode to allow for continuous recording of afferents during flight. Although experimental difficulties precluded the completion of the entire original plan, the perfection and utilization of the sieve electrode was achieved.

Launch Date 12/5/2001 **Landing Date** 12/17/2001

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Development and Function of the Avian Otolith System in Normal and Altered Gravity Environments

Science Discipline
Neurophysiology

Investigator / Institute
J.D. Dickman — University of Washington, St. Louis

Co-Investigator(s) / Institute
Lysakowski, A. — University of Illinois at Chicago

Research Subject(s)
Coturnix coturnix japonica (Japanese quail egg)

Ground-Based Controls
Delayed Synchronous Control

Key Flight Hardware
Avian Development Facility (ADF)

Selected Publications
Dickman, J.D.; Lysakowski, A.; Huss, D.; and Price, S.: Vestibular receptor development during spaceflight and hypergravity. American Society for Gravitational and Space Biology Bulletin, vol. 16, 2002, p. 57.

Dickman, J.D.; Huss, D.; and Lowe, M.: Morphometry of Otoconia in the Utricle and Saccule of Developing Japanese Quail. Hearing Reseach, vol. 188, 2004, pp. 89–103.

Objectives/Hypothesis
The objective of this experiment was to determine the differences in anatomical properties of the receptor otolith organs in quails that develop in either normal gravity (1-G) or microgravity during spaceflight environments. The experiment directly tested the primary hypothesis that gravity acts as a trophic factor in the development of the otolith organs in the vestibular system. The first stage of development occurs during embryogenesis when major portions, but not all, of the vestibular gravity sensing receptors and their afferents are differentiating and forming terminal contacts. Specific morphological features of the otolith organs and their innervating afferent fibers were examined, quantified, and compared across animals raised in the different gravity environments.

Approach or Method
For the flight experiments, two gravity conditions were directly compared including microgravity and normal 1-G controls. The 1-G control environment received simultaneous exposure to spaceflight conditions along with the microgravity animals, but was provided constant 1-G centrifugation on a second carousel in the Avian Development Facility (ADF). Microgravity animals were not exposed to centrifugation, but otherwise housed identically to the 1-G control group. The flight lasted 12 days, which represents 75% of total embryonic development. Additional quails were exposed to 2-G hypergravity during 12 days of development using a specially designed egg incubator ground-based centrifuge. Anatomical characterization of developing vestibular otolith afferents, otoconia formation, and synapse formation between afferent and receptor hair cell were quantified for three groups of 12-day-developing embryonic quail (E12), including microgravity, 1-G flight, and 2-G hypergravity animals.

Results
Findings from our spaceflight experiments indicate that type I hair cells in E12 quail embryos raised in microgravity had more synaptic ribbons, but were less well developed, than 1-G spaceflight controls. Confirmation at the EM level of attenuated type I hair cell development agrees well with our innervation and light microscopic observations for delayed calyceal afferent formation in microgravity raised embryos. Interestingly, an even greater number of synaptic ribbons were observed in a limited sample of 2-G E12 embryos. Differences in the type of ribbons were also found, with elongated linear ribbons noted for type II hair cells and round clustered ribbons seen in type I hair cells. Significant questions remain regarding the emergence of synapses during embryogenesis and post-hatch development in normal 1-G animals, and the effects of different gravity exposures throughout the development continuum.

Foton / M2

Launch Date 6/20/2005 **Landing Date** 7/5/2005

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Structure and Function of the Snail Statocyst System After a 16-Day Flight on Foton-M-2

Science Discipline
Neurophysiology

Investigator	Institute
R. Boyle	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
None	

Research Subject(s)
Helix aspersa, *Helix lucorum* (Snail)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
BB boxes (provided by IMBP)

Selected Publications
Ilyin, E.A.; Tairbekov, M.G.; Vasques, M.F.; and Skidmore, M.G.: Foton-M2 Russian/US Biology Experiments, Development, Implementation, and Operations. Journal of Gravitational Physiology, vol. 13, no. 1, July 2006, pp. 177–180.

Balaban, P.M.; Malyshev, A.Y.; Zakharov, I.S.; Aseev, N.A.; Bravarenko, N.I.; Ierusalimsky, V.N.; Samarova, A.I.; Vorontzov, D.D.; Popova, Y.; and Boyle, R.: Structure and Function of the Snail Statocycst System After a 16-Day Flight on Foton-M-2. Journal of Gravitational Physiology, vol. 13, no. 1, July 2006, pp. 201–204.

Objectives/Hypothesis
The objectives were as follows: Determine the regulation of expression of the preproHPep gene (gene that is expressed in the primary statocyst receptor cells) as a consequence of spaceflight and during the readaptation to Earth's gravity. Regulation of this gene might signal how the statocyst receptor is "tuned" by the gravity vector. Specify the changes in excitability of the gravireceptors of the statocyst organ during the readaptation period following spaceflight. Investigate the potential changes in intersensory interaction between the photosensory and olfactory pathways and the statocyst receptors. Determine the mechanical and electrical excitability of the statocyst receptor during the readaptation period. Measure the changes in the internal Ca2+ concentration in the statocyst receptors in response to mechanical stimulation.

Approach or Method
This study investigated the function of the gravisensing statocyst receptor as a consequence of spaceflight in the readaptation phase after landing.

Twenty small (3–8 gm) *Helix aspersa*, and 15 large (12–18 gm) snails, *Helix lucorum*, were flown on Foton-M2. The juvenile snails were divided into two groups for pedal peptide analysis using messenger ribonucleic acid (mRNA) expression analysis, and the first half of the samplers were prepared upon receipt of the snails at 30 hours after landing. The other half were processed 12 hours later (two time periods). The adult snails were weighed, then behavioral tests were conducted to measure their timed negative gravitaxis to tilt. After the behavioral tests, an electrophysiological study was conducted to evaluate the intersensory interaction between the photo- and statoreeceptors using natural light and a tilt stimulus in the isolated central nervous system preparation, and an electrophysiology study to evaluate the firing rate modulation of the statorecptor to tilt. Conventional electrophysiological techniques were used.

Results
The behavioral "negative gravitaxis" responses of the snails to a sudden shift in orientation revealed that the flight snails in general responded faster than their control counterparts. The flight snails were faster in their response to pitch stimulation at each phase. These results suggest the existence of changes in the statocyst of the postflight snails.

A change, upregulation, in the expression of a peptide (Pedap peptide) associated with the statocyst was observed. Significant changes were also observed in the intersensory interaction between the photo- and statoreceptors, and orientation selectivity of statocyst responses. The results obtained in this simple animal model open the possibility for identifying other neurobehavioral responses and revealing subcellular processes affected by the space environment.

Foton / M3

Launch Date 9/14/2007

Landing Date 9/26/2007

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Structure and Function of the Snail Statocyst System After Flight on Foton-M-3

Science Discipline
Neurophysiology

Investigator
R. Boyle

Institute
NASA Ames Research Center (ARC)

Co-Investigator(s)
None

Institute

Research Subject(s)
Helix aspersa, *Helix lucorum* (Snail)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
BB boxes (provided by IMBP)

Selected Publications
Balaban, P.M.; Malyshev, A.Y.; Ierusalimsky, V.N.; Aseyev, N.; Korshunova, T.A.; Bravarenko, N.I.; Lemak, M.S.; Roschin, M.V.; Zakharov, I.S.; Popova, Y.; and Boyle, R.: Structure and Function of the Snail Statocyst System After Orbital Missions on Foton M-2 and M-3. Journal of Gravitational Physiology, vol. 15, no. 1. July 2008, pp. 273–276.

Balaban, P.M.; Malyshev, A.Y.; Zakharov, I.S.; Aseev, N.A.; Bravarenko, N.I.; Ierusalimsky, V.N.; Samarova, A.I.; Vorontzov, D.D.; Popova, Y.; and Boyle, R.: Structure and Function of the Snail Statocycst System After a 16-Day Flight on Foton-M-2. Journal of Gravitational Physiology, vol. 13, no. 1, July 2006, pp. 201–204.

Objectives/Hypothesis
Reflight of Foton-M2 experiment to confirm results, improve research techniques, and expand the areas of inquiry based on the Foton-M2 results. Major objective: to study the impact of unmanned orbital flight on: (1)the whole animal behavior (*Helix lucorum L.*); (ii) the statoreceptor responses to tilt in an isolated neural preparation (*Helix lucorum L.*); and iii) the differential expression of the Helix pedal peptide (HPep) and the tetrapeptide FMRFamide genes in neural structures (*Helix aspersa L.*).

Approach or Method
Experiments were performed 13–42 hours after return to Earth. Changes after a 16-day (Foton M-2) and a 12-day (Foton M-3) exposure to microgravity were studied in behavior, neural responses to adequate motion stimulation, intersensory interactions between the photosensory pathways and the statocyst receptors, and in expression of the HPeP and FMRFa genes in the primary statocyst receptor cells.

Results
In postflight snails, the latency of body reorientation to sudden 90° head-down pitch was significantly reduced in postflight snails indicating an enhanced negative gravitaxis response. Statoreceptor responses to tilt in postflight snails were independent of motion direction, in contrast to a directional preference observed in control animals. Positive relation between tilt velocity and firing rate was observed in both control and postflight snails, but the response magnitude was significantly larger in postflight snails indicating an enhanced sensitivity to acceleration. A significant increase in messenger ribonucleic acid (mRNA) expression of the gene encoding HPep, a peptide linked to ciliary beating, in statoreceptors was observed in postflight snails; no differential expression of the gene encoding FMRFamide, a possible neurotransmission modulator, was observed.

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Synaptic Plasticity in Mammalian Utricle

Science Discipline
Neurophysiology

Investigator	Institute
L. Hoffman	University of California, Los Angeles

Co-Investigator(s)	Institute
Schweizer, F.	UCLA School of Medicine
Zampighi, G.	UCLA School of Medicine

Research Subject(s)
Mus musculus (B6.SJL-Ptprca Pepcb/BoyJ mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
An understanding of adaptations to changes in the ambient gravity environment by the nervous system is critical to managing human exploration and survivability during long-term spaceflight. The goals were to expand the study of the cells of the inner ear vestibular system to a broader region of the inner ear that is devoted to sensing changes in the surrounding gravity field. The purpose was to determine whether the earlier observations could be more broadly extended to the entire gravity-sensing structure. In addition, the sites of communication between sensory cell sand neurons were investigated in far greater detail by using new technology to determine whether there are changes in the internal structure of these communication sites, which are known as synapses.

Approach or Method
Specimens were obtained from mice exposed to microgravity aboard STS-131 and from control specimens. Temporal bone specimens were obtained shortly after landing at Kennedy Space Center. These were shipped to the PI's laboratory at UCLA, where they were microdissected to remove the inner ear vestibular epithelia. They were then processed for transmission electron microscopy. Ultrastructural analyses were completed that produce estimates of synaptic ribbon density (ribbons per hair cell). Conical electron tomography was also conducted in order to obtain three-dimensional reconstructions for quantitative analyses of the pools of neurotransmitter vesicles. Additional specimens obtained from STS-131 have enabled completion of a immunohistochemical analysis of synapse density, which complements our ultrastructural findings.

Results
At the time of publication data analysis is still in progress.

STS-133 / BSP

Launch Date 2/24/2011 **Landing Date** 3/9/2011

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Inner Ear Otoconia Response to Microgravity

Science Discipline
Neurophysiology

Investigator / Institute
R. Boyle — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Simulated Flight Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Boyle, R.; Popova, Y.; and Varelas, J.: Functional and Structural Changes in Otolith Structures from Micro- to Hyper-Gravity in Vertebrates. In NASA Human Research Program Investigators' Workshop, Houston, TX, February 11-13, 2014, Abstract 3062.

Objectives/Hypothesis
Does exposure to long-duration spaceflight lead to neural structural alterations, and does this remodeling impact cognitive and functional performance? A widely considered mechanism by which the nervous system responds to a change in gravity load is a change in the weight-lending otoconia. This experiment examined the hypothesis that weightlessness over a significant period of time triggers a compensatory mechanism that leads to a constructive process of ion deposition and an increase of otoconia mass. To address this risk there was one specific aim, namely to specify the structural integrity of otoconia as a result of short- and long-duration exposures to altered gravity conditions. Results from previous microgravity experiments with mice have demonstrated alterations in topographical features of otoconia.

Approach or Method
Scanning and transmission electron microscopy and microstructural-crystallographic techniques are being utilized to evaluate the possible mechanisms of otoconia restructuring in response to gravity loading.

Results
At the time of publication data analysis is still in progress. The results are of limited importance due to the small sample size of control animals and the 1-day delay after landing as dictated by the principal study protocol. Nevertheless, they offer a reliable data point and thus are valuable. The results will be included in the overall study in a complimentary hypergravity study and the Mouse Drawer System results.

Launch Date 2/24/2011 **Landing Date** 3/9/2011

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Spaceflight Effects on the Mouse Retina: Histological, Gene Expression and Epigenetic Changes After Flight (Hindlimb Suspension (HS) as an Analog Model of Ocular Alterations Associated With Cephalad Fluid Shifts: Resveratrol as a Countermeasure)

Science Discipline
Neurophysiology

Investigator	Institute
S. Zanello	Universities Space Research Association (USRA)

Co-Investigator(s)	Institute
Boyle, R.	NASA Ames Research Center (ARC)

Research Subject(s)
Mus musculus (Mouse)
Rattus norvegicus (Rat)

Ground-Based Controls
Simulated Flight Control, Hindlimb Suspension (HS) Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Zanello, S.B.; Theriot, C.; Prospero-Ponce, C.; and Chevez-Barrios, P.: Spaceflight effects and molecular responses in the mouse eye: Observations after Shuttle Mission STS-133. Gravitational and Space Research, vol. 1, no. 1, 2013, pp. 30-46.

Objectives/Hypothesis
This project aims at testing the overall hypothesis that cephalad fluid shifts elicited by either microgravity or hindlimb suspension (HS) represent a stress factor that induces optic disc neuroanatomical changes, as well as retinal cell deterioration and loss via oxidative stress. To date, there have been limited flight animal studies aimed at studying the effects of microgravity on the retina (Tombran-Tink, Adv Exp Med Biol, 2006; Grigoryan, Adv Space Res, 2002). The study will provide data for the new Visual Impairment and Intracranial Pressure risk. The second objective is to investigate the effects of HS in the retina of rats and to identify relevant genes that are significantly modulated by the mechanical stress imparted by the fluid shifts. This is a principal step towards the validation of HS as a model for the study of ocular alterations induced by simulated microgravity.

Approach or Method
The first specific aim of this pilot project is to perform histological and gene expression analysis of retinas collected from mice flown in STS-133 and STS-135 and from their ground control counterparts. The second specific aim is to perform similar analysis in retinas from hindlimb suspended rats, focusing on pathways consistent with cellular and oxidative stress, thus validating this ground analog animal model for the study of retinal stress in microgravity. The third specific aim will explore the potential efficacy of reversatrol and EGCG as a nutritional countermeasures to mitigate the risks or retinal oxidative stress and degeneration in conditions of simulated microgravity.

Results
Upon completion of this task, we have expanded the immunohistopathologic analysis of the rodent retina as far as the effects of and responses to spaceflight observed in the eyes of mice aboard shuttle mission STS-133 and STS-135, focusing, for the first time, on molecular and cellular processes. While the results reported here represent pilot data due to the small sample size, these data provide for the first time, direct evidence suggesting that oxidative stress, neuronal damage, and mechanical injury take place in the retina and optic nerve of rodents flown in low-Earth orbit for a period under 2 weeks. Our work gives a first insight into the impact of space-associated factors on biological processes like cell death, oxidative stress, and probable mechanical injury in the rodent eye.

Moreover, comparative results obtained with two different mouse strains (albino versus pigmented mice) suggest that susceptibility to stress factors is determinant of the extent of the stressors effects. While individual variability is not regularly encountered with genetically homogenous mouse strains, we observed some individual susceptibility variation, which may be ascribed to a particular stress impact in spaceflight, part of which is stochastic in nature (radiation).

The present work also investigated the contribution of cephalad fluid shift alone in the rat retina by using the well-characterized hindlimb suspension (HS) model, and identified, for the first time, a gene expression response suggestive of mechanical stress imposed by cephalad fluid shift on the eye. In addition, an antioxidant nutritional countermeasure based on a green-tea enriched diet resulted in a decreased stress response, suggesting an association between the possible mechanical stress and oxidative stress genetic circuits.

STS-135 / BSP

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Inner Ear Otoconia Response to Microgravity

Science Discipline
Neurophysiology

Investigator / Institute
R. Boyle — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Boyle, R.; Popova, Y.; and Varelas, J.: Functional and Structural Changes in Otolith Structures from Micro- to Hyper-Gravity in Vertebrates. In NASA Human Research Program Investigators' Workshop, Houston, TX, February 11-13, 2014, Abstract 3062.

Objectives/Hypothesis
The proposed research was a ground-based study utilizing STS-133 and STS-135 mouse tissues and addressed fundamental mechanisms of neural compensation that directly affect crew health and performance during exploration missions and on return to Earth. This study sought to answer the following questions: Are there structural changes in otoconia as a result of experimental altered gravity conditions? If so, is the change due to a constructive or destructive process? And, is the process dependent on length of exposure to altered gravity loading? It was anticipated that this study would produce both a path toward quantification of a crew health and performance risk, and provide the basis for valid ground-based studies for countermeasure development.

Approach or Method
Scanning and transmission electron microscopy and microstructural-crystallographic techniques were utilized to evaluate the possible mechanisms of otoconia restructuring in response to gravity loading.

Results
At the time of publication data analysis is still in progress. The results are of limited importance due to the small sample size of control animals. Despite this limitation, the data do serve as a valuable data point. The results will be included in the overall study in a complimentary hypergravity study and the Mouse Drawer System results.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: NEUROPHYSIOLOGY

Title of Study
Spaceflight Effects on the Mouse Retina: Histological, Gene Expression and Epigenetic Changes After Flight (Hindlimb Suspension (HS) as an Analog Model of Ocular Alterations Associated With Cephalad Fluid Shifts: Resveratrol as a Countermeasure)

Science Discipline
Neurophysiology

Investigator	Institute
S. Zanello	Universities Space Research Association (USRA)

Co-Investigator(s)	Institute
Boyle, R.	NASA Ames Research Center (ARC)

Research Subject(s)
Mus musculus (Mouse)
Rattus norvegicus (Rat)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Theriot, C.A. and Zanello, S.B.: Molecular effects of spaceflight in the mouse eye after Space Shuttle Mission STS-135. Gravitational and Space Research, vol. 2, no. 1, 2014, pp. 3-24.

Objectives/Hypothesis
This project aims at testing the overall hypothesis that cephalad fluid shifts elicited by either microgravity or hindlimb suspension (HS) represent a stress factor that induces optic disc neuroanatomical changes, as well as retinal cell deterioration and loss via oxidative stress. To date, there have been limited flight animal studies aimed at studying the effects of microgravity on the retina (Tombran-Tink, Adv Exp Med Biol, 2006; Grigoryan, Adv Space Res, 2002). The study will provide data for the new Visual Impairment and Intracranial Pressure risk. The second objective is to investigate the effects of HS in the retina of rats and to identify relevant genes that are significantly modulated by the mechanical stress imparted by the fluid shifts. This is a principal step towards the validation of HS as a model for the study of ocular alterations induced by simulated microgravity.

Approach or Method
The first specific aim of this pilot project is to perform histological and gene expression analysis of retinas collected from mice flown in STS-133 and STS-135 and from their ground control counterparts. The second specific aim is to perform similar analysis in retinas from hindlimb suspended rats, focusing on pathways consistent with cellular and oxidative stress, thus validating this ground analog animal model for the study of retinal stress in microgravity. The third specific aim will explore the potential efficacy of reversatrol and EGCG as a nutritional countermeasures to mitigate the risks or retinal oxidative stress and degeneration in conditions of simulated microgravity.

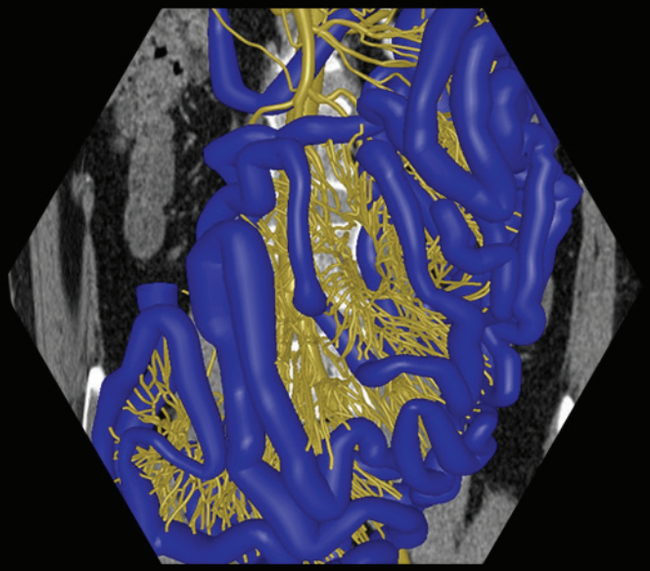
Results
Upon completion of this task, we have expanded the immunohistopathologic analysis of the rodent retina as far as the effects of and responses to spaceflight observed in the eyes of mice aboard shuttle mission STS-133 and STS-135, focusing, for the first time, on molecular and cellular processes. While the results reported here represent pilot data due to the small sample size, these data provide for the first time, direct evidence suggesting that oxidative stress, neuronal damage, and mechanical injury take place in the retina and optic nerve of rodents flown in low-Earth orbit for a period under 2 weeks. Our work gives a first insight into the impact of space-associated factors on biological processes like cell death, oxidative stress, and probable mechanical injury in the rodent eye.

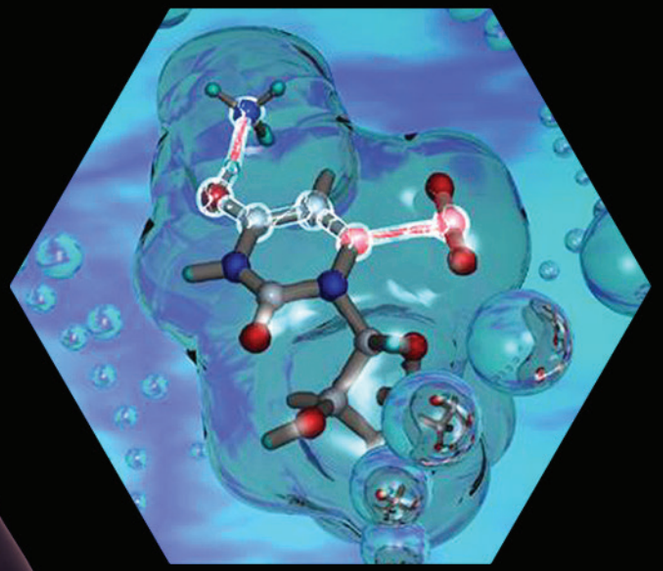
Moreover, comparative results obtained with two different mouse strains (albino versus pigmented mice) suggest that susceptibility to stress factors is determinant of the extent of the stressors effects. While individual variability is not regularly encountered with genetically homogenous mouse strains, we observed some individual susceptibility variation, which may be ascribed to a particular stress impact in spaceflight, part of which is stochastic in nature (radiation).

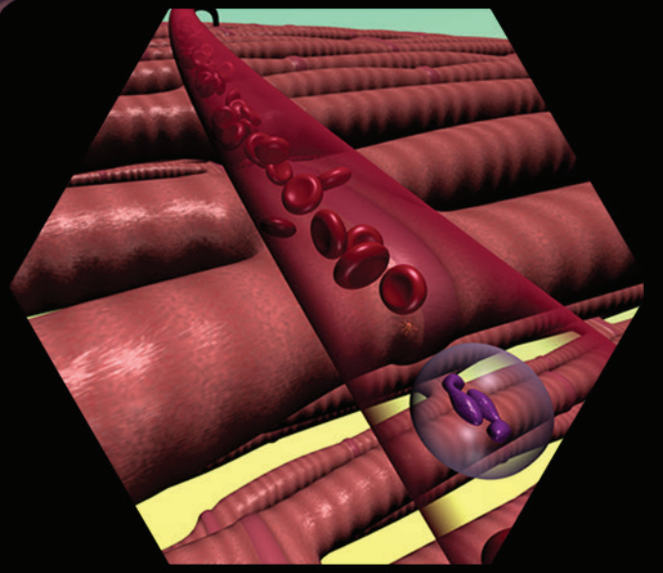
The present work also investigated the contribution of cephalad fluid shift alone in the rat retina by using the well-characterized hindlimb suspension (HS) model, and identified, for the first time, a gene expression response suggestive of mechanical stress imposed by cephalad fluid shift on the eye. In addition, an antioxidant nutritional countermeasure based on a green-tea enriched diet resulted in a decreased stress response, suggesting an association between the possible mechanical stress and oxidative stress genetic circuits.

Regulatory Physiology

Chronobiology
Endocrinology
Hematology
Metabolism & Nutrition







Regulatory Physiology Introduction

Charles Wade, Ph.D., Center for Translational Injury Research (CeTIR)
Daniel C. Holley, Ph.D., San Jose State University

Regulatory physiology focuses on the ability of living systems to maintain homeostasis. Homeostasis is the capacity of a system to regulate and maintain a relatively constant and stable internal environment by acclimating and adapting to changes in the highly variable external environment. All known life forms have evolved in the presence of a constant gravitational force. Furthermore, in some organisms the gravitational force may serve as a cue on which to base regulation. Spaceflight has afforded the opportunity to study and assess the influence of gravity on the regulatory mechanism of living systems, as well as the influence of other unique aspects of the spacecraft environment.

The research area addressed in this section focuses on systems that serve as integrators. The endocrine system is a humoral communication system with information transferred between cells via the interstitial fluid or the blood stream employing specialized substances (bioregulators) to encode the message [Hymer et al., 1996] [STS-46 / PHCF, p. 412]. The endocrine system, coupled with the nervous system, integrate cellular response to changes in the environment including the behavior of an organism, as well as regulation of metabolism, nutrition, and chronicity [Holley et al., 1991].

Over the past half century, access to spaceflight for studies of regulatory physiology has been limited, and there have been a wide range of issues to overcome, including housing of the specimens, controlling for interfacing with the spaceflight environment and flight crews, and accounting for the impact of launch and landing profiles, and subsequent recovery of the specimens for analysis [Cosmos 1887 / Bion 8, p. 399]. The studies that have been done spanned the breadth of biological specimens from single-cell organisms (slime mold) [STS-69 / BRIC-06, p. 380], to insects (beetles) [Hoban-Higgins, 2003] [NASA-Mir / NASA 5, p. 391], amphibians (newts) [Almeida et al., 2006] [Foton / M2 and M3, p. 383, 385], rodents (rats and mice), and monkeys. The ability to perform studies across species allows the unique aspects of each species to be addressed and the responses integrated into an overall hypothesis of the impact of the spaceflight environment and how the responses may translate to humans in space or on the ground.

Chronobiology

There have been numerous reports of sleep abnormalities by astronauts [Gundell et al., 1997]. Subsequent controlled studies on orbit confirm shorter sleep duration, and in some instances, changes in sleep structure, circadian phase, and amplitude [Dijk et al., 2001]. The circadian timing system (CTS) has evolved under the influence of the fluctuating lighting of the solar day to allow organisms to anticipate and prepare for fluctuations in the environment [Hoban-Higgins et al., 2003]. It is well known that the light/dark cycle is the main time-setter of the CTS. Numerous circadian studies in space have been performed on several animal species including beetles, rats, mice, and monkeys. The combined results indicate that gravity too can influence the CTS. Bion 11 flight monkeys showed phase delay in their deep body temperature (DBT) circadian rhythm, which indicates a basic alteration in the endogenous circadian oscillator of these animals [Alpatov et al., 2000] [Bion 11, p. 390]. Beetles flown on Mir and studied on the ground in hyper G (centrifuge) studies revealed gravity-induced changes in the underlying period of the endogenous circadian oscillator and a gravity-specific influence of light sensitivity of the biological clock mechanism. The monkeys on Cosmos 1514 / Bion 6, Cosmos 2044 / Bion 9, and Cosmos 2229 / Bion 10 showed possible gravity-induced internal rhythmic dissociation of rhythms believed to be controlled by separate components/pacemakers of the CTS (temperature and activity).

It was also suggested that a weakening of the coupling between the internal circadian pacemaker and the external light-dark (LD) (cycle) synchronizer had occurred in flight animals of Bion 6 / Cosmos 1514 [p. 387] [Sulzman et al., 1992]. Rats in constant conditions (light-light (LL)) on the STS-90 / Neurolab flight [p. 392] showed changes in the free running rhythms (Tau) that were different from ground controls; and flight animals had a phase delay in the body temperature rhythm. Additionally, ground based hyper-gravity studies in rats and mice confirm a gravity effect on the CTS (Fuller et al., 1994; Holley et al., 2003).

Endocrinology

Because of its key role in regulating multiple hormonal systems involved in the response to stress, metabolic homeostasis, water and electrolyte balance, and reproductive physiology, the pituitary function of rats on Cosmos 782 / Bion 3 [p. 394] was assessed. Plasma immunoassayable growth hormone (iGH) was decreased, and by inference of adrenal hypertrophy it was suggested that adrenocorticotropic hormone (ACTH) secretion was increased and indicative of a stress response. No change in melanocyte-stimulating hormone (MSH) secretion was detectable. Decreased iGH was confirmed on subsequent flights, and bioassayable GH (bGH) was shown to be 50 to 60 percent of ground controls [Grindeland et al., 2005]. It was also shown that anterior pituitary gland cells from flight animals could not support growth when transplanted into the brains of rats with their pituitary glands removed, whereas control animal anterior pituitary cells could [Hymer et al., 1991]. It was shown in rats flown on Cosmos 1887 / Bion 8 [p. 400] that both growth hormone- (GH-) releasing hormone and GH-release-inhibiting hormone (somatostatin) were decreased by spaceflight. Pituitary cells from rats flown on STS-46 and subsequently grown in cell culture showed decreased release of bGH in response to GH-releasing hormone [STS-46 / PHCF, p. 412]. In two monkeys flown on Cosmos 2229 / Bion 10, postflight plasma GH was 50 percent and 90 percent lower than that of controls, and remained depressed for up to 17 days postflight. Further support for increased secretion of the hypothalamic-pituitary-adrenal cortical axis comes from the rats flown on STS-54 / PARE.02 [p. 414]. Anterior pituitary cells (adenohypophyseal cells) were examined using immunohistochemical and genetic techniques. Corticotrophs showed ultrastructural evidence of enhanced secretory activity. The expression of proopiomelanocortin messenger ribonucleic acid (POMC mRNA), the transcript for the precursor of ACTH, was also increased. These are consistent with increased secretion of corticosterone. Posterior pituitary content of both oxytocin and vasopressin were decreased in rats flown on Cosmos 1887 / Bion 8 and Cosmos 2044 / Bion 9.

Rats on STS-90 / Neurolab [p. 382] had normal kidney vasopressin receptor number and functional ability to stimulate adenylate cyclase thus ruling out kidney vasopressin receptor mediated change in urine output seen in astronauts both during and after flight. The rats on the Neurolab flight also had depressed plasma levels of the thyroid hormone T3 with little change in T4. It was hypothesized that the difference was due to a decrease in T4 deiodinase activity. The reduction in plasma T3 in the Cosmos 2229 / Bion 10 monkeys immediately postflight was 80 percent of controls and still low (30 percent) at recovery day 3. It has been shown that astronauts on Spacelab had decreased testosterone secretion even though plasma luteinizing hormone (LH) was elevated. This was investigated in rats on Cosmos 1887 / Bion 8 and Cosmos 2044 / Bion 9. In all flight animals, plasma T was lowered and in some studies the testicular T was elevated, suggesting decreased secretion from the testes. Testes weight of flight animals was decreased, as were spermatogonial cell counts. Testosterone levels in postflight (Cosmos 2229 / Bion 10) monkeys were 50 percent of ground controls, and these values returned to normal within 3 days. Female mice flown on STS-131,

133, and 135 / BSP had reduced ovary weights and had fewer corpora lutea, and estrogen and progesterone receptor expression were both lower than ground controls. A possible mechanism for the testicular and ovarian responses is an increase in pineal melatonin secretion. It was shown in both Cosmos1887 / Bion 8 [p. 398] and Cosmos 2044 / Bion 9 [p. 404] rats that melatonin synthesis precursors 5-HIAA and 5-HT were elevated, thus inferring increased melatonin secretion. Melatonin is known to be "anti-gonadal" and may contribute to the testicular response noted in animals and astronauts (Holley et al., 1991.)

Hematology

To elucidate the consistent finding in astronauts of decreased red blood cell mass (RBCM) and plasma volume (PV), the red blood cell physiology of rats on the STS-40 / SLS-1 [p. 423-424] mission was assessed. On landing day, both the RBCM and PV, when normalized for body mass, were significantly decreased in the spaceflight animals. During an 8-day postflight observation period, iron incorporation into circulating red blood cells was diminished in the flight animals. During the first 4 days postflight, increases in reticulocyte counts were significantly smaller in the flight animals than the control animals. Fewer erythropoietin-responsive progenitor cells were recovered from the bone marrow of flight animals after landing than control rats. Serum erythropoietin (EPO) levels were the same in both groups (Udden et al., 1995). Changes in erythrocyte and lymphocyte membrane composition noted in rats on STS-58 / SLS-2 [p. 378] and the reports of increased hemolysis in rats on Cosmos 782 / Bion 3 [p. 422] may help to explain the abnormal red blood cell structure and anemia, and compromised immune function noted in astronauts. In studies using newts, tissue regeneration may be altered and the effect was tissue specific (Almeida, 2006) [Foton / M2, p. 383 and Foton / M3, p. 385]. This has significance for understanding slower wound healing reported in astronauts.

Metabolism and Nutrition

Humans after long-term duty (128–195 days) onboard the International Space Station (ISS) consumed 80 percent of their recommended energy intake, and on landing day their body weight was less than before flight [Smith, 2005]. Animal studies in space have attempted to assess the mechanisms associated with these observations. A review of the effects of spaceflight on human metabolism including protein, carbohydrate lipid and vitamin metabolism, and blood enzyme activity and red blood cell metabolism has been provided by Ushakov and Popova [1996]. The temperature and heart rate data collected from monkeys on Cosmos 1514 / Bion 6 suggested a decrease in heat loss and metabolism. This was followed up by monkey studies on two Bion flights in which double labeled water was used to measure metabolism [Bion 9 / Cosmos 2044, p. 434] [Bion 10 / Cosmos 2229, p. 438]. Overall energy expenditure was lower in spaceflight than in ground control experiments as shown by turnover of doubly labeled water in the monkeys flown on Cosmos 2044 / Bion 9 and Cosmos 2229 / Bion 10 [Stein, 1996]. However, this response was not seen in Bion 11 monkeys [p. 442] also using the doubly labeled water technique [Hoban-Higgins, 2000]. Supporting a probable decrease in metabolism was the observations that there was a general vasoconstriction and a decrease in heart rate in flight animals, suggesting a decreased metabolic rate [Hoban-Higgins, 2000]. Additionally, most of the Bion monkeys studied have shown a decrease in body temperature in flight, however, thermoregulatory ability was not compromised [Klimovitsky, 2000] [Bion 11, p. 381]. The STS-58 / SLS-2 flight rats were dissected on orbit and samples returned for analysis. This showed hypoclycemia on orbit followed by hyperglycemia on return to earth. On orbit the Krebs cycle intermediate enzyme isocitrate hydrogenase was decreased yet glycolysis and adenosine triphosphate (ATP) production were increased. Bion 10 monkeys had depressed serum Insulin Like

Growth Factor 1 (IGF-1) until day 11 postflight (in one animal; longer in the second monkey) [p. 413].

In attempting to accommodate a variety of species, extensive efforts have gone into flight and ground control habitat development and validation. Through these efforts, though not specifically included in the flight experiment description provided herein, extensive knowledge was gained on the day-to-day functional activities of specimens, and the impact of the housing environment on the species. This knowledge led to the employment of not only vivarium controls, but also to control groups housed under the same habitat conditions as flight animals with similar lighting, airflow, and temperature conditions. As the experiments progressed over time, the fidelity, complexity, and sophistication of the control groups have increased (e.g. STS-90 / Neurolab).

To access space frequently, studies have necessarily been conducted in a range of spacecraft with varying environmental conditions, as well as launch profiles, flight durations, and landing and recovery conditions. In addition, experiments have been conducted under a wide range of periods of exposure to post-landing reexposure to the gravity of Earth. These periods have been as short as 4 hours after landing and up to 2 days postflight to assess the acute effects of spaceflight. This variance makes cross-comparison and analysis of these experiments challenging. Given the constraints of the spaceflight conditions, investigators have adapted their experiments to take advantage of the rare flight opportunities, and maximize scientific returns and advancement of our knowledge of the effects of spaceflight on complex biological systems/organisms.

Scientific investigation is a reiterative process. A major issue in spaceflight experiments in regulatory physiology has therefore been the inability to systematically address findings and issues raised in the initial flight experiment in subsequent experiments conducted under identical conditions. This challenge includes both constraints on the opportunity of repeated spaceflight experiments and the employment of sufficient ground-based experiments conducted post flight to address and expand on flight observation. While interesting findings have been identified as noted above, many need to be confirmed. Thus, when considering the findings of regulatory physiology space flight experiments, the influence of the specific flight environment, other than the gravity effects, must be considered [Merrill et al., 1992] [Grindeland et al., 1990].

The present cadre of experiments represents the first steps in elucidating the effect of spaceflight on the maintenance of homeostasis of living systems. Valuable lessons have been learned about the conducting experiments in the spaceflight environment that will refine and focus research activities. The present studies provide a baseline and direction for future studies in specific disciplines with the ultimate goal of understanding mechanisms resulting in the negative effects noted in astronauts and development of countermeasures.

List of referenced flight experiments:

Cosmos 782 / Bion 3, R.E. Grindeland, Effects of Spaceflight on Plasma and Glandular Concentrations of Pituitary Hormones

Cosmos 782 / Bion 3, H. Leon, Alterations in Erythrocyte Survival Parameters in Rats After 19.5 Days Aboard Cosmos 782

Cosmos 1514 / Bion 6, Frank Sulzman, Synchronization of Primate Circadian Rhythms in Space

Cosmos 1887 / Bion 8(a), D. Holley, Pineal Physiology in Microgravity: Relation to Rat Gonadal Function

Cosmos 1887 / Bion 8(b), L. Keil, The Effect of Spaceflight on Pituitary Oxytocin and Vasopressin Content of Rats

Cosmos 1887 / Bion 8, Richard E. Grindeland, Growth Hormone Regulation, Synthesis, and Secretion in Microgravity: I. Somatotroph Physiology

Cosmos 2044 / Bion 9, C.A. Fuller, Biological Rhythm and Temperature Regulation: I. Biological Rhythms and Temperature Regulation

Cosmos 2044 / Bion 9, D. Holley, Pineal Physiology in Microgravity: Relation to Rat Gonadal Function

Cosmos 2229 / Bion 10, C.A. Fuller, Circadian Rhythms and Temperature Regulation in Rhesus Monkeys During Spaceflight

Cosmos 2229 / Bion 10, Richard E. Grindeland, Plasma Hormone Concentration in Rhesus Monkeys After Spaceflight

NASA-Mir / NASA 5, Tana Hoban-Higgins, Effects of Gravity on Insect Circadian Rhythm

Bion 11, Charles A. Fuller and Alexei M. Alpatov, Circadian Rhythms of Macaca mulatta during Space Flight

Bion 11, Charles A. Fuller, T. Peter Stein, and V. I. Korolkov, Energy Metabolism of Macaca mulatta during Space Flight

Foton / M2, E. Almeida, Spaceflight Effects on Regeneration in Lower Vertebrates: Molecular-Biology and Cytochemistry Examinations

Foton / M3, E. Almeida, Spaceflight Effects on Regeneration in Lower Vertebrates: Molecular-Biology and Cytochemistry Examinations

STS-40 / SLS-1, R.D. Lange, Regulation of Erythropoiesis During Spaceflight

STS-40 / SLS-1, C.P. Alfrey, Regulation of Blood Volume During Spaceflight

STS-46 / PHCF, Wesley C. Hymer, Microgravity-Induced Effects on Pituitary Growth Hormone Cell Function (PHCF): A Mechanism for Muscle Atrophy in Manned Space Flight

STS-54 / PARE.02, A. Mortimer, Effects of Spaceflight on Morphology of the Rat Adenohypophysis

STS-58 / SLS-2, S.M. Ivanova, Cellular Homeostasis in Microgravity: Energy and Structure

STS-69 / BRIC-06, I. Block, Cellular Signal Perception and Signal Transduction

STS-90 / Neurolab, C.A. Fuller, CNS Control of Rhythms and Homeostasis During Space Flight

STS-90 / Neurolab, C. Wade, Reduction in Density and Expression of Vasopressin Receptors in the Kidneys of Rats Following Spaceflight

Literature references:

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Alpatov, A.M.; Hoban-Higgins, T.M.; Klimovitsky, V.Y.; Tumurova, E.G.; and Fuller, C.A.: Circadian Rhythms in Macaca mulatta Monkeys During Bion 11 Flight. J. Gravitational Physiology, vol. 7, no. 1, 2000, pp. S119–123.

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Fuller, C.A.; Hoban-Higgins, T.M.; Griffin, D.W.; and Murakami, D.M.: Influence of Gravity on the Circadian Timing System. Advances in Space Research, vol. 14, 1994, pp. 399–408.

Grindeland, R.E.; Iyin, E.A.; Holley, D.C.; and Skidmore, M.G.: International Collaboration on Russian Spacecraft and the Case for Free Flyer Biosatellites. Experimentation With Animal Models in Space. G. Sonnenfeld (Ed.), 2005, Elsevier B.V., DOI: 10.1016/S1569-2574(05)10003-3.

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Klimovitsky, V.Y.; Alpatov, A.M.; Hoban-Higgins, T.M.; Utekhina, E.S.; and Fuller, C.A.: Thermal Regulation in Macaca mulatta during Space Flight. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S149-S152.

Merrill, A.H.; Wang, E.; LaRocque, R.; Mullins, R.E.; Morgan, E.T.; Hargrove, J.L.; Bonkovsky, H.L.; and Popova, I.A.: Differences in Glycogen, Lipids, and Enzymes in Livers From Rats Flown on Cosmos 2044. J. Applied Physiology, suppl., vol. 73, no. 2, 1992, pp. 142S–147S.

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Sulzman, F.M.; Ferraro, J.S.; Fuller, C.A.; Moore-Ede, M.C.; Klimovitsky, V.; Magedov, V.; and Alpatov, A.M.: Thermoregulatory Responses of Rhesus Monkeys During Spaceflight. Physiology & Behavior, vol. 51, no. 2, Mar. 1992, pp. 585–591.

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Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: REGULATORY PHYSIOLOGY

Title of Study
Tissue Fluid-Electrolyte Composition

Science Discipline
Regulatory Physiology

Investigator	Institute
Y.V. Natochin	Institute of Evolutionary Physiology and Biochemistry

Co-Investigator(s)	Institute
Serova, L.V.	Institute of Biomedical Problems
Snetkova, E.V.	Institute of Biomedical Problems
Shakhmatova, E.I.	Sechenov Institute
Lavrova, E.A.	Sechenov Institute

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Serova, L.V.; Natochin, Y.V.; Keil, L.; Shakhmatova, E.I.; Lavrova, E.A.; Snetkova, E.V.; and Ivanova, S.Y.: Weightlessness Effect on Water and Electrolytes in the Animal Body. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, p. 36.

Objectives/Hypothesis
Calcium loss that occurs in a prolonged spaceflight, negative calcium balance, and continuous hypercalcemia have been serious problems related to long-duration space missions. The purpose of this study was to accumulate new data about mechanisms of changes in fluid-electrolyte metabolism of mammals during spaceflight.

Approach or Method
The samples studied included: right lobe of the liver, left kidney, heart from apex of ventricles, ventral skin, skeletal muscle from the right hamstring, and right humerus bone without marrow. Contents of water, sodium, potassium, calcium, magnesium, copper, zinc, and manganese were measured. Samples were weighed, put into quartz tubes, and dried at 105°C to reach a constant weight in order to evaluate water content. The dried samples were ashed by concentrated nitric acid. Sodium and potassium were measured by means of a propane-air mixture; calcium and magnesium were measured in an atomic absorption spectrophotometer.

Results
Results indicate that fluid-electrolyte homeostasis of animal tissues remained stable immediately and 9 days after return to the Earth. The differences between the flight and control animals were insignificant, and the changes detected were probably caused by water and electrolyte redistribution between various tissues and organs. A decrease in water and sodium content of the skin, as well as a decrease in the water, sodium, and potassium content of the heart was observed. No changes were observed in other tissues. The changes observed in SLS-1 rats were very close to those seen after a 7-day Cosmos-1667 flight and greater than those reported after 14-day Cosmos flights, reflecting what may be an acute stage of adaptation.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY

Title of Study
Cellular Homeostasis in Microgravity: Energy and Structure

Science Discipline
Regulatory Physiology

Investigator	Institute
S.M. Ivanova	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Ross, M.D. and Tomko, D.L.: Effect of Gravity on Vestibular Neural Development. Brain Research Review, vol. 28, nos. 1–2, 1998, pp. 44–51.

Objectives/Hypothesis
Analysis of previously reported biochemical parameters of spaceflight have shown that an animal's metabolic status is greatly affected when it undergoes a transition from 0 to 1 g. The importance of this study was the opportunity to examine biosamples isolated and fixed in space. This allows a better understanding of metabolic balance in microgravity and its qualitative and quantitative changes upon return to the Earth's gravitational field. For this purpose, enzyme activities were measured in plasma as well as subcellular fractions, and isolated by differential centrifugation of liver homogenates.

Approach or Method
Samples were taken from three groups of rats decapitated on flight day 13, 4 hours after landing or 14 days after landing. The liver was removed and a mitochondrial supernatant was prepared and analyzed using commercial Boeringer mannheim test kits. Blood samples were taken and studies were performed on isolated eryrocytes. Metabolic parameters were measured using a spectrophotometer. The results were calculated per one gram hemoglobin, measured by means of the cyan methemoglobin procedure. Membrane lipids and phospholipids were determined by thin-layer chromatography. Mitochondrial enzymes were evaluated by quantitative cytochemistry.

Results
Glucose and isocitrate dehydrogenase (ICDH) levels were decreased while glycolysis and adenosine triphosphate (ATP) synthesis were increased in flight rats. Immediately after recovery, hypoglycemia was relpaced with hyperglycemia. Some enzymes—aspartate transaminase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK)—returned to preflight levels, while alkaline phosphatase went down and acid phosphatase went up. Nitrogen metabolism changes occurred during flight and persisted immediately after flight, leading to a higher level of creatine in the blood and lower activity in the Krebs cycle. Study of activity of hepatic subcellular fractions gives evidence that the recovery of metabolic balance in blood challenges biochemical processes in the liver: at R+14 aminotransferases in the cytoplasm are in the hypercompensation state. Changes in basic metabolic parameters in erythrocytes and lymphocytes were evidently produced by changes in the structure and function of their membranes. This is shown by lipid and phospholipid composition of membranes.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY

Title of Study
Kidneys and Fluid-Electrolyte Homeostasis

Science Discipline
Regulatory physiology

Investigator	Institute
L.V. Serova	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Natochin, Y.V.	Sechenov Institute of Evolutionary Physiology and Biochemistry
Shakhmatova, E.I.	Sechenov Institute of Evolutionary Physiology and Biochemistry

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Serova, L.V.; Natochin, Y.V.; and Shakhmatova, E.I.: Kidneys and Fluid-Electrolyte Homeostasis. SLS-2 Final Report, 1996.

Objectives/Hypothesis
Previous studies have examined the effects of spaceflight on the water, sodium, potassium, calcium, and magnesium concentrations in the skin, bone, liver, kidneys, heart, and reproductive organs. However, the specific effects of microgravity could be masked by the effects of readaptation to the Earth's environment. The purpose of this experiment was to study the kidney and fluid-electrolyte homeostasis of rats dissected in flight.

Approach or Method
The kidney was isolated into its components (medulla, papillae, and cortex) and dried for water measurement. The dried samples were ashed using nitric acid at 80°C. Sodium and potassium levels were measured using a photometer. Calcium and magnesium were measured using an atomic absorption spectrophotometer. The results were analyzed using Student's t-test.

Results
Data analysis was difficult due to significant variations within the controls, however, the following results were obtained. Rats dissected in flight (IF) had water and sodium contents in the renal component identical to the controls. Potassium content in the medulla and cortex were identical, while in the papillae, it was lower in flight rats. Calcium was decreased in the cortex, and magnesium was decreased in the cortex and papillae. Rats dissected immediately postflight (R+0) showed a decrease in water content in the cortex and papillae. The concentrations of electrolytes were identical to those of the controls. Rats dissected 14 days postflight (R+14) had water and electrolyte concentration unchanged in the medulla and papillae, while water and electrolyte concentrations in the cortex increased.

STS-69 / BRIC-06

Launch Date 9/7/1995 **Landing Date** 9/18/1995

Physiological/Science Discipline: REGULATORY PHYSIOLOGY

Title of Study
Cellular Signal Perception and Signal Transduction

Science Discipline
Regulatory Physiology

Investigator	Institute
I. Block	Deutschen Zentrum für Luft- und Raumfahrt (DLR)

Co-Investigator(s)	Institute
Hemmersbach, R.	Deutschen Zentrum für Luft- und Raumfahrt (DLR)
Sauer, H.W.	Texas A&M University

Research Subject(s)
Physarum polycephalum (acellular slime mold)

Ground-Based Controls
24-Hour Asynchronous Ground Control using the Orbiter Environment Simulator (OES)

Key Flight Hardware
BRIC-60 (2), GN_2 Freezer

Selected Publications

Block, I.; Ivanova, K.; Wolke, A.; Rabien, H.; and Briegleb, W.: Graviperception and Signal Transduction in Physarum. Sixth European Symposium on Life Sciences Research in Space, June 16–20, 1996, Trondheim, Norway, European Space Agency SP-390, 1996, pp. 199–202.

Block, I.; Rabien, H.; and Ivanova, K.: Involvement of the Second Messenger cAMP in Gravity-Signal Transduction in Physarum. Thirty-First COSPAR Scientific Assembly, July 14–21, 1996, Birmingham, United Kingdom, Advances in Space Research, vol. 21, nos. 8–9, 1998, pp. 1311–1314.

Objectives/Hypothesis
Cellular signal processing in all organisms is probably based on fundamentally similar mechanisms. The stimulus interacts with a primary receptor in order to initiate a response, mediated by signal transduction pathways. In the case of gravity as a stimulus, it has been shown that free-living single eukaryotic cells, like slime mold, often use this vector for their spatial orientation (gravitaxis) and, in addition, shows distinct gravisensitivities. This experiment used slime mold cells to locate the gravireceptor, and determine the interaction between signal perception and the response of the cell (signal transduction and processing).

Approach or Method
To investigate the acceleration-stimulus signal transduction chain a gravisensitive *Myxomycete*, *Physarum polycephalum* (acellular slime mold) was used. With its ameboid locomotion it represents one of the two major types of cellular motility (the other major type being the microtubule-based locomotion). The plasmodia, giant cells, display a distinct gravitaxis, and their intrinsic rhythmic contraction activity and cytoplasmic streaming are modulated by gravity. To determine the gravity influence on cell function acceleration deprivation (i.e., near weightless “0g” in space) was used. Thirty-six petri plates (18 in each BRIC-60 canister) were flown on the Shuttle for 11 days. Petri plates were removed from the BRIC and frozen in the GN2 freezer at two different points in the flight, nine petri plates after 2 days in space and after 3 days in space. The remaining 18 petri plates were returned to Earth and frozen after recovery. These samples were then analyzed and compared with the ground control.

Results
Previous flight studies under variable gravity found that a plasmodia immersed in microgravity was still able to respond to acceleration changes, which proved that the gravity response in Physarum is based directly on gravity. This gravity response is relayed via parts of the cell that have a higher density than the rest of the system, such as the nuclei or the mitochondria. This BRIC-06 study indicates that the acceleration-stimulus signal transduction may use a second messenger pathway involving cAMP.

Bion 11

Launch Date 12/24/1996

Landing Date 1/7/1997

Physiological/Science Discipline: REGULATORY PHYSIOLOGY

Title of Study
Thermoregulation in Macaca mulatta during Space Flight

Science Discipline
Thermoregulation

Investigator	Institute
C.A. Fuller	University of California, Davis
V.Y. Klimovitsky	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Alpatov, A.M.	Institute of Biomedical Problems
Demaria-Pesce, V.H.	College de France

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Klimovitsky, V.Y.; Alpatov, A.M.; Hoban-Higgins, T.M.; Utekhina, E. S.; and Fuller, C.A.: Thermal Regulation in Macaca mulatta during Space Flight. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S149-S152.

Objectives/Hypothesis
Non-thermal effects of microgravity on the body, such as gravitational and postural unloading of skeletal muscles, changes in muscle structure and function, headward blood shift, and changes in fluid and electrolyte homeostasis, may themselves cause thermal changes in the body. These thermal effects, such as changes in heat production, transfer, and release, may cause variations in body temperature and thermoregulatory disorders. This experiment examined the effects of microgravity on deep body temperature and skin temperature.

Approach or Method
A thermistor implanted in the brain measured deep body temperature. Thermistors attached to the leg, ankle, and head measured skin temperature. Ambient temperature in the capsule was also measured. All data were recorded every 10 seconds. Available flight monkeys were used for control studies at 17 and 45 days postflight.

Results
Data were pooled across six Bion missions, giving a total of 12 subjects. In eight out of 12 animals, a gradual decrease in mean deep body temperature (DBT) of 0.3-0.8 °C was observed. DBT reached a minimum on flight days 4-8, then began to increase. Values of skin temperature at the head and leg were not significantly different in space and on the ground. Out of the three skin temperatures measured, only ankle temperature suggested that the skin region participates in physiologically controlled heat release as it usually varied inversely to DBT. An unusually high increase in ankle skin temperature versus DBT was seen at night during space flight: this may be due to blood redistribution in microgravity. Overall, the pooled data shows that the system of thermal regulation is adequate to support the normal range of DBT, even during high ambient temperatures.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: REGULATORY PHYSIOLOGY

Title of Study
Reduction in Density and Expression of Vasopressin Receptors in the Kidneys of Rats Following Spaceflight

Science Discipline
Regulatory Physiology

Investigator	Institute
C. Wade	NASA Ames Research Center (ARC)
D. Brooks	SmithKline Beecham Pharmaceuticals

Co-Investigator(s)	Institute
None	

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Brooks, D.P.; Nambi, P.; Laping, N.J.; Olson, B.A.; Pullen, M.; and Wade, C.E.: Renal Vasopressin Receptor Expression and Function in Rats Following Spaceflight. Journal of Applied Physiology, vol. 88, no. 4, Apr. 2000, pp. 1316–1320.

Objectives/Hypothesis
A number of studies have demonstrated that renal function is altered during and immediately following spaceflight. It has been suggested there is a decreased renal responsiveness to vasopressin following spaceflight and that this may be the mechanism for the increased urine flow that is observed following return to normal gravity. This experiment measured vasopressin receptor expression and activity in kidneys taken from rats 1 and 14 days following spaceflight of 15-days duration.

Approach or Method
Twelve male Fischer 344 rats were individually housed in the Research Animal Holding Facility (RAHF) during spaceflight. Ground controls consisted of rats similarly kept in the RAHF and another 12 rats maintained in standard vivarium cages. Flight animals were killed within 5 hours of landing and real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) was used to determine messenger ribonucleic acid (mRNA) levels for the vasopressin V2 and V1a receptors. The proportion of V2 and V1a receptors was determined by measuring 3H-labeled arginine vasopressin binding to kidney membranes.

Results
Measurements of renal vasopressin V2 and V1a receptor mRNA expression by quantitative RT-PCR demonstrated little difference at either 1 day or at 14 days following return from space. Evaluation of 3 H-labeled arginine vasopressin binding to membranes prepared from kidneys indicated that the majority of the vasopressin receptors were V2 receptors. Furthermore, the data suggested that binding to vasopressin V2 or V1a receptors was unaltered at 1 day and 14 days following spaceflight. Similarly, the ability of vasopressin to stimulate adenylate cyclase suggested no change in vasopressin V2 receptor activity in these animals. These data suggest that, whatever changes in fluid and electrolyte metabolism are observed following spaceflight, they are not mediated by changes in vasopressin receptor number or vasopressin-induced stimulation of adenylate cyclase.

Foton / M2

Launch Date 6/20/2005

Landing Date 7/5/2005

Physiological/Science Discipline: REGULATORY PHYSIOLOGY

Title of Study
Spaceflight Effects on Regeneration in Lower Vertebrates: Molecular-Biology and Cytochemistry Examinations

Science Discipline
Regulatory Physiology

Investigator / Institute
E. Almeida — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Pleurodeles waltl (Newt)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Russian "Triton" spaceflight habitat

Selected Publications

Almeida, E.A.C.; Roden, C.; Phillips, J.A.; Yusuf, R.; Globus, R.K.; Searby, N.; Vercoutere, W.; Morey-Holton, E.; Tairbekov, M.; Grigoryan, N.; Domaratskaya, E.; Poplinskaya, V.; and Mitashov, V.: Analysis of Cell Proliferation in Newt (*Pleurodeles Waltl*) Tissue Regeneration During Spaceflight in Foton M-2. Journal of Gravitational Physiology, vol. 13, no. 1, July 2006, pp. 185–188.

Grigoryan, E.; Almeida, E.; Domaratskaya, E.; Poplinskaya, V.; Aleinikova, K.; Tairbekov, M.; and Mitashov, V.: Experiment "Regeneration" Performed Aboard the Russian Spacecraft Foton M-2 in 2005. Journal of Gravitational Physiology, vol. 13, no. 1, July 2006, pp. 189–192.

Objectives/Hypothesis
The objective of this experiment was to identify molecular-biological mechanisms of spaceflight-stimulating effects on cell proliferation and tissue regeneration in newts *Pleurodeles waltl*.

Approach or Method
The experimental procedure involved the administration of the immunodetectable nucleotide analog bromedeoxyuridine (BrdU) in the water available in the triton habitat, followed by recovery of available tissue samples for measurement of nucleotide incorporation in specific cell types. Delivery of BrdU was performed using a miniature osmotic pump programmed to start delivery after the animals reach orbit. Programming was achieved by layering the BrdU containing solution with a layer of inert oil that delayed drug delivery for as long as necessary for preflight animal loading into the launch vehicle. Overall proliferation, morphology and gene expression in flight tissues compared to 1-g controls, as well as in cells that express stem cell markers specific to tissues of interest, were measured.

Results
Cells in spaceflight tail regenerates proliferated at a slightly slower rate and were more undifferentiated than those in ground synchronous controls. In addition, the size of regenerating tails from spaceflight was smaller than synchronous controls. However, onboard temperature recordings show that the temperature in the spaceflight was about 2°C lower than ground synchronous controls, possibly explaining the observed differences. (See results for Foton-M3.)

Foton / M2

Launch Date 6/20/2005

Landing Date 7/5/2005

Physiological/Science Discipline: REGULATORY PHYSIOLOGY

Title of Study
The Effect of Microgravity on the Morphology and Function of the Nervous System, Skeleton, and Endocrine Organs of the Gecko

Science Discipline
Musculoskeletal

Investigator / Institute
E. Almeida — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Pachydactylus turneri (Gecko)

Ground-Based Controls
Basal Control, 48-Hour Delayed Synchronous Control

Key Flight Hardware
Gecko housing unit

Selected Publications
Ilyin, E.A.; Tairbekov, M.G.; Vasques, M.F.; and Skidmore, M.G.: Foton-M2 Russian/US Biology Experiments, Development, Implementation, and Operations. Journal of Gravitational Physiology, vol. 13, no. 1, July 2006, pp. 177–180.

Almeida, E.A.C.; Roden, C.; Phillips, J.A.; Yusuf, R.; Globus, R.; Searby, N.; Vercoutere, W.; Morey-Holton, E.; Gulimova, V.; Saveliev, S.; Tairbekov, M.; Iwaniec, U.T.; McNamra, A.J.; and Turner, R.: Development of the Gecko (Pachydactylus Turneri) Animal Model During Goton M-2 to Study Comparative Effects of Microgravity in Terrestrial and Aquatic Organisms. Journal of Gravitational Physiology, vol. 13, no. 1, July 2006, pp. 193–196.

Objectives/Hypothesis
The objective of the experiment was to perform histological examinations of the central nervous system, peripheral organs of senses (visual, auditory, vestibular, olfactory and vomero-nasal systems), musculo-skeletal system (bones, tendons, ligaments), and endocrine and reproductive systems of geckos in order to detect cell growth and morphological tissue changes.

Approach or Method
The gecko *Pachydactylus turneri* was used as a spaceflight model organism due to its robustness in adapting to long periods of fasting and xeric conditions with limited water, and its ability to adhere to wall surfaces in spacefight habitat. The animals are poikilothermic, have tissue regenerative ability, and are adapted to moderate periods of fasting and can survive prolonged periods without water. One male and four females were housed in each Gecko housing unit. One unit with 5 animals was flown. A second unit with 5 animals was used as a basal control, and a third unit with 5 geckos served as a 48-hour delayed control. Approximately 30 hours after the 16-day flight, without food or water, the geckos were recovered, examined, and euthanized for histological and other analyses.

Results
The Geckos showed no apparent negative health effects. Detailed analysis of bone mass and architecture showed that both synchronous controls and spaceflight animals lost significant amounts of cancellous bone in the distal femur and humerus. In addition cell cycle analysis of 30-hour postflight liver tissue revealed a shift of DNA content from G2 and S to G1, both in spaceflight and synchronous controls. These results suggest that housing conditions alone induce rapid catabolism of cancellous bone and reduced normal tissue regeneration. (See results for Geckos on Foton-M3.)

Foton / M3

Launch Date 9/14/2007

Landing Date 9/26/2007

Physiological/Science Discipline: Regulatory Physiology

Title of Study
Spaceflight Effects on Regeneration in Lower Vertebrates: Molecular-Biology and Cytochemistry Examinations

Science Discipline
Hematology

Investigator / Institute
E. Almeida — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Pleurodeles waltl (Newt)

Ground-Based Controls
Synchronous Control, 48-Hour Delayed Control

Key Flight Hardware
BB boxes (provided by IMBP)

Selected Publications
Domaratskaya, E.; Almeida, E.A.C.; Butorina, N.N.; Nikonova, T.M.; Grigoryan, E.N.; and Poplinskaya, V.A.: Spaceflight Effects on the Hematopoietic Tissue of Ribbed Newts. Journal of Gravitational Physiology, vol. 15, no. 1, July 2008, pp. 281–284.

Grigoryan, E.N.; Poplinskaya, V.A.; Domaratskaya, E.; Novikova, Y.P.; Aleinikova, K.S.; Dvorochkin, N.; and Almeida, E.A.C.: Tissue Regeneration in Urodela on Foton-M3. Journal of Gravitational Physiology, vol. 15, no. 1, July 2008, pp. 277–280.

Objectives/Hypothesis
Tissue regeneration in Urodeles is an important and well-established experimental model to study the effects of spaceflight factors, such as microgravity and radiation, on the long-term viability of biological organisms in space. Spaceflight experiments with the newt Pleurodeles waltl, aboard Foton-M3 in 2007, used new technical approaches to validate experiments on tissue regeneration in space. In these experiments newt lens, limb, and tail regeneration were investigated with respect to cell proliferation, apoptosis, morphology, and growth factor regulation. Results from space-flown newts were compared with a synchronous (1 g), and two aquarium controls. Progress of tissue regeneration was evaluated by comparison to basal controls operated 10 days prior to launch. Lens regeneration in space-flown animals was more uniform than synchronous controls and about 0.5 to 1 stage more advanced. In both groups, lens regeneration was accompanied by the growth of the retinal radial glia cell population and by an increase of stress protein (HSP90) expression. In the growth zone of regenerating eyes, expression of FGFβ, a key cytokine in lens regeneration, increased simultaneously with cell mitotic activity in the lens regenerate.

Approach or Method
A total of 16 newts were flown in 2 separate habitats for 12 days on Foton-M3 in 2007. Prior to the flight, all the animals underwent surgery to remove their lenses, toe, and tail tips for regeneration studies.

During flight (F) and synchronous control (SC) experiments video recording, temperature, and radiation monitoring, as well as BrdU, nucleotide analog delivery, to detect cell proliferation occurring in space, were performed. Post-flight differential blood counts and histological analysis of the liver were conducted. In blood, neutrophils, eosinophils, basophils, lymphocytes, and monocytes were counted.

Results
Tissue expression HSP90 and FGFβ were higher in space-flown animals than in synchronous controls. Forelimb toe regeneration proceeded to a comparable early stage of wound healing in both space-flown and synchronous control animals. At this stage no specific signs of toe formation could be detected. Tail regeneration in space-flown and synchronous control animals reached regeneration stage IV to V. Some tail size parameters, such as area, were also unchanged, while others, such as length, showed a decrease in spaceflight. In contrast, remarkable changes in tail tip morphology were found between synchronous, flight, and neutrally buoyant aquaria animals. Computer morphometric analysis showed that flight and aquarium-control tail regenerates were identical in shape, while synchronous controls developed distinct dorso-ventral asymmetry. These results, together with findings from earlier flights, suggest that tissue regeneration in Urodeles exposed to spaceflight is altered in a tissue-specific manner and can result in abnormally fast or slow regenerative tissue growth and differentiation.

Biosatellite III

Launch Date 6/28/1969 **Landing Date** 7/7/1696

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Chronobiology

Title of Study
Circadian Rhythms of the Pig-Tailed Monkey in Biosatellite III

Science Discipline
Chronobiology

Investigator	Institute
W.R. Adey	University of California, Los Angeles

Co-Investigator(s)	Institute
Hahn, P.M.	University of California, Los Angeles
Hoshizaki, T.	University of California, Los Angeles

Research Subject(s)
Macaca nemestrina (Pig-Tailed Monkey)

Ground-Based Controls
Laboratory (Flight Backup Subjects), Flight Simulated (to 30 days)

Key Flight Hardware
Primate Life Support System; Primate Physiological Sensors

Selected Publications

Hahn, P.M.: Circadian Rhythms of the Macaca nemestrina Monkey in Biosatellite III. BIOSPEX: Biological Space Experiments, NASA TM -58217, 1979, p. 109.

Hahn, P.M.; Hoshizaki, T.; and Adey, W.R.: Circadian Rhythms of the Macaca nemestrina Monkey in Biosatellite III. Aerospace Medicine, vol. 42, 1971, pp. 295-304.

Hoshizaki, T.; Hahn, P.M.;, and Adey, W.R.: Circadian Rhythms and Sleep/Wake Activity in the Biosatellite Monkey. Physiologist, vol. 16, 1973, pp. 202-208.

Objectives/Hypothesis
The rhythmicity of activity levels, metabolism, excretion rates, thermoregulation, and cardiovascular measures persist in terrestrial laboratory conditions where environmental factors such as light, temperature, and humidity are kept in con- stant and unvarying conditions. It is believed that if these circadian processes become arrhythmic or desynchronized a deterioration of the organism can result. As the possibility of desynchronosis of the circadian rhythm and its consequences in the space environment is of great concern, this experiment was designed to study the effect of weightlessness on circadian rhythms.

Approach or Method
A variety of parameters measured inflight were analyzed and compared to simi- larly maintained ground-control subjects in order to determine if desynchronosis occurred. Telemetry included implanted sensors for EEG, EMG, ECG, and respiration, vascular catheters to monitor venous and arterial pressures, temperature sensors in the brain, and general environmental parameters. Computer programs and plotting techniques were used to estimate periodicity. Due to the rapid changes in parameters recorded during the last thirty hours, only 7.5 cycles of 24-hour rhythms were used in analysis from the 8.8-day flight. Day averaging was the most common method: data obtained during the flight were interpolated to fixed 1.5-hour intervals; an average for a four-day period was obtained; and deviations were plotted to give the parameter a cyclic representation. Time displacement of two such tracings was an indication of an altered circadian rhythm.

Results
All physiological sensors functioned well throughout the flight, and the subject displayed a define desynchronosis in some physiological processes. The pCO2, brain and body temperatures and heart rate were well correlated and indicated a rhythm of greater than 25 hours; however arterial blood pressure remained at 24 hours. Such internal desynchronization of temperature, cardiac, and respiratory cycles from the blood pressure and the external desynchronization from the imposed 24-hour daily routine may have been detrimental to the well-being of the flight subject. The derangement of the cardiovascular system suggested as a concomitant of space flight, and the desynchronization found in the flight subject, may well have acted together to bring about its rapid deterioration. There was no evidence of this desynchronosis in any ground controls, including Biosatellite simulations lasting up to thirty days. This suggests the existence of a gravity dependent mechanism in the control of circadian rhythm.

Cosmos 1514 / Bion 6

Launch Date 12/14/1983 **Landing Date** 12/19/1983

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Chronobiology

Title of Study
Synchronization of Primate Circadian Rhythms in Space

Science Discipline
Chronobiology

Investigator	Institute
F. Sulzman	State University of New York

Co-Investigator(s)	Institute
Fuller, C.A.	University of California, Riverside
Moore-Ede, M.C.	Harvard Medical School
Klimovitsky, V.Y.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Primate-BIOS, Circadian Rhythm Experiment Hardware, Cosmos 1514 Russian Hardware Suite

Selected Publications
Sulzman, F.M.; Ferraro, J.S.; Fuller, C.A.; Moore-Ede, M.C.; Klimovitsky, V.; Magedov, V.; and Alpatov, A.M.: Thermoregulatory Responses of Rhesus Monkey During Spaceflight. Physiology and Behavior, vol. 51, no. 3, 1992, pp. 585–591.

Sulzman, F.M.; Fuller, C.A.; Moore-Ede, M.C.; Klimovitsky, V.; Magedov, V.; and Alpatov, A.M.: Synchronization of Primate Circadian Rhythms in Space. Final Reports of U.S. Monkey and Rat Experiments Flown on the Soviet Satellite Cosmos 1514. R.C. Mains and E.W. Gomersall, eds., NASA TM-88223, 1986, pp. 71–102.

Objectives/Hypothesis
In natural conditions, mammalian circadian rhythms are normally externally and internally synchronized. Whereas many researchers feel that the circadian timekeeping system is endogenous, others argue that the timing system is exogenous, with an organism perceiving daily timing signals such as slight variations in gravity associated with the rotation of the Earth. A test of this hypothesis would be to determine if circadian rhythms persist outside of the Earth's environment.

Approach or Method
Activity was monitored via a U.S.-developed sensor attached to the monkey's restraint jacket, and totaled over 16-minute intervals and fed into a data collection unit. Auxiliary temperature was monitored with a Soviet biotelemetry system; transmitter output was recorded at 16-minute intervals on another data collection unit. Ankle skin temperature was measured by thermistors also developed by the U.S. and recorded on the digital data collection unit. Data were analyzed by computer and plotted digitally. To evaluate the underlying repetitive circadian characteristics of the data, a waveform reduction was performed.

Results
The most significant differences from pre- and postflight values were detected in auxiliary and skin temperatures. However, it is clear that the circadian rhythms of activity and auxiliary temperature persist in space; there may be changes in mean level and amplitude, but as expected, rhythmicity is still quite evident. Ankle skin temperature displayed very little day/night variations. The auxiliary temperature of monkeys in space appears to be maintained at an average of 0.5°C and 1.0°C a lower level than on the ground. The ankle skin temperature is also low in space, and maintained just above ambient temperature. This probably reflects a decrease in cutaneous blood flow in space. The results of the period analyzed also suggest that entrainment to the light-dark cycle is not as strong in space as it is on Earth. It is possible that the non-24-hour cycle could reflect a changing waveform in space or an alteration in the phase relationship to the light cycle, or could indicate some sort of stress induced desynchronization.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Chronobiology

Title of Study
Biological Rhythm and Temperature Regulation: I. Biological Rhythms and Temperature Regulation

Science Discipline
Chronobiology

Investigator	Institute
C.A. Fuller	University of California, Davis

Co-Investigator(s)	Institute
Alpatov, A.M.	Institute of Biomedical Problems
Klimovitsky, V.Y.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Primate-BIOS

Selected Publications
Sulzman, F.M.; Ferraro, J.S.; Fuller, C.A.; Moore-Ede, M.C.; Klimovitsky, V.; Magedov, V.; and Alpatov, A.M.: Thermoregulatory Responses of Rhesus Monkey During Spaceflight. Physiology and Behavior, vol. 51, no. 3, 1992, pp. 585–591.

Objectives/Hypothesis
This study examined the influence of microgravity on temperature regulation and circadian timekeeping systems in two Rhesus monkeys. Animals were exposed to 14 days of microgravity while constantly monitoring the circadian patterns, temperature regulation, heart rate, and activity. This experiment extended results from Cosmos 1514, as well as providing insights into the physiological mechanisms that produce these changes.

Approach or Method
Four monkeys (3.5–4.0 kg), two serving as ground controls, were implanted with auxiliary temperature/electrocardiogram (ECG) transmitters. The ECG was hardwired; the cathode ray tube (CR/T) equipment received pulses corresponding to R-waves that were sensed and converted to heart rate within the CR/T signal processor. Motor activity rhythms were monitored via piezoelectric sensors attached to the monkey's restraint jacket. Skin sensors recorded temperatures from the ankle, proximal leg, and head. Ambient temperatures at the top and bottom of the primate restraint system were recorded. Data were collected at 5-minute intervals and stored on a battery operated logger. Experiment data were transferred to a microcomputer for waveform and period analysis, and storage.

Results
It is clear that circadian rhythms in the various parameters studied persisted in the subjects. However, data indicate that the microgravity environment has a significant influence on both temperature regulation and circadian timing. In general, there is a tendency for a reduction in skin temperatures, and a possible reduction in metabolism and a concomitant reduction in deep body temperature. The circadian timing system appears to be more liable in the microgravity environment, even in the presence of a 24-hour light/dark cycle. Activity-related rhythms appear to be maintained with a 24-hour period, while thermoregulatory rhythms are more variable in the periodic responses and tend to show a greater variability in phase. Observations suggest that the two or more central pacemakers, which compose the circadian timing system, show differential responses to the microgravity environment. Heart rate and motor activity evidence appropriate 24-hour rhythms in a 24-hour light/dark cycle, while the pacemaker for body temperature does not show such stability.

Cosmos 2229 / Bion 10

Launch Date 12/29/1992 **Landing Date** 1/10/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Chronobiology

Title of Study
Circadian Rhythms and Temperature Regulation in Rhesus Monkeys During Spaceflight

Science Discipline
Chronobiology

Investigator	Institute
C.A. Fuller	University of California, Davis

Co-Investigator(s)	Institute
Hoban-Higgins, T.M.	University of California, Davis
Griffin, D.W.	University of California, Davis
Klimovitsky, V.Y.	Institute of Biomedical Problems
Alpatov, A. M.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications

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. Journal of Applied Physiology, vol. 81, no. 1, 1996, pp. 188–193.

Fuller, C.A.; Hoban-Higgins, T.M.; Griffin, D.W.; and Murakami, D.M.: Influence of Gravity on the Circadian Timing System. Advances in Space Research, vol. 14, no. 8, 1994, pp. 399–408.

Fuller, C.A.: The Effects of Gravity on the Circadian Timing System. Journal of Gravitational Physiology, vol. 1, 1994, pp. P1–P4.

Objectives/Hypothesis
Living organisms have evolved under the unvarying level of Earth's gravity. Physiological and behavioral responses to changes in gravity are not completely understood. Exposure to altered gravitational environments has profound effects on physiological and behavioral systems, including body temperature regulation and circadian rhythms. One objective of this study was to examine the influence of microgravity on temperature regulation and circadian timekeeping systems in Rhesus monkeys. Another objective was to find insights into the physiological mechanisms that produce these changes.

Approach or Method
Two male Rhesus monkeys were used in the experiment. The animals were studied in a 3–5 day baseline control experiment verifying all procedures and collecting baseline data prior to the flight of the biosatellite. The animals were flown for 11 days and 16 hours, and subsequently studied in a 3-day postflight experiment that began 13 days after flight. Six weeks after recovery, a second, longer control study was performed. In all studies, monkeys were housed in a 24-hour light/dark cycle. The lights were on for 16 hours and off for 8 hours. The atmosphere in flight was maintained at sea level partial pressure and barometric pressure. The following parameters were measured: brain temperature, axillary temperature, head skin temperature, ankle skin temperature, heart rate, motor activity, and ambient temperature at the upper portion of the chair. Brain temperature measurements were recorded at 1-minute intervals. All other measurements were recorded at 10-minute intervals.

Results
Circadian rhythms persisted in both subjects during preflight, in flight, and postflight. The phase of the brain temperature (Tbr) rhythm was delayed in flight compared to the control while the amplitude and mean Tbr were similar. The phase of the axillary temperature (Tax) rhythm was delayed during flight. The Tax rhythm amplitude was larger during flight than control; there was no difference in mean Tax between flight and controls. The mean heart rate (HR) decreased in flight, compared to controls. The amplitude of the HR rhythm was also lower in flight. The phase of the activity rhythm was also later in flight than in the postflight control study.

Bion 11

Launch Date 12/24/1996 **Landing Date** 1/7/1997

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Chronobiology

Title of Study
Circadian Rhythms of *Macaca mulatta* during Space Flight

Science Discipline
Chronobiology

Investigator	Institute
A.M. Alpatov	Institute of Biomedical Problems
C.A. Fuller	University of California, Davis

Co-Investigator(s)	Institute
Hoban-Higgins, T.M.	University of California, Davis

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Alpatov, A.M.; Hoban-Higgins, T.M.; Klimovitsky, V.Y.; Tumurova, E. G.; and Fuller, C.A.: Circadian Rhythms in Macaca mulatta Monkeys during Bion 11 Flight. Journal of Gravitational Physiology, vol. 7, no.1, Jan 2000, pp. S119-S123.

Objectives/Hypothesis
The Circadian Timing System (CTS) coordinates physiological processes within an organism. The CTS ensures that activities occur at the most favorable time. Human health and performance can be adversely affected by CTS dysfunction. Research has shown that altering the gravitational force, such as during exposure to microgravity during space flight, can affect circadian rhythms. This study attempts to analyze the effects that exposure to microgravity has upon the circadian rhythms.

Approach or Method
Animals were flown on the 14-day Bion 11 mission in BIOS-Primate capsules. While on a light-dark cycle of 16 hours of light followed by 8 hours of darkness, the animals were offered food twice a day and given performance tests 4 times a day. Brain temperature, head skin temperature, ankle skin temperature, motor activity, heart rate, and ambient temperature within the capsule were all recorded at 10-second intervals and stored in a digital memory unit.

Results
Analysis of the data supports the hypothesis that the gravitational environment affects circadian rhythms. The circadian rhythm of brain temperature of flight animals showed a significant phase delay compared to the control animals. This was evident in both the later rise to day time levels as well as the delayed drop to night time levels. No free-running rhythms were detected; each animal had an average period of 24 hours. This suggests that the available time cues may have masked the changes in circadian period. The distinct peaks in head skin temperature corresponding to meals and performance tests provide additional support for this hypothesis.

Launch Date 5/15/1997 **Landing Date** 9/25/1997

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Chronobiology

Title of Study
Effects of Gravity on Insect Circadian Rhythm

Science Discipline
Chronobiology

Investigator	Institute
T.M. Hoban-Higgins	University of California, Davis

Co-Investigator(s)	Institute
Alpatov, A.M.	Institute of Biomedical Problems

Research Subject(s)
Trigonoscelis gigas (Black bodied beetle)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Beetle Kit

Selected Publications
Hoban-Higgins, T.M.; Alpatov, A.M.; Wassmer, G.T.; Rietveld, W.J.; and Fuller, C.A.: Gravity and Light Effects on the Circadian Clock of a Desert Beetle, Trigonoscelis Gigas. Journal of Insect Physiology, vol. 49, no. 7, July 2003, pp. 671–675.

Hoban-Higgins, T.M.: Gravitational Biology on Mir. Journal of Gravitational Physiology, vol. 5, no. 1, July 1998, pp. P181–P184.

Alpatov, A.M.; Hoban-Higgins, T.M.; Fuller, C.A.; Lazarev, A.O.; Rietveld, W.J.; Tschernyshev, V.B.; Tumurova, E.G.; Wassmer, G.; and Zotov, V.A.: Effects of Microgravity on Circadian Rhythms in Insects. Journal of Gravitational Physiology, vol. 5, no. 1, July 1998, pp. P1–P4.

Objectives/Hypothesis
Previous studies have demonstrated that microgravity affects the circadian timing system (CTS), but scientists do not understand exactly how or why these changes take place. The response of the CTS to prolonged spaceflight is of prime interest to scientists because alterations in the CTS could affect astronaut performance. This experiment was designed to study how a simple organism, in this case the black-bodied beetle (*Trigonoscelis gigas Reitter*), responds to changes in time cues that influence the CTS in microgravity.

Approach or Method
Individual beetles were housed in beetle activity monitors (BAMs) that recorded their movement on solid-state data loggers. Two beetle kits (BKs) containing 32 BAMs each were flown on the mission. The BK1 experiment studied the ability of a light pulse to phase shift the beetles' CTS at different times of day while the BK2 experiment examined the effect of light intensity on the period of the clock. To collect baseline data, all subjects were first exposed to 12 hours of light followed by 12 hours of darkness (LD 12:12). For the BK1 experiment, 10 days of LD 12:12 were followed by 17 days of constant darkness (DD). On the 7th day of constant darkness, a 6-hour light pulse was given. Half the animals received the light pulse early in their active period and half were pulsed late in their active period. This 27-day protocol was repeated for the duration of the mission with the timing of the light pulse switched between groups. For the BK2 experiment, 20 days of LD 12:12 was followed by 20 days of constant conditions. Half the animals were placed in DD and half in constant light (LL). This 40-day protocol was repeated for the duration of the mission with animals crossed over to the opposite constant condition.

Results
Thirty-nine days after the beetle kits were activated, a Progress supply ship collided with the Mir Space Station during a docking sequence. While only the first protocol segment of the experiment was uninterrupted for both BK1 and BK2, activity data were collected for the entire 4-month mission. For BK1, analysis of the available results revealed that both phase delays and advances can be induced in a microgravity environment. It is known that light intensity affects the period of the CTS; comparison of the BK2 data with ground experiments could reveal if exposure to altered force environments also affects the period of the clock. The completed ground work showed that the period of the beetle clock was shorter in flight than in a hyperdynamic field produced via centrifugation. There were no differences in activity levels between flight and ground studies, suggesting that the ambient force environment may directly affect the clock.

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Chronobiology

Title of Study
CNS Control of Rhythms and Homeostasis During Spaceflight

Science Discipline
Chronobiology
Neurophysiology

Investigator	Institute
C.A. Fuller	University of California, Davis

Co-Investigator(s)	Institute
Hoban-Higgins, T.M.	University of California, Davis
Murakami, D.M.	University of California, Davis

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Control, Vivarium Control, Simulated Flight Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Homick, J.L.; Delaney, P.; and Rodda, K.: Overview of the Neurolab Spacelab Mission. Acta Astronautica, vol. 42, nos. 1–8, Jan.–Apr. 1998, pp. 69–87.

Campbell, M.R.; Williams, D.R.; Buckey, J.C. Jr.; and Kirkpatrick, A.W.: Animal Surgery During Spaceflight on the Neurolab Shuttle Mission. Aviation, Space, and Environmental Medicine, vol. 76, no. 6, June 2005, pp. 589–593.

Objectives/Hypothesis
These experiments examined the effects of spaceflight on the physiology of the circadian timing system (CTS) and the homeostatic control system of animals, specifically: 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.

Approach or Method
Rats were implanted with biotelemetry units to allow the collection of body temperature, heart rate, and activity data. Eating and drinking was also monitored. Rats were maintained in either a 24-hour light/dark cycle (LD); 12 hours of light (30 lux) then 12 hours of darkness, or constant light (LL). Data were analyzed to determine the circadian rhythms and daily mean of body temperature, heart rate, feeding, drinking, and activity. Subsets of rats were sacrificed on flight day (FD) 2 and FD 14 and comparable days postflight. At each time point, one group of rats received a 1-hour pulse of light during the dark cycle. Another group was not exposed to a pulse of light. The brains and eyes from both groups were then removed and sections of the hypothalamus were histochemically stained for c-Fos reactive neurons.

Results
The LL flight rats exhibited an increase ($p < 0.001$) in free-running period of body temperature and heart rate relative to controls. The periods returned to preflight values after landing. The LL flight animals maintained internal phase angle relationships between rhythms compared with controls. The LD flight rats remained entrained to the LD cycle; however, they evidenced a pronounced phase delay in body temperature, suggesting an increase in period, compared to controls. The LD flight rats also demonstrated a decrease in body temperature and a change in the daily waveform compared to controls. Both the LD and LL flight rats and controls exhibited an increase in heart rate, suggesting a possible caging effect. Finally, the FD 2 flight animals demonstrated a reduced sensitivity to light as evidenced by highly attenuated c-Fos immunoreactivity in the suprachiasmatic nucleus (SCN) compared to controls. The sensitivity to light of the flight animals returned to preflight and control levels by FD 14. These findings demonstrate that microgravity affects the circadian clock, including the clock's ability to maintain temporal organization and to properly entrain to an external LD cycle.

Biosatellite III

Launch Date 6/28/1969 **Landing Date** 7/7/1696

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Urine Excretion Rates of Calcium, Creatine, and Creatinine in the Test Monkeys and Flight Monkey Used for Biosatellite III

Science Discipline
Regulatory Physiology

Investigator	Institute
N. Pace	University of California, Berkeley

Co-Investigator(s)	Institute
Grunbaum, B.W.	University of California, Berkeley
Rahlmann, D.F.	University of California, Berkeley
Smith, G.D.	University of California, Berkeley
Kiepert, D.W.	University of California, Berkeley
Rho, J.H.	Jet Propulsion Laboratory (JPL)
Spaeth, E.A.	Jet Propulsion Laboratory (JPL)

Research Subject(s)
Macaca nemestrina (Pig-Tailed Monkey)

Ground-Based Controls
Laboratory (Flight Backup Subjects)

Key Flight Hardware
Primate Life Support System; Urine Analyzer

Selected Publications
Pace, N. et al.: Urine Excretion Rates of Calcium, Creatine, and Creatinine in Test Monkeys and Flight Monkey Used for NASA Biosatellite III. NASA CR-114425, 1971.

Objectives/Hypothesis
Among other Biosatellite III objectives, the urine analyses, together with appro- priate analysis of feces and a knowledge of quantity and composition of food, were to permit computation of the calcium balance of the animal in weightless- ness, thereby allowing assessment of the degree of possible skeletal demineral- ization. Also, measurement of the excretion rate of creatinine and creatine was expected to shed some light on the question of whether or not significant disuse atrophy of the musculature occurs as a consequence of space flight.

Approach or Method
The flight Urine Analyzer included a case which contained a urine sample accumulator, a calcium analyzer, a creatinine-creatine analyzer, reagent storage bags, logic sequencers, a data handling system and a power converter. Once every six hours during flight urine sample aliquots were analyzed and telemetered to the ground and correlated with laboratory animals. Collections for flight and control animals began seventeen days preflight, and continued through flight termination. Bladder and vascular catheters were surgically implanted thirteen, twelve, and eight days preflight.

Results
Three of the flight candidate animals, including the flight animal, experienced a profound hypocalciuria in association with the preflight surgery, which was tran- siently reversed but then recurred ten to fourteen days after initial occurrence. This obscured any possible effects of weightlessness on urine calcium excretion rate in the flight animal. On a more positive side, the development of a fully automated urine analyzer which permitted continuous measurement of these three substrates (calcium, creatinine, and creatine) during the flight ranks as an out- standing accomplishment. Other results suggest that while anorexia occurred in the flight monkey, there was no evidence of diuresis, and that the urine excretion rate of creatinine is depressed in monkey and man, in the weightless state.

Cosmos 782 / Bion 3

Launch Date 11/25/1975 **Landing Date** 12/15/1975

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Effects of Spaceflight on Plasma and Glandular Concentrations of Pituitary Hormones

Science Discipline
Endocrinology

Investigator	Institute
R.E. Grindeland	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Keil, L.C.	NASA Ames Research Center (ARC)
Parlow, A.F.	University of California, Los Angeles
Ellis, S.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Grindeland, R.E.: Space Weightlessness and Hormonal Changes in Human Subjects and Experimental Animals. Space Gerontology, vol. 6, 1982, pp. 55–57.

Keil, L.C.; and Severs, W.B.: Reduction in Plasma Vasopressin Levels of Dehydrated Rats Following Acute Stress. Endocrinology, vol. 100, 1977, pp. 30–38.

Objectives/Hypothesis
The important role played by hormones in regulation of metabolic parameters affected by spaceflight suggests that altered endocrine function may contribute to other observed metabolic changes. The aim of this study was to clarify the effects of spaceflight on pituitary function by measuring plasma and glandular concentrations of pituitary hormones and, where feasible, evaluating the status of the appropriate target tissue.

Approach or Method
At the conclusion of the flight, all rats were weighed and inspected. Anterior and posterior-intermediate lobes were collected, weighed, and individually frozen for assays. Plasma and pituitary growth hormone (GH), and prolactin were immunoassayed using GH and prolactin purified in-house as standards. Thyrotropin was assayed by a double antibody method, and luteinizing and follicle stimulating hormones by procedures employing National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD) rat standards. Adrenocorticotropic hormone (ACTH) was immunoassayed using the Third International Standard (porcine). Vasopressin was measured by a radioimmunoassay procedure and melanocyte stimulating hormone (MSH) by the bioassay method.

Results
Although of modest extent, some perturbations of endocrine function were found in this investigation. The low plasma GH titers of flight animals either reflects the unusual sampling conditions at recovery or the effects of weightlessness. The larger adrenals of flight rats suggests they were secreting increased quantities of corticosterone over a significant part of the flight, which may have contributed to the smaller flight body weights by its protein catabolic effect. The adrenal hypertrophy of flight rats suggests these animals were secreting more ACTH and corticosterone during flight. There were no changes in either pituitary or plasma MSH suggesting that either the type or degree of stress was not adequate to stimulate MSH secretion.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study

Morphometric Studies of Atrial Granules and Hepatocytes: I. Morphometric Study of the Liver

Science Discipline

Endocrinology

Investigator / Institute

L. Kraft — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute

Popova, I.A. — Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Wistar rat)

Ground-Based Controls

Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications

Kraft, L.M.; Keil, L.C.; and Popova, I.A.: Morphometric Studies of Atrial Granules and Hepatocytes: I. Morphometric Study of the Liver. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 279–290.

Objectives/Hypothesis

The goal of the present study was to characterize the vacuoles, to obtain data with which to evaluate the gross and microscopic differences, and, if possible, to explain the increased liver weight of the flight group from microscopic morphometric findings. In addition to the use of general histological techniques, morphology of the hepatocyte nuclei and intracytoplasmic vacuoles was undertaken using light microscopic computer assisted image analysis.

Approach or Method

Light microscopic computer assisted morphometry was performed with an image analysis system of the hepatocyte nuclei and intracytoplasmic vacuoles of rat liver. Nuclei in 25 fields of view were measured in stained 4-μm embedded sections. Care was taken to include representative fields from all lobular regions. These were selected at random but were included only if scanning indicated that fixation was adequate. To ascertain the relationship between the increase in liver weight of flight animals and the results of this study, an assumption was made that the specific gravity of the vacuolar contents was similar to that of the other extranuclear components of the hepatocyte.

Results

On that basis, calculations showed that the elevated vacuolar volume density in the flight group did not cause the increased liver weight in those animals, but that the non-nuclear, non-vacuolar parenchymal compartment did contribute significantly. Because only rare vacuoles were stained in all groups, severe glycogenic infiltration was regarded as the most likely cause of the pale appearance of the flight livers. Supporting this conclusion is the fact that glycogen would have been dissolved in the aqueous fluids used in processing, leaving empty spaces such as those seen in preparations. Regardless of the correctness of these assumptions, there is little doubt that changes in nuclear and vacuolar components were only minor contributors to the increased liver weight of the flight animals, while the remainder of the hepatocytic cytoplasm contributed the major portion of the increase.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Morphometric Studies of Atrial Granules and Hepatocytes: II. Atrial Granular Accumulations

Science Discipline
Endocrinology

Investigator	Institute
L. Kraft	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Keil, L.C.	NASA Ames Research Center (ARC)
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Kraft, L.M.; and Keil, L.C.: Morphometric Studies of Atrial Granules and Hepatocytes: II. Atrial Granular Accumulations. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 291–296.

Objectives/Hypothesis
Because electrolyte imbalance and fluid shifts have been experienced by humans (and animals) during spaceflight, it seemed appropriate to examine the atrial natriuretic factor (ANF) granulated regions in the atria of rats from the Cosmos 1887 flight, and to ascertain, by means of morphometric and stereological methods, if quantitative changes occurred in flight animals.

Approach or Method
Light microscopic computer assisted morphometry of the atria was performed with an image analysis system. To make measurements for the area and perimeter, an editing function was used to outline the granular regions projected on the image monitor; because the reference area did not always fill the monitored field, the same method was used to delineate the pertinent reference regions when necessary. From values for object (granular accumulation) area, object perimeter, and reference field area, the stereology program calculated volume density and mean volume of the objects. Numerical density was calculated as the number of objects per unit reference area x103.

Results
Those of the flight group had a significantly greater volume density than the synchronous or vivarium control groups, while the controls did not differ in this respect. The number of granular accumulations per unit reference area was also increased in flight animals. Mean volume on the individual granulated regions did not differ among the three groups. The increase in the flight group was therefore due to an increase in the number of granular regions rather than their size. No differences were seen between right and left atria of any groups. Likely causes for the increase in volume density of the granular regions in the flight group include reduced blood volume and/or body water content, but an exhaustive attempt to interpret these findings in terms of physiological importance would be speculative, especially because no chemical determinations of ANF were made.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
The Effect of Cosmos 1887 Flight on Spermatogonial Population and Testosterone Level in Rat Testes

Science Discipline
Endocrinology

Investigator	Institute
D.E. Philpott	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Kato, K.	NASA Ames Research Center (ARC)
Sapp, W.	Tuskegee University
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Philpott, D.E.; Kato, K.; Stevenson, J.; Vasques, M.; Sapp, W.; Williams, C.; Popova, I.A.; and Serova, L.V.: The Effect of Cosmos 1887 Flight on Spermatogonial Population and Testosterone Level in Rat Testes. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 345–358.

Sapp, W.J.; Philpott, D.E.; Williams, C.S.; Williams, J.W.; Kato, K.; Miquel, J.M.; and Serova, L.: Comparative study of spermatogonial survival after X-ray exposure, high let (HZE) irradiation or spaceflight. Advances in Space Research, vol. 12, Issues 2–3, 1992, pp. 179-189.

Objectives/Hypothesis
Immobilization, applied for a short or long period, is considered a form of physiological stress. Most reports indicate that stress decreases testosterone levels, but does not cause any morphological changes in the seminiferous tubules. On the other hand, irradiation, depending on the dosage, can result in the depletion of all spermatogonial cells except a few stem cells, while testosterone levels do not seem to be affected either in serum or intratesticular tissue. This experiment was to further understand the effects of spaceflight on the testes.

Approach or Method
The left and right testes provided material for weight determination, testosterone assay, and spermatogonial cell loss quantification. Two-micron cross sections were cut on an ultramicrotome. Alternate sections containing maturation stage six were used to count surviving spermatogonial cells. Testosterone measurements were made on the rat plasma samples.

Results
When the mean weights of the flight testes, normalized for weight of 100 g, were compared to the vivarium controls, they were 6.7% lighter; although the difference was not significant. Counts of spermatogonial cells from five animals in each group revealed a 4% decrease in flight compared to vivarium controls. In both cases the t-test significance was $0 < 0.02$. The serum testosterone levels of all animals (flight, synchronous, and vivarium) were significantly below the basal controls. Stress-related gonadal dysfunction and possible galactic radiation exposure, along with other possible factors, apparently contribute to the significant decrease in spermatogonial cell numbers observed in rats flown in space.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Pineal Physiology in Microgravity: Relation to Rat Gonadal Function

Science Discipline
Endocrinology

Investigator	Institute
D. Holley	San Jose State University

Co-Investigator(s)	Institute
Soliman, M.R.I.	Florida A&M University
Markley, C.	San Jose State University
Krasnov, I.B.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Holley, D.; Soliman, M.R.I.; Kaddis, F.; Markley, C.; and Krasnov, I.: Pineal Physiology in Microgravity: Relation to Rat Gonadal Function. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 371–385.

Objectives/Hypothesis
It is now known that the pineal organ can interact with many endocrine and nonendocrine tissues in a regulatory fashion. In view of the fact that the pineal is an important link to the environment, it is conceivable that exposure to microgravity and spaceflight might alter the function of this gland and, in turn, affect the various physiological functions including the circadian timing system. Given the link between exposure to microgravity and perturbation of calcium metabolism, and the fact that the pineal is apparently one of the only "soft tissues" to calcify, this study examined pineal calcium content following spaceflight.

Approach or Method
Given its key role in the regulation of melatonin synthesis, its high concentration, and the fact that its levels may persist longer than the more rapidly changing melatonin, it was thought that serotonin might give a more accurate assessment of the effects of microgravity on pineal function. 5-hydroxyindole acetic acid (5-HIAA), a major metabolite of serotonin, was also measured. Serotonin and 5-HIAA were analyzed in the filtered homogenates by high-performance liquid chromatography (HPLC); melatonin content was determined by radioimmunoassay. Total calcium content of the pineal homogenates was determined by atomic absorption spectrophotometry using an electrothermal atomizer equipped with a carbon rod.

Results
Pineal melatonin content for individual glands showed no significant differences among the three test groups as determined by one-way analysis of variance. Serotonin and 5-HIAA content results from two animals (one flight and one synchronous) were considerably different from other values within their respective groups. If the two values are removed from the analysis, then both flight group serotonin and 5-HIAA are significantly greater than controls. The plasma concentration of 5-HIAA in all groups was below the detectable sensitivity of the HPLC machine used in the analysis, which indicates the plasma concentrations were less than 2.0 ng/ml. One-way analysis of variance of calcium determinations indicated no statistical differences among groups. In summary, it was concluded that the spaceflight resulted in a stress response as indicated by adrenal hypertrophy, that gonadal function was compromised, and that the pineal may be linked as part of the mechanisms of the responses noted.

Launch Date 9/29/1987 **Landing Date** 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
The Effect of Spaceflight on Pituitary Oxytocin and Vasopressin Content of Rats

Science Discipline
Endocrinology

Investigator	Institute
L.C. Keil	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Evans, J.	NASA Ames Research Center (ARC)
Grindeland, R.E.	NASA Ames Research Center (ARC)
Krasnov, I.B.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Keil, L.C.; Evans, J.; Grindeland, R.E.; and Krasnov, I.: The Effect of Spaceflight on Pituitary Oxytocin and Vasopressin Content of Rats. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 387–392.

Objectives/Hypothesis
Disturbances in fluid and electrolyte balance have been noted in humans exposed to spaceflight. Similar postflight fluid-electrolyte and hormone responses have been observed in rats that were exposed to microgravity. Microscopic examination of the hypothalamus and posterior pituitary gland of flight rats exposed to spaceflight shows changes indicative of increased activity (e.g., increased hormone synthesis and secretion). The purpose of this investigation was to measure levels of pituitary oxytocin (OT) and vasopressin (AVP) as possible indicators of changes in fluid-electrolyte balance during spaceflight.

Approach or Method
Pituitary levels of OT and AVP were measured in space-flown rats and ground-based controls. An aliquot of the homogenate was diluted 1:200,000 in 0.5 M phosphate assay buffer for radioimmunoassay of OT and AVP. After the hormone levels were determined, protein concentrations were measured in aliquots from each homogenate, and then calculated as a function of total protein for each posterior pituitary homogenate.

Results
Both neural lobe hormone levels were significantly reduced in the flight animals when compared to either set of controls. When expressed in terms of pituitary protein content, the results still indicate a significant reduction in pituitary OT and AVP compared to either control group by both parametric and nonparametric tests. Pituitary OT in the flight group was 33.6% lower compared to synchronous controls and 37.3% lower than vivarium controls. Pituitary AVP was 20.7% lower in the flight group compared to that in synchronous controls, and 29.2% lower than in vivarium controls. The reduced levels of pituitary OT and AVP may have resulted from the combined effects of water deprivation (during reentry) and the stress of the novel microgravity environment. The flight animals were dehydrated, and this led to a significant reduction in both pituitary OT and AVP. Results also show that pituitary OT was reduced to a greater extent than AVP, and perhaps this decrease was in response to increased stress or motion sickness encounter during flight and/or recovery.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study

Growth Hormone Regulation, Synthesis, and Secretion in Microgravity: I. Somatotroph Physiology

Science Discipline

Endocrinology

Investigator	Institute
R.E. Grindeland	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Hymer, W.C.	Pennsylvania State University
Kaplansky, A.S.	Institute of Biomedical Problems
Victorov, I.	Brain Research Institute, Moscow

Research Subject(s)

Rattus norvegicus (Wistar rat)

Ground-Based Controls

Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications

Grindeland, R.E.; Vale, W.; Hymer, W.; Sawchenko, P.; Vasques, M.; Krasnov, I.; Kaplansky, A.; Popova, I. and Victorov, I.: Growth Hormone Regulation, Synthesis, and Secretion in Microgravity: I. Somatotroph Physiology. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 419–458.

Hymer, W.C. et al.: Life Sciences, Biotechnology, and Microgravity. Aerospace Century XXI: Space Sciences, Applications, and Commercial Developments. Proceedings of the 33rd Annual AAS International Conference, Boulder, Colo., Oct. 26–29, 1986, Univelt, Inc., 1987, pp. 1333–1345

Objectives/Hypothesis

Because the pituitary growth hormone (GH) controls the activity of both muscle and bone, effects of space flight on GH cell function have received some attention. Taken together, results suggested that GH cells from flight animals had experienced a partial shutdown in hormone secretion. The overall objective of this experiment was to determine if results from the SL-3 experiment were repeatable. Additionally, this study was to extend the earlier findings in light of the longer duration of flight. Finally, the design of the methodology was modified to permit statistical analysis of the GH secretion data.

Approach or Method

The design of this study was dictated by the following considerations: five pituitary glands were available for study; ~2 x 1,000,000 cells could be prepared from each gland; and a number of structure function tests, each requiring different numbers of cells, was possible. In order to accomplish experimental goals, some cells from each gland were cultured individually while the remaining cells from each gland were then pooled with others from the same treatment groups for subsequent morphological analyses and transplantation study.

Results

The percentages of GH cells prepared from glands in the different groups, based on counts of 50,000 cells/treatment group, did not differ. However, the staining intensity of the GH cells in the flight group was two times greater than that of cells in the synchronous group. The increased intensity of specific cytoplasmic GH fluorescence was also documented by the morphological appearance of cells. These results suggest, but do not prove, that there was more GH/cell in the flight group. Cell culture data revealed that: 1) levels of secreted GH were, in the case of serumless medium, ~70% of those in serum-containing medium; 2) relative to the first 3-day culture period, levels of hormone in the synchronous group were two to three times greater during the second 3-day culture period; and 3) flight cells did not show the same corresponding increase in GH during the second 3-day period. Results support the contention that there is a secretory lesion in pituitary GH cells of flight animals.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study

Growth Hormone Regulation, Synthesis, and Secretion in Microgravity: II. Immunohistochemical Analysis of Hypothalamic Hormones

Science Discipline

Endocrinology

Investigator	Institute
C.E. Cann	The Salk Institute, La Jolla

Co-Investigator(s)	Institute
Sawchenko, P.E.	The Salk Institute, La Jolla
Krasnov, I.B.	Institute of Biomedical Problems
Grindeland, R.E.	NASA Ames Research Center (ARC)

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Synchronous Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Sawchenko, P.E.: Evidence for Differential Regulation of CRF and Vasopressin-Immunoreactivities in Parvocellular Neurosecretory and Autonomic-Related Projections of the Paraventricular Nucleus. Brain Research, vol. 437, 1987, pp. 253–263.

Sawchenko, P.E.; Swanson, L.W.; Rivier, J.; and Vale, W.W.: The Distribution of Growth Hormone-Releasing Factor (GRF)-Immunoreactivity in the Central Nervous System of the Rat: An Immunohistochemical Study Using Antisera Directed Against Rat Hypothalamic GRF. Journal of Comparative Neurology, vol. 237, no. 1, 1985, pp. 100–115.

Objectives/Hypothesis

It was originally anticipated that, for this experiment, blocks of hypothalamic tissue would be prepared for radioimmunoassay of hypophysiotropic hormones mediating somatic growth (growth hormone-releasing factor, somatostatin) and stress-related corticotropin secretion (corticotropin-releasing factor). Even within the hypothalamus, however, each is also rather broadly distributed in cell bodies and/or axons that bear no ostensible relationship to their hypophysiotropic functions. Because of this, it was decided to attempt to employ immunohistochemical methods to better localize effects of spaceflight on these neuropeptide systems.

Approach or Method

The fixation protocol employed was based on preliminary studies in which investigators attempted to maximize antigenicity and morphologic preservation in fresh frozen samples. A conventional indirect immunofluorescence method was used for staining. Complete series of sections through the hypothalamus of each member of the flight and synchronous group were incubated in primary antisera. All primary antisera were localized with an affinity purified, fluorescein-conjugated, goat anti-rabbit IgG.

Results

In summary, consistently lesser staining intensities for both somatostatin-28 and growth hormone-releasing factor were observed in flight tissues, relative to synchronous controls. No such alterations were noted in staining for arginine vasopressin and corticotropin-releasing factor. The fact that staining for both of the principles involved most directly in the central regulation of growth hormone secretion appeared to be affected somewhat selectively may suggest a specific neuroendocrine dysfunction within the central nervous system. The sub-optimal fixation protocol, and the (presumably associated) diffuse staining of fibers and terminals in the median eminence, must temper any interpretation of this data. Finally, the fact that both the stimulatory and inhibitory principles appear driven in the same direction is perplexing, and could represent a compensatory or counter-regulatory response of one system to a perturbation in the other.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Growth Hormone Regulation, Synthesis, and Secretion in Microgravity: III. Plasma Analysis

Science Discipline
Endocrinology

Investigator	Institute
R.E. Grindeland	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Popova, I.A.	Institute of Biomedical Problems
Vasques, M.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Grindeland, R.E.; Popova, I.A.; Vasques, M.; and Arnaud, S.B.: Cosmos 1887 Mission Overview: Effects of Microgravity on Rat Body and Adrenal Weights and Plasma Constituents. FASEB Journal, vol. 4, no. 1, 1990, pp. 105–109.

Objectives/Hypothesis
Plasma hormone and biochemical analyses were performed either at NASA Ames Research Center or by a clinical laboratory. Results of these tests were made available to all U.S. investigators to facilitate their evaluation of animal physiological status and interpretation of their data. For example, phosphorus, calcium, and alkaline phosphatase values are considered with bone studies; plasma proteins and albumin concentrations are discussed with the liver enzyme studies; and testosterone titers are discussed with the testes and pineal gland investigations.

Approach or Method
Trunk blood was collected after decapitation into tubes containing 50-ml ammonium heparin. Blood biochemical measurements were determined in automated analysis. Plasma immunoactive growth hormone was determined in-house by radioimmunoassay. Testosterone and corticosterone were assayed using immuno-assay kits.

Results
The increased plasma glucose concentration in flight rats appears to be a response to microgravity, but the mechanisms are uncertain. Plasma calcium was lower in flight than in vivarium or basal rats, but not different from synchronous rats, suggesting a dietary regimen or caging effect. In contrast, phosphorus concentrations were higher in flight than synchronous animals, similar to those of vivarium and less than those of basal. Alkaline phosphate values were 50% higher in the flight animals than synchronous controls, consistent with changes in bone and mineral metabolism. Plasma sodium concentrations, immunoreactive growth hormone measurements, and total protein and albumin concentrations were similar for all groups of rats. If hemoconcentration occurred in flight rats, any decrease in protein could be obscured by the loss of plasma volume. Corticosterone levels did not differ between flight and synchronous groups; flight rats had decreased levels of testosterone compared to synchronous controls, but similar concentrations as compared to vivarium rats.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Rat Testis Morphology and Physiology

Science Discipline
Endocrinology

Investigator	Institute
R. Amann	Colorado State University

Co-Investigator(s)	Institute
Serova, L.V.	Institute of Biomedical Problems
Deaver, D.R.	Pennsylvania State University
Zirkin, B.R.	Johns Hopkins University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspension Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Amann, R.P.; Deaver, D.R.; Zirkin, B.R.; Grills, G.S.; Sapp, W.J.; Veeramachaneni, D.N.; Clemens, J.W.; Banerjee, S.D.; Folmer, J.; and Gruppi, C.M.: Effects of Microgravity or Simulated Launch on Testicular Function in Rats. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S174–S185.

Deaver, D.R.; Amann, R.P.; Hammerstedt, R.H.; Ball, R.; Veeramachaneni, D.N.; and Musacchia, X.J.: Effects of Caudal Elevation on Testicular Function in Rats: Separation of Effects on Spermatogenesis and Steroidogenesis. Journal of Andrology, vol. 13, 1992, pp. 224–231.

Objectives/Hypothesis
There is seemingly contradictory evidence on the effects of spaceflight on testicular function in mammals. The reason for abnormalities of the endocrine and possible exocrine (spermatogenic) functions of the testes are unknown, but could be related to altered function of the hypothalamus or adenohypophysis, altered fluid distribution in the body, and/or restricted blood and lymph flow within the testes. In this study, histology and testicular gene expression were studied to evaluate effects of spaceflight on testicular function.

Approach or Method
Using light microscopy, testicular tissue was evaluated for normalcy of both seminiferous epithelium and interstitial tissue. In addition, 2-micrometer sections were stained with toluidine blue for enumeration of nuclei of spermatogonia and Sertoli cells. The number of homogenization resistant spermatids per gram of tissue was determined to measure the efficiency of sperm production. Other operations included Northern-blot analysis for expression of selected genes (hsp70 and hsp90), quantification of testosterone and receptors of luteinizing hormone (LH), and morphometric analysis of Leydig cells by electron microscopy.

Results
Two of five flight and three of five vivarium rats had abnormal testes before launch; their data were excluded. Diameter of seminiferous tubules and numbers of germ cells per tubule cross section were lower in flight rats; however, ratios of germ cells to each other, or to Sertoli cells, and number of homogenization resistant spermatids did not differ from control values. Neither was there an effect on normal expression of testis-specific hsp gene products, or evidence for production of stress-inducible transcripts of hsp70 or hsp90 genes. Concentration of receptors for rLH in testicular tissue, and surface densities of smooth endoplasmic reticulum and peroxisomes in Leydig cells, were similar in flight and controls animals. However, concentrations of testosterone in flight testicular tissue and peripheral blood were significantly decreased, by up to 20%.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Pineal Physiology in Microgravity, Relation to Gonadal Function

Science Discipline
Endocrinology

Investigator	Institute
D. Holley	San Jose State University

Co-Investigator(s)	Institute
Krasnov, I.B.	Institute of Biomedical Problems
Soliman, M.R.I.	Florida A&M University
Asadi, H.	San Jose State University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspension Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Holley, D.C.; Asadi, H.; Krasnov, I.; and Soliman, M.R.I.: Pineal Physiology in Microgravity: Relation to Rat Gonadal Function. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 2, J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-108802, 1994, pp. 85–99.

Objectives/Hypothesis
Because the pineal is an important link to the environment, it is conceivable that exposure to spaceflight might alter the function of this gland and, in turn, affect various physiological functions including the circadian timing system and reproduction. Given the link between microgravity exposure and perturbation of calcium metabolism, and that the pineal is apparently one of the only "soft tissues" to calcify, this study examined pineal calcium content along with serotonin metabolism.

Approach or Method
Serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA), a major metabolite of serotonin, were analyzed in filtered homogenates injected into a reverse phase column of high-pressure liquid chromatograph. The melatonin contents were determined by radioimmunoassay using "ultraspecific" melatonin antiserum. Total calcium content was determined by atomic absorption spectrophotometry using an electrothermal atomizer equipped with a carbon rod. Groups were analyzed by one-way analysis of variance and other statistical methods within a 95% confidence limit.

Results
Pineal serotonin and pineal 5-HIAA contents of flight and tail-suspended rats were significantly higher than the synchronous controls, indicating that the space environment did have an effect on pineal 5-HT content and its turnover. This would be consistent with increased melatonin secretion during the spaceflight, which may have been involved in noted antigonadal activity. The adrenal hypertrophy in flight rats as evidenced by a significant increase in relative adrenal weights and plasma corticosterone levels would indicate a chronic stress response. Past studies suggest that the deposition of calcium may be related to polypeptide secretion by the pineal; pineal calcium levels were elevated in flight and tail-suspended rats.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study

The Effect of Spaceflight on Pituitary Oxytocin and Vasopressin Content

Science Discipline

Endocrinology

Investigator	Institute
L.C. Keil	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Evans, J.	NASA Ames Research Center (ARC)
Grindeland, R.E.	NASA Ames Research Center (ARC)
Krasnov, I.B.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Vivarium Control, Synchronous Control, Tail-Suspension Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Keil, L.C.; Evans, J.; Grindeland, R.E.; and Krasnov, I.: Pituitary Oxytocin and Vasopressin Content of Rats Flown on Cosmos 2044. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S166–S168.

Objectives/Hypothesis

Disturbances in fluid and electrolyte balance have been noted in humans exposed to spaceflight, as shown by a loss of plasma volume and increased excretion of sodium and potassium. Upon return to Earth, these imbalances are quickly corrected with rehydration and increased renin-angiotensin-aldosterone activity. The purpose of this investigation was to measure levels of pituitary oxytocin (OT) and vasopressin (VP) as possible indicators of changes in fluid-electrolyte balance during flight.

Approach or Method

An aliquot of the pituitary homogenate (1 ml of 0.1 N HCL) was diluted 1:200,000 in a 0.05-m phosphate assay buffer for radioimmunoassay of OT and VP. To eliminate interassay variability, flight and control aliquots were measured within the same OT or VP radioimmunoassay. After hormone levels were determined, protein concentrations were measured by assay with bovine serum albumin as the standard. Hormone concentrations were then calculated as a function of total protein for each posterior pituitary homogenate.

Results

The VP content of flight rats was 32%, 32%, and 20% lower than synchronous, vivarium, and tail-suspended animals, respectively, indicating that the animals may have been dehydrated during flight, or, alternatively, pituitary stores of the hormones may have been reduced due to decreased synthetic activities of the magnocellular neurons. However, increased hemotocritis in flight rats also raises the possibility that blood volume was reduced, which would also be stimulus for VP secretion. Because it has been pointed out that OT may be released in response to “neurogenic” stress, whereas VP is released in response to “physical” stress, perhaps the observed decrease in pituitary OT may be attributed to a chronic neurogenic stress the rats experienced during flight in their efforts to adapt to microgravity.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study

Growth Hormone Regulation, Synthesis and Secretion in Microgravity: I. Secretion of Growth Hormone

Science Discipline

Endocrinology

Investigator	Institute
R.E. Grindeland	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Hymer, W.C.	Pennsylvania State University
Vale, W.	The Salk Institute, La Jolla
Sawchenko, P.E.	The Salk Institute, La Jolla

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Synchronous Control, Tail-Suspension Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Hymer, W.C.; Grindeland, R.E.; Krasnov, I.; Victorov, I.; Motter, K.; Mukherjee, P.; Shellenberger, K.; and Vasques, M.: Effects of Spaceflight on Rat Pituitary Cell Function. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S151–S157.

Objectives/Hypothesis

Changes in the musculoskeletal, immune, vascular, and endocrine system of the rat occur as a result of short-term spaceflight. Since pituitary growth hormone (GH) plays a role in the control of these systems, and because an earlier spaceflight (Spacelab-3) showed that GH cell function was compromised in a number of postflight tests, the Spacelab experiment was repeated and extended in two subsequent flights: Cosmos 1887 and Cosmos 2044, the latter including a tail-suspension control model.

Approach or Method

Cells were prepared from individual pituitary glands by trypsinization. Concentrations of immunoreactive GH and prolactin (PRL) in culture media and extracts were determined by established enzyme immunoassays. Immunocytochemistry for GH cells was done using a diaminobenzidine procedure; an optical analysis system was used to quantify the area of GH-specific cytoplasmic staining. Pooled cells were incubated in antisera overnight, counterstained with propidium iodide, and analyzed with a flow cytometer for determinations of cell percentages, intensity of hormone fluorescence, and cytoplasmic granularity.

Results

There was a marked reduction in flight levels of biologically active GH (bGH). The intracellular concentrations of iGH before or after the culture tended to be similar between all groups; however, intracellular concentrations of bGH in the flight and tail-suspended groups were always significantly lower than controls. In general, the levels of iGH in media of the first and second 3-day cultures were not significantly different. The results provide further insight to the hypothesis that exposure to microgravity could alter the secretory activity of a subpopulation of GH cells, perhaps mediated through changes in intracellular packaging of the hormone molecule. Data show that GH cells have not recovered their ability to secrete bGH up to 2 weeks postflight, the longest period studied thus far.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study

Growth Hormone Regulation, Synthesis and Secretion in Microgravity: II. Hypothalamic GH-Releasing Factor, Somatostatin Immunoreactivity, and mRNA Levels

Science Discipline

Endocrinology

Investigator	Institute
P.E. Sawchenko	The Salk Institute, La Jolla

Co-Investigator(s)	Institute
Vale, W.	The Salk Institute, La Jolla
Arias, C.	The Salk Institute, La Jolla
Krasnov, I.B.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls

Synchronous Control, Tail-Suspension Control

Key Flight Hardware

Cosmos Rodent-BIOS

Selected Publications

Sawchenko, P.E.; Arias, C.; Krasnov, I.; Grindeland, R.E.; and Vale, W.: Effects of Spaceflight on Hypothalamic Peptide Systems Controlling Pituitary Growth Hormone Dynamics. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S158–S165.

Objectives/Hypothesis

Immunohistochemical analysis from a previous investigation (Cosmos 1887) suggested preferential effects on hypophysiotropic principles involved in the regulation of growth hormone secretion and synthesis. To provide an additional, more penetrating analysis, this study attempted to complement immunohistochemical analysis of growth hormone-releasing factor (GRF) and somatostatin (SS) staining with quantitative, in situ assessments of messenger ribonucleic acids (mRNAs) encoding the precursors for both these hormones.

Approach or Method

Longitudinally bisected hypothalami were sectioned 20 μm thick and stained with a conventional avidin-biotin-immunoperoxidase procedure. For GRF, a polyclonal antiserum raised in rabbits against synthetic rat GRF[1-43] was used; for SS an antiserum against SS-28 was employed. For in situ hybridization, plasmid was linearized, and labeled antisense probes were generated using SP6 RNA polymerase and 35-S-UTP. Sections were dehydrated and exposed to x-ray films, later coated with a liquid autoradiographic emulsion, then finally developed. Cell and grain counting procedures were used to compare the strengths of mRNA signals on autoradiographic material.

Results

The results complement and extend analyses of the previous study, which showed roughly comparable decrements in both SS and GRF-IR in the median eminence of flight animals; although in this study, flight hypophysiotropic fibers were more severely depleted. In comparison to synchronous and tail-suspended animals, the ppGRF mRNA signal in the arcuate nucleus of flight animals was significantly reduced, while ppSS mRNA levels were not significantly altered. While this effect on indices of ppGRF mRNA levels may be representative of an influence exerted at the level of ppGRF gene transcription, alternate explanations (e.g., effects on mRNA stability) cannot be discounted.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Growth Hormone Regulation, Synthesis and Secretion in Microgravity: III. Plasma Analysis Hormone Measurements

Science Discipline
Endocrinology

Investigator	Institute
R.E. Grindeland	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Popova, I.A.	Institute of Biomedical Problems
Grossman, E.	NASA Ames Research Center (ARC)
Rudolph, I.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control, Tail-Suspension Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Merrill, A.H. Jr.; Wang, E.; Mullins, R.E.; Grindeland, R.E.; and Popova, I.A.: Analyses of Plasma for Metabolic and Hormonal Changes in Rats Flown Aboard Cosmos 2044. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S132–S135.

Objectives/Hypothesis
Plasma from spaceflight and tail-suspended animals was analyzed for a number of constituents in order to evaluate the metabolic status and endocrine function of the specimens. Plasma electrolytes, proteins, glucose nitrogenous products of metabolism, and cholesterol were analyzed, as well as various plasma hormones involved in regulation of calcium metabolism. This experiment was concerned chiefly with plasma hormone measurements.

Approach or Method
Corticosterone, thyroxine, and testosterone were measured by radioimmunoassay using kits. Prolactin and growth hormone were measured by double antibody immunoassays using hormones and antisera prepared at NASA Ames Research Center. Data were evaluated by analysis of variance.

Results
Corticosterone concentrations varied nearly 14-fold between groups, with a level of 3 μg/dl in basal rats, to values of 41 μg/dl in the flight animals, a level similar to that found for highly stressed rats under Earth laboratory conditions. Thyroxine levels were significantly lower in flight rats than in controls and were suggestively lower ($p < 0.08$) than in tail-suspended specimens. The cause of the decreased thyroxine levels is unknown; it appears not to be attributable to either dietary iodine insufficiency or to excessive metabolic demands. A deficit apparently occurs at the thyroid gland, pituitary, or hypothalamic level. Testosterone concentrations were decreased in response to spaceflight and tail suspension. The fact that tail-suspended and flight rats both showed decreases in plasma bioassayable growth hormone would argue that the decreased secretion is due to weightlessness and/or hypodynamia and not to reentry stress.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Mechanisms of Changes in the Exocrine Functions of the Pancreas

Science Discipline
Endocrinology

Investigator	Institute
K.V. Smirnov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Pechyonkina, R.A.	Institute of Biomedical Problems
Goncharova, N.P.	Institute of Biomedical Problems
Lacy, P.	Washington University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Smirnov, K.V.; Lazi, R.; Pechyonkina, R.A.; and Goncharova, N.P.: Mechanisms of Changes in the Exocrine Function of the Pancreas. Spacelab Life Sciences-1 Final Science Report, NASA TM-4706, Aug. 1995, p. 33.

Objectives/Hypothesis
Previous space experiments demonstrated a progressive increase in the acidic-peptic potential of the stomach, as well as a simultaneous decline in the function of the pancreas. The purpose of this experiment was to conduct a biochemical investigation of the exocrine compartments of the pancreas of rats after spaceflight.

Approach or Method
Amylase, lipase, and trypsinogen were measured biochemically in the pancreas of space-flown rats, and the results were compared with those from appropriate controls. Amylase activity was measured by the photocolorimetric method, and lipase activity was measured using the spectrophotocolorimetric method.

Results
Investigation of the functional status of the pancreas after spaceflight revealed complex changes of digestive enzymes. Amylolytic activity of the pancreas was still significantly increased at R+9. No marked effect was seen in the level of trypsinogen. On R+9 there was a significant fall of lipase activity. The existence of a relative pancreatic insufficiency during spaceflight requires further study. Gastrointestinal tract activity was characterized by continuity of the processes of food substance hydrolysis. The interaction of the stomach, pancreas, and small intestine during readaptation to gravity is an example of self-regulation in the distribution of enzymatic activities.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Determination of ANF Receptors and of Particulate Guanylate Cyclase from Rats Flown in Weightlessness

Science Discipline
Endocrinology

Investigator / Institute
R. Gerzer — Deutsche Forschungsanstalt für Luft und Raumfahrt

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF), Animal Enclosure Module (AEM)

Selected Publications
Heim, J.-M.; Montigel, U.; and Gerzer, R.: ANF-Sensitive Guanylyl Cyclase Activity in Rat Liver Tissues Flown on SLS-1. Proceedings of the Fifth European Symposium on Life Sciences Research in Space, Arachon, France, 1993.

Objectives/Hypothesis
The major stimulus for secretion of atrial natriuretic factor (ANF) is increased pressure in the cardiac atria. Because weightlessness induces a fluid shift from the lower to upper parts of the body, the secretion of ANF may be enhanced. The objective of the experiment was to determine possible alterations of ANF regulation in weightlessness.

Approach or Method
The responsiveness of the ANF-sensitive guanylate cyclase system was studied. Guanylyl cyclase activity was measured in the liver, and enzymatic activity was determined in response to various ANF analogs. Formed cyclic guanosine monophosphate (GMP) was measured by radioimmunoassay.

Results
The activity of ANF-sensitive guanylyl cyclase was unaltered. Stimulation with various ANF analogs showed the same pattern response for all three groups: twofold increase with ANF as well as with urodilatin, and slight increases with C-type natriatic peptide and ANF-AP 1. These patterns indicate that there is no apparent altered receptor subtype distribution during weightlessness.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Effects of Spaceflight on Anterior Pituitary Receptors

Science Discipline
Endocrinology

Investigator / Institute
R.E. Grindeland — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
None — None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Conrol, Delayed Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF), Animal Enclosure Module (AEM)

Selected Publications
Woodman, C.R.; Tipton, C.M.; Evans, J.; Linderman, J.K.; Gosselink, K.; and Grindeland, R.E.: Metabolic Responses to Head-Down Suspension in Hypophysectomized Rats. Journal of Applied Physiology, vol. 75, no. 6, Dec. 1993, pp. 2718–2726.

Hymer, W.C. and Grindeland, R. E.: The Pituitary: Aging and Spaceflown Rats. Experimental Gerontology, vol. 26, nos. 2–3, 1991, pp. 257–265.

Objectives/Hypothesis
It has been found that the secretion of growth hormone (GH) is decreased during exposure to actual or simulated microgravity. GH is secreted by somatotrophs in the anterior pituitary and is the primary regulator of growth. The release of GH is under the control of two hypothalmic peptides, stimulatory growth hormone releasing factor (GRF) and the inhibitor somatostatin (SS). GRF acts on the specific cell receptors of somatotrophs, thus activating the release of GH. Increased numbers of GH content and GH granules in somatotrophs after spaceflight suggest that the decrease in GH secretion is not due to a decrease in synthesis of GH but rather a decrease in secretion. The hypothesis of this experiment was that spaceflight causes an alteration in the number and/or affinity of GRF receptors, which accounts for the decrease in GH secretion.

Approach or Method
Pituitaries harvested from SLS-1 and stored at –70°C for 2.5 years were homogonized in a Tris buffer (pH 7.4) to a final concentration of 20 mg pituitary tissue/ml of buffer. These homogenates were then assayed using iodinated GRF (human) as the radioligand and decreasing concentrations of cold GRF (rat) as the cold competitor. Concentrations of cold GRF were 10^{-6} M, 10^{-8} M, 10^{-10} M, and 10^{-12} M. Tubes containing the homogenate, the iodinated GRF, and the cold GRF were incubated for 2 hours and immersed in ice-cold water to stop the reaction. Samples from each tube were centrifuged, and the resulting pellets were counted using a Packard Gamma counter. Assays were performed using five groups of glands from SLS-1, as well as fresh glands for comparison purposes.

Results
Total binding from glands of SLS-1 rats and glands from fresh rats were comparable suggesting total protein content was similar. However, specific binding was not seen in any of the SLS-1 rat homogenates. Assays from fresh glands showed a dose-response curve indicating binding of iodinated GRF to the pituitary receptors is specific and inhibited in a dose-dependent manner by increasing concentrations of cold GRF. The failure of flight samples to show specific binding sites suggests that the flight samples were compromised and no conclusions can be reached regarding alterations in GRF receptors due to exposure to microgravity.

STS-46 / PHCF

Launch Date 7/31/1992 **Landing Date** 8/8/1992

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Microgravity-Induced Effects on Pituitary Growth Hormone Cell Function (PHCF): A Mechanism for Muscle Atrophy in Manned Spaceflight

Science Discipline
Endocrinology

Investigator	Institute
W.C. Hymer	Pennsylvania State University

Co-Investigator(s)	Institute
Grindeland, R.E.	NASA Ames Research Center (ARC)
Morrison, D.R.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Culture Vials, Delayed Synchronous Culture Vials

Key Flight Hardware
Ambient Temperature Recorder (ATR)

Selected Publications
Hymer, W.C.; Grindeland, R.E.; and Salada, T.: Experimental Modification of Rat Pituitary Growth Hormone Cell Function During and After Spaceflight. Journal of Applied Physiology, vol. 80, no. 3, Mar. 1996, pp. 955–970.

Hymer, W.C.; Shellenberger, K.; and Grindeland, R.: Pituitary Cells in Space. Advances in Space Research, vol. 14, no. 8, 1994, pp. 61–70.

Objectives/Hypothesis
Spaceflight has been found to induce a number of changes in the structure and function of pituitary growth (GH) hormone cells. These changes may be important due to the regulatory effect pituitary GH has on other organ systems such as musculoskeletal, immune, and endocrine. This experiment flew cell cultures taken from rat pituitary glands. The objectives of this study were: 1) to establish the effect of microgravity on storage, synthesis, and secretion of GH by rat pituitary cells; 2) to study the effects of hydrocortisone and hypothatamic GH-releasing hormone (GHRH) on GH cells; 3) to determine whether these changes persist in vitro after flight; 4) to determine whether microgravity affects the molecular form of GH; and 5) to determine the effects of microgravity on the ultrastructure of the somatotroph and whether there is a difference in somatotrophs from the dorsal or ventral area of the pituitary gland.

Approach or Method
Cells were taken from the entire anterior pituitary gland or the dorsal or ventral regions of Sprague Dawley rats and dissociated into single cell suspensions 19 hours before launch. Cells were separated into the five following groups; low-density cells from the entire pituitary, high-density cells from the entire pituitary, all cell types from the entire pituitary, all cell types from the dorsal section of the gland, and all cell types from the ventral section of the gland. There were six sealed cell-containing vials for each experimental group, a total of 165 vials were flown. Three identical sets of 165 were used as ground controls. After flight, cultures were tested for their responsiveness to a synthetic GHRH. Concentration of immunoreactive GH (iGH) released from the cells into the culture media was determined by enzyme immunoassay. Concentrations of biologically active GH in the culture media and extracts were measured using bioassays. High performance liquid chromatography was used to determine the molecular weights of GH released from flight and ground control cells. Immunocytochemistry was used to identify GH cells and flow cytometry was used to study their cell morphology.

Results
Image analysis of GH cells from mixed- and high-density groups showed an increase in cytoplasmic areas of flight cells. There were no differences in area of the low-density group. Microgravity did not affect the release of iGH during flight or during the 6-day postflight period. Release of bGH was reduced in the high-density flight cells, but treatment with hydrocortisone raised the levels to that of the ground controls. Hydrocortisone had the opposite effect on low-density and mixed-density flight cells. A greater fraction of high molecular weight iGH was found in flight samples, but in general, neither microgravity or steroids had an effect on the size distribution of the hormone. Flight cells were less sensitive to GHRH than ground cells. No changes were seen in release of iGH between flight and ground-control cells of the dorsal or ventral regions, however, the release of bGH was reduced by half in ventral flight cells as compared to ventral ground-control cells. In summary, data show that changes in chemical and cellular makeup of cell cultures affect GH cells response to microgravity.

Cosmos 2229 / Bion 10

Launch Date 12/29/1992

Landing Date 1/10/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Plasma Hormone Concentration in Rhesus Monkeys After Spaceflight

Science Discipline
Endocrinology

Investigator	Institute
R.E. Grindeland	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Dotsenko, R.	Institute of Biomedical Problems
Mukku, V.R.	Genentech, Inc.
Gosselink, K.L.	NASA Ames Research Center (ARC)

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware
Bion 10 Hardware Suite

Selected Publications
Grindeland, R.E.; Mukku, V.R.; Gosselink, K.L.; and Dotsenko, R.: Plasma Hormone Concentrations in Rhesus Monkeys After Spaceflight. Final Reports of the U.S. Experiments Flown on the Russian Biosatellite Cosmos 2229. J.P. Connolly, M.G. Skidmore, and D.A. Helwig, eds., NASA TM-110439, Apr. 1997, pp. 355–371.

Objectives/Hypothesis
Previous studies have shown changes in plasma concentrations of several hormones in humans and rats after spaceflight. In order to further understand the effects of spaceflight on endocrine function, the circulating levels of growth hormone, insulin-like growth factor I (IGF-I), thyroid hormones, cortisol, and testosterone in young male Rhesus monkeys were investigated following 12.5 days in space.

Approach or Method
Cortisol, testosterone, and thyroid hormones were measured using commercially obtained radioimmunoassay (RIA) kits. Serum IGF-I was measured by RIA using recombinant human IGF-I as standard, and growth hormone was measured by an in vitro bioassay. Blood samples were obtained about 7 weeks preflight; at R+0, R+3, R+11, and R+17 (recovery from flight + days); and at similar times following a spaceflight simulation beginning 45 days after recovery from space. Hormone values for flight or simulation animals falling outside the control mean ±2SE were considered significant ($p < 0.05$).

Results
Due to limited serum sample at R+0, cortisol was not measured. At R+3 and R+11, cortisol was decreased from control but returned to control at R+17. Cortisol levels from all other sample times were similar to preflight or control values. Testosterone levels in control animals were low, as expected, in the sexually immature monkeys. At R+0, testosterone was 50% less in flight monkeys, but at all other times was similar to controls. At R+0 and R+3, thyroxine (T4) concentrations in flight animals were similar to controls. At R+11, T4 levels fell for unknown reasons. Following the R+45 simulation, control and simulation animals had 25% lower T4 levels, but control animals showed a prompt return to usual values whereas simulation animals did not. Triiodothyronine (T3) concentrations were reduced by 80% immediately after flight and by 30% 3 days later. At R+11, T3 had returned to control levels. Growth hormone levels were reduced by 50% and 92% in the two flight animals at R+0 and remained at suppressed levels at the last postflight sampling time of R+17. IGF-I levels were also reduced in both monkeys after flight and returned to normal in one monkey after 11 days but not in the other.

Launch Date 1/13/1993 **Landing Date** 1/19/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Effects of Spaceflight on Morphology of the Rat Adenohypophysis

Science Discipline
Regulatory Physiology

Investigator / Institute
A. Mortimer — Canadian Space Agency

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Delayed Synchronous Controls

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Thapar, K.; Kovacs, K.; Horvath, E.; Stefaneanu, L.; Chambers, E.; and Mortimer, A.J.: Effects of Spaceflight on Morphology of the Rat Adenohypophysis. Journal of Applied Physiology, vol. 77, no. 3, Sept. 1994, pp. 1411–1420.

Objectives/Hypothesis
Spaceflight has been shown to have a profound effect on homeostasis in both humans and rats. The endocrine system plays a significant role in homeostasis and is particularly sensitive to the effects of spaceflight. The function of the adenohypophysis may have a strong influence in these endocrine changes. Given that changes in adenohypophyseal functions may lead to endocrinological changes, the purpose of this study was to comprehensively examine the morphological response of adenohypophyseal cell types to spaceflight.

Approach or Method
Immunochemistry was performed on both flight and synchronous control rats. Antibodies for growth hormone (GH), prolactin (PRL), adrenocortropic hormone (ACTH), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), and luteinizing hormone (LH) were used. Gonadotroph and corticotroph sizes were measured, and corticotroph cells were counted. Semithin sections of pituitary tissue were stained, and the ultrastructure was examined using transmission electron microscopy. Cellular distribution of messenger ribonucleic acids (mRNAs) for GH, PRL, and proopiomelanocortin (POMC) was determined by in situ hybridization.

Results
Significant enlargement of corticotrophs was seen in flight rats. Nuclear and cytoplasmic size increased in equal magnitudes. Corticotrophs showed ultrastructural evidence of enhanced secretory activity. The expression of POMC mRNA, the transcript for the precursor of ACTH, was also increased. Gonadotrophs also exhibited a significant increase in size, but did not show ultrastructural evidence of enhanced secretory activity. No changes were seen in the overall cell type of any singular adenohypophyseal cell type. No evidence of cell necrosis or injury was seen in adenohypophyseal secretory or vascular elements. In conclusion, changes were seen in only 2 of the 5 secretory cells of the anterior pituitary, indicating that the adenohypophysis is resistent to the effects of spaceflight. Changes in cortiotrophs and gonadotrophs appear to be a result of compensatory changes in the endocrine system, rather than pathological damage to the anterior pituitary.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Functional State of Thyroid and Calcitonin Producing System of Rat Thyroid Gland in Microgravity

Science Discipline
Endocrinology

Investigator / Institute
V.I. Loginov — Institute of Biomedical Problems

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
None

Objectives/Hypothesis
Previous experiments on SLS-1 and Cosmos missions have indicated that microgravity causes activity of the thyroid gland and the thyroid parenchyma to decrease. These studies have also shown the quantity and activity of calcitonin producing cells (C-cells) to decrease. The purpose of this experiment was to perform histological and immunocytochemical examinations of thyroid glands from rats sacrificed both during and after spaceflight. This was necessary to differentiate thyroid changes produced by microgravity from changes produced from the stress of the return to the Earth's gravitational field.

Approach or Method
Thyroid glands were fixed in Bouin's fixative and embedded in histoplast. Horizantal sections were stained with hematoxylin and eosin. Thyroid sections were also stained to measure iodinated thyrogloblins in follicular colloid. Iodinated thyroglobulins were colored blue, and non-iodinated throglobulins were colored yellow. Thyroid function was evaluated in terms of the follicular epithelium height, thyrocyte nuclear volume, and percentage of follicles stained yellow, yellow-blue, or blue in thyroid sections. An immunoperoxidase technique with avidin-bioin complex was used to provide ommunocytochemical detection of C-cells. The size, nuclear volume, and total number of C-cells were determined. Percentages of C-cells in active state (type 1), in synthesis and hormone accumulation state (type 2), and in active calcitonin secretion were also determined.

Results
Histological examinations showed a lower follicular epithelium height and a smaller number of resorption vacuoli in the colloid, indicating inhibition of resorption activity in the tyroid paranchyma. A smaller size of throcyte nuclei indicated an inhibition of their synthetic activity. Resorption disorders were predominant in thyroid glands of flight animal as seen by their enlarged follicular lumen filled with a dense stratified colliod. The number of type 3 C-cells was decreased, indicating a decrease in secretion activity of C-cell populations. A significant reduction in the size of C-cells and their nuclei suggests an inhibition of biosynthesis. Observations give evidence that C-cells developed hypotrophy and secretory and biosynthetic activity declined in microgravity. As early as 2 days after recovery, marked hyperplasia and hypertrophy of C-cells were observed. By 14 days after recovery, functional activity of the cells had returned to normal.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Morphological Analysis of Pituitary Somatotrophs of SLS-1 and SLS-2 Rats

Science Discipline
Endocrinology

Investigator / Institute
E.I. Alekseyev — Institute of Biomedical Problems

Co-Investigator(s) / Institute
None

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
None

Objectives/Hypothesis
In order to gain a better understanding of the mechanisms involved in metabolic disorders in response to microgravity, it is important to study the somatotrophic function of the pituitary gland controlling growth and anabolic processes in mammals. Previous in-flight experiments have shown a progressive minimization of endocrine regulatory function and inhibition of growth hormone (GH) production and secretion with increased flight time. The objective of this study was to study the histological and cytokaryometric changes in the somatotroph cells of rats flown on SLS-2.

Approach or Method
Pituitary glands were fixed in Bouin's fixative and embedded in paraffin. Horizontal 4-mm-thick sections were stained with paraldehyde fuchsin and Halmi's mixture to identify both basophilic cells and somatotrophs. The major ingredient of Halmi's mixture selectively stainspituitary somatotroph cells. Cytokaryometric examination of somatotroph cells was performed. In the pituitary of each flight and control rat, 100 somatotroph cells and their nuclei located along capillaries and between capillaries in glandular areas were outlined. Diameters and volumes of the cells and their nuclei were measured and statistically treated by means of routine morphometric methods. The somatotrophic cell status was determined with respect to cytokaryometric data and visual evaluation of growth hormone in the cytoplasm.

Results
Pituitary glands of rats sacrificed 5 hours after spaceflight had a greater GH concentration than those of the controls. The pericapillary somatotroph cells had a high GH concentration but no change in cell size, while intervascular glandular somatatroph cells had a low GH concentration and a smaller cell size. Both populations exhibited drastically reduced and dense maculae. These observations indicate that spaceflight diminishes the functional activity of somatotroph cells. During readaptation to 1 g, the secretory and biosynthetic function of the cells recovered and their activity returned to normal.

Bion 11

Launch Date 12/24/1996 **Landing Date** 1/7/1997

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Fluid Electrolyte Metabolism and its Regulation in Primates in Microgravity

Science Discipline
Endocrinology

Investigator	Institute
R.E. Grindeland	NASA Ames Research Center (ARC)
M.A. Dotsenko	Institute of Biomedical Problems

Co-Investigator(s)	Institute
None	

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Grindeland, R.E.; Dotsenko, M.A.; Mukku, V.R.; Bigbee, A.J.; and Bengtson, S.G.: Rhesus Monkey Hormonal Responses to Microgravity. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S143-S144.

Objectives/Hypothesis
Knowledge of the hormonal responses to space flight is necessary to predict how changes in endocrine function in response to long-term microgravity exposure may affect human growth, metabolism, and reproductive function. Several endocrine studies have been performed postflight in rats, Rhesus monkeys and humans. This experiment will continue the study of microgravity-induced endocrine changes using the Rhesus monkeys flown on Bion 11. Specifically, it examines the effect of space flight and restraint on plasma concentrations of several hormones regulating metabolism and protein synthesis.

Approach or Method
Blood samples were taken from flight, restraint control, and vivarium monkeys. Commercial radioimmunoassay (RIA) kits were used to measure growth hormone (GH), testosterone, thyroxine (T4), triiodothyronine (T3), cortisol, and insulin-like growth factor-I (IGF-I).

Results
Immediately postflight, GH, T3 and testosterone levels were significantly suppressed. In contrast, cortisol values were doubled immediately postflight, and T4 showed no change in response to space flight. All hormones returned to control levels by R+6. Flight simulation monkeys showed no changes in GH, inconsistent changes in T4 and testosterone, and suppressed cortisol and T3 levels. Suppression of T3 in restraint controls was only half of that observed after space flight. The decreased levels of GH, T3, and testosterone are consistent with those seen postflight in monkeys from the Bion 10 flight, as well as in rats and humans after long duration space flight. The increase in cortisol levels immediately postflight is most likely due to reentry stress, not microgravity. So far, the physiological mechanisms controlling the suppression of GH, T3, and testosterone are not established. Reduced growth hormone releasing factor may result from reduced proprioceptive input, and decreased testosterone is now known to be related to reduced secretion of luteinizing hormone, but the mechanisms remain to be established.

STS-90 / Neurolab

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Effect of Spaceflight on Adrenal Medullary Function

Science Discipline
Endocrinology

Investigator	Institute
P. Lelkes	University of Wisconsin Medical School

Co-Investigator(s)	Institute
Unsworth, B.R.	Marquette University

Research Subject(s)
*Rattus norvegicus (*Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control, Vivarium Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
None

Objectives/Hypothesis
It is hypothesized that microgravity conditions during spaceflight alter the expression and specific activities of the adrenal medullary catecholamine synthesizing enzymes (CASE). Previous examination of adrenals from six rats flown for 6 days aboard STS-54 showed that microgravity induced a decrease in the expression and specific activity of rat adrenal medullary tyrosine hydroxylase, the rate-limiting enzyme of catecholamine (CA) synthesis, without affecting the expression of other CASE. Analyzing the catecholamine contents and the enzymatic activities of catecholamine synthesizing enzymes in adrenals from the STS-90 Neurolab mission was intended to confirm and extend previous findings.

Approach or Method
Adrenals from 3 Space Shuttle missions (PARE.03, SLS-2, and Neurolab) were examined as follows: Tissue catecholamine content was determined by high-performance liquid chromatography (HPLC) and the levels of immunoreactive CASE were evaluated using Western blotting and quantitative reverse transcription polymerase chain reaction (RT-PCR). In addition, the enzymatic activities of two critical enzymes in this cascadetyrosine hydroxylase (TH), the rate-limiting enzyme of the catecholamine synthesis cascade, and phenylethanolamine-N-methyl transferase (PNMT), the enzyme catalyzing the production of epinephrine from norepinephrine, were analyzed by radioenzymatic assay.

Results
Exposure to microgravity during spaceflight led to a decrease in the levels of tissue catecholamines and CASE expression, evident in the samples from PARE.03, SLS-2, and Neurolab, collected at 24 hours after return to earth. For example, the levels of norepinephrine (NE) are decreased by $15 \pm 7\%$, ($p < 0.05$), while the levels of epinephrine (E) decreased by $34 \pm 13\%$, ($p < 0.05$). The levels of immunoreactive TH decreased by $28 \pm 11\%$, ($p < 0.01$), while PNMT levels decreased by $12 \pm 5\%$, ($p < 0.05$). Similar data were obtained for the expression of the genes for TH and PNMT. These findings seem to support a recent theory that gravitational alterations in circulating catecholamines levels might, in part, be responsible for physiological consequence of weightlessness and cardiovascular abnormalities upon return from space. Thus, the findings are consequential for spaceflight in general and might provide new pharmacological approaches for the management of physiological imbalances during spaceflight and upon readaptation to 1 G.

Launch Date 4/5/2010 **Landing Date** 4/20/2010

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Long-Term Spaceflight Impacts on Female Reproductive Health

Science Discipline
Cell and Molecular Biology

Investigator / Institute
J. Tash — University of Kansas Medical Center

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (B6.SJL-Ptprca Pepcb/BoyJ mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Ronca, A.E.; Baker, E.S.; Bavendam, T.G.; Beck, K.D.; Miller, V.M.; Tash, J.S.; and Jenkins, M.: Effects of Sex and Gender on Adaptations to Space: Reproductive Health. J. Womens Health, 2014, vol. 23., no. 11, pp. 967-974.

Objectives/Hypothesis
The STS-131 BSP program was the first opportunity to determine the effects of spaceflight on normal adult rodent ovary/uteri. Originally selected as part of the BION M1 mission, this project has had the opportunity to be part of the Biospecimen Sharing Program (BSP) for STS-131, STS-133, and STS-135. These additional opportunities have enabled the expansion of flight research to include investigations into the impact of spaceflight on normal female reproductive health in female mice. Previous spaceflight experiments involving female rodents have been limited to neonates, ovariectomized, pregnant, or nursing females. No flight data for normal adult estrous-cycling female rodents has been published. Furthermore, female astronauts take medications to stop estrous cycles during spaceflight. As a result, no human data on effects of spaceflight on normal ovarian function is available.

Approach or Method
Experimental subjects: 15 adult C57Bl/6J female mice were flown on STS-131 for 15 days. Sixteen mice were maintained in ground Animal Enclosure Modules (AEMs).

Approach: Identify specific histopathology and dysfunction of follicle development, ovaries, and uterine horns in normal cycling adult female mice exposed to LEO for 12 days (as determined by mission duration) and compare to ground-based controls. Determine if immune-challenged mice show alterations in follicle, ovarian, and uterine horn structure/function compared to non-challenged controls, and whether microgravity in LEO for 12 days exacerbates or reduces the response to an immune challenge. Determine dysregulation in gene transcription of markers of ovarian and uterine regulation and inflammatory markers in ovary and uterine horns from mice exposed to LEO for 12 days and compare to ground-based controls.

Results
The ovaries were smaller ($P < 0.001$) in flight (4.04+1.53 mg) compared to ground controls (5.52+0.89 mg). There were significantly fewer ($P < 0.002$) corpora lutea in the flight ovaries (1.57±0.61 mg) versus ground (3.75±0.59 mg). Most of the growing follicles in flight ovaries were atretic, indicating blocked estrous cycle. There was no significant difference in number of uterine glands, but there was a trend toward smaller uteri in flight mice. Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) revealed that expression of estrogen receptors ERa and ERb were significantly lower (> 20 fold) in uteri from flight compared to ground animals. Progesterone receptor Pgr-AB expression was down-regulated (3–4 fold) in flight ovaries, but not uteri, while Pgr-B was lower in flight-group ovaries. Lactoferrin messenger ribonucleic acid (mRNA) expression is regulated by ERa and ERb levels in mice uterus and was consistently low (> 20 fold) during spaceflight. Corticosterone releasing hormone receptor CRH-R2 mRNA levels increased, though not significantly in flight-mice uteri.

The unique opportunity offered by the STS-131 Immune BSP program was the first opportunity to examine the effects of spaceflight on adult ovarian and uterine structure/function for rodent models on any flight platform. As such, these experiments are the first to directly assess a potential long-term health risk in animal and human models.

Launch Date 2/24/2011 **Landing Date** 3/9/2011

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Long-Term Spaceflight Impacts on Female Reproductive Health

Science Discipline
Cell and Molecular Biology

Investigator / Institute
J. Tash — University of Kansas Medical Center

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Simulated Flight Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Ronca, A.E.; Baker, E.S.; Bavendam, T.G.; Beck, K.D.; Miller, V.M.; Tash, J.S.; and Jenkins, M.: Effects of Sex and Gender on Adaptations to Space: Reproductive Health. J. Womens Health, 2014, vol. 23., no. 11, pp. 967-974.

Objectives/Hypothesis
Originally selected as part of the BION M1 mission, this project has had the opportunity to be part of the Biospecimen Sharing Program (BSP) for STS-131, STS-133, and STS-135. These additional opportunities have enabled expansion of flight research to include investigations into the impact of spaceflight on normal female reproductive health in female mice. Previous spaceflight experiments involving female rodents have been limited to neonates, ovariectomized, pregnant, or nursing females. No flight data for normal adult estrous cycling female rodents has been published. Furthermore, female astronauts take medications to stop estrous cycles during spaceflight. As a result, no human data on effects of spaceflight on normal ovarian function is available.

Approach or Method
STS-133 presented severe statistical robustness limitations because there were only two untreated (non-respiratory syncytial virus (RSV) infected) flight animals at each time point postflight. Balb/c mice were the research subjects on this flight. The specific aims were as for STS-131:

Specific aim 1: Identify specific histopathology and dysfunction of follicle development, ovaries, and uterine horns in normal cycling adult female mice exposed to LEO for 12 days (as determined by mission duration) and compare to ground-based controls.

Specific aim 2: Determine if immune-challenged mice show alterations in follicle, ovarian, and uterine horn structure/function compared to non-challenged controls, and whether microgravity in LEO for 12 days exacerbates or reduces the response to an immune challenge.

Specific aim 3: Determine dysregulation in gene transcription of markers of ovarian and uterine regulation and inflammatory markers in ovary and uterine horns from mice exposed to LEO for 12 days and compare to ground-based controls.

Results
At the time of publication data analysis is still in progress. Preliminary analysis of data from both STS-133 and STS-135 confirms the results obtained from STS-131. For STS-133, even though the untreated n's were lower than for STS-131 and STS-135, the flight ovaries for STS-133 still showed a highly significant decline in corporal lutea (to 0, $P < 0.02$). In summary, studies on STS-131, STS-133, and STS-135 confirm that spaceflight significantly and negatively affects ovarian physiology, subsequently comprising the reproductive potential. The potential impact on other estrogen-regulated systems such as bone, muscle, wound repair, and immune functions, and whether ERa signaling is a common mechanism underlying spaceflight's effects on these systems, needs to be determined.

Taken together, the mouse data suggest that decrements in gonadal endocrine function and steroid hormone receptor signaling may be an underlying mechanism in spaceflight-induced changes on wider physiological systems. Research in both flight and ground-based systems is exploring the mechanisms that involve these space-related changes, how they relate to the normal aging process, and common mechanisms that could be used to develop countermeasures to ameliorate spaceflight's effects on these systems and also provide solutions to improve the lives and reduce the health risks of the aging individual on Earth.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Endocrinology

Title of Study
Long-Term Spaceflight Impacts on Female Reproductive Health

Science Discipline
Cell and Molecular Biology

Investigator	Institute
J. Tash	University of Kansas Medical Center

Co-Investigator(s)	Institute
None	

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications

Tash, J.S.; Gupta, V.; Holets, L.; and Roby, K.F.: STS-131: Spaceflight Has Negative Impacts on the Morphology, Follicle Health, and Steroid Hormone Receptors in Ovaries and Uterine Horns in C57Bl/6J Mice. Proceedings of the 18th IAA Humans in Space Symposium, Houston, Tex., April 11–15, 2011.

Ronca, A.E.; Baker, E.S.; Bavendam, T.G.; Beck, K.D.; Miller, V.M.; Tash, J.S.; and Jenkins, M.: Effects of Sex and Gender on Adaptations to Space: Reproductive Health. J. Womens Health, 2014, vol. 23., no. 11, pp. 967-974.

Objectives/Hypothesis
Originally selected as part of the BION M1 mission, this project has had the opportunity to be part of the Biospecimen Sharing Program (BSP) for STS-131, STS-133, and STS-135. These additional opportunities have enabled expansion of flight research to include investigations into the impact of spaceflight on normal female reproductive health in female mice. Previous spaceflight experiments involving female rodents have been limited to neonates, ovariectomized, pregnant, or nursing females. No flight data for normal adult estrous cycling female rodents has been published. Furthermore, female astronauts take medications to stop estrous cycles during spaceflight. As a result, no human data on effects of spaceflight on normal ovarian function is available.

Approach or Method
On STS-135 a larger n, similar to STS-131, provided an excellent opportunity to replicate this study in C57Bl6 as on STS-131. However, an error in a group assignment spreadsheet caused half of the animals that the BSP team received to be drug treated. Regarding the potential impact of the Amgen anti-bone loss drug treatment on these experiments, the target for the Amgen drug is not of regulatory significance in ovary or uterus. A blind analysis of the distribution of appearance and weight of the tissues that were received was performed and resulted in no identification of a bimodal distribution of either parameter within the flight or ground controls, respectively. Therefore, the samples in question were kept.

Specific aim 1: Identify specific histopathology and dysfunction of follicle development, ovaries and uterine horns in normal cycling adult female mice exposed to LEO for 12 days (as determined by mission duration) and compare to ground-based controls.
Specific aim 2: Determine if immune-challenged mice show alterations in follicle, ovarian, and uterine horn structure/function compared to non-challenged controls, and whether microgravity in LEO for 12 days exacerbates or reduces the response to an immune challenge.
Specific aim 3:Determine dysregulation in gene transcription of markers of ovarian and uterine regulation and inflammatory markers in ovary and uterine horns from mice exposed to LEO for 12 days and compare to ground-based controls.

Results
Preliminary analysis of the data from both STS-133 and STS-135 confirms the results obtained from STS-131. For STS-133, even though the untreated n's were lower than for STS-131 and STS-135, the flight ovaries for STS-133 still showed a highly significant decline in corporal lutea (to 0, $P < 0.02$).
In summary, the studies on STS-131, STS-133, and STS-135 confirm that spaceflight significantly and negatively affects ovarian physiology, subsequently comprising the reproductive potential. The potential impact on other estrogen-regulated systems such as bone, muscle, wound repair, and immune functions and whether ERa signaling is a common mechanism underlying spaceflight effects on these systems needs to be determined.

Taken together, the mouse data suggest that decrements in gonadal endocrine function and steroid hormone receptor signaling may be an underlying mechanism in spaceflight-induced changes on wider physiological systems. The research in both flight and ground-based systems is exploring the mechanisms that involve these space-related changes, how they relate to the normal aging process, and common mechanisms that could be used to develop countermeasures to ameliorate both spaceflight effects on these systems, and also provide solutions to improve the lives and reduce the health risks of the aging individuals on earth.

Cosmos 782 / Bion 3

Launch Date 11/25/1975 **Landing Date** 12/15/1975

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Hematology

Title of Study
Alterations in Erythrocyte Survival Parameters in Rats After 19.5 Days Aboard Cosmos 782

Science Discipline
Hematology

Investigator	Institute
H. Leon	NASA Ames Research Center (ARC)

Co-Investigator(s)	Institute
Landaw, S.A.	Syracuse VA Hospital
Cummins, J.	Northrop Services, Inc.
Serova, L.V.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications

Leon, H.A. and Landaw, S.A.: Attenuation of Hemolytic Action of Oxygen by Nitrogen. Proceedings of the Annual Scientific Meeting of the Aerospace Medical Association, Houston, Tex., 1971, p. 41.

Leon, H.A.; Serova, L.V.; Cummins, J.; and Landaw, S.A.: Alterations in Erythrocyte Survival Parameters in Rats After 19.5 Days Aboard Cosmos 782. Aviation, Space, and Environmental Medicine, vol. 49, 1978, pp. 66–69.

Landaw, S.A.; Leon, H.A.; and Winchell, H.S.: Effects of Hyperoxia on Red Blood Cell Survival in the Normal Rat. Aerospace Medicine, vol. 41, no. 1, 1970, pp. 48–55.

Objectives/Hypothesis
The results of the Skylab flights demonstrate that prolonged weightlessness causes a significant decrease in red cell mass of astronauts that is not related to oxygen partial pressure. The objective of this experiment was to determine if the complex factors associated with relatively prolonged spaceflight interact to alter survival parameters of preformed red blood cells in rats.

Approach or Method
Based on the amount of exhaled carbon-14 labeled carbon monoxide, survival parameters of a cohort of erythrocytes labeled 15.5 days preflight were evaluated upon return from orbit and compared to control rats injected at the same time. Breath samples were taken for 3 hours at the same time each day to minimize potential circadian fluctuations. The 14CO generated was trapped in ethanolamine/ 2-Ethoxyethanol and triplicate aliquots were counted by liquid scintillation. Using a computer, the obtained data points were fitted into an equation relating red blood cell survival to 14CO production.

Results
Statistical evaluation indicates that all survival parameters were altered by the spaceflight. The mean potential red blood cell life span, which was 62.4 days in control rats, was decreased to 59.0 days in the flight rats, and random hemolysis was increased three-fold in the flight rats. The measured size of the cohort was decreased, lending further support to the idea that hemolysis was accelerated during some portion of the flight. Factors that might be contributory to these changes include forces associated with launch and reentry, atmospheric and environmental parameters, diet, radiation, and of course, weightlessness.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Hematology

Title of Study
Regulation of Erythropoiesis During Spaceflight

Science Discipline
Hematology

Investigator	Institute
R.D. Lange	University of Tennessee Medical Center

Co-Investigator(s)	Institute
Ichiki, A.T.	University of Tennessee Medical Center
Jones, J.B.	University of Georgia, Athens

Research Subject(s)
*Rattus norvegicus (*Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF), Animal Enclosure Module (AEM)

Selected Publications
Allebban, Z.; Ichiki, A.T.; Gibson, L.A.; Jones, J.B.; Congdon, C.C.; and Lange, R.D.: Effects of Spaceflight on the Number of Rat Peripheral Blood Leukocyte and Lymphocyte Subsets. Journal of Leukocyte Biology, vol. 55, 1994, pp. 209–213.

Udden, M.M.; Driscoll, T.B.; Gibson, L.A.; Patton, C.S.; Pickett, M.H.; Jones, J.B.; Nachtman, R.; Allebban, Z.; Ichiki, A.T.; and Lange, R.D.: Blood Volume and Erythropoiesis in the Rat During Spaceflight. Aviation, Space, and Environmental Medicine, vol. 66, no. 6, June 1995, pp. 557–561.

Objectives/Hypothesis
One of the most consistent findings observed in men exposed to orbital spaceflights has been a decrease in the total circulating number of red blood cells (RBCs). The objective of this study was to gain an understanding of the regulatory parameters that modulate RBC production and destruction. Peripheral blood and spleen lymphocytes were studied to ascertain the immunodeficiency of the flight animals. Another objective was to determine if the rat is an appropriate animal model to study these mechanisms.

Approach or Method
Using in-flight food and water consumption data, the role of nutrition (energy balance) and hemoconcentration was assessed in the rat's erythropoietic response to spaceflight. Measurements included erythropoietin (Epo) levels, changes in hematocrit, and the rate of erythropoiesis and red blood cell production. The effect of spaceflight on erythropoietin responsive cell cultures was investigated. Red blood cell survival was accomplished through both reticulocyte counts and radio assays provided by other studies.

Results
The results of these studies indicated that on R+0 there was a significant decrease in the number of Epo-responsive erythroid progenitor cells. Peripheral blood showed a significant decrease in the total number of white blood cells (WBCs) and in the absolute number of lymphocytes, monocytes, and eosinophils. Immunophenotyping studies of peripheral blood lymphocytes indicated a significant decrease in the absolute number of B-cells, T-helper cells, and T-suppressor cells. All values returned to the control levels by R+9. No significant differences between flight and control animals were observed in the red blood cells parameters (RBC, Hgb, Hct), serum erythropoietin level, and reticulocyte counts.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Hematology

Title of Study
Regulation of Blood Volume During Spaceflight

Science Discipline
Hematology

Investigator	Institute
C.P. Alfrey	Baylor College of Medicine

Co-Investigator(s)	Institute
Driscoll, T.B.	Baylor College of Medicine
Nachtman, R.G.	Baylor College of Medicine
Udden, M.M.	Baylor College of Medicine

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF), Animal Enclosure Module (AEM)

Selected Publications
Udden, M.M.; Driscoll, T.B.; Gibson, L.A.; Patton, C.S.; Pickett, M.H.; Jones, J.B.; Nachtman, R.; Allebban, Z.; Ichiki, A.T.; and Lange, R.D.: Blood Volume and Erythropoiesis in the Rat During Spaceflight. Aviation, Space, and Environmental Medicine, vol. 66, no. 6, June 1995, pp. 557–561.

Objectives/Hypothesis
In spaceflights as short as 7 days, an 8- to 15-percent reduction in red blood cell mass (RBCM) has been measured in astronauts upon landing. Current theories regarding the regulation of erythropoiesis would require an increased rate of red blood cell (RBC) destruction to produce such changes in RBCM. However, data from humans has indicated that RBC survival time is unchanged during spaceflight. The objective of this experiment was to evaluate whether the rat is a suitable animal model for researching the mechanism responsible for the RBCM loss observed in humans.

Approach or Method
Radioactive tracers were administered to animals pre- and postflight. Plasma volume (PV) was measured using 125I-labeled albumin, and 51Cr-labeled donor RBCs were used to measure RBCM and RBC survival. RBCM and PV were measured 8 days before launch (L-8), at recovery (R+0), and 8 days postflight (R+8). 51Cr RBC survival studies were from L-7 to R+0, and R+1 to R+8. Blood samples fixed with 0.5-percent glutaraldehyde were coded, and the proportion of cells that had echinocytic morphology were determined in a blind fashion. To study iron kinetics, 59Fe was injected on R+0 and incorporation into RBCs was followed over the next 8 days. Serum ferritin levels and 51Cr spleen-to-liver ratios were determined on R+0 and R+9.

Results
Because no statistical difference could be attributed to housing conditions, measurements from single- and group-housed animals were combined. No flight-related changes were found in hematocrit values, number of echinocytes, or 51CR spleen-to-liver ratios. Upon landing, mean RBCM of flight rats was significantly less than controls, both when expressed as absolute volume or volume normalized for body mass. PV, normalized for body mass, was also significantly decreased at R+0. The 51Cr survival data did not suggest an increased RBC destruction rate as the cause of the decreased RBCM. Instead postflight decreases in 59Fe incorporation could indicate a decrease in RBC production in response to spaceflight or to decreased food intake and weight gain postflight.

Cosmos 782 / Bion 3

Launch Date 11/25/1975 **Landing Date** 12/15/1975

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Absence of Gastric Ulceration in Rats After Flight on the Cosmos 782

Science Discipline
Metabolism and Nutrition

Investigator / Institute
P. Brown — NASA Ames Research Center (ARC)

Co-Investigator(s) / Institute
Vernikos-Danellis, J. — NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications
Brown, P.A.; Brown, T.H.; and Vernikos-Danellis, J.: Histamine H2 Receptor: Involvement in Gastric Ulceration. Life Sciences, vol. 18, no. 3, 1976, pp. 339–344.

Brown, P.A.; Sawrey, J.M.; and Vernikos-Danillis, J.: Attenuation of Salicylate and Stress-Produced Gastric Ulceration by Metiamide. Proceedings of the Western Pharmacology Society, vol. 18, 1975, pp. 123–127.

Objectives/Hypothesis
A variety of environmental stressors can be shown to produce gastric ulceration in rats. Several converging lines of research have shown that endogenous histamine plays an essential role in stress-produced gastric ulceration. It has been demonstrated, as well, that the mechanism specifically involves the H2 receptor for histamine. Whether the actual stresses associated with a long-term spaceflight are sufficient to produce gastric ulceration in rats is uncertain, and were the focus of this study.

Approach or Method
Stomachs from the flight animals were compared with stomachs removed from animals in synchronous and vivarium control groups. The stomachs were examined microscopically for gastric ulceration using the following classification: a) petechial ulcers: minute superficial erosions involving only the mucosa; b) punctate ulcers: discrete punched-out ulcerations measuring at least 1 mm; and c) longitudinal ulcers: continuous linear ulcerations larger than 1 mm. Tissue for histological examination was taken from the rumen, corpus, and antrum; hematoxylin and eosin sections were examined microscopically for evidence of superficial erosion of the mucosa.

Results
None of the animals examined from the flight or control groups evidenced gastric ulcers or pronounced mucosal erosion, a not unexpected result. In conjunction with other experiments, most of the animals in flight and synchronous groups were maintained on the antibiotic Declomycin. A broad spectrum of antibiotics can result in a deficiency of pyridoxal phosphate, a required co-factor in the biosynthesis of histamine. A histamine depletion could afford protection against experimental ulceration, as would the 6-hour feeding intervals, as a minimum of 24 hours of food deprivation is usually required to produce stress ulcers in rats. Possibly, during the initial phase of the flight, the animals did ulcerate but recovered prior to return.

Cosmos 936 / Bion 4

Launch Date 8/13/1977

Landing Date 8/22/1977

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
The Effects of Spaceflight on Some Liver Enzymes Concerned with Carbohydrate and Lipid Metabolism in the Rat

Science Discipline
Metabolism and Nutrition

Investigator	Institute
S. Abraham	Bruce Lyon Memorial Research Lab.

Co-Investigator(s)	Institute
Klein, H.P.	NASA Ames Research Center (ARC)
Lin, C.Y.	Bruce Lyon Memorial Research Lab
Volkmann, C.	NASA Ames Research Center (ARC)

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Centrifuged Control

Key Flight Hardware
Cosmos Rodent Cages

Selected Publications

Abraham, S.; Lin, C. Y.; Klein, H. P.; and Volkmann, C.: The Effects of Spaceflight on Some Liver Enzymes Concerned with Carbohydrate and Lipid Metabolism in Rats. Physiologist, vol. 21, no. 4, 1978, pp. 199 -217.

Abraham, S.; Klein, H.P; Lin, C.Y.; and Volkmann, C.: The Effects of Spaceflight on Some Rat Liver Enzymes Regulating Carbohydrates and Lipid Metabolism. Advances in Space Research, vol. 1, no. 14, 1981, pp. 199–217.

Objectives/Hypothesis
During early manned spaceflights, a specific physiological enigma concerning caloric requirements was noted. While theoretically in the absence of gravitational force less energy should be required for life-sustaining functions, on the initial flights where caloric intake was restricted, a marked in-flight loss in body weight resulted. It is important to study a key organ like the liver in order to determine whether any regulative enzymes of carbohydrate and lipid metabolism might have been affected; that is, whether the capabilities of the organ to produce these cellular constituents are, in fact, altered in spaceflight.

Approach or Method
In the rat liver, the activities of about 30 enzymes concerned with carbohydrate and lipid metabolism were examined according to well-established methods or modifications of currently used techniques. In addition, the levels of glycogen and of the individual fatty acids in hepatic lipids were determined. The livers from rats subjected to near weightlessness spaceflight and rats subjected to a 1-g force via an onboard centrifuge were compared to appropriate ground-based controls. Several key enzymes of lipid biosynthesis, many of which are known to be regulative, were studied in this experiment, including acetyl-CoA carboxylase, fatty acid synthetase, palmitoyl-CoA synthetase, and stearoyl-CoA desaturase.

Results
In rats sacrificed at recovery, statistically significant decreases in the activity levels of a-glycerol-phosphate acyltransferase, diglyceride acyltransferase, aconitase, and 6-phosphogluconate dehydrogenase were noted in the flight-weightless group versus flight-centrifuged animals. The flight-weightless group also contained, on average, more than twice the amount of glycogen than did flight-centrifuged livers, and a remarkable shift in the ratio of palmitate to palmitoleate was noted. As a possible explanation, it was observed that although glycogen synthetase activity was about the same in all groups, its glycogen phosphorylase activity was significantly less. The observed alterations, particularly those that involved increased glycogen levels and decreased activities of the acyl transferases concerned with lipid synthesis, appear unique to the weightless condition. Most of the enzyme activities, though, did not show any significant differences between weightless and centrifuged rats, and all parameters had returned to normal by 25 days postflight.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979

Landing Date 10/14/1979

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study

Studies of Specific Hepatic Enzymes and Liver Constituents Involved in the Conversion of Carbohydrates to Lipids in Rats Exposed to Prolonged Spaceflight

Science Discipline

Metabolism and Nutrition

Investigator	Institute
S. Abraham	Bruce Lyon Memorial Research Laboratory

Co-Investigator(s)	Institute
Klein, H.P.	NASA Ames Research Center (ARC)
Lin, C.Y.	Bruce Lyon Memorial Research Laboratory
Tigranyan, R.A.	Institute of Biomedical Problems

Research Subject(s)

Rattus norvegicus (Wistar rat)

Ground-Based Controls

Vivarium Control, Synchronous Control

Key Flight Hardware

Cosmos Rodent Cages

Selected Publications

Abraham, S., et al.; Biochemical Changes in Rat Liver After 18.5 Days of Spaceflight. Proceedings of the Society for Experimental Biology and Medicine, vol. 172, 1983, pp. 334–339.

Abraham, S.; Lin, C.Y.; Klein, H.P.;Volkmann, C.; Tigranyan, R.A.; and Vetrova, G.: Studies of Specific Hepatic Enzymes Involved in the Conversion of Carbohydrates to Lipids in Rats Exposed to Prolonged Spaceflight Aboard Cosmos 1129. Gravitational Physiology, vol. 19, 1981, pp. 71-77.

Objectives/Hypothesis

Examination of liver, blood, muscle, and skeletal tissues from rats aboard earlier Cosmos flights indicated changes in the lipid and carbohydrate levels of these tissues in response to spaceflight. These metabolic alterations, both in enzyme levels and in hepatic constituents, appeared to be unique to the weightless condition. The present study was designed to reinvestigate some of these earlier observations and to extend the range of inquiry to include additional hepatic microsomal and mitochondrial enzymes, as well as other liver constituents (total lipids, triglycerides, phospholipids, and sterols) not included in the original Cosmos 936 protocol.

Approach or Method

The activities of 26 enzymes concerned with carbohydrate and lipid metabolism in hepatic tissue were investigated. These activities were measured in the various hepatic cell compartments (i.e., cytosol, mitochondria, and microsomes). In addition, the levels of glycogen, total lipids, phospholipids, triglycerides, cholesterol, cholesterol esters, and the fatty acid composition of the rat livers were examined and quantified.

Results

The activities of most of the hepatic enzymes and liver constituents were unaffected by spaceflight. In confirmation with Cosmos 936 results, a significant difference was again found in the ability of flight animals to complex long-chain fatty acids. Thus, both microsomal diglyceride acyltransferase and microsomal cholinephosphotransferase of the flight rats showed reduced activities when compared with the synchronous controls; however, these decreased enzyme activities did not appear to affect the hepatic lipid values. The livers of the flight rats had 30% more glycogen than those of synchronous controls, yet flight animals showed no significant changes in the activities of glycogen synthesis or of phosphorylase that could fully explain the increased glycogen contents. It would appear that weightlessness can indeed affect metabolic pathways concerned with lipid and carbohydrate metabolism, and that such metabolic changes are in some cases independent of stresses, other than weightlessness, that are involved in spaceflight.

Cosmos 1129 / Bion 5

Launch Date 9/25/1979 **Landing Date** 10/14/1979

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Effects of Weightlessness on Body Composition in the Rat

Science Discipline
Metabolism and Nutrition

Investigator	Institute
G. Pitts	University of Virginia

Co-Investigator(s)	Institute
Ushakov, A.S.	Institute of Biomedical Problems
Pace, N.	University of California, Berkeley
Smith, A.H.	University of California, Davis

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Rodent Cages, Cosmos 1129 Russian Hardware Suite

Selected Publications
Pitts, G.C.; Ushakov, A.S.; Pace, N.; Smith, A.H.; Rahlmann, D.F.; and Smirnova,T.A.: Effects of Weightlessness on Body Composition in the Rat. American Journal of Physiology, vol. 244, no. 3, 1983, pp. R332–R337.

Ushakov, R.S.; Smirnova, T.A.; Pitts, G.C.; Pace, N.; Smith, A.H.; and Rahlmann, D.F.: Body Composition of Rats Flown Aboard Cosmos-1129. Physiologist, supl., vol. 23, no. 6, Dec. 1980, pp. S41–S44.

Objectives/Hypothesis
While organisms have evolved defenses against changes in temperature, altitude, osmotic pressure, etc., they have had no need or opportunity to evolve defenses against changes in the chronic acceleration field (ΔG, gravity). Thus, it comes as no surprise that ΔG perturbs body composition to a degree not seen with changes in most other environmental factors. As weightlessness represents a virtual extinction of the acceleration field, it is of basic physiological interest and has obvious applicability to space medicine. This study sought to characterize the body composition responses of the adult rat to weightlessness of 18.5 days duration.

Approach or Method
Dissection involved separation and weighing of 15 individual organs and systems. Aliquots were analyzed for nitrogen, potassium, phosphorus, magnesium, and sodium. Determination of water content (freeze-drying) and fat content (Soxhlet extraction) were carried out separately on three major body compartments: skinned, eviscerated carcass (musculoskeletal system); skin; and all other components pooled designated “viscera.” The following calculations were also employed: net body mass = total body mass – fur, gut content, and urine; intra-cellular water content = 0.73 x body cell mass; extracellular water content = total body water – intracellular water; body protein = 6.25 x body nitrogen; body cell mass = 8.9 x body potassium; and bone mineral = 2.93 x body calcium. Statistical significance between groups was evaluated with the T test.

Results
In comparison to synchronous controls, the flight group showed a 6.7% reduction in total body water probably attributed to a 36.2% reduction in the extracellular compartment; reductions of 6.6% in musculoskeletal water and 17.2% in skin water; an apparent shift of some water from skin to viscera; and a 20% reduction in bone mineral mass. Among organ fresh masses, there was a 7.5% increase in kidneys and a 14.0% decrease in spleen. The results support the validity of the rat as an experimental model for gravitational studies and the usefulness of the body composition approach to gravitational physiology. It appears highly probable that by the end of the 18.5-day exposure there was a steady state in body composition, potentially regulated and characteristic of the new g level. While the reported changes might have been greater if recorded just before reentry (instead of 32–36 hours postflight), it is believed that they reflect true effects of weightlessness. It appears reasonable, though, that the flight animals might have regained 15–20% of their live mass in the 1.5 days being considered here.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987

Landing Date 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Hepatic Function in Rats After Spaceflight

Science Discipline
Metabolism and Nutrition

Investigator	Institute
A.H. Merrill	Emory University School of Medicine

Co-Investigator(s)	Institute
Hoel, M.	Emory University School of Medicine
Hargrove, J.	Emory University School of Medicine
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Merrill, A.H.: Altered Carbohydrate, Lipid, and Xenobiotic Metabolism by Liver From Rats Flown on Cosmos 1887. FASEB Journal, vol. 4, no. 1, 1990, pp. 95–100.

Objectives/Hypothesis
Among the changes that have been observed during and after spaceflight are elevated adrenal steroid secretion and altered concentrations of various lipids and carbohydrates in blood and other tissues. Because the liver is an important site of nutrient and xenobiotic metabolism, further study of the effects of spaceflight on this organ appear warranted. Based on previous findings, it was proposed that several aspects of hepatic function are altered by spaceflight. In particular, these include key enzymes of cholesterol and sphingolipid biosynthesis and drug metabolism (e.g., cytochrome P-450). This study was to provide additional data from analyses of liver and serum samples from space-flown rats that support this hypothesis.

Approach or Method
To determine the possible biochemical consequences of prolonged weightlessness on liver function, tissue samples from flight and control rats were analyzed for hepatic protein, glycogen, and lipids, as well as the activities of a number of key enzymes involved in metabolism of these compounds and xenobiotics.

Results
Among the parameters measured, the major differences were elevations in the hepatic glycogen content and HMG-CoA reductase activities of the flight rats, and a decrease in the amount of microsomal cytochrome P-450 and the activity of aniline hydroxylase, a cytochrome P-450-dependent enzyme. Decreases in these two indices of the microsomal mixed-function oxidase system indicate that spaceflight may compromise the ability of the liver to metabolize drugs and toxins. The higher HMG-CoA reductase correlated with elevated levels of serum cholesterol. Other changes included somewhat higher blood glucose, creatinine, serum glutamic oxalocetic transaminase (SGOT), and much greater alkaline phosphatase and blood urea nitrogen (BUN). These results generally support the earlier observation of changes in these parameters. The importance of these alterations is not known; however, they have the potential to complicate long-term spaceflight.

Cosmos 1887 / Bion 8

Launch Date 9/29/1987 **Landing Date** 10/11/1987

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Structural Changes and Cell Turnover in the Rat's Small Intestine Induced by Spaceflight

Science Discipline
Metabolism and Nutrition

Investigator	Institute
R. Phillips	Colorado State University

Co-Investigator(s)	Institute
Sawyer, H.R.	Colorado State University
Smirnov, K.V.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Wistar rat)

Ground-Based Controls
Vivarium Control, Synchronous Control

Key Flight Hardware
Cosmos Rodent-BIOS, Cosmos 1887 Russian Hardware Suite

Selected Publications
Phillips, R.W.; Sawyer, H.R.; and Smirnov, K.V.: Structural Changes and Cell Turnover in the Rat's Small Intestine Induced by Spaceflight. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 1887. J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-102254, 1990, pp. 359–363.

Sawyer, H.R.; Moeller, C.L.; Phillips, R.W.; and Smirnov, K.L.: Effects of Spaceflight on the Proliferation of Jejunal Mucosal Cells. FASEB Journal, vol. 4, no. 1, 1990, pp. 92–94.

Objectives/Hypothesis
The purpose of this study was to test the hypothesis that the generalized, whole-body decrease in synthetic activity associated with microgravity conditions of spaceflight as evidenced by negative nitrogen balance and muscle atrophy, as well as inhibited lymphocyte proliferation, would be evident in cells characterized by a rapid rate of turnover. As a model it was decided to study the turnover of mucosal cells lining the jejunum of the small intestine, because these cells are among the most rapidly proliferating in the body.

Approach or Method
The mitotic index was determined for mucosal cells lining the proximal, middle, and distal regions of the jejunum in rats from three treatment groups (synchronous control, vivarium control, and flight); the depth of the crypts of Lieberkuhn was measured; and the length of villi present in each of the three jejunal regions sampled. To accurately determine the mitotic index for each region, at least 2,000 cells per region per animal were examined. Prior to evaluation, all slides were coded so that the technician reading the slides did not know the region or treatment group being examined. To determine villus length and crypt depth, at least 25 villi and crypts per region per animal were measured using a computerized image analysis system coupled to a bright field microscope.

Results
The number of mitotic figures observed in the proximal jejunum of the flight animals was higher compared to either the synchronous or vivarium animals. Conversely, in the middle and distal jejunum, both the synchronous controls and flight animals had an increased number of mitotic figures compared to vivarium controls. The height of jejunal villi in flight animals was not significantly different from that observed in animals included in the synchronous or vivarium groups. With respect to region by treatment interactions, the only marked difference was that the crypt depth in the proximal jejunal region in the flight animals was less than that measured in the synchronous animals. However, because there were no consistent differences between animals in the flight group and those in synchronous and vivarium control groups, it appears that any effects of microgravity on the turnover of jejunal mucosal cells were short-lived and rapidly returned to normal.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Hepatic Function in Rats After Spaceflight: I. Analyses of Selected Parameters of Carbohydrate, Amino Acid, Lipid, and Xenobiotic Metabolism

Science Discipline
Metabolism and Nutrition

Investigator	Institute
A.H. Merrill	Emory University School of Medicine

Co-Investigator(s)	Institute
Popova, I.A.	Institute of Biomedical Problems
Wang, E.	Emory University School of Medicine
Mullins, R.E.	Emory University School of Medicine

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Merrill, A.H. Jr.; Wang, E.; LaRocque, R.; Mullins, R.E.; Morgan, E.T.; Hargrove, J.L.; Bonkovsky, H.L.; and Popova, I.A.: Differences in Glycogen, Lipids, and Enzymes in Livers from Rats Flown on Cosmos 2044. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. 142S–147S.

Merrill, A.H. Jr.; Wang, E.; Mullins, R.E.; Grindeland, R.E.; and Popova, I.A.: Analyses of Plasma for Metabolic and Hormonal Changes in Rats Flown Aboard Cosmos 2044. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. 132S–135S.

Objectives/Hypothesis
Because the liver is basically a central “clearing house” for the metabolism of most nutrients, drugs, and other foreign compounds, further studies of the effects of spaceflight on the organ appeared warranted. Previous findings suggest that the xenobiotic metabolism is altered by spaceflight. In an attempt to further these findings, additional data from analyses of liver samples and serum samples from space-flown rats are described in this report.

Approach or Method
Liver and plasma samples were analyzed, with established methods, for hepatic protein (with bovine serum albumin as standard), glycogen (rabbit liver glycogen standard) and lipids, as well as the activities of a number of key enzymes involved in metabolism of these compounds and xenobiotics. DNA was quantified using calf thymus DNA as standard. Cytochrome P-450 was measured spectrophotometrically, and by the standard assays of testosterone metabolism and immunochemistry. Other enzyme and serum analyses were conducted using an analyzer.

Results
The major differences between the flight group versus the synchronous control were: 1) elevations in microsomal protein, liver glycogen content, tyrosine aminotransferase, and tryptophan oxygenase; and 2) reductions in sphingolipids and the rate-limiting enzyme of heme biosynthesis, delta aminolevulinic acid synthase. These results support the hypothesis that spaceflight alters liver function; however, results with these samples differed notably from those of Skylab 3 and Cosmos 1887, presumably due to the conditions of spaceflight and/or the postflight recovery period.

Launch Date 9/15/1989

Landing Date 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Hepatic Function in Rats After Spaceflight: II. Glycogen Studies

Science Discipline
Metabolism and Nutrition

Investigator	Institute
S. Cormier	University of Louisville

Co-Investigator(s)	Institute
Racine, R.N.	University of Louisville
Popova, I.A.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Racine, R.N. and Cormier, S.M.: Effects of Spaceflight on Rat Hepatocytes: A Morphometric Study. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S136–S141.

Objectives/Hypothesis
Research from previous spaceflights has shown marked differences in hepatic glycogen and lipid levels; however, the findings have been somewhat contradictory in some instances. Because dietary effects were held relatively constant, alterations in lipid or glycogen storage due to spaceflight may indicate important metabolic alterations in liver function. This study used morphometric techniques to examine glycogen and lipid levels in flight and ground-control animals.

Approach or Method
The caudate lobe of each liver was removed and cut into small pieces. Semi-thin (1.0 micrometers) plastic sections (Epon) were stained for glycogen; another section from the same sample was stained with toluidine blue and analyzed for lipid. Twenty-five areas were enumerated for each specimen compartment (lipid, glycogen, nucleus, etc.) and the average percent calculated. Analyses were performed with an image analysis system equipped with a monochrometer. Kupffer cells, hepatocyte nuclei, and fluid-filled spaces were measured from stained 5-micrometers paraffin sections.

Results
Glycogen levels in flight rats were found to be significantly elevated over controls. Lipid was also higher, but not significantly different. Hepatocytes appeared to be larger in flight animals due to area attributed glycogen. Sinusoids were less prominent in flight animals, and flight Kupffer cell population appeared to be reduced per total area, possibly reflecting changes in liver immune function. Alterations in the storage of glycogen and number of Kupffer cells suggest an important effect of spaceflight that may have important implications for long-term flight.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Structural Changes and Cell Turnover in the Rat's Small Intestine Induced by Spaceflight.

Science Discipline
Metabolism and Nutrition

Investigator	Institute
R. Phillips	Colorado State University

Co-Investigator(s)	Institute
Smirnov, K.V.	Institute of Biomedical Problems
Moeller, C.L.	Colorado State University
Sawyer, H.R.	Colorado State University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Synchronous Control, Tail-Suspended Control

Key Flight Hardware
Cosmos Rodent-BIOS

Selected Publications
Sawyer, H.R.; Moeller, C.L.; Phillips, R.W.; and Smirnov, K.L.: Proliferation of Jejunal Mucosal Cells in Rats Flown in Space. Journal of Applied Physiology, supl., vol. 73, no. 2, 1992, pp. S148–S150.

Objectives/Hypothesis
The purpose of this project was to test the hypothesis that the generalized, whole-body decrease in synthetic activity due to microgravity conditions encountered during spaceflight would be demonstrable in cells and tissue characterized by a rapid rate of turnover. Jejunal mucosal cells were chosen as a model because these cells are among the most proliferative in the body.

Approach or Method
Sections 1 μm thick were cut from each of the five sample segments from each of the three jejunal regions per animal and stained with toluidine blue. To accurately determine the mitotic index for each respective region, at least 2,000 cells per region per animal were examined. To determine villus length and crypt depth, at least 20 villi and crypts were measured per region per animal using a computerized image analysis system coupled to a bright field microscope equipped with a 4x objective and video camera. All data were statistically analyzed by analysis of variance, and differences between means were detected.

Results
The percentage of mitotic cells present on the crypts of Lieberkuhn in the proximal, middle, and distal regions of flight rats did not differ significantly from controls. Although the ability of jejunal cells to divide by mitosis was not impaired in the flight group, there was, however, a reduction in the length of villi and depth of crypts. Because villi in flight rats were lined by normal mucosal cells, the concomitant reduction in villi height and crypt depth probably reflects changes (e.g., shrinkage) in the connective tissue core of villi and is not due to an impairment in the migration of newly proliferated cells needed to replace those desquamated.

Cosmos 2044 / Bion 9

Launch Date 9/15/1989 **Landing Date** 9/29/1989

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Biological Rhythm and Temperature Regulation: II. Metabolism

Science Discipline
Metabolism and Nutrition

Investigator	Institute
C.A. Fuller	University of California, Davis

Co-Investigator(s)	Institute
Dotsenko, M.A.	Institute of Biomedical Problems
Korolkov, V.I.	Institute of Biomedical Problems
Stein, T.P.	University of Medicine and Dentistry

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Cosmos Primate-BIOS

Selected Publications
Fuller, C.A.; Dotsenko, M.A.; Korolkov, V.I.; Griffin, D.W.; and Stein, T.P.: Circadian Rhythms and Temperature Regulation During Spaceflight: II. Metabolism. Final Reports of the U.S. Experiments Flown on the Soviet Biosatellite Cosmos 2044. Vol. 2, J.P. Connolly, R.E. Grindeland, and R.W. Ballard, eds., NASA TM-108802, 1994, pp. 361–383.

Objectives/Hypothesis
In this experiment, energy expenditure was measured in Rhesus monkeys using the doubly labeled water (2H218O) method. Determinations were made during spaceflight and during postflight ground control. The doubly labeled water method is the only method available for continuously measuring energy expenditure during spaceflight considering the severely restricted conditions of spacecraft. Therefore, this study focused on the development and use of this procedure on nonhuman primates during spaceflight.

Approach or Method
When doubly labeled water is given orally, it mixes with body water in about 3 hours. The two isotopes then leave the body at different rates; 2H leaves as water, mainly in urine, whereas 18O leaves both as water and exhaled CO2. Thus, the turnover rate of isotopic hydrogen and oxygen labeled differ, the difference being proportional to the rate of CO2 production. Monkeys were dosed (3–5 ml) 3 days preflight. Urine samples (3 ml) were collected following the dose to determine body water pool size, as well as pre- and postflight. Animals were redosed with 18O postflight to determine any changes in body protein and fat content.

Results
The data from this study demonstrate the viability of this technique as an in-flight measure of metabolism, although accuracy could be enhanced by collecting more urine samples. The total body water (TBW) in the flight study of 2.986 corresponds to 77.76% of the animal's body weight, suggesting that the animal was in a dehydrated state when the measurements were made. The flight energy expenditure value of 266 kcal/monkey/day or 69.3 kcal/kg/day appears physiological. Due to a problematic sample, the postflight TBW could not be determined.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Mechanism of Formation of the Gastric Hypersecretory Syndrome of the Stomach

Science Discipline
Metabolism and Nutrition

Investigator	Institute
K.V. Smirnov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Pechyonkina, R.A.	Institute of Biomedical Problems
Goncharova, N.P.	Institute of Biomedical Problems
Phillips, R.	Colorado State University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Smirnov, K.V.; Phillips, R.; Pechyonkina, R.A.; and Goncharova, N.P.: Mechanism of Development of the Hypersecretory Syndrome of the Stomach. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, p. 33.

Objectives/Hypothesis
During exposure to spaceflight factors there are significant alterations in the morphofunctional status of the digestive system. Previous experiments revealed a progressive increase in the acidic-peptic potential of the stomach, and a simultaneous stimulation of the gastrin mechanism of regulation of the chief and parietal cells of the stomach. The purpose of this experiment was to conduct a biochemical investigation of the mucous membrane of the stomach of rats after spaceflight.

Approach or Method
The stomach was removed and opened along the greater curvature, and the contents emptied. The mucous homogenate was used to measure pepsin characterizing the activity of the chief cells of the stomach. Pepsin activity was determined by the absorption-colorimetry method.

Results
A study of the functional status of the rat stomach revealed increased peptic potential of the stomach, which was more marked on day 9 of readaptation. The hypersecretory gastric syndrome, as evidenced in flight animals, is characterized by a higher activity of the chief gastric pepsinogen-producing cells and an increased gastric level of hydrochloric acid during the interdigestive period. The growth of the gastric acid/peptic potential in the flight animals was correlated with an increased level of gastrin, the main physiologic activator of gastric epithelial cells. This series of alterations created prerequisites for increased aggression of gastric juice in relation to gastric mucosa and possible ulceration.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Study of the Digestive Transportation Function of the Small Intestine

Science Discipline
Metabolism and Nutrition

Investigator	Institute
K.V. Smirnov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Pechyonkina, R.A.	Institute of Biomedical Problems
Goncharova, N.P.	Institute of Biomedical Problems
Phillips, R.	Colorado State University

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Control, Vivarium Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Smirnov, K.V.; Phillips, R.; Pechyonkina, R.A.; and Goncharova, N.P.: Study of the Digestion-Transport Conveyor in the Small Intestine. Spacelab Life Sciences-1 Final Report, NASA TM-4706, Aug. 1995, p. 33.

Objectives/Hypothesis
Previous experiments have revealed an increase in the acidic-peptic potential of the stomach and a decline of the functional capability of the pancreas. The purpose of this experiment was to conduct a morphological and biochemical investigation of changes in the mucous membrane of the small intestine after spaceflight.

Approach or Method
The duodenum, jejunum, and ileum sections of the small intestine were removed from flight and control animals, and the mucosa was examined with an electron microscope. Enzymes involved in cavity and membrane digestion (carbohydrases, peptidases, monoglyceride lipase, alkaline phosphatase) were investigated biochemically.

Results
The investigation of the functional status of the small intestine revealed complex changes of enzyme activities. In the system of protein membrane hydrolysis there was a shift of proximodistal gradient dipeptidase activity indicating the compensatory nature of the changes. Analysis of processes of lipid digestion revealed a number of alterations in the digestive pattern manifested as a significant decrease of nonglyceridelipase activity, and an increase of alkaline phosphatase activity in the proximal segment of the small intestine, again reflecting the compensatory and adaptational nature of the alterations. In the carbohydrase enzymatic change no significant alterations were found. Changes of digestive/transport hydrolysis of proteins, fats, and carbohydrates were reversible and functional in nature. The adaptive nature of the rearrangements of membrane digestion was demonstrated by the self-regulatory activity of the digestive system in the distribution of enzyme activities.

Launch Date 6/5/1991 **Landing Date** 6/14/1991

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Effect of Microgravity on the Relations Between Microbiological and Epithelial Tissue and Functions of the Gastrointestinal Tract

Science Discipline
Metabolism and Nutrition

Investigator	Institute
O. Szylit	National Institute for Agronomic Research

Co-Investigator(s)	Institute
Nugon- Baudon, L.	National Institute for Agronomic Research
Amdrieux, C.	National Institute for Agronomic Research
Ravisse, R.	Institut Pasteur

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF), Animal Enclosure Module (AEM)

Selected Publications
Szylit, O.; Nugon-Baudon, L.; Andrieux, C.; Rabot, S.; Meslin, J.C.; Viso, M.: Influence of Microgravity on Different Metabolic Potentials of Intestinal Microflora. Life Sciences Research in Space, Proceedings of the Fifth European Symposium held 26 September - 1 October, 1993 in Arcachon, France. Edited by H. Oser and T.D. Guyenne. ESA SP-366. European Space Agency, 1994, p.433-438.

Objectives/Hypothesis
Intestinal microflora have versatile enzymatic potentials that can interact directly or through its products with the host. Minor modifications of diet or digestive physiology may alter the intestinal microflora equilibrium. Spaceflight conditions may lead to an imbalance in the digestive microflora, thus leading to nutritional and physiological modifications. The objective of this study was to assess the bacterial and endogenous metabolic potentials of intestinal microflora, and to test the hypothesis that the digestive physiology and the detoxication system are altered after spaceflight.

Approach or Method
Cecal contents were collected and pH was measured. Short chain fatty acids (SCFAs) were analyzed using gas-liquid chromatography. Glycosidase activities were expressed as the rate of p-nitrophenol released from its specific precursor. Histochemical analysis was performed on stained duodenum and ileum samples. From these samples, neutral, acid, and sulfomucin containing cells were counted. Mucus-containing cells (MCCs) were counted for 20 crypts and villi in each specimen. Xenobiotic metabolizing enzymes were studied through the determination of microsomal and cytosolic protein concentrations. The activity of glutathione-S-transferases was assayed in duplicate in both microsomal and cytolitic fraction using spectrophotometry with 1-chloro-2,4-dinitrobenzine as a substrate.

Results
There was a slight decrease of pH in the flight group and a significantly enhanced total SCFA concentration. This was due to increases in valerate and branched-chain acids. Effects did not last after a 9-day postflight recovery period. Of the microbial glycolytic activities that were investigated, none were modified by spaceflight. MCC numbers were increased for all types of mucin, with some exceptions. Nine days postflight, a further increase of the number of acid MCC in the villi, and of sulfated MCC in the crypts and the villi, occurred in the duodenum. The specific activity of microsomal glutathione-S-transferase in the flight rats was three-fold enhanced and persisted to a lesser extent in the specimens that underwent a 9-day post-flight recovery period.

Cosmos 2229 / Bion 10

Launch Date 12/29/1992

Landing Date 1/10/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study

Rhesus Monkey Metabolisn During Spaceflight: Measurement of Energy Expenditure Using the Doubly Labeled Water (2H218O DLW) Method

Science Discipline

Metabolism and Nutrition

Investigator	Institute
C.A. Fuller	University of California, Davis

Co-Investigator(s)	Institute
Stein, T.P.	University of Medicine and Dentistry of New Jersey
Griffin, D.W.	University of California, Davis
Dotsenko, M.A.	Institute of Biomedical Problems
Korolkov, V.I.	Institute of Biomedical Problems

Research Subject(s)

Macaca mulatta (Rhesus monkey)

Ground-Based Controls

Synchronous Control, Delayed Synchronous Control, Vivarium Control

Key Flight Hardware

Bion 10 Hardware Suite

Selected Publications

Stein, T.P.; Dotsenko, M.A.; Korolkov, V.I.; Griffin, D.W.; and Fuller, C.W.: Energy Expenditure in Rhesus Monkeys (*Macaca mulatta*) During Spaceflight Using Doubly Labeled Water. Journal of Applied Physiology, vol. 81, no. 1, July 1996, pp. 201–207.

Hoban-Higgins, T.M.; Robinson, E.L.; and Fuller, C.A.: Primates in Space Flight. Advances in Space Biology and Medicine, vol. 10, 2005, pp. 303–325.

Objectives/Hypothesis

In theory, the energy requirements of larger mammals should be decreased in the microgravity spaceflight environment. The objective of this study was to determine the effect of spaceflight on mean daily energy expenditure in Rhesus monkeys.

Approach or Method

The doubly labeled water method for measuring energy expenditure is simple, noninvasive, and highly accurate. If doubly labeled water is given orally, it mixes with the body water in about 3 hours. The two isotopes then leave the body at different rates. (Labeled hydrogen leaves as water, mainly in the urine, whereas labeled oxygen leaves both as water and exhaled labeled carbon dioxide). Thus the turnover rate of isotopic hydrogen and oxygen-labeled water differ, and that difference is proportional to the rate of carbon dioxide production. Urine was used to sample body water. Energy expenditure was measured for the two flight monkeys over a 4-day period preflight. Three days preflight, the monkeys were redosed with doubly labeled water. The urine was sampled again preflight and immediately postflight.

Results

In this experiment, the values for mean in-flight energy expenditure were significantly less than the preflight values. The approximate 30% decrease between the flight subjects and ground controls was found to be statistically significant.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Stressogenic Effect of Microgravity

Science Discipline
Metabolism and Nutrition

Investigator	Institute
A.S. Kaplansky	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Popova, I.A.	Institute of Biomedical Problems
Durnova, G.	Institute of Biomedical Problems

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Basal Controls, Vivarium Controls, Synchronous Controls

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Grigoriev, A.I.; Kaplansky, A.S.; Durnova, G.N.; and Popova, I.A.: Biochemical and Morphological Stress-Reactions in Humans and Animals in Microgravity. Acta Astronautica, vol. 40, no. 1, 1997, pp. 51–56.

Kaplansky, A.S.; Durnova, G.N.; Hinds, W.; and Vorobyova, V.N.: Experimental Morphological Investigation of Stress-Inducing Effects of Microgravity in Rats Flown Aboard SLS-2 (in Russian). Aerospace and Environmental Medicine, vol. 30, no. 2, 1996, pp. 16–20.

Objectives/Hypothesis
The question of whether microgravity has a stressogenic effect on mammals still remains open, because morphological and biochemical manifestations of an acute gravitational stress detected in rats after flight can mask changes induced by spaceflight factors, including microgravity. This experiment examined the sole effects of microgravity stress through dissection of the adrenal and thymus glands midflight. After flight, stress was evaluated through histological and histomorphometric analysis of the glandular tissues.

Approach or Method
Ground control rats were dissected simultaneously with corresponding flight groups. The left adrenal and thymus were dissected, weighed, fixed, and sectioned. Serial adrenal and thymus sections were stained and examined histologically and histomorphometrically. The following parameters were analyzed in the central adrenal sections: total adrenal area; cortical and medullary areas; ratio of the cortical area to the medullary glomerular area; areas of the reticular zones of cortex; and size of cellular nuclei of the fascicular zone of the cortical and medullary portions. The thymus was examined histologically and histomorphometrically. The right adrenals were weighed and analyzed for lipid composition with thin-layer silica gel chromatography. In chromatograms, free fatty acids (FFA), triglycerides (TG), phospholipids (PL), free cholesterol (FCS), and cholesterol esters (CHE) were measured in absolute numbers and as a percentage of total lipids.

Results
In rats dissected in flight (IF), adrenal area, areas of adrenal cortex and medulla, the ratio of cortical and medullary areas, the relation of individual regions of cortex, and sizes of nuclei in cells of the fascicular regions in cortex and medulla were equal to their values in the ground control. Some increase was found in the number of dividing thymocytes in the thymus of IF rats. Yet, F+0 rats exhibited signs of moderately expressed acute stress reaction in adrenal and thymus. In F+0 rats, hypertrophy of adrenals with an expansion of the fascicular region in the adrenal cortex and enlargement of nuclei of its cells was found. Sites of depositing free and phagocyted nuclear detritus and depression of mitotic activity of thymocytes were seen in the thymus cortex. Lipid analysis showed that IF rats, sacrificed during flight, had decreased total lipids, FFA, TG, and FCS.

Launch Date 10/18/1993 **Landing Date** 11/1/1993

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Influence of Microgravity on Rat Digestive Physiology and Xenobiotic Metabolizing System-Interactions With Intestinal Microflora Alterations

Science Discipline
Metabolism and Nutrition

Investigator	Institute
O. Szylit	National Institute for Agronomic Research

Co-Investigator(s)	Institute
Rabot, S.	National Institute for Agronomic Research
Nugon- Baudon, L.	National Institute for Agronomic Research
Meslin, J.-C.	National Institute for Agronomic Research

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Synchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications

Grigoriev, A.I.; Kaplansky, A.S.; Durnova, G.N.; and Popova, I.A.: Biochemical and Morphological Stress-Reactions in Humans and Animals in Microgravity. Acta Astronautica, vol. 40, no. 1, 1997, pp. 51–56.

Kaplansky, A.S. et al.: Experimental Morphological Investigation of Stress-Inducing Effects of Microgravity in Rats Flown Aboard SLS-2 (in Russian). Aerospace and Environmental Medicine, vol. 30, no. 2, 1996, pp. 16–20.

Objectives/Hypothesis
Intestinal microflora possess an extremely versatile enzymatic potential that can interact directly or via its products with the overall body physiology. Spaceflight conditions, which are known to generate modifications of the gastrointestinal and hepatic functions, may also alter functions of the digestive microflora. The objective of this work was to assess the influence of a 14-day spaceflight on several parameters of digestive physiology and microbial fermentation.

Approach or Method
Rats were sacrificed by decapitation. The cecal content, the mucosa of the small intestine and the colon, and the right lobe of the liver were immediately collected and stored under appropriate frozen conditions until analyses. In the cecum, pH was measured and short-chain fatty acids (SCFA), ammonia, urea, and histamine were assayed. The concentration of total cytochrome P-450 (CYP450) was determined in liver microsomes. Glutathione-S-transferase (GST) activity was assayed in both microsomal and cytosolic fractions of the liver and the small intestine. In the colonic mucosa, variations of the number of neutral, acid, and sulfated mucus-containing cells (MCC) were investigated.

Results
The 14-day spaceflight induced a slight acidification of the cecal content ($p < 0.05$) and a 60% decrease of cecal SCFA concentration ($p < 0.05$). Among SCFA, acetate greatly increased (+14%) at the expense of butyrate (–7%) and cumulated valerate, caproate, and isoacids (–7%), whereas propionate remained stable. Cecal ammonia, urea, and histamine were not modified. Spaceflight did not alter GST activity either in the small intestine or in the liver, whereas concentration of hepatic CYP450 was significantly lowered ($p < 0.05$). In the colon, spaceflight led to a 20% reduction of the number of neutral MCC ($p < 0.05$). Microgravity temporarily affected the microbial fermentation and the histochemical structure of the mucosa in the large intestine, because modifications occurring in the flight (RF) group were not observed in the R+14 group, i.e., at the end of the 14-day recovery period. On the contrary, the decrease of hepatic CYP450 observed in RF rats persisted in the R+14 group. These findings, together with those obtained in a previous flight (SLS-1), should help to predict the alterations of digestive physiology likely to occur in astronauts and suggest that microgravity may durably disturb host responses to toxics and drugs.

Launch Date 11/3/1994 **Landing Date** 11/14/1994

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Fluid Electrolyte Metabolism

Science Discipline
Metabolism and Nutrition

Investigator	Institute
L.V. Serova	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Natochin, Y.V.	Institute of Evolutionary Physiology and Biochemistry
Shakhmatova, E.I.	Institute of Evolutionary Physiology and Biochemistry

Research Subject(s)
*Rattus norvegicus (*Sprague-Dawley rat)

Ground-Based Controls
Vivarium Control, Delayed Synchronous Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Serova, L.V.; Nosovsky, A.M.; Natochin, Y.V.; Shakmatova, E.I.; Savelyev, S.V.; Besova, N.V.; and Fast, T.: NIH.R1: The Effect of Microgravity on Embryonic Electrolytes and Skeleton [abstract]. American Society for Gravitational and Space Biology Bulletin, vol. 9, no. 1, 1995, p. 97.

Serova, L.V.; Natochin, Y.V.; Nosovsky, A.M.; Savelyev, S.V.; Chelnaya, N.A.; Shakmatova, E.I.; and Fast, T.: Effect of Weightlessness on the Mother-Fetus System (Results of Embyrological Experiment NIH-R1 Aboard the "Space Shuttle") (in Russian). Aviakosm Ekolog Med, vol. 30, no. 6, 1996, pp. 4–8.

Objectives/Hypothesis
Previous experiments have revealed that an adult organism may well remain functional, and a developing fetus may also develop normal functions in a space environment. However, changes were also observed in space-flown fetuses and animals. In this experiment it was assumed that a longer flight and exposure to microgravity may aggravate these changes. The purpose of this experiment was to accumulate new data concerning the metabolism of water and electrolytes in the placentas and fetuses that developed during spaceflight.

Approach or Method
Animals were laparotomized on the 8th gestational day to measure implantation rate, and unilaterally hysterectomized on the 20th gestation day to remove one uterine horn. Animals were flown from the 9th through the 20th gestational day. One fetus and placenta from the flight, laparotomized vivarium controls, and synchronous groups were analyzed. Fetus organs were dissected, dried to a constant weight at 105°C to measure content, and then ashed in concentrated nitric acid at 90°C. Sodium and potassium were measured in air propane flames using a Corning-410 photometer; calcium, copper, iron, and magnesium were measured in air-acetylene flame using a Hitachi atomic absorption spectrophotometer. Placentas were also analyzed for the same parameters.

Results
Water, sodium, potassium, magnesium, and iron in placentas of the flight dams did not differ from those in synchronous and vivarium dams. Calcium in the placenta of the flight dams was lower than in synchronous dams and identical to that in vivarium controls, whereas copper in the placentas of the flight dams was higher than in the vivarium controls and very similar to that in synchronous dams. Water, sodium, and magnesium in fetuses of the flight group did not differ significantly from those in the synchronous controls and were lower than in the vivarium controls. Potassium, calcium, and copper in fetuses of the three groups were identical. Iron in the fetuses of the flight group was higher than in the synchronous group and identical to that in the vivarium controls. The data obtained allow the conclusion that no changes in water, sodium, potassium, iron, or copper in the fetuses and placentas were observed that can be attributed to the effect of microgravity. Minor changes seen in the flight fetuses and placentas were also observed in the synchronous group.

Bion 11

Launch Date 12/24/1996 **Landing Date** 1/7/1997

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Energy Metabolism of *Macaca mulatta* during Space Flight

Science Discipline
Metabolism

Investigator	Institute
C.A. Fuller	University of California, Davis
T.P. Stein	University of Medicine & Dentistry
V. I. Korolkov	Institute of Biomedical Problems

Co-Investigator(s)	Institute
Hoban-Higgins, T.M.	University of California, Davis
Dotsenko, M.A.	Institute of Biomedical Problems

Research Subject(s)
Macaca mulatta (Rhesus monkey)

Ground-Based Controls
Vivarium Control, Asynchronous Control

Key Flight Hardware
Bion 11 Hardware Suite

Selected Publications
Hoban-Higgins, T.M.; Stein, T.P.; Dotsenko, M.A.; Korolkov, V.I.; and Fuller, C.A.: Energy Metabolism of Macaca mulatta during Spaceflight. Journal of Gravitational Physiology, vol. 7, no. 1, Jan 2000, pp. S145-S148.

Objectives/Hypothesis
Larger mammals should theoretically expend less energy in the microgravity space flight environment than they expend on Earth since they are relieved of the burden of opposing gravity. However, results of human studies have been mixed. This current study was undertaken to determine if energy metabolism is affected by the microgravity environment.

Approach or Method
The doubly labeled water method was used to determine energy expenditure. This method is non-invasive and highly accurate. A baseline urine sample was collected and animals were given doubly-labeled water 72 hours prior to placement in the Bion capsule. Preflight and postflight urine samples were collected and analyzed.

Results
Energy expenditure for the Bion 11 flight animals was slightly higher than had been seen in previous studies conducted in rhesus monkeys inflight. During the Cosmos 2229 flight, the decrease in energy expenditure was correlated with a decreased heart rate compared to the ground controls. In contrast, the Bion 11 flight animals had an increased heart rate during flight compared to the subsequent ground control experiment. Pooled data from Cosmos 2044, Cosmos 2229, and Bion 11, reveals that energy expenditure is significantly lower during space flight than in ground-based controls.

Launch Date 4/17/1998 **Landing Date** 5/3/1998

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Apoptosis in Microgravity-Sensitive Cells After Spaceflight

Science Discipline
Metabolism and Nutrition

Investigator	Institute
W. Rhoten	Marshall University School of Medicine Huntington WV

Co-Investigator(s)	Institute
Parkash, J.	Marshall University School of Medicine Huntington WV

Research Subject(s)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous Control

Key Flight Hardware
Research Animal Holding Facility (RAHF)

Selected Publications
Sergeev, I.N.; Rhoten, W.B.; Chaudhry, M.A.; and Norman, A.W.: Regulation of Intracellular Calcium in Simulated Microgravity. Vitamin D Endocrine System: Structural, Biological, Genetic and Clinical Aspects. A.W. Norman, R. Bouillon, and M. Thomasset, eds., University of California, Riverside, 2000, pp. 719–722.

Sergeev, I.N.; Rhoten, W.B.; and Carney, M.D.: Calbindins Decreased After Space Flight. Endocrine, vol. 5, no. 3, Dec. 1996, pp. 335–340.

Objectives/Hypothesis
The objective of this experiment was to better understand the mechanism of action of calbindins, Ca2+-binding proteins that are involved in regulating biological functions such as gene expression, muscle contraction, bone remodeling, glucose metabolism, and apoptosis. TASK 1: to quantify in situ levels of calbindins in the small intestine, hypothalamic nuclei, kidney, and pancreas. TASK 2: to evaluate apoptosis in the small intestine, hypothalamic nuclei, kidney and pancreas. TASK 3: to determine if levels of renal calbindin-D28k depleted by microgravity are restored to normal after 9 and 14 days at Earth's gravity. TASK 4: to determine pancreatic levels of insulin.

Approach or Method
In situ levels of calbindins, insulin, and glucagon from 10% formalin-fixed tissues of flight rats and their controls were measured by quantitative immunocytochemistry (QICC) and digital video image analysis. Cellular localization of calbindin-D28k was carried out using a monoclonal antibody directed against chicken intestinal calbindin-D28k (clone CL-300, Sigma) and an indirect peroxidase method. Cellular localization of calbindin-D9k was carried out with a rabbit anti-rat intestinal calbindin-D9k. Monoclonal antibodies were used to localize insulin and glucagon. A quantitative in situ immunocytochemical analysis was done to establish differences in percentages of reactive cells and intensity of the reaction product in specific cells, an indication of differences in gene expression for proteins under microgravity conditions. QICC was performed blindly, i.e., without knowing initially the protocol for the groups from which the tissue was derived. The insulin and glucagon content in the pancreas was also determined by using standard biochemical Enzyme-Linked Immunosorbent Assay (ELISA) protocol.

Results
Task 1: Kidney: A significantly higher (by one-way analysis of variance) level of calbindin-D28k was observed in animals in space for 16 days on STS-90 plus 1 day (R+1) compared to asynchronous ground-control rats. Only the flight animals at 1 day postflight exhibited statistically significant differences from the other groups. Duodenum: Levels of calbindin-D28k were consistent across groups. No statistically significant differences were found. Hypothalamus: The flight controls showed weaker neurophysin II immunoreactivity than flight animals; however, the flight group exhibited considerable variability in the density of reaction product. Pancreas: There was little or no expression of calbindin-D28k in Beta-cells of normal pancreatic islets of the rat and no observed differences in calbindin-D28k levels in the pancreatic islets of flight and control animals. Task 2: Duodenum: Apoptosis ranged from 5 to about 11 apoptotic nuclei per 3 microvillus profiles over all experimental and control groups. There was no statistically significant difference by analysis of variance (ANOVA) in apoptotic rates between any groups. Pancreas: Apoptosis was expressed as the number of FITC-positive (apoptotic) beta-cell nuclei per pancreatic islet. The apoptotic rate based upon Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) staining and morphological criteria was low for all islets examined (0–3). There was no statistically significant difference by ANOVA in apoptotic rates between any groups. Hypothalamus: Apoptotic nuclei were only infrequently observed in nerve cells of the hypothalamus in flight and control specimens. There was no statistically significant difference by ANOVA in apoptotic rates between any groups. Kidney: Distal convoluted tubules exhibited few apoptotic nuclei and there was no statistically significant difference in apoptotic rates between any groups. Task 3: ELISA of calbindin-D28k did not confirm the reduction in calbindin-D28k found in an earlier study (Sergeev, et al. 1996). Task 4: insulin concentration in the pancreas head increased following 9 and 14 days of exposure to microgravity. No differences in tail content were found in flight versus control animals.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Effect of Spaceflight on Expression of Metabolic Enzyme Genes in Mice

Science Discipline
Cell and Molecular Biology

Investigator / Institute
V.E. Wotring — Johnson Space Center (JSC)

Co-Investigator(s) / Institute
None

Research Subject(s)
Mus musculus (Mouse)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
None

Objectives/Hypothesis
The objective of this experiment was to investigate the effect of radiation on the liver metabolism of administered medications by identifying liver metabolic enzymes altered during spaceflight or radiation exposure that mimics the spaceflight environment. Crew health could be affected if the actions of medications are altered during spaceflight.

Specific Aim 1: To determine the effect of short-duration spaceflight on expression of liver genes involved in oxidative stress and repair, and those involved in drug metabolism.
Specific Aim 2: In affected genes, determine relationships between protein expression and gene expression.
Specific Aim 3: To compare results from Aims 1 and 2 above with the results of previous pilot study of liver gene expression after ground exposure to gamma irradiation.

Approach or Method
Mice were exposed to either 137 Cs (cesium) (controls, 50 mGy (milligray), 6 Gy, or 50 mGy + 6 Gy separated by 24 hours) or 13 days of spaceflight on STS 135. Animals were anesthetized and sacrificed at several time points (4 hours, 24 hours, or 7 days) after their last radiation exposure, or within 6 hours of return to Earth for the STS-135 animals. Livers were removed immediately and flash-frozen in liquid nitrogen. Tissue was homogenized, ribonucleic acid (RNA) extracted, purified and quality-tested Complementary DNA (cDNA) was prepared from high-quality RNA samples, and used in real-time polymerase chain reaction (RT-qPCR) experiments to determine relative expression of a wide variety of genes involved in general metabolism and drug metabolism.

Results
Results of the ground radiation exposure experiments indicated that ~65 genes of the 190 tested were significantly affected by at least one of the radiation doses. Many of the affected genes are involved in the metabolism of drugs with hydrophobic or steroid-like structures, maintenance of redox homeostasis, and repair of DNA damage. Most affected genes returned to near control expression levels by 7 days post-treatment. Not all recovered completely by the final time point tested: with 6 Gy exposure, metallothionein expression was 132-fold more than control at the 4 hour time point, and continued to fall with increasing time. In contrast, there were other genes whose expression was altered and remained relatively constant through the 7-day period we tested. One example is Cyp17a1, which showed a 4-fold elevation at 4 hours after exposure and remained constant for 7 days after the last treatment. Spaceflight samples were evaluated with similar methods and comparisons were made between the radiation-treated groups and the spaceflight samples. Conclusion: It appears likely that radiation exposure triggers homeostatic mechanisms, which could include alterations of gene expression. Better understanding of these pathways could aid in optimizing medication doses given to crew members who require treatment and eventually, to development of new countermeasures to ameliorate or prevent radiation-induced damage to cells and tissues.

Launch Date 7/8/2011 **Landing Date** 7/21/2011

Physiological/Science Discipline: REGULATORY PHYSIOLOGY Metabolism/Nutrition

Title of Study
Evaluation of the Effect of Short-Duration Spaceflight on Hepatic Nutrition, Oxidative Damage, and Colon Microflora

Science Discipline
Metabolism and Nutrition

Investigator	Institute
S.M. Smith	NASA Johnson Space Center (JSC)

Co-Investigator(s)	Institute
Zwart, S.R.	Universities Space Research Association (USRA)
Morgan, J.L.L.	Oak Ridge Associated Universities (ORAU)

Research Subject(s)
Mus musculus (Mouse)
Rattus norvegicus (Sprague-Dawley rat)

Ground-Based Controls
Asynchronous 48-Hour Delayed Ground Control

Key Flight Hardware
Animal Enclosure Module (AEM)

Selected Publications
Morgan, J.L.L.; Theriot, C.A.; Wu, H.; Smith, S.M.; and Zwart, S.R.: A Comparison of Nutritional Status and Markers of Oxidative Stress in Rats Exposed to Both High Dietary Iron and Radiation With Mice Flown on a 14-d Space Shuttle Mission (STS-135). NASA Human Research Program Investigators' Workshop, Houston, Texas, Feb. 15, 2012, #4149.

Objectives/Hypothesis
During space flight astronauts are exposed to both increased radiation and increased body iron (Fe) stores. Increased body Fe results from a decrease in red blood cell mass and the typically high Fe content of the food system. Radiation exposure and increased Fe status independently cause oxidative damage that can result in oxidation of protein, lipid, and DNA. Here we report two studies evaluating oxidative stress and nutritional status, the first with rats exposed to high dietary Fe and radiation, and the second with mice flown on a 14-day Space Shuttle mission (STS-135).

Approach or Method
In the rat study we investigated the combined effects of radiation exposure (0.375 Gy of Cs-137 every other day for 16 days, a total of 3 Gy) and high dietary Fe (650 mg Fe/kg diet versus 45 mg Fe/kg for controls) on male Sprague-dawley rats (n=8/group). We also investigated the effects of space flight on C57BL/6 female mice (ground control, n=15, and flight, n=7). Blood and liver samples were collected from the rats and liver samples were collected from the mice for analysis of oxidative defense systems and oxidative damage indices, along with Fe and related mineral analyses.

Results
Rats
Liver and serum Fe were significantly increased in the high dietary Fe groups, as expected. Liver selenium (Se) remained the same in all four groups, but serum Se tended to decrease in the radiation-treated group. Likewise, radiation exposure increased serum ferritin and Fe concentrations. Hematocrit decreased in the group exposed to radiation, providing a possible mechanism for the shift in Fe indices after radiation exposure. Markers of oxidative stress were also affected by both radiation and high dietary Fe, evidenced by increased liver glutathione peroxidase (GPX) and serum catalase (CAT) as well as decreased serum GPX. The treatments had no effect on serum or liver superoxide dismutase (SOD) or liver CAT.

Mice
There was no difference in liver CAT or SOD between ground control and flown mice. There was also no difference in liver Fe concentration between the two groups. Liver Se decreased significantly in the flight mice. Selenium is integral to cellular oxidative defense mechanisms, including GPX.

Publications Index

Abraham, S., et al.; Biochemical Changes in Rat Liver After 18.5 Days of Spaceflight. Proceedings of the Society for Experimental Biology and Medicine, vol. 172, 1983, pp. 334–339.

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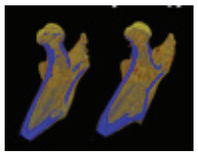
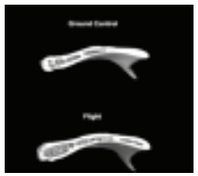
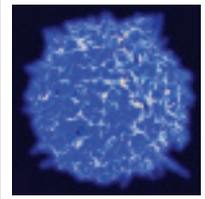
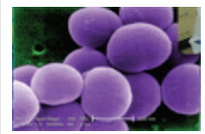
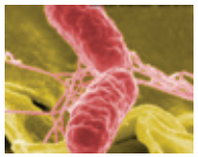
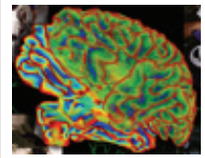
Author and Investigator Index

Image Index

Bone Physiology (p. 21)	
	Rodent Habitat developed at Ames Research Center (ARC) and flown on SpaceX 4. Credit: NASA, Photographer: Dominic Hart
	Bone image from Eduardo Almeida lab, NASA Ames Research Center. Credit: NASA
	Bone image from Eduardo Almeida lab, NASA Ames Research Center. Credit: NASA
	Astronaut Nicole Stott, Expedition 20 flight engineer, installs hardware in the Kibo laboratory of the International Space Station while Space Shuttle Discovery (STS-128) remains docked to the station. Credit: NASA
Cardiovascular/Cardiopulmonary (p. 109)	
	Image of Astronaut Sunita L. Williams, Expeditions 14 and 15 flight engineer, conducts a Surface, Water and Air Biocharacterization (SWAB) air sampling in the Destiny laboratory of the International Space Station. Credit: NASA
	HRP Glenn Harness - New Glenn Harness - The harness was developed by researchers in the Human Research Program's Exercise Countermeasures Project to improve the comfort and loading for crewmembers. Credit: NASA
	The Braslet-M Occlusion Cuff is owned and issued by Russian Medical Operations and is approved for use through their partnership with this SDTO. The Braslet-M is an operational countermeasure that is designed to help redistribute the circulating blood volume, temporarily reducing the amount of blood in the central circulation.
Developmental Biology (p. 138)	
	Public domain photos from the NIH image bank at https://imagebank.nih.gov/
Immunology (p. 199)	
	Blue image in background is an SEM photo of a human T-cell from the NIH imagebank at https://imagebank.nih.gov/
	Sitting at the microscope is Space Biology Principal Investigator, Dr. Millie Hughes-Fulford Credit: NASA
	Bacteria *Staphylococous aureus* from the CDC Public Health Information Database at http://www.cdc.gov/HAI/organisms/staph.html
	Doctor and little girl from the CDC PHIL database at http://phil.cdc.gov/phil/home.asp

Microbial Growth & Virulence (p. 228)	
	Background photo of Salmonella bacterium from the NIH imagebank at https://imagebank.nih.gov/
	Space Biology Principal Investigator Dr. Sheila Neilsen-Preiss preparing her flight samples for the Micro-5 experiment. Credit: NASA
	Space Biology Principal Investigator Dr. Cheryl Nickerson who studies microbial virulence in space. Credit: NASA
Muscle Physiology (p. 246)	
	Astronaut Nicole Stott, a flight engineer who flew on the space station in September, 2009, runs on the Treadmill Vibration Isolation System (TVIS) in the Zvezda Service Module of the International Space Ssytem. Credit: NASA
	Astropnaut Commander Peggy Whitson exercises in the Destiny laboratory on the International Space Station. Credit: NASA

Neurophysiology (p. 308)	
	Colorized image of the neural networks of the brain and fluorescent green nerves from the NIH image bank at https://imagebank.nih.gov/
	Payload Specialist Jay Buckey retrieves support hardware from a locker. Mission Specialist Rick Linnehan trains on the Virtual Environment Generator (VEG). Mission Specialist Dave Williams prepares the rotator chair for operation with alternate Payload Specialist Alex Dunlap as the subject. The crew is training in the Spacelab mockup. Credit: NASA
	Astronaut Robert Thirsk is performing the Canal and Otolith Integration Studies (COIS) experiment. Thirsk is wearing the Binocular Optokinetic Stimulus Goggles to elicit and investigate eye movements associated with nystagmus. Credit: NASA
	Eye stimulation and measurement recording subsystem head unit; the flight hardware used in the Spatial Orientation of the Vestibulo-Ocular Relfex and Velocity Storage experiment flown on the STS-90 shuttle mission. Credit: NASA
	Susan J. Helms, Payload Commander, measures the distance between Jean-Jacques Faviers head and the luminous torque, used for the Canal and Otolith Interaction Study (COIS) on the Life and Microgravity Spacelab (LMS-1) mission. This view shows the Voluntary Head Movement (VHM) segment of the experiment. Credit: NASA
Regulatory Physiology (p. 370)	
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