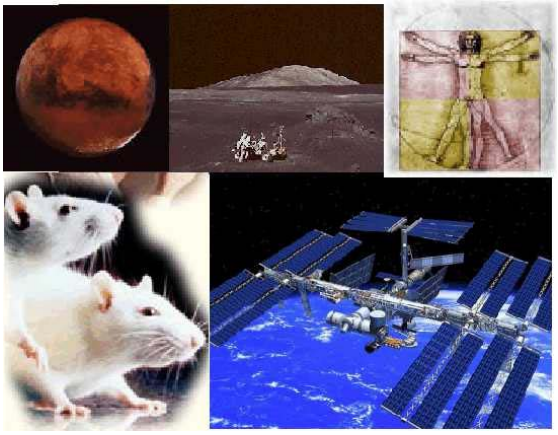

Meeting Report: Animal Research in Support of Human Space Exploration

April 12-16, 2004

A workshop at the
J. Erik Jonsson Center of the
National Academy of Sciences,
Woods Hole, Massachusetts

Co-Chaired by Charles Sawin and Kenneth Souza

Report authored by Diana Jennings, Marine Biological Laboratory, and Kenneth Souza, NASA Ames Research Center

Table of Contents

1. Introduction

1.1 Meeting Overview

In April 2004, a meeting was convened in Woods Hole to determine the necessity and identify the nature of animal research needed to support human space exploration. Using NASA's Bioastronautics Program Critical Path Questions as a guide, participants considered those risks associated with long-term human exposure to spaceflight, especially microgravity. The thirty-five biomedical risks identified in the Bioastronautics Critical Path Road Map (BCPR) were specifically considered. The meeting was structured to address the following questions:

- What animal research is needed to support human space exploration?
- When is it needed?
- How much is needed?
- How can it be done?

Forty-eight individuals gathered for this meeting, which was held at the J. Erik Jonsson Center of the National Academy of Sciences, Woods Hole, MA and sponsored jointly by NASA's Fundamental Space Biology and Bioastronautics Programs. Participants were leading scientists and science managers directly involved with human research and a similar cadre of scientists and science managers that utilize animal models, primarily, but not limited to, rodents. Their home institutions included NASA, universities, medical institutions, and the Russian Space Agency (Institute of Medical and Biological Problems, Moscow). Kenneth A. Souza from NASA's Ames Research Center and Charles Sawin from NASA's Johnson Space Center co-chaired the meeting.

The general format of the meeting was to provide morning tutorials on the human and animal response to spaceflight in such discipline areas as bone, muscle, immunology and microbiology, neuroscience including behavior and performance, nutrition and metabolism and the cardiovascular system. Presentations on the first day covered the use of animal models in biomedical research both on the ground and in space. Afternoon breakout sessions discussed specific research areas and questions where animal research is needed, to determine feasible timelines for the research, appropriate animal models, and general roadmaps to direct the course of the research. Discussion of the radiation hazard associated with spaceflight was not a focus of this meeting, both because of the broad scope of the problem and because similar meetings are planned for this topic.

Science presentations covered the following areas: Animal contributions to human space exploration; Needs for animal research in the following areas: Musculoskeletal, Sensory Motor, Immunology and Disease, Wound Healing, Cardiovascular, Nutrition and Metabolism, Pharmacotherapeutics. Discussion groups focused on these sections but related areas of concern also arose. The presentations, discussions and recommendations of the participants are summarized in this report.

1.2 Summary of Main Recommendations

The overall conclusion reached by the group is that **animal research is an integral tool for understanding and ameliorating the known and yet-to-be-discovered impacts of spaceflight upon the human body. This tool needs to be deployed in the context of a long-term, multi-disciplinary, team-based research program including ground-based and flight-based platforms. Science, and the relevance of a given model system to human physiology, must drive the choice of the research platform and the animal model system to be used.**

Other over-arching conclusions are summarized as follows:

- Animal research was an acknowledged high-priority requirement for all disciplines except nutrition and psychology/behavior
- Research priority is for ground-based studies using a research team approach and saving flight opportunities for high priority and high payoff experiments
- Animal models should be matched to the discipline and science objectives; species, gender, and age must be considered
- Ground-based analogues of flight should be utilized and validated with flight experiments
- Artificial Gravity research with animals both on the ground and inflight is essential to understanding the risks associated with altered gravity environments. Hypogravity research (1/6 g, 3/8 g) experiments in space will be critical to predicting human responses to long term stays on the Moon and Mars
- Animal research and human research should be tightly coupled, e.g. bedrest studies and similar animal model studies should utilize comparable testing regimes
- All flight platforms should be utilized to maximize flight data return: science objectives and the animal model dictate the platform; tissue sharing is highly encouraged and should be worked out before flight
- ISS research facilities should be utilized for experiments requiring crew intervention, and/or specialized ISS facilities, e.g. 2.5M centrifuge, and/or long duration spaceflight.

2. Presentation summaries and Recommendations

NASA's exploration vision was presented to the conferees from two perspectives: that of astronaut Dafydd Williams, and from the Agency's perspective by David Tomko.

Keynote speaker Dafydd Williams presented a first-hand account of the physical challenges of spaceflight and the vagaries of carrying out experimental research on animals in Neurolab (STS-90). Neurolab was NASA's most complex life sciences flight ever, with twenty-six separate experiments. Williams described what it was like to adapt physically to the spaceflight environment, and listed for the group a number of the exploration challenges facing the life sciences community. Some of these challenges are clinical in nature and are further described in section 3.1. Williams noted the different kinds of human and animal studies done on the flight and indicated that many aspects of the spaceflight environment, for example microgravity, are novel and must be overcome to meet both science and operational medicine agendas. On orbit tasks such as animal dissections and human trauma care, including surgery, present unusual challenges in the microgravity environment.

David Tomko presented the Exploration Vision documented in A Renewed Spirit of Discovery, The President's Vision for U.S. Space Exploration. Consistent with the President's Vision for U.S. Space Exploration, NASA has set a new course for exploration and discovery, as summarized in the exploration roadmap, The Vision for Space Exploration . Tomko summarized organizational changes, budget projections, facilities expectations, including International Space Station (ISS) status and configuration, international cooperation, and the agency's aspirations for future Moon and Mars exploration.

Tomko continued by describing the Organizing Questions of the Roadmap prepared by NASA's Office of Biological and Physical Research. Organizing questions 1 and 2 were of particular importance to this conference:

Question 1: How can we assure the survival of humans traveling far from Earth?
Question 2: How does life respond to gravity and space environments?

A description of the Bioastronautics Research Program and the Critical Path questions followed. Animal research can address these questions, so Tomko issued these challenges to the animal research community:

- Apply knowledge and skills to answer high priority questions required to protect human explorers
- Push promising findings to higher Countermeasure Readiness Levels
- Make compelling arguments to NASA management for the rationale for conducting key experiments that answer critical questions

With the Exploration theme set before the group, the meeting Agenda continued with the focus on animal research.

2.1 Animal contributions to Human Space Exploration

For centuries humans have observed animals in order to understand aspects of human biology and function. In modern times, animal research is the gold standard for basic biology and medicine. With animal subjects it is possible to control and reproduce environmental conditions, subject state and protocol, a set of advantages that are often difficult if not impossible to achieve with human subjects. There are needs for sufficient sample size and appropriate experimental timelines that can only be addressed using particular animal systems. Many studies require post-mortem analysis. Moreover, ethical considerations surrounding invasive procedures necessitate the use of non-human subjects.

The use of animals in space biology and medicine began with early suborbital rocket flights and matured with the Russian flight of the dog, Laika, in 1957. As richly shown at this meeting by Eugene Ilyin for the Russian space program, clinical and experimental observations in animals provided the foundation for later human spaceflight. Early spaceflights with animals defined the risks to humans. Subsequently, more sophisticated spacecraft, both U.S. and Russian, were developed to enable more complex scientific investigations. The Bion flights included many with Rhesus monkeys as passengers. Bion flights revealed a deconditioning syndrome of widespread structural, functional and metabolic changes in muscles, bones, cardiovascular, neuroendocrine, neurosensory systems in microgravity. Sounding a theme repeated throughout the rest of the meeting, Ilyin described early studies using rats that showed that artificial gravity as provided by onboard centrifuges countered aspects of deconditioning. Using Bion it was possible as well to provoke and study the development of and recovery from radiation sickness in rats. Ilyin described the community's hopes for future Bion flights; international cooperation and data sharing will be a hallmark of these flights as indeed it has been historically.

Charles Wade focused on the role of animals in biomedical research, stating some of the considerations and requirements governing their use. Animals must receive every consideration for their comfort, being properly housed, fed, and their surroundings kept in sanitary conditions. Experimenters are mandated to minimize discomfort and pain. When using an animal model it must be the case that the model mimics the clinical scenario observed in humans. Wade reminded the audience

> Animals also serve as sentinels in the exposure of humans to novel environments or experimental conditions. In the conduct of experimental investigations, observations made in animals can lead to a better understanding of subsequent risk to humans. For example, the short life span of lower organism often elucidates long-term effects that can aid in the protection of humans.

The assumptions and requirements outlined by Wade regarding the proper use of animals were fundamental to discussions throughout the workshop.

In discussion participants frequently cited the kinds of experiments that would ethically and logistically require use of animals. These would include e.g. bone fracture studies, radiation exposure, and partial gravity/hypergravity studies. Other remarks and recommendations regarding the proper use of animals as experimental models can be summarized as follows:

- All areas considered in this workshop here have animal models that could be used in flight at the present time but many require further ground-based study to refine flight experimental protocols.

- Animal models should be matched to the discipline and science objectives; species, gender, and age must be considered. There is no "one size fits all" animal model. Immunologists for example prefer to use the mouse because the immunological functions and reagents available are closest to those of humans and there are a great number of genetically defined strains available. Numerous bone studies utilize the rat as rat bone shows the same mechanisms for gains and losses and the same response to loading, hormones, et cetera as does human bone (see Frost and Jee, 1992). Muscle biologists make heavy use of the rat; cardiovascular biologists often use the pig heart; sensorimotor studies may best be run with monkeys. One potential area of concern is the model for "adult humans"—many of the rats that have been flown were young rats due primarily to available flight cage size. Their physiology in areas of bone and muscle will be different and as such they may not be as appropriate a human model as adult animals.

- Animal research and human research should be tightly coupled, e.g. bedrest studies and similar animal model studies, e.g., hindlimb unloaded (HU) models, should utilize comparable testing regimes. Throughout the meeting participants called for standard dependent measure test batteries similar to those used for human subjects (such as the Integrated Test Regimen, ITR or Clinical Status Evaluation, CSE). These test batteries would need to be developed and required for animal models.

- Participants emphasized that especially in flight experiments, experimental planning should maximize the science return for every animal. A robust tissue sharing program is needed. Proposals for Biospecimen sharing should be solicited before flight so that recipients of tissue can integrate their experiment objectives into the overall science protocol and thereby get the most science return from each flight animal.

2.2 Science presentations: Musculoskeletal animal research

Since the Skylab missions of the 1970s, it has been known that human muscle changes during even short stays in the microgravity environment (Fitts, Riley and Widrick, 2001). This clinical observation in humans is replicated in experimental models in flight, such as rats (Fitts, Riley, and Widrick, 2000; Tischler et al, 1993), and in ground analogues such

as hindlimb suspension (rat) (Morey et al, 1979) and human bedrest studies (LeBlanc et al, 1992). The most critical biomedical risks associated with muscle changes in spaceflight are specified within BCPR risks numbers 13 and 14.

As reviewed for the conferees by Ken Baldwin, in the spaceflight environment muscle tissue undergoes reductions in mass and strength, shows a shift from slow to fast contractility, and becomes more easily fatigued. These muscle changes can impact ability to complete extravehicular activity (EVA) and lead to muscle injury upon reloading or emergency egress. It is not entirely clear whether these muscle losses are fully reversible. Exercise is a partial countermeasure but which exercise modalities are most beneficial is still being debated.

The number of human subjects with flight experience is relatively limited, so Baldwin and colleagues have developed a rat model that can be used to observe the effects of specific types of contractile exercise on anti-gravity muscles. The protocol introduces resistance training at the initial stages of the unloading stimulus, hindlimb unloading (HU), a widely used model system for the study of various effects of the spaceflight environment. Animals are subjected to specific contractile modes of exercise at the outset of HU in order to prevent or slow down the atrophy process. Specific success in heading off atrophy has been observed (see abstract) and these promising findings in rat are being studied at the tissue, cellular and molecular levels. Baldwin advocates aligning these data with those obtained in bed-rest studies and in flight to develop a better understanding of the mechanisms of atrophy and the development of pharmacological countermeasures for muscle atrophy.

Dan Feeback reviewed for the group historical and recent data involving measurements of human muscle capacity in astronauts in shuttle sorties and long duration missions on Mir and ISS. Many of the muscle deficits seen in flight are observed also in the bedrest model. Feeback outlined numerous exercise or "loading" countermeasures developed by both Russian and US scientists. Feeback also raised the specter of loss of neuromuscular control accompanying muscle atrophy, and described an experimental apparatus that will test motor control in humans.

In his abstract, Feeback states

> Development of truly novel approaches to countering the effects of microgravity on human physiology will require a more complete understanding of the processes that underlie them. Hypothesis driven research using mechanistic approaches will be required to advance the state of knowledge and to provide answers to many of the enabling questions contained within the BCPR. To this end, both animal studies using models of skeletal muscle unloading and cellular/molecular paradigms are fertile ground.

In breakout sessions participants agreed with this assessment.

Recommendations included a reinforcement of the need for studies of muscle atrophy in adult as well as growing rats. Young rats are usually flown owing to restricted

experimental space in spaceflight environments. An additional suggestion was made that time course information for astronauts without the use of countermeasures would be useful, though it is obviously difficult to obtain.

2.3 Science presentations: Sensory Motor Research

In his keynote presentation Dave Williams described a number of neurovestibular studies carried out in Neurolab and also described the disorientation of conducting surgery on rats in the microgravity environment.

Microgravity affects almost all components of sensorimotor system including proprioception, integration, neuromuscular transmission and the muscles themselves. Inessa Kozlovskaya identified specific sensorimotor deficits encountered in microgravity, including deficit and distortion of specific information necessary for building motor programs; deficit and distortion of specific information necessary to control the execution of movements (feedback and feedforward mechanisms); and deficit of nonspecific information necessary to activate specific and nonspecific motor control mechanisms. These and related neurologically related risks are captured within BCPR risks 15, 16, 29 and 30.

She described work in Rhesus monkeys flown on BION flights; the physiological profiles developed from these subjects contributed to better countermeasures for human space crews.

William Paloski continued on the theme of sensory-motor disturbances with a description of his work in human postural and balance control. Upon return to earth, postural stability is disrupted in all crewmembers. This effect of the spaceflight environment will be particularly pronounced following a flight to Mars and subsequent entry into its partial gravity field. Paloski presented to the group an extensive description of artificial gravity, its physics, and potential ways to implement it. First artificial gravity must be validated as an effective countermeasure for effects of the spaceflight environment. It will be important to establish how much AG is needed to maintain physiological function/ performance. Moreover since a rotation will likely be used to generate AG, acceptable and/or optimal ranges for radius and angular velocity of a rotating space vehicle or centrifuge must be identified.

In discussions and breakout sessions participants explored the potential animal models for the study of sensory-motor systems in microgravity. Though many in this conference felt that invasive testing of sensory-motor integration and adaptation would require Rhesus monkeys, others noted that there are ways in which rats could also be used. Russian scientists have investigated and documented adult rat instability and balance problems on unstable platforms (Cosmos 936, Gurovsky et al, 1980); this is one model system that could be extended. Those advocating Rhesus monkey research advocated the use of free-fliers, such as those used in Bion. Prior to flight studies all reasonable data obtainable from ground-based studies should be collected and analyzed. Russians, for example, conduct sensory-motor studies using Rhesus and the dry-immersion platform,

ie., suspending the animal in a water bath within a protective sleeve that keeps the animal dry while floating.

With the focus on neuroscience, Charles Fuller presented data indicating a role of the vestibular system in mediating microgravity-induced changes in the circadian timing system. The removal of a loading stimulus to vestibular structures could result in disrupted sleep patterns, BCPR risk number 31. It should be mentioned that Michael Delp reported changes in blood circulation to vestibular portions of rat brain in hindlimb-suspended rats; and Gay Holstein reported anecdotally the finding of dead cells in vestibular portions of brain from rats flown on the Neurolab mission Fuller also discussed artificial gravity as a follow on to previous mentions of this potential countermeasure; by providing a vestibular stimulus it would be hypothesized that artificial gravity would alleviate disruptions of circadian activity. This countermeasure could be assessed in flight using rats.

Conference participants repeatedly advanced the idea that animal research remains the best avenue for understanding the effects of changes in gravitational force. Artificial gravity, partial gravity (e.g. Mars and Moon gravities) and transitions in gravitational force are all parameters that are most conveniently and ethically studied first in animals, especially rat and rhesus monkeys. A critical facility for investigating all these factors is the ISS 2.5 M centrifuge contained within Centrifuge Accommodation Module, or CAM.

2.4 Science Presentations: Bone

Bone mass loss, increased fracture risk, and impaired fracture healing (BCPR risks 1, 2 and 3) were phenomena considered by Emily Morey-Holton. She summarized both human and animal research on the effects of the microgravity environment on bone tissue. In essence, bones that experience the highest loads on Earth are most directly impacted by unloading, whether the unloading occurs in space, HU in rats, or bedrest in humans. Bone integrity can be weakened in part by muscle atrophy. Vertebral disk composition is altered (rats, Foldes et al, 1996). With widespread adaptations of the system the individual is more likely to sustain risk for fractures, torn muscles and ligaments, and herniated vertebral disks upon reloading.

The certainty of bone loss poses a potentially grave risk to mission scenarios that involve extended exposure to microgravity followed by rapid reloading, as in traveling to Mars and then landing on the Martian surface with its 3/8 Earth gravity field. Morey-Holton advocated accelerated research for development of countermeasures for bone loss. An integrated test regime should be developed for animal subjects; she proposes that adult, not juvenile rats be used and that measurements in animals should track those used for humans.

Very little is known about the nature of bone healing in microgravity. Only two flight studies (e.g. Kirchen et al, 1995) have been conducted and the fractures were initiated before flight. Both studies indicate that bone healing is impaired. Morey-Holton advocates the immediate use of HU studies in rats to investigate bone healing, and that

such studies should involve rats already adapted to HU. Her abstract (see Appendix 5) describes a number of ground-based investigations that should be conducted in preparation for spaceflight studies, potentially involving the CAM as well to determine whether lunar gravity could maintain bone tissue health.

The group as a whole considered her presentation and concluded that long duration missions require development of proper countermeasures to bone loss, including consideration of artificial gravity.

2.5 Science Presentations: Immunology and Disease

Several speakers addressed risks associated with immunology and disease, and the conferees generally found animal research to be particularly necessary in this area. Immunology and disease risks are cited in the BCPR as numbers 8,8,10,11, and 12.

The spaceflight environment is a major challenge to mammalian immune systems as evidenced by studies monitoring astronauts, and using animal and cell culture models. As Clarence Sams reviewed, closed environmental systems, recycled air and water, limited personal hygiene, and stress combine with direct and indirect effects of microgravity on pathogen growth and human physiology to result in greater risk of infection.

In flight, rats and rhesus monkeys are currently the primary animal species for immunological studies; on ground, mice are used as well. Gerald Sonnenfeld reviewed basic space immunology. A number of effects are found, such as disruptions of leukocyte blastogenesis, cytokine production, and natural killer cell activity. Possible mechanisms for these effects are numerous: exposure to microgravity, stress, radiation, disruption of circadian rhythms and other systems. Sonnenfeld described ground-based work with mice in his laboratory, using hindlimb unloading to mimic the spaceflight environment with Swiss/Webster strain mice. Measurements of various immune system parameters in this model mimic spaceflight results; Sonnenfeld has gone on to show the efficacy of at least one nutritional countermeasure, Active Hexose Coordinated Compound.

Clarence Sams described work in human subjects including that conducted in the spaceflight environment, in submarine crews, and Antarctic over-wintering individuals. Focusing on spaceflight, Sams noted that during flight investigators have measured decreased cell-mediated immune function, reactivation of latent viruses, and altered in vitro lymphocyte activation & migration. Sams advanced the hypothesis that deficits like these are directly the result of activation of stress responses mediated through the pituitary-adrenal hormones CRF, ACTH, cortisol and others. He concluded by advocating that direct controlled infection of animals is needed to understand the degree of increased risks.

Daila Gridley noted that challenging animals with multiple stressors will mimic the effect of the spaceflight environment on the immune system. She particularly advocated the mouse, which is widely used for immunological research. Animal research, she noted, has resulted in resolution of numerous immune-related medical challenges, including

anaphylaxis, phagocytosis, importance of histocompatibility antigens, monoclonal antibody production, pathogenesis of oncogenic viruses and the curative effects of penicillin and other drugs. With these successes it is clear that animal models have a track record in identifying mechanisms and curative agents for immune challenges in humans, and spaceflight should be no exception.

The immune system compromised during space flight could result in enhanced development of tumors due to impaired host defenses. This, combined with exposure to new forms of radiation in interplanetary missions, could result in increased risk to crews of long-term tumor development. Although this would not compromise mission performance or outcome, it could have a long-term negative effect on crew health. Animal models could be effectively used to study the combined effects of radiation and space flight conditions on the immune system and resistance to development of tumors.

While microgravity negatively affects the immune system it appears to also directly enhance microbial growth and microbial virulence. Mark Ott presented work related to the estimation of risk of infectious disease noting that astronauts on long duration missions will have an increased risk for microbial contamination "due to the increased use of regenerated water and air systems, an inability to thoroughly disinfect the vehicle, and the difficulty in timely identification of microbial contaminants and infectious agents" (see abstract). Identification of the microbial inhabitants of Mir, for example, include dust mites, protozoa, spirochetes, and a variety of bacterial species (Ott et al, 2004), while investigations of the International Space Station show the presence of *E. coli, S. marcesens*, and other medically important organisms such as *Pseudomonas* and *Staphlococcus* (Castro et al, 2004). Ott and his colleagues have used cell culture in the rotating wall vessel to evaluate changes in gene expression of diverse microorganisms, such as *Salmonella.* In the final analysis, however, virulence and risk are the outcome of interactions between the organism, the environment and the host animal. Thus animal models are critical for understanding risk factors related to microbial contaminations.

Microbial virulence was also the theme of the presentation by David Niesel, with a focus on opportunistic bacteria. The virulence of *Streptococcus pneumoniae*, commonly cultured from crew members before flight, was enhanced following culture in the bioreactor, a microgravity analogue, as shown by animal studies in mice. Gene expression is upregulated for parts of the *Streptococcus* genome that confer antibiotic resistance and produce adhesin, a molecule that mediates adhesion to cell surfaces. Concludes Niesel (see abstract):

> It is becoming clear that low shear forces and gravity represent mechanical force environmental signals that bacteria "sense" which can lead to adaptive activities requiring altered gene and protein expression and subsequently to new properties. Significant questions remain to be answered to understand the behavior of microbes in the space environment. We do not know the full virulence potential of opportunistic bacteria during long term space flight or during the habitation of space… the significant question of the interaction of these microbes with host cells in microgravity environments remains to be rigorously examined.

2.6 Science presentations: Wound healing

Because of the ethical considerations involved the area of wound healing (BCPR risk 20) emerged as a particularly strong candidate for animal research. Ray Vanderby reviewed direct (e.g. Davidson et al (1998); Kaplansky, et al (1991); Stauber, et al (1992); Turner (2000), Ilyina-Kajueva and Burkovskava (1991)) evidence that wound repair in microgravity is impaired. Indirect evidence from ground-based clinical observations are also of import:

> Reduced mechanical stimulation, poor perfusion, inadequate immune response, sepsis, etc. altered peripheral nerve activity are all associated with delayed wound healing, non-healing, or excessive fibrosis. All of these conditions exist, to some extent, during spaceflight…Each of the above microgravity-induced, physiologic compromises has been shown to degrade wound healing in ground-based or clinical studies. It is likely that a combination of these factors contribute to wound-healing problems associated with spaceflight. So, a limited strategy of addressing only one (e.g. only mechanical or only immune deficits) may prove unsatisfactory. More comprehensive treatment strategies may be necessary, but they will take more effort to formulate. *Such strategies will require the extensive use of animal models*(see abstract, emphasis added).

While much is unknown about wound healing in flight, at least one broadly-used ground model is of relevance to this critical risk. Hindlimb unloading in rat produces a number of the factors that are known to inhibit wound healing. Vanderby presented evidence from his own laboratory regardingligament repair. Rat ligament is surgically acceptable, convenient to test mechanically, and shows soft tissue behaviors typical for wound healing. Ruptured ligaments in suspended animals showed disorganized healing patterns. Countermeasures, such as growth factors, can easily be screened in this assay.

Meeting participants urged that animal models be put to use as soon as possible to look at wound healing in a broad array of injuries, including burns, cuts, fractures and contusions. A variety of research questions were considered, e.g. whether wound healing would be compromised the longer the time spent in space, i.e. would wound healing occur differently if the wounding occurred early rather than late in flight? The ISS was considered by the group to be an important platform for flight-based wound healing research as it is the only platform in which long duration stays in the microgravity environment are currently possible.

2.7 Science presentations: Cardiovascular

Cardiac deconditioning in microgravity affects the astronaut in various ways and is thus characterized within critical path risks numbers 5 and 6. Cardiac effects include orthostatic intolerance, cardiac dysrhythmias, and diminished aerobic and work capacity as well as less obvious and ubiquitous effects on bone, muscle, pharmacodynamics, the nervous system, and others (see Delp, abstract).

Cardiovascular risks reviewed by Jan Meck included orthostatic hypotension and presyncope. Multiple factors play into the expression of these phenomena, including readily addressable ones such as dehydration, and more complex factors such as loss of neural control of blood pressure, gender, flight duration and others. Longer duration flights are more likely to result in orthostatic hypotension and presyncope; female astronauts are more prone to problems in this area than are males. The importance of animal models became particularly clear when Meck reviewed multiple lines of evidence indicating that hindlimb suspended rats provide useful information on mechanisms subserving neural control of blood pressure (e.g. Moffitt et al, 2002).

A new risk has been identified in recent years following extended duration flights first on Mir and the later on ISS. Changes in cardiac function including the development of abnormal cardiac rhythms have been noted in several astronauts. For investigations aimed at mitigating this risk, Meck suggests that HU pigs may actually serve as a more compelling animal model than the rat. The pig heart is anatomically similar to the human heart and is presently used in studies of ischemic heart disease (Swindle and Adams, 1988).

Other cardiovascular changes in spaceflight are of concern as well. Michael Delp described data indicating disrupted vascular function in HU rats. Cardiac output changes and blood flow redistributes, critical for understanding pharmacodynamics. Changes in peripheral vasoconstriction can impact wound healing. Delp reported currently unpublished observations of lower cerebral blood flow to brain regions involved in controlling circadian rhythm, gait and vision. These dsyfunctions need to be validated in flight and moreover cannot be imaged in humans with current technologies. Delp also reported that skeletal vascular alterations occur which could impact fracture healing. The group was very interested in Delp's overall proposal that many changes might be secondary to vascular changes. There are important changes noted on ground in small blood vessels but little is known about changes in these vessels in flight. All agreed that there is a strong need to understand these systemic circulatory effects and their impact on a wide array of critical risks. They also agreed that animal research would play key role in reducing the critical risks.

2.8 Science Presentations: Nutrition/Metabolism

Nutritional deficiencies (BCPR risk 18) play an increasingly important role both directly and indirectly as mission durations increase, pointed out T. Peter Stein. The negative energy balance typically seen in long duration spaceflight will have direct impacts on physical performance, wound healing, muscle growth and repair, immune function, and other areas. Notes Stein, "The total phenomenon has not been reproduced in any ground based model – human or animal" (see abstract). Effective countermeasures are required but will be difficult to develop because nutritional findings tend to be mission specific. Stein sees no role for animals in developing nutritional countermeasures.

Animals do have a role in elucidating the endocrine response to space flight. Endocrine relationships have a crucial role in regulating metabolism and pharmacological interventions are often targeted at the endocrine system. In discussion groups there was support for animal research in endocrine and metabolic aspects of spaceflight.

2.9 Science Presentations: Pharmacotherapeutics

Lakshmi Putcha presented data on the use of drugs in space by crew members, and identified multiple areas of concern about drug efficacy in the spaceflight environment. Putcha et al (1999) observed that 94% of astronauts take some kind of drug in flight. Evidence of altered delivery, actions and metabolism has been reported (Srinivasan et al, 1994). For example, sedation efficacy is reduced (Bagian and Ward, 1994), and drugs have altered bioavailability (Tietze and Putcha, 1994).

Drug effects depend upon rates of absorption, distribution, metabolism and elimination, but space flight-induced changes in blood flow (e.g. those described by Delp) and changes in organ function have largely unknown implications for pharmaceutical efficacy (Czarnik and Vernikos, 1999). Another area of concern is the stability of pharmaceuticals in the face of exposure to radiation.

In discussion conferees agreed that this area would benefit from further animal research, noting that fairly straightforward studies with rats could be conducted first on ground and then on the shuttle and ISS. Based on the widespread impacts of the microgravity environment some concern was expressed about the pharmaceutical efficacy, and some called also for an investigation into the effects of radiation on frequently-used drugs using the facilities at Brookhaven National Laboratory.

3. Other areas of Discussion and Recommendations

3.1 Operational Medicine

A key challenge for longer term off-world missions is the inevitable need for emergency medical care, and with that need is evidence that such care will be difficult to provide and not as effective as it is on Earth. Physiological changes suggest an impaired ability to withstand major systemic trauma (Kirkpatrick et al, 1997). Testing in parabolic flight suggests increased danger with standard surgical techniques (Campbell et al, 2002). Dave Williams, himself a trauma physician, described some of the difficulties in carrying out surgery during the Neurolab flight, and mentioned quirks of the microgravity environment that makes surgery riskier, e.g. the tendency towards formation of air bubbles in intravenous lines. Others have called for the qualification of equipment and protocols requiring evaluation in continuous microgravity (McCuaig and Houtchens, 1992).

Williams indicated several challenges in the arena of operational medicine:

- Determination of level of clinical capability required to support exploration class missions
- Evolution of space medicine from experience based practice to evidence based practice with defined clinical skill set
- Development of novel technologies to enhance clinical capability of space surgeons

Based on descriptions of difficulties executing medical procedures and the documented impact of microgravity on various biological phenomena, including wound healing, the conferees recommended that animal models be used to develop improved clinical procedures for medical emergencies.

3.2 Policy issues

The Bioastronautics Critical Path Roadmap (BCPR)

Bioastronautics is the study and management of the effects of exposure to space flight on humans, and the Bioastronautics Critical Path Roadmap (BCPR) has been developed to address several areas:

- It provides the framework to identify the most important risks to crews exposed to the hazardous environments of space
- It guides the implementation of research and technology development strategies to prevent or reduce those risks
- It will enable a formal critical path analysis in the future

Charles Sawin described the BCPR to the participants, outlining its history and stating the assumptions behind the list of critical risks and their associated questions. He presented to the group a spreadsheet that lists the critical risks and associated issues/parameters for each, including an estimate as to whether reducing each risk requires animal research.

Throughout the meeting participants referred to and discussed the spreadsheet, a portion of which is attached in Appendix 3. Conferees expressed the broad concern about the flexibility of the Critical Path Roadmap document, noting that it should be a living document even after reviews such as the current Institute of Medicine Review. Participants thought that risk status should be modifiable, and that risks could be added or retired.

Some commented on the split between radiation and other potential causes of disease, for example in the case of cancer, which could be due to immune system failure or radiation (risks 9, 31). As noted earlier, a discussion of the radiation hazard was not a focus of this meeting though it was acknowledged as an area in which animal models can play a particularly important role.

NASA's relationship with the Life Sciences Community

Throughout the meeting participants reflected on how the changing vision of the Agency affected the life sciences community. Early on participants expressed that this community has to be made more aware of the changes being made in the Agency, that a paradigm shift has occurred, i.e., a shift to greater emphasis on research directly applicable to the Human Exploration Initiative.

Breakout groups questioned and commented on the mechanisms for conducting human space exploration-based research. Among the questions they posed in a general way were:

What is the role of fundamental versus directed research in the agency?
How does NASA envision running these directed programs? The sense was that the NIH R01 process would not work. Some commented that investigator-initiated research groups should be formed, cross or within discipline, let that group formulate its research with NASA providing the overall mission and direction. One group commented:

> We really need to have a team approach i.e. that espoused by Williams including scientists, flight surgeons and engineers. We need more general team-based simulation studies on the ground in order to teach us how to do these team-generated studies in flight.

Others expressed concern about meeting the timeline of the President's vision given the engineering constraints, and the loss of up/down mass with decommissioning of Shuttle in 2010 with no alternative for getting adequate equipment and specimens up and down.

3.3 Flight Platforms

Shuttle. The group noted that there are some areas of research that can benefit from the short mission durations of the Shuttle. Again, the scientific goals should specify the platform to be used.

The group commented very frequently about the limited flight opportunities given the present shuttle grounding and expressed, as indicated above, their deep concern about Shuttle decommissioning in 2010.

Free Flyers. Because of the potential for extended flight times and also because of the exposure to radiation, a known risk of spaceflight, and because of the long and successful history of free flyer lines such as Cosmos, Bion, Photon and others, the group strongly emphasized the utility of this platform.

It was suggested that the free flyer be considered as a "proof of concept" analogous to that needed for FDA licensure procedures in order to go forward with human research, i.e. validate the preclinical ground-based studies in animals, cells and tissues.

Though most felt that mice and rats would be suitable for Bion flights, others advocated non-human primate flights using rhesus monkeys and examining a number of physiological parameters. One group advocated that following a well-designed set of ground-based studies, a minimum of 6 flights, with 2 monkeys per flight would be needed. The first two subjects would be considered as "learning curve" subjects, leaving the remaining flights with an N of 10 for analysis. The group felt that it is very unlikely that monkeys will be flown on the ISS in the foreseeable future. Therefore, they argued that the Bion flights would be excellent for experiments requiring monkeys, e.g., primarily sensory-motor disturbances. Free flyers are also an excellent venue for the study of infectious disease as there would be no risk of exposure to crewmembers.

The ISS. Louis Ostrach presented a short report on the status of the rodent facilities for the ISS. The Life Sciences Advisory Sub-committee was requested to convene an ad hoc Task Force to address issues about development of the rodent habitat and generate a recommendation for consideration by the Fundamental Space Biology Division Director. This Task Force endorsed the use of adult rats for the initial studies on ISS, strongly recommended the building of a mouse facility, and advised NASA to implement a strategic plan to continue to fly both rats and mice on Shuttle middeck lockers. Moreover, the task force advised that NASA should build or negotiate use of a freeflyer. The participants of this conference concurred with these recommendations.

In general, the group acknowledged that because of present limitations on up/downmass there would need to be some selection criteria in place to prioritize research on station. These would include experiments that require long time spans, experiments requiring human intervention over long periods of time, gravity dose experiments assuming availability of the CAM, centrifuge, and integrative studies.

3.4 Prioritization

For policy and planning purposes, the discussions and presentations offered at this workshop present an opportunity to prioritize animal research studies though such a prioritization was not a specific charge to the workshop participants. At the conclusion of the workshop a subset of the original participants talked in greater detail about the specific priorities for animal research with respect to the BCPR. The comments of this group, when combined with the presentations and discussions throughout the meeting, provide the basis for the Summary of Needs for Animal Research shown in Appendix 4.

4. Conclusions

Based on the presentations and discussions of this group of participants, animal research is an essential part of the program of life sciences research that will be required to enhance the safety of humans as they venture off-world to extended stays, on the ISS, the

Moon and extended travel to Mars. All areas of inquiry would benefit from the tools that have already been developed for the study of life processes in animals. All areas of inquiry will benefit from the scientific rigor that can be imposed on studies involving animal, as opposed to human, subjects in both the spaceflight and ground-based environments. Moreover, this workshop clearly demonstrated the value of meetings in which representatives of NASA's full life sciences program, including operational medicine, share their knowledge and experience, their research data, and their opinions. NASA is encouraged to continue to sponsor focused multidisciplinary workshops like this one and to initiate regular full program meetings in order to promote communication throughout the space biology and biomedical communities.

5. References

Bagian, JP, Ward, DF. (1994) A retrospective study of promethazine and its failure to produce the expected incidence of sedation during space flight. Journal of Clinical Pharmacology, 34:649-651.

Bush, George W. (2004) A Renewed Spirit of Discovery: The President's Vision for Space Exploration. Available at http://www.whitehouse.gov/space/renewed_spirit.html.

Kirkpatrick AW, Nicolaou S, Campbell MR, Sargsyan AE, Dulchavsky SA, Melton S, Beck G, Dawson DL, Billica RD, Johnston SL, Hamilton DR. (2002) Percutaneous aspiration of fluid for management of peritonitis in space. Aviation and Space Environmental Medicine. 73(9):925-30.

Castro, VA, AN Thrasher, M Healy, CM Ott, and DL Pierson. 2004. Microbial characterization during the early habitation of the International Space Station Microbial Ecology (In Press)

Czarnik, TR, Vernikos, J. (1999) Physiological changes in spaceflight that may affect drug action. Journal of Gravitational Physiology, 6(1):161-164.

Davidson JM, Aquino AM, Woodward SC, Wilfinger WW. (1999) Sustained microgravity reduces intrinsic wound healing and growth factor responses in the rat. The FASEB Journal. 13:325-329.

Fitts, RH, Riley DR, and J. Widrick. (2000) Microgravity and skeletal muscle. Journal of Applied Physiology, 89: 823-839.

Fitts, RH, Riley DR, and JJ Widrick. (2001) Functional and structural adaptations of skeletal muscle to microgravity. The Journal of Experimental Biology, 204: 3201-3208.

Foldes I, Kern M, Szilagyi T, Oganov VS. (1996) Histology and histochemistry of intervertebral discs of rats participated in spaceflight. Acta Biologica Hung. 47(1-4):145-56.

Frost, HM and WSS Jee. (1992) On the rat model of human osteopenias and osteoporoses. Bone Mineral 18: 227-236.

Gurovsky NN, Gazenko OG, Adamovich BA, Ilyin EA, Genin AM, Korolkov VI, Shipov AA, Kotovskaya AR, Kondratyeva VA, Serova LV, Kondratyev YuI. (1980). Study of physiological effects of weightlessness and artificial gravity in the flight of the biosatellite Cosmos-936. Acta Astronauta 7(1): 113-121

Ilyina-Kakueva IE, Burkovskaya TE. (1991) The microgravity effect on a repair process in M. soleus of the rats flown on Cosmos-2044. Physiologist 34: S141-2.

Kaplansky AS, Durnova GN, Burkovskaya TE Vorotnikova. (1991) The effect of microgravity on bone fracture healing in rats flown on Cosmos-2044. Physiologist 34: S196-9

Kirchen ME, O'Connor KM, Gruber HE, Sweeney JR, Fras IA, Stover SJ, Sarmiento A, Marshall GJ. Effects of microgravity on bone healing in a rat fibular osteotomy model. Clinical Orthopedics. 318:231-42

Kirkpatrick AW, Campbell MR, Novinkov OL, Goncharov IB, Kovachevich IV. Blunt trauma and operative care in microgravity: a review of microgravity physiology and surgical investigations with implications for critical care and operative treatment in space. Journal of the American College of Surgeons 184(5):441-53.

LeBlanc, AD, Schneider, VS, Evans, HJ, Pientok, C, Rowe, R, and E. Spector. (1992) Regional changes in muscle mass following 17 weeks of bed rest. Journal of Applied Physiology, 73:2172-2178.

McCuaig KE, Houtchens BA. (1992) Management of trauma and emergency surgery in space. Journal of Trauma. 33(4):610-25.

Moffitt JA, Heesch CM, Hasser EM. (2002) Increased GABA(A) inhibition of the RVLM after hindlimb unloading in rats. Am J Physiol Regul Integr Comp Physiol 283(3):R604-14

Morey ER, Sabelman EE, Turner RT, Baylink DJ. (1979) A new rat model simulating some aspects of space flight. Physiologist 22(6):S23-4.

O'Keefe, S. (2004) The Vision for Space Exploration. Available at http://www.nasa.gov/pdf/55583main_vision_space_exploration2.pdf

Ott, CM, RJ Bruce, and DL Pierson. 2004. Microbial characterization aboard spacecraft: Evaluation of the Mir space station Microbial Ecology (In Press)

Putcha, L. (1999) Pharmacotherapeutics in space. Journal of Gravitational Physiology 6(1):165-8.

Srinivasan RS, Bourne DW, Putcha L. (1994) Application of physiologically based pharmacokinetic models for assessing drug disposition in space. Journal of Clinical Pharmacology. 34(6):692-8.

Stauber WT, Fritz VK, Burkovskaya TE, Ilyina-Kakueva EI. (1992) Effect of spaceflight on the extracellular matrix of skeletal muscle after a crush injury. Journal of Applied Physiology. 73(2 Suppl):74S-81S

Swindle, M.M., Adams, R.J, eds. (1988) Experimental surgery and physiology: Induced animal models of human disease. Baltimore: Williams and Wilkins, pp. 74-113.

Tietze KJ, Putcha L..(1994) Factors affecting drug bioavailability in space. Journal of Clinical Pharmacology. 34(6):671-6.

Tischler, ME, Henriksen, EJ, Munoz, KA, Stump, CS, Woodman, CR and CR Kirby. (1993) Spaceflight on STS-48 and Earth-Based Unweighting produce similar effects on skeletal muscle of young rats. Journal of Applied Physiology, 74:2161-2165.

Turner RT. (2000) What do we know about the effects of spaceflight on bone? J Appl Physiol 89(2): 840-7

6. Appendices

1. Agenda and Notes on Workshop Structure
2. List of participants
3. BCPR critical risks
4. Summary of Needs for Animal Research
5. Abstracts

Appendix 1: Agenda and Workshop Format

Animal Research in Support of Human Space Exploration

AGENDA

Unless noted, all events will take place on the campus of the J. Erik Jonsson Center for the National Academy of Sciences (NAS)

Monday, April 12th:

7:30-10:00 p.m.	RECEPTION	Meigs Room, Swope Conference Center

Tuesday, April 13th:

7:30 a.m.	BREAKFAST	Main House, NAS
8:30 a.m	Welcome: meeting purpose and plan *C. Sawin, K. Souza, D. Jennings*	Carriage House, NAS
9:00 a.m.	Spaceflight: The Human Experience *D. Williams*	
9:45 a.m.	The Exploration Initiative: What is the role of animal research? *D. Tomko*	
10:30 a.m.	BREAK	
10:45 a.m.	Animal Models for Biomedical Research *C. Wade*	
11:30 a.m.	Animal Contributions to Human Spaceflight *E. Ilyin*	
12:15 p.m.	LUNCH	Main House, NAS
1:15 p.m.	Report on the Rodent Advisory Team for AAH: Design Path for Rodent Habitat *L. Ostrach*	Carriage House, NAS

1:30 p.m.	Critical Path Roadmap: Critical Questions *C. Sawin*	
2:15 p.m.	Breakout group assignments and charge Co-chairs *Leads: Gerald Sonnenfeld, William Paloski, and Charles Wade*	
2:30 p.m.	Breakout group discussions	Main House, NAS
3:45 p.m.	BREAK	Carriage House, NAS
4:45 p.m.	Breakout leads report	
5:15 p.m.	Adjourn	
5:30 p.m.	MIXER	Main House
6:30 p.m.	DINNER	Main House

Wednesday, April 14

7:30 a.m.	BREAKFAST	Main House, NAS
8:30 a.m.	Spaceflight Effects on the Musculoskeletal System *K. Baldwin*	Carriage House
9:15 a.m.	Muscle Research and Human Space Exploration: Current Progress and Future Challenges *D. Feeback*	
9:45 a.m.	Bone Research and animal support of Human Space Exploration: Where do we go from here? *E. Holton*	
10:15 a.m.	BREAK	
10:30 a.m.	Spaceflight effects on the Sensory-motor System *I. Kozlovskaya*	
11:15 a.m.	Sensory-Motor Adaptation to Space Flight: Human Balance Control and Artificial Gravity *W. Paloski*	
11:45 a.m.	Integrative Countermeasure Development: Neural Regulation and Animal Models *C. Fuller*	
12:15 p.m.	LUNCH	Main House, NAS

1:15 p.m.	The use of Animal models to Study the Effects of Space Flight on the Immune System *G. Sonnenfeld*	Carriage House
2:00 p.m.	Focused abstract – human immunological research *C. Sams*	
2:30 p.m.	The Immune System: Value of Animal Research *D. Gridley*	
3:00 p.m.	Charge to Breakout groups and afternoon break Co- chairs	
3:15 p.m.	Breakout discussions	Main House, NAS
5:00 p.m.	Breakout Leads report	Carriage House
5:45 pm.	Adjourn	
6:00 p.m.	MIXER	Main House,NAS
6.30 p.m.	DINNER	

Thursday, April 15

7:30 a.m.	BREAKFAST	Main House
8:30 a.m.	The Use of Animal Models for the Microbial Risk Assessment of Long Duration Spaceflight *M. Ott*	Carriage House
9:15 a.m.	*Streptococcus pneumoniae* Gene expression and Virulence Potential in Low Shear and Modeled Microgravity Environments *D. Niesel*	
9:45 a.m	Wound Healing in Microgravity: Issues and Animal Models *R. Vanderby*	
10:15 a.m.	BREAK	
10:30 a.m.	Nutrition & Metabolism: an Overview *P. Stein*	
11:15 a.m.	Pharmacotherapeutics in Space *L. Putcha*	

11:45 a.m.	LUNCH	Main House
1:00 p.m.	Cardiovascular risks: Overview *J. Meck*	Carriage House
1:45 p.m.	The Pervasive Effects of Microgravity on the Cardiovascular System: The Necessity of Animal Studies *M. Delp*	
2:15 p.m.	Breakout group charge & afternoon break	
2:30 p.m	Breakout discussions	Main House
4:30 p.m.	Breakout lead reports	Carriage House
5:15 p.m.	Final remarks & future plans *Co-chairs*	
6:00 p.m.	Adjourn	
6:00 p.m	DINNER	Main House

Friday, April 16 NOTE: EXECUTIVE SESSION ONLY

8:30 a.m. Executive session: co-chairs, session leads and recorders, CASSLS organizer (Sawin, Souza, Paloski, Sonnenfeld, Wade, Skidmore, Hill, Jennings)
Review of meeting, Discussion of next steps and schedule

10:30 Adjourn

Notes on Workshop Structure

The goal of the first day of presentations was to equip participants with a broad-based understanding of the agency's new vision, the Bioastronautics Critical Path questions, and a historical view of contributions of animal research to human spaceflight. These historical perspectives were supplied from both US and Russian speakers. A keynote presentation by astronaut Dave Williams gave participants a first-hand account of one human's experience as both an experimental subject and an experimenter using animals in the microgravity environment.

On Day 2 the meeting focused specifically on risks to musculoskeletal, immune and sensorimotor systems. Broad talks on spaceflight and/or ground modeled impacts on these systems gave way later in the day to focused research presentations. Following these presentations the breakout groups reconvened to consider whether compelling cases had been made for the value of the use of animals in research addressing these phenomena.

A similar format was used for Day 3, which considered issues of microbial virulence, wound healing, nutrition and pharmacodynamics.

Appendix 2: List of Participants

Name		*Organization Name*	*Email Address*
Baer	David	US Army Institute of Surgical Research	david.baer@cen.amedd.army.
Baldwin	Ken	University of California, Irvine	kmbaldwi@uci.edu
Becker	Jeanne	Nat. Space Biomedical Research Institute	jbecker@bcm.tmc.edu
Dawidowicz	Lenny	Marine Biological Laboratory	ldawidow@mbl.edu
Delp	Michael	Texas A&M University	mdd@hlkn.tamu.edu
Emond	John	NASA Headquarters	john.l.emond@nasa.gov
Feeback	Daniel	Johnson Space Center	daniel.l.feeback1@jsc.nasa.gov
Fuller	Charles	University of California, Davis	cafuller@ucdavis.edu
Girten	Beverly	NASA Ames Research CTR	bgirten@mail.arc.nasa.gov
Gridley	Daila	Loma Linda University	dgridley@dominion.llumc.edu
Halloran	Bernard	University of California, San Francisco	bhallor@itsa.ucsf.edu
Hill	Esther	NASA Ames Research Ctr	ehill@mail.arc.nasa.gov
Holstein	Gay	Mount Sinai School of	holstg01@doc.mssm.edu
Holton	Emily	NASA Ames Research Ctr	eholton@mail.arc.nasa.gov
Ilyin	Eugene	Institute of Biomedical	ilyine@imbp.ru
Jennings	Diana	CASSLS at the Marine Biological Laboratory	djennings@mbl.edu
Jones	Jeffrey	Johnson Space Center	jajones@3ems.jsc.nasa.gov
Kozlovskaya	Inessa	Institute of Biomedical	ikozlovs@imbp.ru
Liskowsky	David	NASA Headquarters	dliskowsky@nasa.gov
Lomax	Terri	NASA Headquarters	tlomax@mail.hq.nasa.gov
Meck	Janice	Johnson Space Center	janice.v.meck1@jsc.nasa.gov
Niesel	David	University of Texas Galveston	dniesel@utmb.edu
Oganov	Victor	Institute of Biomedical	oganov@imbp.ru
Ostrach	Louis	NASA Headquarters	lostrach@mail.hq.nasa.gov
Ott	Mark	Johnson Space Center	cott@ems.jsc.nasa.gov
Paloski	William	Johnson Space Center	william.h.paloski@nasa.gov
Pellis	Neal	Johnson Space Center	neal.r.pellis@nasa.gov

Name		*Organization Name*	*Email Address*
Peterson	Barry	Northwestern University	b-peterson2@northwestern.edu
Putcha	Lakshmi	Johnson Space Center	lakshmi.putcha1@jsc.nasa.gov
Riley	Danny	Medical College of Wisconsin	dariley@mcw.edu
Sams	Clarence	Johnson Space Center	csams@ems.jsc.nasa.gov
Sawin	Charles	Johnson Space Center	csawin@ems.jsc.nasa.gov
Schneider	Victor	NASA Headquarters	vschneider@nasa.gov
Skidmore	Michael	NASA Ames Research Ctr	Michael.G.Skidmore@nasa.gov
Sonnenfeld	Gerald	Morehouse School of	sonneng@msm.edu
Souza	Ken	NASA Ames Research Ctr	ksouza@mail.arc.nasa.gov
Stein	T. Peter	University of Medicine and Dentistry New Jersey	tpstein@umdnj.edu
Tash	Joseph	University of Kansas Medical Center	jtash@kumc.edu
Tomko	David	NASA Headquarters	dtomko@mail.hq.nasa.gov
Tou	Janet	Wyle Laboratories	jtou@mail.arc.nasa.gov
Turner	Russell	Mayo Clinic College of	Turner.Russell@mayo.edu
Tverskaya	Galina	NASA Ames Research Ctr	gtverskaya@mail.arc.nasa.gov
Vanderby	Ray	University of Wisconsin	vanderby@surgery.wisc.edu
Wade	Charles	NASA Ames Research Ctr	cwade@mail.arc.nasa.gov
Wagner	Erika	Mars Gravity Program/MIT	erika@mit.edu
Wainwright	Norman	Marine Biological Laboratory	nwainwri@mbl.edu
Williams	Dafydd	Canadian Space Agency	david.r.williams1@jsc.nasa.gov

Appendix 3: BCPR Critical Risks
in Assessing Importance of Animal Research for Biomedical Risks

Risk Number	Risk Title
1	Accelerated Bone Loss and Fracture Risk
2	Impaired Fracture Healing
3	Injury to Joints and Intervertebral Structures
4	Renal Stone Formation
5	Occurrence of Serious Cardiovascular Dysrhythmias
6	Diminished Cardiac and Vascular Function
7	Define Acceptable Limits for Contaminants in Air and Water
8	Immunodeficiency / Infection
9	Virus-Induced Lymphomas and Leukemia's
10	Anemia, Blood Replacement & Marrow Failure
11	Altered Host-Microbial Interactions
12	Allergies and Autoimmune Diseases
13	Skeletal Muscle Atrophy Resulting in Reduced Strength and Endurance
14	Increased Susceptibility to Muscle Damage
15	Vertigo, Spatial Disorientation and Perceptual Illusions
16	Impaired Movement Coordination Following G-Transitions
17	Motion Sickness
18	Inadequate Nutritional Requirements
19	Monitoring & Prevention
20	Major Illness & Trauma

Risk Number	Risk Title
21	Pharmacology of Space Medicine Delivery
22	Ambulatory Care
23	Return to Gravity/Rehabilitation
24	Insufficient Data/Information/Knowledge Management & Communication Capability
25	Skill Determination and Training
26	Palliative, Mortem, and Post-Mortem Medical Activities
27	Human Performance Failure Due to Poor Psychosocial Adaptation
28	Human Performance Failure Due to Neurobehavioral Problems
29	Mismatch between Crew Cognitive Capabilities and Task Demands
30	Human Performance Failure Due to Sleep Loss and Circadian Rhythm Problems
31	Carcinogenesis
32	Acute and Late CNS Risks
33	Other Degenerative Tissue Risks
34	Heredity, Fertility and Sterility Risks
35	Acute Radiation Syndromes

Appendix 4: Summary of Needs for Animal Research

Summary of Needs for Animal Research

Area	Model	Ground	Shuttle[1]	Flight ISS[1]	Free Flyer[1]	CPR Risk
Muscle	rat, mouse, monkey	High	Low	High	High	13,14
Bone	rat, mouse, monkey	High	Low	High	High	1,2,3
Sensory Motor	monkey, rat	High	Low	Low	High[2]	15,16, 29,30
Immunology & Disease	mouse, rat, monkey	High	Med/High[4]	High	High	8,9,10,11,12
Wound Healing	rat, mouse, monkey	High	Low	High	Med	20
Cardiovascular	rat, mini-pig, monkey	High	tbd	tbd	tbd	5,6
Nutrition & Metab	none identified	Low	Low	Low	Low	18
Pharmacotherapeutics	rat, mouse, monkey	High	High	High	Low	21
Surgical Techniques[3]	rat, tbd	Low	Low	Low	Low	20,25
Artificial Gravity	rat, mouse, monkey	High	Low	High	Medium	Cross cutting
Radiation[3]	mouse, rat	High	Low	Low	Medium	31,32,33,34,35

1. Rating is based on one or more of the following: science need, applicability of platform to science, availability of onboard technology to achieve science; likelihood of making significant contributions on platform within 5 years.
2. High rating applies to rhesus monkey only.
3. Topic was not formally presented; it was acknowledged as an important area for animal research in small group discussions.
4. High rating pertains to pathogen virulence changes that can be studied on sortie flights

Appendix 5: Abstracts as Submitted

The Effects of Spaceflight on Skeletal Muscle: The Role of a Rodent Model For Studies on Resistance Exercise As a Countermeasure For Muscle Atrophy.

K. M. Baldwin, F. Haddad, and G.R. Adams
Department of Physiology & Biophysics
University of California, Irvine; Irvine,

Previous studies on both human and animal subjects clearly suggest that the hallmarks of exposure to a microgravity environment such as spaceflight involve the following alterations: 1) muscle atrophy, chiefly involving the extensor muscle groups of the lower extremity; 2) a concomitant reduction in muscle strength (force generating capability) that often exceeds the degree of atrophy; 3) a change in contractile phenotype (slow to fast), which may increase the fatigability of the affected muscles; 4) a deficit in motor function, which may impair the fidelity of movement skill; and 5) a proneness to soft tissue injury due the weakening of muscle fibers including the connective tissue infrastructure. The extent of these alterations varies from subject to subject depending on the effectiveness of those countermeasures that have been used to counteract these deficiencies. To date, these have focused on treadmill/cycling aerobic exercise and some form of resistance exercise performed under less optimal conditions. Based on the available evidence, the consensus is that there has not been an overarching prescription of activity/nutritional/pharmacological paradigms, the collection of which, that are considered effective in maintaining the homeostasis of skeletal muscle at the level typically seen in healthy individuals maintained at 1G.

The goal of this presentation is to lay a foundation as to how rodents can be used as experimental subjects in order to identify paradigms of different contraction modes that are effective in inducing an anabolic state in skeletal muscle in order to induce muscle hypertrophy and then to test these paradigms in the setting of hindlimb unloading in order to identify an effective resistance exercise (RE) prescription that can be translated to human subjects for eventual validation and verification in the spaceflight environment. In addressing this topic it is recognized that RE may, in the final analyses, be only one of many countermeasure strategies to improve the homeostasis of skeletal muscle when used along with other strategies such as improved nutrition, pharmacological interventions (micronutrient/antioxidant supplementation and protease inhibitors) and even artificial gravity playing significant roles.

What have we learned thus far about RE paradigms involving rodent? In a recent study performed at UC Irvine, we compared three different contraction modes of training a) isometric action training (ISO); b) concentric (shortening action training; CON), and c) eccentric (lengthening action training, ECC) using a novel egometer system in which the target muscle group is electrically activated via stimulation of the sciatic nerve under carefully controlled conditions, e.g., all contraction modes are performed under the same degree of stimulation frequency (60 Hz) and the duration for each contraction cycle is for 2 seconds. In comparing the three contraction modes the accumulation of the force integral was as follows EEC>ISO>CON. However, all three training modes produced similar degrees of hypertrophy (based on muscle weight and protein accumulation analyses) which amounted to ~12% following 12 training sessions. This level of hypertrophy is consistent with other studies using both animal and human subjects. Additional analyses suggested that activation of growth factor genes such as IGF-1 and MGF appeared to be greater in either the CON and ISO mode relative to the ECC mode suggesting the ECC actions which produce higher force integrals on the muscle do not produce greater anabolic stimuli that the other contraction modes. These findings suggest the ECC actions

may not necessarily produce the key type and magnitude of stimuli to induce anabolic adaptation in skeletal muscle compared to other contraction modes as commonly thought.

Based on the above findings we have initiated additional studies to investigate the effectiveness of isometric contractions in blunting the acute atrophy response that occurs in the rodent hindlimb suspension (SUS) model. In this model within five days of unloading the triceps surae (focal point being the MG muscle) complex, the muscle undergoes significant atrophy. Initial findings suggest that a modest isometric resistance training paradigm which consisted of 40 2-scond contractions per day to the left hind limb was effective in maintaining normalized muscle mass similar to ambulatory control animals; whereas by 5 days there was significant atrophy on the suspended non trained side of the suspended group. In order to gain insight concerning the factors responsible for maintaining the muscle mass, we examined a variety of factors involved in protein balance. Compared to the suspended control side, the RE induced higher levels of total RNA, which was used as an index protein translational capacity since the majority of the total RNA is ribosomal. Also we observed higher levels of IGF-1 mRNA in the trained versus non trained muscle, as well as higher levels of total and phosphorylated p70 S6 kinase, which is a regulator of translational processes involving mRNAs encoded for the ribosomal machinery. These changes occurred in the face of blunting the increased gene expression of two E3 ubiquitin ligases, atrogin and MURF-1, which have been shown to become elevated and thus playing a regulatory role in almost all models of muscle wasting. Collectively, these results suggest that the RE paradigm utilized in this study was able to maintain the muscle in an anabolic state to effectively inhibit atrophy processes; whereas, the untrained unloaded muscles were clearly in a catabolic state.

In conclusion, the findings reported herein suggest that the rodent model of resistance exercise can be a useful modality to study mechanisms of hypertrophy as well as in defining optimal conditions of selected countermeasures designed to minimize the atrophy that occurs in response to limb unloading. This research was supported in part by NSBRI Grant: NCC9-58-70.

THE PERVASIVE EFFECTS OF MICROGRAVITY ON THE CARDIOVASCULAR SYSTEM: THE NECESSITY OF ANIMAL STUDIES

Michael D. Delp
Departments of Health and Kinesiology and Medical Physiology, and the Cardiovascular Research Institute, Texas A&M University, College Station, TX

INTRODUCTION

It appears that the precision in which the cardiovascular system regulates arterial pressure and cerebral perfusion during the upright posture is due in part to the capacity of the cardiovascular system to appropriately adapt to the prevailing mechanical environment, which on Earth is largely determined by the head-to-foot hydrostatic pressure gradient created by force of gravity. Consequently, when the head-to-foot gravitational vector is removed during spaceflight, there is a headward fluid shift and a putative elimination of arterial pressure gradients. Such alterations in fluid volume and pressure distribution are thought to trigger structural and functional adaptations within the cardiovascular system. Although there is the potential for serious heart rhythm disturbances and cardiac atrophy with long-duration spaceflight, there is currently little evidence to suggest that overt cardiovascular function is greatly compromised in microgravity, or that structural and functional adaptations of the cardiovascular system to microgravity are inappropriate. Nevertheless, adaptive responses to a weightless environment are inappropriate for return to 1G and possibly to partial gravity conditions, such as would be experienced on Lunar or Mars expeditions. These "mal-adaptations" of the cardiovascular system are manifest primarily as orthostatic hypotension and reduced aerobic capacity in 1G, and have been well characterized in the human population exposed to a microgravitational environment. Other more subtle cardiovascular alterations are also likely to occur with exposure to microgravity. These less overt cardiovascular alterations are pervasive and affect multiple organ systems. Furthermore, investigation of these more subtle cardiovascular alterations has largely been elucidated through ground-based animal studies.

OVERT CARDIOVASCULAR ALTERATIONS: ORTHOSTATIC INTOLERANCE

Studies of humans following spaceflight and bedrest indicate that one of the predominant mechanisms underlying orthostatic intolerance is hypotension that results from an inability to adequately elevate peripheral vascular resistance (Arbeille et al. *Acta Astronautica* 36, 1996; Buckey et al. *JAP* 81, 1996; Mulvagh et al. *J Clin Pharm* 31, 1991; Vernikos et al. *J Clin Pharm* 31, 1991; Waters et al. *JAP* 92, 2002). This inadequate elevation of peripheral vascular resistance has been proposed to occur through both neural and vascular mechanisms. For example, several studies provide evidence in humans of impaired vasoconstriction of peripheral arteries (Schmid et al. *Hypogravic and Hypodynamic Environments*, p. 211-223 (SP-269) 1971; Shoemaker et al. *JAP* 84, 1998; Whitson et al. *JAP* 79, 1995). To address possible dysfunction of neural and intrinsic vascular control mechanisms of arterial pressure, the hindlimb unloaded rat has been used as the primary ground-based model of microgravity, and the results of these studies demonstrate impaired vasoconstriction of arteries (Delp et al. *JAP* 75, 1993; Purdy et al. *JAP* 85, 1998), arterioles (Delp, *JAP* 86, 1999) and veins (Dunbar et al. *JAP* 89, 2000). Furthermore, central processing of baroreceptor afferent information has been shown to adversely affect neural mechanisms for blood pressure regulation (Moffett et al. *Am J Physiol* 274, 1998; Moffett et al. *Am J Physiol* 277, 1999). The mechanisms underlying these vascular and neural effects have not been clearly delineated.

SUBTLE CARDIOVASCULAR ALTERATIONS: ANIMAL STUDIES

The problem of orthostatic intolerance and putative cardiac dysrhythmias and cardiac atrophy, the primary cardiovascular risks outlined in the Bioastronautics Critical Path Roadmap (BCPR) risk assessment document, have been investigated in both humans and animals. The effects of long-term microgravity on orthostatic intolerance and cardiac function will require continued investigation to characterize and elucidate mechanisms underlying these effects with animals. In contrast to the abovementioned cardiovascular risks associated with weightlessness, other, more subtle forms of cardiovascular alterations and dysfunction have almost exclusively been described in ground-based animal studies. Moreover, these "subtle" forms of cardiovascular alterations appear in the BCPR document under discipline areas other than the Cardiovascular discipline and, consequently, have received relatively little attention. These cross risk disciplines include the Nervous (Neurovestibular) System, Musculoskeletal System (Bone Loss and Muscle Alterations & Atrophy), Immune System, Nutrition, Pharmacodynamics/kinetics, Reproductive Status, and Emergency Medicine and Rehabilitation. Examples of the pervasive effects of microgravity on the cardiovascular system in cross risk areas include, 1) altered cerebral perfusion of discrete regions in the brain that are associated with the maintenance of equilibrium (vestibular nuclear area, caudate putamen, cerebellar vermis and floccular lobe), circadian rhythm regulation (suprachiasmatic nucleus and pineal gland), vision (superior colliculus) and cardiorespiratory control (insular cortex, hypothalamus, red nuclei, and the ventrolateral medulla) (unpublished observations); 2) diminished bone and marrow perfusion and the associated bone loss through putative vascular coupling mechanisms with osteoblast and osteoclast activities (Colleran et al. *JAP* 89, 2000), as well as possible impaired angiogenesis and fracture healing capacity; 3) functional lymphatic impairment (Gashev et al., *FASEB J.* 17, 2003) and a possible associated immune dysfunction, as well as potential impairments of angiogenesis and wound healing; 4) loss of muscle endurance and an associated vascular remodeling and diminished blood flow capacity in high oxidative muscle (McDonald et al. *JAP* 72, 1992; Delp et al. *Am J Physiol* 278, 2000); 5) the impact of nutrition and caloric balance on heart mass and cardiac function (Ray et al. *JAP* 91, 2001); 6) the contribution of altered cardiac output and blood flow distribution on putative changes in the dynamics/kinetics of pharmaceutical agents; 7) the adverse effects of simulated microgravity on smooth muscle function in arteries, veins and lymphatics may likewise negatively impact smooth muscle function in the uterus (Burden et al. *J Grav Physiol* 5, 1998; Serova et al. *Physiologist* 27, 1984) and gastrointestinal system (Gazenko et al. *Kos Biol Aviak Med* 21, 1987); and 8) the many issues of the cardiovascular system pertaining to emergency medicine and rehabilitation. Many of these understudied but pervasive effects of microgravity on the cardiovascular system will require the use of animals to characterize and delineate mechanisms of action.

Muscle Research and Human Space Exploration: Current Progress and Future Challenges

Daniel L. Feeback, Ph.D.
Muscle Research Laboratory, Johnson Space Center

Since the beginning of human space flight, there has been serious concern over the exposure of human crewmembers to the microgravity of space due to the systemic effects on terrestrially-evolved creatures that are adapted to Earth gravity. Humans in the microgravity environment of space, within our currently developed space vehicles, are exposed to various periods of skeletal muscle unloading (unweighting). Unloading of skeletal muscle both on Earth and during spaceflight results in remodeling of muscle (atrophic response) as an adaptation to the reduced loads placed upon it. As a result, there are decrements in skeletal muscle strength, fatigue resistance, motor performance, and connective tissue integrity. This normal adaptive response to the microgravity environment is for the most part of little consequence within the space vehicle *per se* but may become a liability resulting in an increased risk of crewmember physical failure during extravehicular activities or abrupt transitions to environments of increased gravity (such as return to Earth or landing on another planetary body).

In the U.S. Space Program the only countermeasure to skeletal muscle functional deficits that has been utilized is physical exercise by means of various modalities. In-flight exercise hardware and protocols have varied from mission to mission as have mission durations. Collective knowledge gained from these missions has aided in the evolution of exercise hardware and protocols to spaceflight-induced skeletal muscle atrophy. Long duration missions and missions with several transitions between gravitational environments present the greatest challenges to risk mitigation and to successful development of countermeasures of proven efficacy. Russian scientists have utilized a variety of exercise hardware and in-flight exercise protocols during long duration space flight (up to 1 year) aboard the Mir Space Station. Such protocols have included aerobic and resistive (both active and passive) exercise using a variety of exercise equipment. On the International Space Station (ISS), a combination of resistive and aerobic exercise has been employed. Outcomes have been acceptable based on current expectations for crewmember performance upon return to Earth. However, for a return to the moon mission, establishment of a lunar base, and interplanetary travel to Mars, the functional requirements for human performance during each specific phase of such missions needs to be well defined and countermeasures developed that meet those performance requirements.

NASA's current approach to identifying, soliciting and prioritizing facilitative research that is directed at mitigating risks to human crewmembers both within low earth orbit (ISS) and beyond is defined by the Bioastronautics Critical Path Roadmap (BCPR --http://criticalpath.jsc.nasa.gov). Recently, the BCPR has been re-evaluated jointly by NASA scientists and external experts and changes made to reflect the current direction of efforts for a crewed return to the moon of longer duration than during the Apollo program and for human interplanetary travel to Mars. The BCPR includes a set of enabling questions to which answers will provide knowledge beneficial in mitigation of risks to crewmembers and to increasing the probability of successful missions. Access to human crewmembers during both short and long duration missions for the study of skeletal muscle adaptation to microgravity and the efficacy of countermeasures is a limited resource thus requiring the use of ground-based models for conduct of both fundamental and applied skeletal muscle research. Various models for which sufficient data have been collected were reviewed recently (Adams GR, Caiozzo VJ, and Baldwin KM; J Appl Physiol 95:2185, 2003). Such models include horizontal or head-down bed rest, dry immersion bed rest, limb immobilization, and unilateral limb suspension. While none of these ground-based models

provides a perfect simulation of human microgravity exposure during spaceflight, each is useful for study of certain aspects of muscle unloading and sensory/motor alterations. Future development, evaluation, and validation of novel countermeasures to skeletal muscle unloading will likely employ these same models. Prospective countermeasures may include pharmacologic interventions, innovative exercise hardware providing improved loading modalities, locomotor training devices, passive exercise devices, and artificial gravity either as an integral component of the spacecraft or as a discreet device contained within it. With respect to the latter, the hemodynamic and metabolic responses to increased loading provided by a human-powered centrifuge have been described quite recently (Caiozzo VJ, *et al*., Aviat Space Environ Med, 75:101, 2004). Additionally, use of selected countermeasures during spaceflight will require monitoring of their effectiveness in meeting defined human performance requirements.

Development of truly novel approaches to countering the effects of microgravity on human physiology will require a more complete understanding of the processes that underlie them. Hypothesis driven research using mechanistic approaches will be required to advance the state of knowledge and to provide answers to many of the enabling questions contained within the BCPR. To this end, both animal studies using models of skeletal muscle unloading and cellular/molecular paradigms are fertile ground. Significant resources and efforts should be directed toward such studies; failure to fully exploit such investigations will impede both discovery and advancement of the state of the art.

Integrative Countermeasure Development: Neural Regulation and Animal Models

C.A. Fuller
Section of Neurobiology, Physiology & Behavior
University of California, Davis, CA

Organisms have evolved in Earth's static gravitational environment. It is thus perhaps not surprising that exposure to altered gravitational environments can extensively alter the neural regulation of physiology and behavior. The central nervous system (CNS) integrates information from somatic and neural sensory elements that transduce changes in both gravitational magnitude and the orientation of an organism with respect to gravity. In addition, following integration, the CNS initiates appropriate physiological and behavioral changes to re-establish homeostasis. Data from previous spaceflights are consistent with a significant influence of gravity on CNS regulation. Despite the abundance of data supporting a significant influence of gravity on CNS morphology and physiology, only countermeasures aimed at treating the adaptation of various individual physiological systems (e.g., skeletal muscle, cardiovascular, bone, etc.) to spaceflight are currently under study. Indeed, the integrated regulation of these physiological systems by the CNS has only rarely been considered in the development of countermeasures.
The effects of spaceflight on the CNS are too numerous to detail here. Briefly, however, these changes include: cellular function, CNS development, neuronal metabolism, neurotransmitter levels, vestibular reflexes, and the regulation (e.g., altered CNS efferent outflow) of peripheral physiological systems and behaviors. To this end, much of what we know about CNS changes in altered gravity reflects the use of animal models, including invertebrates, non-mammalian vertebrates, rodents and non-human primates. Although humans are common subjects for neuroscience research during spaceflight, much of the foregoing knowledge could not have been ethically or otherwise satisfactorily collected from human subjects. Lastly, more recent studies have begun to elucidate the precise somatic and neural sensory elements that transduce the gravitational environment to effect CNS regulation.

One specific CNS regulatory system, the circadian timing system (CTS), has been of particular interest to our laboratory. The CTS is responsible for the generation and internal temporal coordination of daily rhythms and for synchronizing these rhythms with the external 24-hour environment, and its regulation is influenced by spaceflight. Individuals with CTS dysfunction often suffer from sleep-wake disturbances, mental health and affect disorders, neuroendocrine dysregulation, and severely compromised long-term health. Recently, our laboratory elucidated a significant and unique role of the vestibular system in mediating the CTS responses to changes in gravity. This novel observation has lead to a paradigm shift in our thinking; that is, vestibular loading will likely be a critical component of an appropriate countermeasure for astronauts suffering CTS dysfunction. Furthermore, an influence of the vestibular system on either CTS entrainment or photic responsiveness will have important implications not only for individuals in altered gravity environments, but also for earth-bound individuals with circadian disorders and potentially, vestibular disorders. By understanding the physiological and molecular mechanisms by which the vestibular and circadian systems interact, therapeutic and diagnostic strategies may be developed for astronauts, who are known to suffer from circadian and sleep-wake disorders and for earth-bound individuals suffering from circadian, vestibular, sleep-wake or aging-related disorders.

Among the many considerations for development of an integrated countermeasure (CM) to protect astronauts in space, gravitational loading is potentially of the greatest physiological value since it replaces the most characteristic terrestrial stimulus lost during spaceflight. Artificial gravity (AG) has been proposed numerous times as a countermeasure for deconditioning related changes as well as for other physiological and behavioral effects of living in the space environment. Thus, while artificial gravity (AG) has been long proposed as a potential CM to

maintain physiological homeostasis of the individual (chiefly the cardiovascular, neurovestibular-sensory-motor, and musculoskeletal systems), its effectiveness has not been experimentally determined. While the use of AG may be appealing, we do not yet understand exactly how AG will be used in space. The most basic considerations have yet to be evaluated including G level, duration and frequency of exposure, and when to time a G stimulus. For example, it is still unknown if intermittent AG will be as effective as continuous exposure. The minimum G level required to counteract microgravity-induced changes is not known. At present we know virtually nothing about the effects of intermediate G levels, for example the 0.38G of Mars or the 0.17G of the Moon. We know from previous lunar missions that intermediate G exposure is survivable and has long-term effects no worse than microgravity exposure, but do not know if intermediate G levels will have beneficial effects or if fields of 1G or greater will be required to prevent deleterious changes.

While acclimation to centrifugal acceleration remains poorly studied in humans, there is a long history of the use of chronic acceleration with animals. Acclimation changes to centrifugation are well known in animals. For example, rhesus monkeys exposed to 2G on a 2.3 m radius centrifuge operating at around 25 rpm acclimate after 2-3 days with no discernable differences in performance compared to a non-rotating environment. This acclimation persists for weeks after cessation of centrifugation, suggesting that pre-training of humans for in-flight centrifugation may also be possible. Similar evidence of acclimation to a centrifuge environment exists for a variety of species, including non-human primates, rodents, and insects. Animal models have provided most of our understanding of the physiological responses to hypergravity and chronic acceleration and may be our best tool for studying the mechanisms underlying microgravity responses.

There are numerous advantages to developing AG countermeasures using animal models. For example, smaller body size requires less cumbersome and expensive facilities and facilitates the use of larger sample sizes. Long-term exposure to hypergravity, over weeks or months, is essentially only practical with animals, as are repeated studies of long durations. Studies of long duration will be necessary to compare the benefits of various regimens for long-term AG exposure. Moreover, long-duration studies will be needed to study the transition from hypergravity to normal 1G as a model of spaceflight deconditioning. After return to normal 1G, hypergravity regimens that are effective in preventing deconditioning from a hypergravity-adapted condition would help identify promising regimens for AG use in the microgravity environment. In this case, it is essential that subjects first be fully acclimated to hypergravity. The most successful regimens in ameliorating de-adaptation might then be evaluated in humans. Furthermore, proposed AG countermeasures will need to be validated in the spaceflight environment. For the foreseeable future the only means of producing an acceleration environment in space will be the animal centrifuge being developed for the International Space Station (ISS). It is unlikely that facilities, particularly for chronic AG, will be available inflight for use with humans for many years. Therefore it is important that ground models of AG countermeasures be developed to include the use of animals for comparison with flight results.

Animals will have a special role in AG countermeasure development, and non-human primates in particular offer unique advantages as a model system for human responses. Non-human primates are close to humans phylogenetically, physiologically and anatomically. Nearly all are diurnal, and the higher primates studied share a high degree of similarity of sleep and circadian organization with humans. Some species, for example the rhesus monkey, are well-characterized and widely used biomedical models. Rodents and other groups are sufficiently similar to humans in their responses to G to serve as models for responses of many organ systems. However for responses to G largely mediated by the central nervous system, for example sleep, behavior and circadian rhythmicity, the greater similarity of non-human primates to humans makes them far more suitable as models for many studies. Enough is known about the responses of rhesus

monkeys to microgravity from the Russian and American Bion data, as well as from ground-based centrifuge studies to validate their use as a human surrogate in AG studies.
In summary, animal models have provided a variety of insights into CNS function in altered gravitational environments, including microgravity. Multiple questions remain regarding how AG might be used as an inflight countermeasure, however. The complexity of the issues, the need for long-duration studies, and the ability to perform initial AG studies on ISS make animal models invaluable for furthering our understanding of how gravity influences CNS function and hence other physiological systems, as well as the development of appropriate and efficacious AG countermeasures. [This research has been supported by NASA grant NAG2-1451 and NSBRI grant NCC9-58233.]

THE IMMUNE SYSTEM: VALUE OF ANIMAL RESEARCH

Daila S. Gridley, Ph.D
Departments of Radiation Medicine, Biochemistry & Microbiology, and Medicine
Loma Linda University and Medical Center, Loma Linda, CA

Brief Overview of Immune System

The immune system consists of a complex and interactive network of cells, tissues, organs and soluble substances. Its three major functions are essential for survival: 1) defense against infectious and noninfectious agents deemed to be foreign; 2) homeostasis, characterized by removal of worn-out or damaged cells of the host; and 3) surveillance, concerned with recognition and removal of mutated or otherwise abnormal cells that appear with considerable frequency over the course of a lifetime. The immune system is highly sensitive to both internal and external environmental changes and does not act alone. Alterations in immune status can impact many body systems. The immune system, in turn, is responsive to perturbations in other body systems. Study of stressor effects on isolated cells in culture, mixtures of various cell types and ex vivo tissues, as well as computer-based modeling, cannot duplicate and thus may not accurately predict the outcome in the intact animal.

Consequences of Immune Depression/Dysfunction

The consequences of immune depression or dysfunction can be serious and sometimes result in death. Overwhelming infection has the potential to rapidly abort space missions that would otherwise be successful. Disease may result from either exogenous or endogenous agents. Bacteria that pose a threat during long-term space missions include *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus* and *Staphylococcus aureus*. Reports suggest that the efficacy of antibiotics may be less than optimal in space. Reactivation of viruses such Epstein-Barr virus (EBV), cytomegalovirus (CMV), and the herpes simplex viruses (HSV) is also a concern, since a high percentage of humans are latently infected and shedding has been reported in astronauts. These viruses are well known to cause debilitating and often life-threatening problems in immunodepressed organ transplant and HIV-1 infected patients. Some also have strong oncogenic potential (e.g. EBV). There is currently no drug that can cure a viral infection and disease resolution largely depends on effective immune defenses.

Immune depression or dysfunction can also increase the risk for cancer. Skin cancers, leukemias, lymphomas, ano-genital cancers and Kaposi's sarcoma are among the most common neoplasms in immunocompromised patients. Furthermore, animal studies have demonstrated that transformed, but benign, cells are more likely to progress to malignancy in an immunodeficient host. It is important to note that pre-malignant and malignant foci are well known to exist in humans (e.g. prostate, breast and thyroid) at a much higher rate than is ever expected to be manifested as clinically relevant disease. The failure of these foci to grow progressively is attributed at least partly to effective immune surveillance. The critical role of T lymphocytes in defense against neoplastic growth has been well documented. The importance of these cells is emphasized by the intense efforts currently being made to recruit their activities as a form of cancer therapy, as well as to develop cancer vaccines.

Adverse changes in immune parameters may also increase risk for other conditions such as autoimmunity, allergic reactions, anemia, bone loss, delayed wound healing, and behavioral abnormalities due to miscommunication between the immune and central nervous systems.

Critical Questions

Spaceflight studies have documented numerous immune aberrations in astronauts, cosmonauts and research animals. Among the major factors that are likely to account for these observations are psychological stress, altered gravity, and radiation. It is not yet known whether the combination of these factors during extended space missions will lead to additive, or perhaps even synergistic, effects that translate into increased susceptibility to infection, cancer, or other diseases. The Critical Questions posed by NASA in the 'Immune System/Microbiology' section are appropriate and highly relevant, given the potentially devastating consequences of immune deficiency. Without animals, it appears likely that at least some of the most important questions will not be answered easily, even a minimal degree of certainly may be lacking and, in the worst case scenario, misleading information may be obtained. This is especially true when contemplating the effects of multiple immune stressors on interactive body systems and when testing agents with potential to serve as countermeasures. Furthermore, if inbred animals are used, it would be prudent if strains of varying genetic backgrounds were evaluated, due to the genetic diversity of humans.

Animal Models in Immunology/Microbiology

Many space-related and other studies of immune mechanisms involve mice. The rationale for this is based at least partly on the following: 1) they are mammals and hence are similar to humans in terms of their immune system components; 2) many mouse and human genes are homologous; 3) they propagate well and age rapidly, thus investigators do not have to wait for years before a large population of a given age is available for study; 4) they are small in size, thereby requiring only minimal space for housing; 5) detailed information is available with respect to the pathological conditions that appear with age; and 6) they are more cost-effective compared to most other mammals. Second in overall frequency of use is the rat. However, the mouse has often been selected over the rat because 1) virtually all reagents needed for extensive immune characterization are available; 2) the mouse is more well defined with respect to effects of radiation, chemical carcinogens and other toxic insults; 3) hundreds of genetically manipulated ("knock-out," "knock-in") mice have been developed for study of specific pathways; 4) the mouse is easier to handle; and 5) a larger number can be easily processed in order to achieve statistically significant differences among groups. In contrast, the rat has been selected over the mouse in the great majority of spaceflight studies, perhaps in large part because of its less odiferous nature.

Brief Highlights of Our Research

In our space-related studies, the long-term goals are to 1) gain a better understanding of immune modulation by factors in the spaceflight environment; 2) determine if the observed changes translate into increased risk for infections and cancer; and 3) identify possible countermeasures. Immunological assays have been performed on mice exposed to radiation (γ-rays, protons, iron, silicon) and hypergravity; similar evaluations have also been done on mice flown on the space shuttle Endeavour (STS-108/UF1). Our current focus is to combine low-dose/low-dose-rate background radiation with solar flare simulated protons and to more fully define T helper (Th) lymphocyte function as manifested by the secretion of immunoregulatory cytokines. Interferon-γ (IFN-γ) is of special interest, because it functions in both innate and adaptive immunity and also has radioprotective, anti-mutagenic, and anti-tumor properties. The C57BL/6 mouse has been utilized throughout most of these studies because it is a well defined general purpose strain, its immune responses to antigen challenge resemble those of humans, and it is a major strain from which genetically engineered mice are derived. In some studies, we have also included the CBA/Ca mouse because, in contrast to the C57BL/6 strain, it is highly susceptible to radiation-induced genomic instability, a condition that predisposes to the development of cancer. Eventually we hope to clearly define immunological deficits in mice simultaneously exposed to radiation and altered gravity, confirm our findings in spaceflight and

identify countermeasures that minimize, if not prevent, deleterious consequences. Our laboratory has also been involved with translational research utilizing new modalities for cancer therapy. A primary goal has been to increase the efficacy of radiotherapy through the use of agents that enhance immunological anti-tumor effects and/or augment tumor cell response to radiation. The animal models have included immunologically competent, as well as athymic, mice and rats with glioma, melanoma and tumors of the lung, colon and prostate. Answers to the questions addressed in all of the above mentioned studies were facilitated through the use of intact mammalian hosts.

Conclusions

Numerous breakthroughs in immunology/microbiology would not have been possible without utilization of animal models. Animals have played, and continue to play, a highly significant role in studies of tumor immunology and immunotherapy, autoimmune diseases, hypersensitivity reactions, immunodeficiencies, organ transplantation and vaccine development. Animal research has led to many new therapies, improvements in old therapies and increased understanding of the mechanisms underlying immune function (anaphylaxis, phagocytosis, and immune tolerance; importance of histocompatibility antigens; techniques for monoclonal antibody production; pathogenesis of oncogenic viruses; and curative effects of penicillin and other drugs). Since the mid 1800s, it is animal research in many scientific disciplines that has been largely responsible for the nearly 30-year increase in the average life span of humans. Furthermore, since animals and humans share more than 250 diseases, the research has also greatly benefited the health and wellbeing of the animals that made so many biomedical discoveries possible.

Bone Research and animal support of Human Space Exploration: Where do we go from here?

Emily R. Morey-Holton, Ph.D., NASA-Ames Research Center

NASA exploration goals include returning humans to the moon by 2015-2020 as a prelude for human exploration of Mars and beyond. The number of human flight subjects available during this very short time period is insufficient to solve high-risk problems without data from animals. This presentation will focus on three questions: What do we know? What do we need to know? Where do we go from here?: roles for animals in the exploration era. Answers to these questions are based on flight and ground-based models using humans and animals.

First, what do we know? Adult humans have spent less than 1% of their lifespan in space while juvenile rats have spent almost 2%. This information suggests that our data are rather meager for projecting to a 30-month mission to Mars. The space platforms for humans have included Skylab, STS/MIR, and STS/ISS and for animals have included the unmanned Bion series and shuttle. The ground-based models include head-down bedrest in humans (BR) and hindlimb unloading in rodents (HU).

We know that as gravity decreases, the impact forces generated by the body during locomotion decrease. For example, on Earth, your legs supports approximately 1 body weight (BW) when standing, 1.33BW when walking, and 3BW when jogging. On Mars, the same activity would generate 0.38BW standing, 0.5BW walking, and 1BW when jogging. In space, no impact load is generated, as gravity is minimal.

The well-known curves depicting physiological systems responses to spaceflight vs. time suggest that each physiological system acclimates to microgravity at a different rate. However, these curves were generated from assumptions based on techniques and timing of samples. For example, the gradual, continuously increasing, loss of bone and calcium is based on metabolic studies and pre/postflight measurements in humans. Current bone scanning procedures, while becoming more sensitive, are not sufficiently sensitive to detect early changes in adult bone that turns over very slowly. Rodent data suggest that bone changes begin very early in flight or during HU. Bone markers in BR and rats (both HU and space flown) indicate that bone formation slows quickly. Biochemical markers from BR and spaceflight in humans indicate an early increase in bone resorption . In adult HU rats, increased bone resorption has been reported. Whether the bone changes stabilize with time is not known.

The musculoskeletal system is composed of bones, muscles, joints, and minor components including connective tissues (ligaments, tendons, vertebral disks), blood vessels and nerves. The shape and size of the musculoskeletal system is determined by mechanical/gravity load, metabolic demand, and function over time. In other words, structural support is only one component of a complex system. Following spaceflight, both rodents and humans have postural problems as postural reflexes have slowed, muscles may be weak and damaged by reloading, and bones may be less supportive. The musculoskeletal system is an integrated system and fixing only one component may not improve the system. Strong muscles could break weak bones, strong bones will not go anywhere with weak muscles, and motion is not possible without appropriate neural input.

Bone is a very complex tissue. You cannot extrapolate from one site to another site in the same bone or from the exterior to the interior of a bone as the tissue remodels according to the loads (metabolic and structural) placed on specific bone sites. Those bone sites experiencing the highest muscle/mechanical loads on Earth are the sites most impacted by unloading. The Bion series of flights have provided multiple insights into flight changes in rodents. The masticatory muscles may change in space as the jaw will no longer be working against gravity. Evidence suggests that bone formation slows in the rat mandible at sites where muscle is not attached. Rat long bones decrease formation at the outer bone surface with increases in lipid droplets in the vessels and abnormal collagen patterns. These changes ultimately decrease the mechanical

strength of the bones. In human crewmembers, trabecular bone appears to be lost faster than cortical bone and the usual mechanical compensation by periosteal expansion may not occur. Leg and spinal muscles lose mass faster and to a greater extent than bone, although recovery upon return to Earth is more rapid in muscle. Exercising may protect certain bone sites, e.g., heel bone and lumbar vertebra. Spinal scans from cosmonauts suggest that muscle atrophy may directly affect bone integrity. Many crewmembers report back pain that may be a function of less curvature and suggests that the disk function may be altered. Space flown rats have smaller lumbar annuli with altered collagen-to-proteoglycan ratios. These data suggest that the musculoskeletal system adapts to the space environment. This adaptation of the entire system makes the individual more susceptible to rips, tears, and fractures upon entering a higher gravity environment. Most data are pre/post flight, thus we do not know if/when bone stabilizes during flight and if there is a mineralization defect that might increase fracture risk or delay fracture repair. Only two flight studies on bone healing have been reported (in rats) and the fractures were initiated 3 or 5d before flight. Both studies suggest that bone healing is impaired. One study suggests that metabolic differences may play a role.

Given these data, we still need more information to mitigate risk of fracture and connective tissue problems for long duration missions. NASA has a limited budget and must maximize science return. This approach will require a new way of doing business. For example, NASA needs to determine the appropriate age, species, and genetic strain of rodents to quickly provide maximal insights into risks and fund only the use of these animals. Many investigators assume that a sexually mature (about 6 wks old) rat is an adult rat. Recent publications suggest that albino rats are not skeletally mature until the epiphyses close which occurs after the rat is 8 (male) or 10 (female) months old. Thus, rats at least one year of age should be considered for bone studies. Given that rats live approximately 3 years and humans 75 years, a one-year-old rat is comparable to a 25yr old human. The Wistar-Hanover outbred rat is a smaller genetic stain that is available across the world, is being used in a number of labs, and should be considered. Mice strains vary greatly in their response to unloading; mice strains with the highest bone mass show the least change while those with the lowest density appear to show the greatest change. The C57B6 mouse appears similar to the human in terms of ageing and unloading responses. In addition to selecting rodent age and genetic strain, NASA should institute an integrated test regime for rodent studies similar to the human countermeasures evaluation and validation program so that all major physiological systems all studied during each experiment. Measurements in animals should be similar to those in humans.

Rodents can make major contributions toward mitigating risks in the exploration era. Immediate studies in HU rodents can investigate bone repair. Animals should be adapted to HU prior to initiation of the fracture using a well-established fracture model. Such studies will determine if the fracture repair proceeds normally during unloading or if the bone changes with unloading impair the healing process. In addition, long duration HU studies should determine if bone alterations continue or stabilize over time and if these changes increase fracture risk or delay fracture repair. Once the process is defined, then exercise paradigms or drugs might be used to minimize critical musculoskeletal changes. Any countermeasure used for bone should maintain metabolic capacity, fluid distribution, muscle/ligament attachments points, disk integrity, and marrow components and not adversely impact any other physiological system. Data from these studies could lead to focused flight experiments. Once the centrifuge is on station, rodents can be used to obtain data suggesting whether moon gravity is sufficient to maintain fitness within acceptable levels.

The NASA vision is to improve life here, to extend life to there, to find life beyond. The exploration era has set impressive goals within a very short time frame. If we are to meet these goals, extensive animal experimentation, starting today, is required to meet the challenge of sending humans back to the Moon and eventually to Mars.

ANIMAL EXPERIMENTS AND HUMAN SPACE MISSIONS

E. A. ILYIN
RF SRC – Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia

Space exploration by humans has helped us gain a better insight into the Universe, as well as expand our knowledge about the huge potential of the human body. The first man in space was Yuri Gagarin who made his historic flight of 108 minutes on April 12, 1961. This March he would have turned 70. We all admire and respect this wonderful person and other fearless astronauts/cosmonauts who followed him. However, we should never forget that the first manned flight was preceded by animal flights on high-altitude probes and Earth's artificial satellites. Later, many more animal experiments were flown, which helped accumulate a large body of data that formed the foundation of space medicine.

The US launched its first animal-carrying rocket on June 18, 1948. Until 1961 the US launched 21 probes that carried chimpanzees and mice.

The Soviet Union began preparing animal space experiments in 1948 and launched its first probe July 22, 1951. Through 1960, the Soviet Union launched 29 rockets each carrying 2 dogs.

After recovery the flown dogs showed no significant changes in their cardiovascular or respiratory functions, behavior or well-being. Animal experiments demonstrated that high G-loads and microgravity were physiologically the most demanding factors. They did not reveal any noticeable effects of space radiation. The experiments also gave evidence that life support systems, catapulting and parachuting systems were adequate. In a word, early animal experiments helped identify physiological responses of animals to short-term exposure to the space environment and facilitate further studies in long-duration flights.

Animal studies performed on Earth's artificial satellites prior to the first man-in-space flight can be viewed as biological verification of future manned spacecraft trajectories. The USSR launched animals onboard the 2nd satellite (the dog Laika launched October 4, 1957) and four unmanned Vostok-type spacecraft that were termed by mass-media spaceships-satellites (1960-1961). The flight experiments were of great importance because they gave an unambiguous answer to the question whether a living being can survive in space. Moreover, they allowed the testing and verification of life support systems, medical monitoring and telemetry systems, etc.

The results of biomedical examinations, analysis of the effects of microgravity and cosmic radiation, as well as ground-based observations allowed the conclusion that humans can go to space.

The era of manned space missions charged space biology with new tasks such as elucidation of the effects of microgravity on fundamental processes, mechanisms of structural and functional changes in various physiological systems, combined effects of microgravity and radiation, and biological effects of artificial gravity.

These problems were investigated in animal flights on BIOS ((USA) and BION (USSR-Russia) biosatellites and on Space-Shuttle spacecraft.

In terms of science return, the longest and most productive animal research program was BION. In 1973-1996, 11 biosatellites were launched, with their flight duration varying from 5 to 22.5 days. The flown experiments were performed on Wistar rats, rhesus monkeys, amphibians, fish, reptiles, insects, worms, amphibian and avian eggs, animal and plant cell cultures, and microorganisms. Obviously, biomedical problems were for the most part addressed in mammalian studies (rats and monkeys).

Bion studies made a significant contribution to our knowledge about animal responses to the space environment. The results of Bion experiments found application in the medical support of manned space missions. For instance, the mere lack of deleterious effects of spaceflight factors on intracellular processes, cells, tissues, organs, physiological systems, and whole

organisms proved that there were no inherent biological limitations to an increasingly longer exposure of humans to the space environment.

Nonetheless, marked structural and functional changes detected in animals required further study of the mechanisms underlying these changes, which may help develop efficient countermeasures. Bion observations were used to design training devices focusing on different muscles and muscle groups and to develop exercise regimens for the tonic component of muscle contractions. This allowed rehabilitation periods following extended manned missions to be shortened significantly. Bion data also helped develop static loading exercise regimens to condition the human bone system. Bion experiments revealed changes in the musculoskeletal system early in flight, which indicated that humans should exercise even in short-duration missions (5-7 days).

Bion neurosensory studies yielded unique data about space motion sickness and motor disorders, as well as vestibular, visual, and proprioceptive interaction in the oculomotor control system. Since the pattern and mechanism controlling gaze fixation of humans and monkeys are identical, the Bion experimental data were used to develop recommendations concerning cockpit instrument panel configuration. For example, visual targets that require immediate response should be located within the central field of vision of the cosmonaut, and control joysticks should be operated without requiring rapid or wide-angle head movements early in flight. Because Bion studies showed motor control changes, it was concluded that cosmonauts should not be required to perform high accuracy/high precision movements during the first flight days. These recommendations were incorporated into cosmonaut training programs and made available to space rocketry designers and manufacturers.

Bion experiments provided evidence that artificial gravity can be used as an efficient countermeasure against the adverse effects of prolonged microgravity. Bion findings were also used in the development of radiation safety standards of manned space missions.

Thus, it is beyond any doubt that the long series of animal investigations has been of great value to both the theory and practice of space biology and medicine.

SENSORY-MOTOR STUDIES IN BION FLIGHTS

Inessa B. Kozlovskaya
RF State Research Center – Institute for Biomedical Problems Russian Academy of Sciences, Moscow, Russia

Physiological data obtained from cosmonauts/astronauts are clinically relevant but not particularly helpful in our understanding of mechanisms that underlie various changes in the human body. The goal of the Bion Primate Program (accomplished in 6 Bion flights) was to use Rhesus monkeys as human surrogates in order to measure, qualitatively and quantitatively, microgravity-induced physiological changes and to elucidate the underlying mechanisms. It was expected that primate data would help better understand the adverse effects of microgravity and develop efficient countermeasures against them. It was also believed that primate observations would expand our knowledge of human physiology as such, especially neurosensory, neuromuscular, bone, and regulatory physiology. It was assumed that primate findings would also help improve prophylactic and therapeutic methods used in the treatment of such human afflictions as neurological disorders, spinal cord injuries, osteoporosis and others.

The Rhesus monkeys flown on Bion 6-11 were trained to perform skilled hand and foot tasks, and were highly instrumented to monitor their vestibular, brain and muscle functions before, during and after flight. Animal tissue, urine and blood samples were collected to study metabolic and hormonal status, as well as specific responses to microgravity. It should be emphasized that a large number of various parameters were measured in the same animals kept under the same strictly controlled conditions. This allowed investigators to compare and correlate their findings across different disciplines. For example, muscle electrical activity and muscle force exertion data were correlated with hormone measurements known to affect muscle function, as well as with metabolic and task performance results. Based on detailed studies by Russian and international PIs, a physiological profile of Rhesus monkeys in space was developed, which contributed to better countermeasures used by space crews in long-duration missions.

One of the most critical effects of spaceflight, especially important for short-term missions, is Space Motion Sickness (SMS). It appears that vestibular sensors are very sensitive to gravity effects. This is why both Russian and US space research programs focused on the human vestibular system in real and simulated microgravity. However the results were very controversial due to many factors. For example, a) it is impossible to standardize work/rest regimens of cosmonauts/astronauts in short- and long-duration missions; b) it is impossible to use regularly the same batteries of vestibular tests in the same individuals; c) it is difficult to perform tests consistently at regular time intervals, etc.

In view of this, the primary goal of Bion experiments was to study: 1) the microgravity effects on different vestibular reactions (which are similar to human responses); 2) the course of adaptation to the space environment and readaptation to 1 G, and 3) the neuronal (basic) organization of these events.

The data obtained in 5 spaceflight experiments (the first two were Russian-only and 3 subsequent were Russian/US joint studies) helped identify vestibular changes, their time course variations and underlying neuronal mechanisms.

Another highly gravity-dependent system is the sensory-motor one. It is known that short- and, particularly, long-duration spaceflights lead to changes in vertical posture, locomotor coordination, loss of voluntary movement accuracy, muscle atonia and atrophy viewed as signs of hypogravitational ataxia. In spite of numerous observations, the nature and the cause and effect of the above changes still remain unclear. This can be attributed to several things. Firstly, microgravity affects almost every component of the sensory-motor system including proprioception, integration processes at different levels (from spinal to cortical), neuromuscular transmission and muscles. Secondly, lack of comprehensive studies in which a systemic

physiological approach can be used together with morphological, biochemical and others methods to analyze the time course of events developing in the same species under the same conditions. Due to this, the goal of Bion primate studies was to perform physiological, biochemical and morphological measurements in neural, muscular and connective tissues related to the adaptation to microgravity.

The results helped clarify processes occurring in the sensory, muscle and CNS control systems during exposure to the space environment and build a well-documented scientific foundation for developing effective countermeasures.

Streptococcus pneumoniae GENE EXPRESSION AND VIRULENCE POTENTIAL IN LOW SHEAR AND MODELED MICROGRAVITY ENVIRONMENTS (LSMMG)

D. W. Niesel, U. Pandya and C. Allen.
Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, TX.

Introduction:

Extended spaceflight and the long-term habitation of the space environment poses a challenge in the protection of humans from infectious disease. There is little information available as to the effect of the space environment or microgravity on the physiology, gene expression, or virulence potential of bacterial pathogens. Previous studies have documented significant changes in the immune system during space flight, which may render personnel more susceptible to microbial infections. *S. pneumoniae* can be a commensal inhabitant of the nasopharyx and is a opportunistic bacterial pathogen in individuals with impaired immune systems. A mediator of serious disease (pneumonia, otitis media, and meningitis), *S. pneumoniae*, is easily spread by aerosols. Carriage rates in healthy individuals range from 5-70% and this microbe has been isolated from crewmembers immediately prior to flight. *S. pneumoniae* and other opportunistic bacterial pathogens could represent a significant threat to personnel during space habitation or during extended spaceflight. It will be important to understand the effects of microgravity and other elements of the space environment on the virulence potential of infectious disease organisms and their interaction with the host immune system to generate a rational risk assessment for infectious disease threats for humans in space.

In our laboratory, we are investigating *S. pneumoniae* virulence and gene / protein expression under conditions of LSMMG and in the space environment. Little is known about bacterial adaptation to the space environment or the effect of microgravity on the virulence activities of bacterial pathogens. Evidence has accumulated that LSMMG acts as an environmental signal to alter bacterial gene / protein expression and virulence activity. Our preliminary studies also show that LSMMG culturing leads to changes in the virulence potential of *S. pneumoniae*. We are conducting experiments to assess gene and protein expression and *S. pneumoniae* virulence activity in microgravity and space flight.

Summary of Results

Using high aspect rotating vessels (HARVs), we have been investigating changes in *S. pneumoniae* growth, protein and gene expression, and virulence activity under LSMMG and normal gravity (1xg) conditions. No changes in growth rates and final cell densities have been observed for 3 different serotypes following growth in THY medium. There were no significant differences in chain length for the serotypes following LSMMG or 1xg culture. Total protein profiles were compared for LSMMG and 1xg cultures. Multiple protein differences were observed by silver staining between the samples. Using two dimensional gel electrophoresis, 12 and19 protein spots were observed to be under expressed and over expressed respectively after LSMMG culture. MALDI-TOF sequencing of two over expressed protein spots has identified a 19kDa protein as dihydrofolate reductase (dfr) and a 37 kDa protein as pneumococcal surface antigen A (PsaA). Significantly, Dfr contributes to trimethoprim resistance in *S. pneumoniae* and PsaA is a major adhesin.

LSMMG was also shown to enhance virulence of *S. pneumoniae* in *in vitro* and *in vivo* models. Adherence of *S. pneumoniae* to lung epithelial cells in culture (A549) was increased three-fold. Additionally, cellular invasion of A549 cells increased by 30% for a serotype 4 strain following LSMMG culture. Recent results have shown that *S. pneumoniae* cultured under LSMMG conditions in HARVs showed enhanced virulence in a murine challenge model. LSMMG culture resulted in a shortened time to death and a lower LD_{50} compared to controls for

highly virulent strains. This is consistent with reports of enhanced virulence in mice of *Salmonella typhimurium* following LSMMG culture. Expression of virulence genes was examined using mRNA extracted from LSMMG and 1xg cultures and probed for expression of mRNA for capsule biosynthetic enzymes (cap3A) and pneumolysin (ply), a major *S. pneumoniae* virulence product. No significant differences were seen in the expression of the mRNA of these virulence products. These results indicate that some but not all *S. pneumoniae* virulence products/activities are enhanced after exposure to LSMMG conditions.

Using a global transcriptional approach, we are investigating *S. pneumoniae* gene expression of this pathogen after LSMMG culture usingTIGR4 cDNA microarrays (2131ORFs) from The Institute for Genomic Research (TIGR) Results to date, show that ~15% of *S. pneumoniae* genes are differentially expressed at 1.5-fold or higher levels with 90% of differentially expressed genes down regulated and 10% showing higher expression levels. Of 323 genes showing expression differences, 30 were up regulated and 293 were down regulated. Differential expression included genes from a wide range of functional groups with the top 3 functional groups being hypothetical proteins (42%), transport and binding proteins (17%) and cell structure and processes (10%). Alterations in gene expression were confirmed by RT-PCR. Interestingly, using a two-fold expression change threshold, 163 and 153 genes from *S. typhimurium* and *S. pneumoniae* respectively were differentially expressed following LSMMG. This represents 4% and 7% of their respective genomes that are differentially expressed under LSMMG.

Conclusions:

It is becoming clear that low shear forces and gravity represent mechanical force environmental signals that bacteria "sense" which can lead to adaptive activities requiring altered gene and protein expression and subsequently to new properties. Significant questions remain to be answered to understand the behavior of microbes in the space environment. We do not know the full virulence potential of opportunistic bacteria during long term space flight or during the habitation of space. This is essential in that infectious diseases are likely to occur in these environments and it will be important to understand the behavior / virulence potential of these bacteria. Using both global transcriptional and proteomic analysis, we should validate LSMMG culture as the microbial culture model for the space environment. This is imperative in light of limited future flight opportunities. Additionally, the significant question of the interaction of these microbes with host cells in microgravity environments remains to be rigorously examined. Finally, because of its pivotal role in host defense, the host innate immune response to these opportunistic bacteria in these environments needs to be examined. These are important considerations, which will need to be addressed to establish a rational risk assessment for infectious diseases during long term flight.

Supported by NASA Cooperative Agreement- NCC 2-1160

ANIMAL EXPERIMENTS HELP US UNDERSTAND MECHANISMS OF HUMAN OSTEOPENIA IN MICROGRAVITY

V. S. Oganov
RF SRC – Institute of Biomedical Problems, Russian Academy of Sciences, Moscow

The patterns and specific features of bone changes in humans exposed to the space environment are discussed. They can hardly be well understood without detailed and strictly controlled animal experiments in real and simulated microgravity allowing the use of biochemical, biomechanical, histomorphometric and other methods.

Regular bone studies of cosmonauts showed bone mass loss (osteopenia) in trabecular structures of the lower body (lumbar vertebrae, proximal epiphysis of the femur and pelvis). They also demonstrated a correlation (R=0.904) between bone mineral density (BMD) or bone mineral content (BMC) loss and position of a skeletal segment relative to the gravity vector, i.e., its weight-bearing level at 1 g. Densitometry measurements also showed a distinct tendency toward an BMC increase in the upper body (skull and cervical vertebrae). This may be attributed to cranial fluid shifts and, consequently, electrolyte accumulation in the cranial-cervical area. As to the lumbar spine, in some cases (about 1/3 cases) lower BMD and in others higher BMD (mostly in spongy bone) were observed. In addition, during the first 3-5 weeks of re-adaptation cosmonauts developed further BMD and/or BMC loss (lumbar vertebrae, pelvis) regardless of their responses in-flight. Clinically, after 6-7-month flights the average BMD loss was within the WHO normal range. There were several cases that were qualified as local osteopenia.

Bone mass loss in space can be ascribed either to biomechanical (unloading) or metabolic changes (cranial fluid shifts). The first hypothesis finds support in the correlation between bone mass loss in a skeletal segment and its gravity-related position. It has also been supported by animal observations: significant morphological, biomechanical and biochemical changes were detected in weight-bearing bones. According to this hypothesis, BMD loss after flight can be explained by enhanced resorption in response to the return to 1 g, which was observed in animals. It is very important to identify the mechanism of BDM loss because it may exacerbate local osteopenia and increase fracture risks, particularly in cosmonauts who had a low BMD when they went into space.

According to the metabolic hypothesis, BMD changes result from different regulatory systems of skeletal metabolism, i.e., bone, extrabone tissue, volume and ion regulation coupled with hormonal regulation of calcium metabolism. This hypothesis is supported by a large number of animal findings, which showed that changes in spongy bone were more significant than in compact bone.

Animal experiments suggested that osteocytic osteolysis could trigger changes in response to a reduction of cyclic deformations. Animal studies showed that osteocytes together with the lacunar-canalicular network they form act as the primary system that senses mechanical loading. A similar role can be played by collagen-crystal links, which serve as the mineral organic composite at the molecular level. Tail suspension experiments revealed collagen-crystal changes, which may affect bone strength. In flown rats, bone strength decreased while bone mineralization remained unchanged. However, lack of deformations and microcracks may, via a feedback loop, diminish tissue stimulation of osteoblast development and, consequently, delay bone formation.

Variable changes in lumbar vertebrae of cosmonauts may also be related to venous congestion in abdominal parenchymatous organs. On the other hand, this phenomenon may be associated with local specificity of changes and mineral redistribution within the same bone segment. At the same time it can be viewed as a manifestation of phenotypical features of metabolism of various bones. This may lead to changes in adaptive remodeling and, probably, bone geometry, as was observed in Cosmos rats. Bone cell culture studies in microgravity seem

to be in agreement with the above hypotheses, underscoring the involvement of local factors of metabolism regulation in bone changes.

Non-bone or tissue regulatory factors (decrease of the blood calcium binding capacity, Ca absorption in the intestine and reabsorption in renal canaliculi) may also play an important part in the hierarchy of calcium regulation. In addition, changes in volume and ion regulation as well as hormonal regulation may also provoke resorption of osteoclasts and osteoblasts.

In summary, it can be ascertained that local osteopenia develops as a result of 1) active resorption of osteocytes as a primary response to the lack of mechanical stress; 2) delayed bone formation in the course of adaptive remodeling, and 3) additional stimulation of resorption triggered by changes in the hierarchy of volume and ion regulation.

The Use of Animal Models for the Microbial Risk Assessment of Long Duration Spaceflight

C. Mark Ott
EASI/Wyle Laboratories

Human presence in space, whether permanent or transient, is accompanied by the presence of microorganisms. Adverse health effects caused by microorganisms in-flight were dramatically demonstrated during Apollo 13, as infectious disease affected both crew selection and in-flight performance. Thus far, the establishment of key programs and procedures such as the Health Stabilization Program has successfully prevented other serious infectious disease outbreaks. However, results from samples acquired during the NASA-Mir Program in the 1990's indicated a need to reevaluate microbial risk on long duration missions. From a microbial perspective, long duration missions, such as the inhabitation of a space station, differ from missions aboard the Space Shuttle due to the increased use of regenerated water and air systems, an inability to thoroughly disinfect the vehicle, and the difficulty in timely identification of microbial contaminants and infectious agents.

Preflight protocols for the crew, food, and environment before spaceflight have been designed to mitigate the risk of infectious disease during a mission. While in-flight evaluation of the microbial ecology aboard the Mir and International Space Station indicates a predominance of common environmental flora, medically significant organisms have been identified. During NASA-Mir missions 6 and 7, free-floating surface condensate was collected and returned to ground for analysis. The analysis revealed the presence of *Escherichia coli*, *Serratia marcescens*, dust mites, and protozoa. While none of these organisms were evaluated for virulence potential, the results demonstrate the potential of unexpected and possibly infectious agents on long duration missions. Moreover, several studies have documented an increased microbial resistance to antibiotics in-flight. When combined with evidence that suggests the human immune system may be altered during flight, the risk of infectious disease during long-duration missions becomes a critical issue to address.

Estimating the risk of infectious disease is difficult, even for earth-based applications. Microorganisms are classified by certain phenotypic or ribosomal characteristics that may not have any correlation with the infectivity or virulence of the organism, as exemplified by *E. coli*, the ubiquitous enteric organism, which can range from a harmless commensal organism to a toxigenic or hemorrhagic pathogen. Spaceflight may create more difficulties in assessing microbial risk. Recent studies investigating the enteric pathogen, *Salmonella typhimurium*, indicate an increased virulence of the organism when grown under modeled microgravity conditions and compared to organisms grown in a normal gravity control. In addition, modeled microgravity-grown *S. typhimurium* displayed increased resistance to environmental stresses (acid, thermal, and osmotic), increased ability to survive within macrophages, and global alterations in gene and protein expression levels. Microarray-based global transcriptional profiling identified 163 *Salmonella* genes whose expression is altered when cultured in this modeled microgravity environment. These genes represented functionally diverse groups including transcriptional regulators, virulence factors, LPS biosynthetic enzymes, iron-utilization enzymes and proteins of unknown function. The mechanism(s) controlling the increase in *Salmonella* virulence is unclear; however, this study suggests the potential of altered virulence characteristics during spaceflight. This potential may also be affected by other understudied factors, such as changes resulting from radiation-induced mutations or decreased antibiotic efficacy.

Because the outcome of infection is determined by the dynamic interactions that occur between the host and pathogen, an adequate risk assessment cannot be determined by only focusing on the microorganism. Changes that occur to the host at both a system level and a cellular level can alter the infection and virulence potential of a microorganism. Several studies

have demonstrated alterations in the immune system in response to spaceflight. However, the systemic response to direct infection has not been measured. Some insight has been provided by evaluating viral reactivation in astronauts. A high percentage of astronauts carry latent viruses, which are not manifested in the host while the immune system is properly functioning. Evaluations of the crew before and after flight indicate increased reactivation of Epstein-Barr virus (EBV), cytomegalovirus (CMV), and varicella zoster virus (VZV). While increased viral reactivation does not provide conclusive evidence, it does suggest an increase susceptibility of the crew to microbial infection. Furthermore, a true picture of infection would also include evaluation of the host-pathogen interaction at a cellular level. Unfortunately, in vivo measurements of cellular response during infection in-flight cannot be practically completed.

Thus, the risk of a given microorganism is best-determined using models as indicators. The most convenient model for many pathogens is the use of cellular monolayers of mammalian cell lines. This conventional methodology has provided basic information about infection by many pathogens; however, cellular monolayers fail to exhibit many in vivo like characteristics, and the information provided during infection analysis is limited and may reflect artifacts resulting from the nature of the cell culturing technique. Advanced three-dimensional cell culture models have shown great utility in the study of infectious disease in that they are more physiologically relevant to the parental tissues from which they were derived. These 3-D cell/tissue constructs provide a high fidelity model for infection studies that display more in vivo like responses. Moreover, 3-D cell models are easy to generate, inexpensive, and may allow investigation of several pathogens that will not propagate in monolayer cultures. While 3-D cell and tissue constructs closely mimic in vivo tissues and thus provide a powerful means for studying infectious diseases, they are still "reductionistic" in that they do not represent a fully integrated host model. To provide a model that incorporates cellular and system function that reflects the entire host response repertoire during infection, animal models must be used. They provide the only ethical and practical method of obtaining cellular and systemic analysis of microbial infection. Animal models of infection are not without inherent limitations; still these models are the cornerstones of infectivity studies, which provide solid scientific results that are well accepted by the scientific and medical communities.

As human exploration of space continues back to the moon and then to Mars, an evaluation of potential risks resulting from changes in the microorganism, the host, and their interaction must be completed to mitigate the potential of infectious disease during flight. This risk assessment can only be completed using appropriate animal models, the gold standard of infectious disease evaluation.

Sensory-Motor Adaptation to Space Flight: Human Balance Control and Artificial Gravity

William H. Paloski,
Human Adaptation and Countermeasures Office, NASA JSC, Houston, TX

Gravity, which is sensed directly by the otolith organs and indirectly by proprioceptors and exteroceptors, provides the CNS a fundamental reference for estimating spatial orientation and coordinating movements in the terrestrial environment. The sustained absence of gravity during orbital space flight creates a unique environment that cannot be reproduced on Earth. Loss of this fundamental CNS reference upon insertion into orbit triggers neuro-adaptive processes that optimize performance for the microgravity environment, while its reintroduction upon return to Earth triggers neuro-adaptive processes that return performance to terrestrial norms.

Five pioneering symposia on The Role of the Vestibular Organs in the Exploration of Space were convened between 1965 and 1970. These innovative meetings brought together the top physicians, physiologists, and engineers in the vestibular field to discuss and debate the challenges associated with human vestibular system adaptation to the then novel environment of space flight. These highly successful symposia addressed the perplexing problem of how to understand and ameliorate the adverse physiological effects on humans resulting from the reduction of gravitational stimulation of the vestibular receptors in space. The series resumed in 2002 with the Sixth Symposium, which focused on the microgravity environment as an essential tool for the study of fundamental vestibular functions. The three day meeting included presentations on historical perspectives, vestibular neurobiology, neurophysiology, neuroanatomy, neurotransmitter systems, theoretical considerations, spatial orientation, psychophysics, motor integration, adaptation, autonomic function, space motion sickness, clinical issues, countermeasures, and rehabilitation. Scientists and clinicians entered into lively exchanges on how to design and perform mutually productive research and countermeasure development projects in the future. The problems posed by long duration missions dominated these discussions and were driven by the paucity of data available. These issues along with more specific recommendations arising from the above discussions will be addressed an upcoming issue of the Journal of Vestibular Research.

Postflight balance control deficits resulting from sensory-motor adaptive responses to the microgravity environment were recognized early on as a potential untoward side effect of orbital space flight. During the First Symposium in 1965, Graybiel and Fregly introduced a "Quantitative Ataxia Test Battery," which was subsequently used to demonstrate balance control deficits in crewmembers returning from orbital missions in the late 1960s and early 1970s. During the Third Symposium in 1967, TDM Roberts introduced the concept of a labyrinthine-generated "behavioral vertical" to explain a critical role the vestibular apparatus plays in providing a dynamic internal reference frame for neuro-motor control of upright stance, and during the Fourth Symposium in 1968 a number of investigators presented data demonstrating the confluence of multi-sensory information in the vestibular nuclei and the cerebellum and detailed anatomical and physiological descriptions of the vestibulo-spinal system. Throughout the series evidence was also provided for adaptive plasticity in sensory-motor function.

Since those days our understanding of terrestrial balance control has progressed rapidly. In parallel, numerous space flight investigators have contributed to our understanding of the characteristics, demographics, and mechanisms underlying the transient loss of balance control following space flight. Human studies of integrated balance control performance, neuro-motor reflex function, proprioceptive function, and visual-perceptive function have been performed on U.S. and Russian Missions since the 1960s. Animal studies of remodeling in the cerebellum and vestibular end organs have also been performed in both programs.

Postflight decrements in sensory-motor control have now been well characterized from both basic science and occupational health perspectives. Early after flight postural stability is disrupted in all

crewmembers. During short duration missions the underlying cause appears to be vestibular system adaptation, but as mission duration increases somatosensory/motor control system adaptation begins to play an important role. The mechanisms of this slower phase of in-flight adaptation are not yet well understood, but understanding them may be critical to the success of extended duration missions beyond low Earth orbit. As mission duration increases there is also an increased incidence of postflight autonomic dysfunction. For example, orthostatic hypotension, which can exacerbate the balance control deficits, may result in part from vestibular autonomic system alterations.

Owing to the untoward effects of these adaptive responses on astronaut performance, their behavioral manifestations have been fairly well characterized; however, the anatomical sites involved and neurological mechanisms responsible have not. Understanding the cellular and molecular bases for these adaptive responses may be among the greatest challenges of modern neuroscience (Pompeiano, 2002), but the payoff may be improved quality of life for aging individuals, improved therapies for spinal cord injuries and vestibular disorders, improved techniques for rehabilitation from sensory-motor injuries, improved training for elite athletes, and safe extension of both time and distance for human space exploration. Recent results from investigations aboard the space shuttle have begun to identify both sites and mechanisms of vestibular system adaptation, and have demonstrated the feasibility of using orbiting laboratories to investigate the fundamental role that gravity plays in neurological processes. Future laboratory capabilities aboard the International Space Station should expand the role of space flight in exploring fundamental questions in neurophysiology, and these answers, in turn, should reduce the limitations of neurophysiology on space exploration.

To facilitate the next steps in human exploration of space, the mechanisms of somatosensory adaptation and the interactions between vestibular adaptation and altered autonomic system function must be more fully understood. Ground-based venues are unlikely to serve as adequate analogs for these investigations, so space flight venues will be required. Also should the functional implications of these long-duration adaptive responses present sufficient risk to the success of future missions, adequate countermeasures must be developed. This too will require space-based experiment platforms, possibly including animal and human centrifuges to provide artificial gravity.

As NASA prepares for unprecedented human missions to the Moon and Mars, the neuro-vestibular/sensory-motor community will face unprecedented challenges in protecting the health, safety, and performance of the crews aboard these missions. Data from six-month low Earth orbit space flight missions suggest that that substantial neuro-vestibular/sensory-motor adaptation will take place during six-month transit missions to and from Mars. Could intermittent or continuous artificial gravity be used to offset these effects? To what degree would the effects of adaptation to this rotational cure affect its potential benefits? Also, little information exists regarding the gravity thresholds for maintaining functional performance of complex sensory-motor tasks such as balance control and locomotion. Will sensory-motor coordination systems adapt to 30-90 days of 1/6 g on the lunar surface or 18 months of 3/8 g on the Martian surface? Would some form of gravity replacement therapy be required on the surface? And, will transitions between 0 g and 1/6 g or 1/3 g present as great a challenge to the vestibular system as transitions between 0 g and 1 g? Answers to these and other related questions will require concerted research and development efforts, the results of which will lead not only to operational countermeasures, but also to an improved understanding of the role that gravity plays in spatial orientation and movement control, and likely to an improved understanding of the stimuli and mechanisms of adaptive neural processes at least in sensory-motor coordination and control.

Pharmacotherapeutic Aspects of Space Medicine

Lakshmi Putcha, Ph.D.
NASA Johnson Space Center Houston TX

Medications are used for a wide variety of indications during space flight. For example, astronauts have taken drugs in flight to ameliorate or prevent symptoms of space motion sickness, headache, sleeplessness, backache, nasal congestion, and constipation. Russian cosmonauts reportedly take medications to prevent metabolic disturbances of the myocardium and intestinal flora, and to optimize their work capacity. Although the discomfort associated with some acute responses to microgravity (e.g., space motion sickness) is expected to diminish with length of time in flight, other responses that have delayed onset (e.g., maintaining nutritional status, bone and muscle strength, and perhaps immune response) may affect health and quality of life during longer missions. Therefore, as the duration of space flights increases, the need for treatment with medications is expected to increase accordingly.

Medications carried on Space Shuttle missions have varied somewhat from flight to flight, depending on the individual needs of the crewmembers. Medications use during Shuttle flights seems to be more prevalent than during earlier programs, perhaps because drugs are provided in easy-to-use forms. In fact, nearly all medications taken to date have been ingested orally in tablet form. However, given that the oral route may not be ideal for those suffering motion-sickness symptoms, intramuscular and intranasal preparations are being tested. For example, intramuscular administration of promethazine hydrochloride (Phenergan®) has been reported to be more effective in alleviating motion-sickness symptoms. The difficulties involved in conducting definitive studies of drug efficacy during U.S. space flights have been compounded by the absence of a systematic approach to determining which drugs were taken by whom and under what circumstances.

The use of some drugs in space has been less efficacious than expected. The onset, intensity, and duration of the response produced by any drug depend upon rates of absorption, distribution, metabolism, and elimination of the drug; space flight-induced changes in blood flow and the function of the gastrointestinal (GI) tract, liver, or kidneys may alter these processes. Another important aspect of clinical efficacy of medications in space is the stability of pharmaceuticals. As the U.S. space program is moving toward extended Space Shuttle flights and beyond, to space station missions and planetary explorations, understanding how space flight affects organ systems and clinical pharmacology is necessary to optimize pharmacotherapeutics in space and ensure adequate safety and health of crewmembers.

The goal of pharmacotherapeutics research at the Johnson Space Center is to provide safe and effective diagnostic and pharmacological intervention products, procedures and strategies in support of successful space medical operations. To achieve this overall goal, research objectives conceived are to: 1) Identify physiologic, pharmacokinetic and pharmacodynamic changes in space; 2) Develop safe and effective non-invasive sustained-release dosage forms and regimens for pharmacological interventions in space, and; 3) Create and maintain a comprehensive space pharmacokinetic, pharmacodynamic and therapeutic database.

Because the physiologic effects of microgravity develop over hours, days, or weeks, it is to be expected that the effects of some medications will change with increasing time spent in space. It should be possible, at least in principle, to assess some of these changes under experimental conditions on Earth. To be significant for space medicine, however, in-flight trials must take place, with replications before and after flight on Earth, in order to assess variations within and between individuals. In view of the heavy workload of flight crews and the present difficulties of performing meaningful pharmacokinetic-pharmacodynamic assessments with humans in space, serious thought should be given to in-flight animal experiments, with concurrent Earth-based controls. Another important aspect of pharmacotherapeutics research should concentrate on the development of

therapeutic drug monitoring and chronic drug delivery technologies that can meet the challenges of remote treatment needs for the Space Station and exploration-class missions, *e.g.*, to the moon and Mars.

In classic ground-based pharmacokinetic studies, estimates of the rates of absorption, distribution, metabolism, and excretion of compounds are calculated from measuring the amount of the drug and its metabolites in plasma as a function of time. Logistical problems (e.g., lack of refrigerated storage, difficulty drawing blood in microgravity), as well as the desire to minimize the number of invasive procedures that the astronauts must undergo, have led to efforts to develop less invasive means such as salivary drug monitoring to examine the fate of drugs in the body.

Although much is known about the processes that constitute the pharmacokinetic characteristics of drugs on Earth, including the molecular basis of xenobiotic transport and biotransformation, much less is known about the mechanisms by which drugs elicit their effects, whether desired or adverse. This lack of knowledge severely limits the ability to predict the pharmacodynamic changes that may occur during space flight. Because changes in the dose-response relationship can be caused by changes in pharmacokinetics, pharmacodynamics, or both, data must be obtained to delineate the dose-concentration from the concentration-effect components. Other potential sources of response-variation include stress, lack of sleep, and changes in chronophysiologic status. This implies the need for in-flight assessment of therapeutic response and optimization of dosage regimens based on that response.

Concurrent with noninvasive pharmacokinetic/pharmacodynamic methods development and assessment, design and development of alternatives to enteral dosage forms are also pursued. Data currently available suggest that space flight affects absorption of orally administered medications and stability of drug formulations. These findings support the need for the development of novel drug delivery systems for acute and chronic treatment in space.

In conclusion, optimization of therapeutics for space exploration requires research and development of enabling technologies and methods for the diagnosis and treatment of acute and chronic ailments encountered by astronauts while in space and upon return to Earth.

The Use of Animal Models to Study the Effects of Space Flight on the Immune System

Gerald Sonnenfeld, Ph.D.
Morehouse School of Medicine

One of the important regulatory biology interactions affected by space flight is the regulation of the immune response. Alterations in the regulation of immunity could have profound effects on the ability of the host to resist infection and development of tumors. Studies on the immune system in space have been carried out on cell cultures, animals, and humans in space and in environments that model some space flight conditions. Access to space flight experiments and the humans that participate in them is both very limited and extremely expensive, and it is difficult, if not impossible, to carry out all types of experiments in humans. Therefore, investigators have turned to animal models in space flight as well as to ground-based models that include some of the conditions that occur during space flight. Results are similar to human studies.

The rat has been the animal used for the bulk of space flight immunological studies. Rats flown in space were shown to have hypoplasia of the thymus. Additionally, proliferation of leukocytes was altered when tested after the rats returned from a US space flight. In experiments designed to determine the effects of space flight on cytokine production, rats were flown in the Space Shuttle for 7 days. After landing, spleens were removed from the rats and placed in individual cell culture. The cultures were then challenged with concanavalin-A to induce interferon-γ. Interferon-γ production by cells taken from flight rats was reduced compared to that by cells obtained from control rats. However, when the same cell cultures were assayed for interleukin-3, another cytokine, interleukin-3 production was normal by the cells from rats that had been flown in space. Experiments undertaken in a Cosmos biosatellite flight were designed to determine the effects of space flight on leukocyte subset distribution. Alterations were observed in the following cell populations from animals that had flown in space compared to ground-based controls: T-lymphocytes, CD8+ T-lymphocytes, and interleukin-2 receptor bearing T-lymphocytes.

Bone marrow cells from rats flown in Cosmos flights were tested for their ability to respond to macrophage colony stimulating factor or granulocyte-macrophage colony stimulating factor. The bone marrow cells from flown rats were inhibited in their ability to form colonies in the presence of M-CSF, indicating a lack of division on the part of those precursor cells. Spleens were removed from rats immediately upon return to earth, and the spleen natural killer cells were tested for ability to kill target tumor cells. The ability of the natural killer cells from flown rats to kill targets was inhibited compared to the ability of natural killer cells from ground-based controls. Other space flight experiments with rats have yielded similar results with respect to leukocyte subset distribution and compartmentalization.

All of the above studies were carried out on tissues obtained from flown rats immediately upon their return to earth. One later study, however, was carried out an experiment where tissue was taken from rats while they were still in the Space Shuttle. In this case, rats were euthanized 1 day prior to landing, and tissues were kept refrigerated until landing and analysis. Control animals were euthanized at the same time on the ground and tissues were maintained under similar conditions for the same duration as the flight samples. Both leukocyte proliferation and natural killer cell activity were inhibited in samples obtained during flight compared to ground controls. These results indicate that the actual in-flight conditions contributed to the effects observed on the immunological parameters.

Experiments were carried out on the Space Shuttle to investigate the effects of space flight on the development of the immune system. In this case, pregnant rats were flown on the Space Shuttle for the majority of the gestation period, and returned to earth immediately before giving birth. Immune responses of dams, fetuses and pups (fetuses and pups obtained after landing) were

determined after landing. Interferon-γ production, proliferation of leukocytes, and the response of immature cells to colony stimulating factor all showed trends toward inhibition in the dams, but were unaffected in the pups and fetuses.

There have been limited studies, carried out on the effects of space flight on the immune responses of rhesus monkeys using animals flown aboard a Cosmos biosatellite. Upon return to earth, the monkeys showed decreases in interleukin-1 production, alterations in receptors for cytokines, and a decrease in the ability of bone marrow cells to respond to exogenous colony stimulating factors compared to ground-based controls.

In a recent series of experiments, mice were flown aboard the Space Shuttle to study the effects of space flight on the murine immune system. Their results were similar to those observed earlier with rats, including altered leukocyte subset populations and altered cytokine production.

The most commonly used model to recreate some aspects of the space flight environment on the ground has been hindlimb unloading. HU allows the development of physiological changes in muscle, bone, fluid-shifts and other parameters that are similar to some changes observed after weightlessness during space flight. Rats maintained in this model have shown involution of the thymus similar to that seen after space flight. No effects were observed on levels of antibody classes in hindlimb-unloaded rats. Maintenance of rats or mice in this model resulted in severe inhibited interferons α, β, and γ production compared to controls. As a control for the stress of confinement and suspension, mice also were restrained with no head-down tilt.

The HU model was validated as an appropriate model for some of the effects of the space flight environment on the immune system when we carried out an HU study in parallel with a Cosmos biosatellite experiment. Similar results were obtained from hindlimb unloaded rats as those seen after space flight with regard to an inhibited response of bone marrow cells to CSF; however, no correlation was observed on the effects of space flight on subpopulations of leukocytes. An additional HU study using mice was carried out to show that hindlimb-unloaded mice had impaired ability to produce superoxide, decreased ability to kill phagocytosed bacteria (*Propionibacterium acnes*), and altered corticosterone levels. Additionally, the ability of HU of rats to model effects of space flight has been confirmed for dynamic functional immune responses, such as cytokine production, but not on non-dynamic immune parameters, such as leukocyte subset distribution .

HU studies have been used to study alterations to resistance to infection. Female Swiss/Webster mice were inoculated with the D variant of encephalomyocarditis virus (EMC-D virus). Females of the Swiss/Webster strain normally are totally resistant to infection with EMC-D virus with the resistance mediated, at least in part, by interferon . Hindlimb-unloaded mice became susceptible to infection, whereas mice restrained in the orthostatic orientation remained resistant. Alterations in resistance to EMC-D virus correlated with differences in interferon production. Mice maintained in the HU model displayed enhanced resistance to primary infection with *Listeria monocytogenes*, an intracellular pathogen. This was probably due to enhanced macrophage function and cytokine production that aided in eliminating the pathogen. However, at the same time that resistance to primary infection was enhanced, the ability to generate long-term immunologic memory to the challenge organism was inhibited. Therefore, although initial resistance to an intracellular organism was enhanced by hindlimb unloading, the ability to develop long-term resistance to subsequent challenge with infectious organisms was compromised. Resistance to infection of hindlimb-unloaded mice to gram negative pathogens, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, was dramatically reduced, but could be prevented by feeding mice Active Hexose Coordinated Compound, a nutritional supplement.

In one early study that used a model other than HU, mice were maintained in an environment in which barometric pressure was altered in a fashion similar to what occurs during space flight. These confined mice were more susceptible to mengovirus infection than were controls.
Current space flight conditions include occupation of the International Space Station in low Earth orbit for extended periods of time. Future plans could include possible human exploratory missions to the Moon and Mars. During extended deep space flights, astronauts will be exposed to chronic

radiation that could influence the immune system. To study this potential problem, investigators have delineated the effects of proton radiation upon immune structure and function in murine models. These reports are particularly pertinent to the issue of solar radiation to long-term space travelers because of the similarity of the type (proton) of radiation. Space, or solar, radiation is acknowledged to comprise photons (X-rays), electrons (γ-rays), protons, neutrons, and heavy metal ions. As protons account for 80 percent of deep space radiation, use of the synchroton accelerator to provide proton radiation is particularly advantageous in determining the effects of space radiation upon astronauts (and mice in the reported experiments). C57BL/6 mice were given 3 Gray (Gy) protons for γ-rays in one dose; some animals also received an immunization with sheep red-blood cells (sRBC). By Days 4-10 after irradiation, there were statistically significant decreases in $CD19^+$ B-cells, $CD3^+$ T-cells, $CD4^+$ T-cells, and $CD8^+$ T-cells . Immunized and irradiated animals showed a delayed and lowered anti-sRBC antibody response. Natural killer (NK) cells were relatively radio-resistant. There was recovery of B-cells by Day 15 and $CD3^+$ and $CD4^+$ T-cells by Day 29, but $CD8^+$ T-cells remained impaired. The authors also discovered that the more conventional γ-radiation yielded similar data. These investigations were extended to demonstrate that proton radiation (and γ-rays) acutely (4 days) decreased erythrocyte, hemoglobin, hematocrit, leukocyte, and monocyte levels, with partial recovery by Day 17. The immunosuppressive cytokine, transforming growth factor-β1 (TGF-β1), was increased by Day 7 in γ-irradiated mice, but then fell in both proton- and γ-ray treated animals by Day 17. In a paired series of experiments in this murine model system, γ-rays acutely (4 days) decreased spleen lymphocytes more than peripheral blood lymphocytes and that higher doses were more toxic and acutely (4 days) decreased IL-2 secretion by activated spleen cells. In summary, these experiments gave ample evidence that space radiation is likely, at least acutely, to affect immune defenses.

NUTRITITION AND METABOLISM – AN OVERVIEW

T.P. Stein, PhD
University of Medicine and Dentistry of New Jersey

As missions increase in duration, any nutritional deficiencies will become progressively more important. Nutritional issues include: (i) low dietary intake and the associated negative energy balance; (ii) the potential for oxidative damage from the increased exposure to ionizing radiation, especially as astronauts venture beyond the earth's protective electromagnetic belts and (iii) the long time scale of recovery.

Protein: Overall there is no evidence to suggest that the in flight protein intake is inadequate. Plasma amino acids are either unchanged or are increased in flight because of the release of amino acids from muscle. The situation may be different post flight. Plasma amino acids are reduced during the recovery process, an observation which is consistent – but does not prove that protein may be limiting. Although astronauts lose weight during space flight, repletion from malnutrition is not an appropriate model for rehabilitation after space flight (or bed rest) because it lacks the muscle atrophy component. The HLS rat is a viable model for studying the recovery process.

Energy: The most serious nutritional problem is the inability to maintain energy balance on missions with high exercise requirements. Dietary intake is reduced in flight with the reductions being the greatest for missions with high exercise requirements. The variation in dietary intake is between missions rather than between subjects. Within a given mission all astronauts appear to eat about the same amount of food suggesting that the decrease is mission specific rather than microgravity specific.

The consequences of a chronic negative energy balance are potentially serious. On the ground, a chronic energy deficit results in progressive weight loss, decreased physical performance, increased fatigability, delayed wound healing and a progressively increasing susceptibility to infection. Why astronauts are unable to maintain energy balance is not known. Comparison of data from various missions suggest that the inadequate intake is not a direct response to microgravity but rather to a combination of the space craft environment and counter-measures to the effect of microgravity. The total phenomenon has not been reproduced in any ground based model – human or animal. Exercise increases the amount of CO_2 and heat that have to be removed. Whether the depressed food intake is secondary to the heat generated or the CO_2 produced, the cause is exercise generated waste products (heat or CO_2) that cannot be disposed of rapidly. Either hypothesis explain the adverse effects of exercise and the observation that the effect is mission dependent rather than subject dependent and not a consequence of living without gravity. The cause is environmental and is related to air flow and purification. In the absence of gravitational induced convection currents, air flow is totally dependent on mechanical means. Although the cachexia can be reproduced in rats subjected to either thermal stress or high environmental CO_2 this does not appear to a fruitful model for pursuit.

Other nutrients: There is no evidence of any problems of consequence with any other nutrients. It might be advisable to limit iron intake because of the propensity to form ROS; calcium is discussed in another report. Vitamin and dietary anti-oxidant deficiencies are unlikely; astronauts routinely take multi-vitamin capsules.

Metabolic effects: A major question is whether microgravity affects metabolism and if so how? There are two aspects of concern; neither of which is related to food processing. Firstly whether drug metabolism is affected and secondly how humans respond to transitions between gravity levels. Rodent models could be useful here.

The best known transitions are entry into orbit and return to earth. The former seems to pass uneventfully, probably because subjects are a peak health. The reverse, return to earth is surprisingly slow. However it is not of any real concern because extensive rehabilitation facilities are available. More important for planetary exploration is the transition from '0' g to the 0.3 g on Mars. If there is a period of weakness on landing there are serious potential problems, for example decreased work capacity. There are complimentary models available, the rodent hind limb suspension model for examining the recovery after a period disuse and secondly the use of hyper-gravity. Hyper-gravity experiments can address the important question of whether there is a continuum in the response to g or if it is it discontinuous for a given parameter. Hypergravity could be a very powerful model for studying the effects of gravity on metabolism. More data is needed to further develop this model before its' utility is known.

Use of rats: Animal models are best used for determining mechanisms. Their utility is because they are cheaper, invasive studies can be done and less complex than humans. Unfortunately the rat is the usual animal model. There are problems with how rat models have been used. Nearly all studies use young, growing rats. Factoring out which effects are due to growth and which to microgravity has to proven to be very difficult. To date there have been no flight studies of adult rats or flight studies of growing humans. A model should not add complexity.

Studies of mechanism for biomedical research have shifted towards mice because of the availability of transgenic and knockout mice. NASA should reconsider the rat as the rodent of choice. Mice are smaller, mature faster and so adult mice can be studied. They are also smaller so more could be flown in a flight experiment.

Exploratory Research: Finally there is the question of waste disposal for very long term missions. NASA has extensive programs of how to process waste products. Some thought should be given to minimizing human waste production. There is a very relevant model; the brown bear. Bears hibernate, they make protein while hibernating, produce urea but do not urinate because they are able to recycle urea N. Humans recycle ~80% of urea N. The remaining ~20% is excreted. Why the difference? And why no muscle atrophy during the long period of disuse. What are the mechanisms? The machinery is likely to be the same but the regulation will be different. Comparison across different models can be very informative.

Wound Healing in Microgravity: Issues and Animal Models

Ray Vanderby

Clinical Science Center, University of Wisconsin

Overview of normal healing

Injury disrupts normal tissue architecture. In higher vertebrates, organs (except bone) fail to regenerate. Instead, scar tissue forms from a fiber-proliferative process. Thus, the organ then is patched with scar tissue rather than being regenerated. If this normal healing process is altered, the outcome is even less desirable. Reduced mechanical stimulation, poor perfusion, inadequate immune response, sepsis, etc. can lead to delayed healing, non-healing, or excessive fibrosis. Wound healing is a dynamic interactive process that is generally organized into three phases: inflammation, proliferation, and tissue remodeling.

The inflammation phase of healing starts immediately after blood vessel injury and deposition of blood constituents into the extra-vascular tissue, and it extends 2 to 5 days. Blood coagulation and platelet aggregation generate fibrin clots to seal severed blood vessels and discontinuities in the damaged tissue. The vessel clot establishes hemostasis, and the clot in the ECM provides a provisional matrix for cell migration. Platelets facilitate formation of the hemostatic plug, and they secrete mediators and growth factors helping to activate parenchymal cells. Together the cells generate vasoactive mediators and chemotactic factors, and they recruit leukocytes.

The proliferation phase (2 days to 3 weeks) includes epithelialization (*i.e*. reestablishing a cutaneous cover) and formation of granulation tissue (i.e. restablishing dermal integrity). Epithelialization of a wound normally begins within hours of injury. Epithelial cells from residual epithelial structures move quickly across the wound to form a barrier. The epithelial barrier reduces morbidity and mortality. Concomitant with migration, cells change their phenotype to have better motility over the wound. As re-epithelialization occurs, basement membrane proteins reappear in the margin of a wound and move inward. Then, granulation tissue (new stroma) begins to form approximately four days after injury. The tissue contains new blood vessels, granulation tissue, macrophages, fibroblasts, and loose connective tissue. The macrophages provide a continued source of cytokines to simulate fibroplasia and angiogenesis, fibroblasts construct new ECM to support cell and blood vessel ingrowth. Blood vessels carry oxygen and nutrients to sustain cell metabolism.

Finally, tissue remodeling phase (3 weeks to 2 years) replaces the provisional matrix with collagenous scar. ECM remodeling, cell maturation, and cell apoptosis then form the third stage of wound healing. ECM remodeling and maturation of the neoepidermis and neovasculature begin in the wound margin even before granular tissue has invaded the wound's center. Once neoepidermis covers the wound, fibroblasts transform into myofibroblasts, contracting the wound, and epidermal cells differentiate to form a permeability barrier. Endothelial cells undergo apoptosis first, then the myofibroblasts, and a relatively acellular scar is gradually formed. The ECM gradually replaces granulation tissue. The first ECM to be deposited around the fibrin clot (*i.e*. the provisional matrix) contains many proteins including fibronectin, vitronectin, von Willebrand factor, and thrombospondin. The provisional matrix promotes a fibroblast phenotype that lyses the fibrin clot and forms granulation tissue. After clot lysis, fibronectin is deposited as a second provisional matrix, followed by type III collagen, and finally type I collagen as the ECM continues to remodel. Hyaluronan is a major component of early granulation tissue to promote cell movement. Its concentration then falls to a level that remains fairly constant as glycoaminoglyans increase. Proteogylcans help in the organization of the newly forming extracellular matrix and are continually remodeled during wound healing like other matrix molecules. After synthesis and deposition of type I, type III, and type VI collagens, myofibroblasts contract and remodel the ECM.

Continued synthesis and catabolism occurs. Collagenase enzymes from granulocytes, macrophages, and fibroblasts control the process. Wounds gain about 20% of their final strength by the third week,

during which fibrillar collagen has been rapidly synthesized and remodeled. Thereafter, wounds gain tensile strength at a slower rate to reach a maximum of 70 to 80% of the original tissue.

Effects of Microgravity

Relatively few studies have been performed that define the effects of microgravity on wound healing. Direct studies of bone (Kaplansky *et al*. 1991), muscle (Ilyina-Kakueva *et al*. 1991), and connective tissue healing (Davidson *et al*., 1998) suggest the microgravity environment is problematic. Indirectly, spaceflight is known to diminish bone mass, muscle strength, ligament strength, and immune function. Spacefight also compromises hemodynamics with fluid shifts and reduced microcirculation, and microgravity alters peripheral nerve activity with altered muscle function.

Diminished bone mass and bone turnover is associated with fracture healing problems in osteoporotic patients. An appropriate (osteogenic) mechanical stimulus accelerates fracture healing and bone remodeling. Reduced muscle loads result in atrophy and reloading produces inflammation and membrane damage. Unloaded joints have compromised ligaments and ligament insertions, and they have compromised ligament healing (Provenzano *et al*. (2003). Mechanical loading from amulation can enhance ligament and tendon healing.

The immune system also plays a critical role in the wound healing process. Through the generation of bioactive substances, macrophages orchestrate the complex process of cell proliferation and functional tissue regeneration. Wound macrophages produce chemo-attractants to recruit and activate additional macrophages. Together they secrete growth factors to promote cell proliferation and synthesis of proteases and ECM molecules. Macrophages also secrete factors that restrain tissue growth once repair is complete.

Compromises in peripheral vascularity and/or innervation are also detrimental to healing. Consider for example, wound healing in diabetics or wound healing in other peripheral neuropathies. Delayed healing in these wounds produce a higher incidence of sepsis.

Each of the above microgravity-induced, physiologic compromises has been shown to degrade wound healing in ground-based or clinical studies. It is likely that a combination of these factors contribute to wound-healing problems associated with spaceflight. So, a limited strategy of addressing only one (*e.g.* only mechanical or only immune deficits) may prove unsatisfactory. More comprehensive treatment strategies may be necessary, but they will take more effort to formulate. Such strategies will require the extensive use of animal models.

Animal Models

Animal models provide data that cannot be gathered in humans. Highly invasive studies can be undertaken, entire tissues and organs can be retrieved, and experimental conditions can be more carefully controlled. The subject population is more uniform and can be selected or "engineered' to optimally address a specific question or hypothesis. "Markers" for bone or ECM remodeling, cell mitosis, gene expression, etc. can be used for specific *ex vivo* assays.

There are space flight animal models and ground-based models. In space, the growing rat is the primary animal model. Rhesus monkey has also been used as well as chicken embryo. Relatively few animals have been flown at one time and no longitudinal studies have been performed (Turner, 2000). Most studies are more observational in nature and short in duration (4-18 days) compared to human orbiting laboratories and planetary exploration (many months).

Ground-based animal models include various methods to immobilize or unweight limbs, but tail suspension of rodents is by far the most dominantly used model. Since its inception at NASA Ames in the mid 1970's, rodent hindlimb unloading has become and accepted and widely used model. Morey-Holton and Globus (2002) cite more than 800 references that have used this model. They summarize the value of ground-based animal models as: Experiments can be scheduled without concern for crew time. Manipulations can be performed without extensive precautions (*e.g*. triple containment of chemicals and specimens). The experimental duration can be varied within a single

experiment. Experiments can be easily repeated or extended. But, more importantly, ground based experiments are cost effective.

Summary

Reduced mechanical stimulation, poor perfusion, inadequate immune response, sepsis, etc. altered peripheral nerve activity are all associated with delayed wound healing, non-healing, or excessive fibrosis. All of these conditions exist, to some extent, during spaceflight. The implications and combined effect of these factors for healing during spaceflight remains unclear. Animal models could be of great value. All of these factors can be controlled in ground-based animal experiments. Increases in morbidity could be better defined. Then, if wound healing is shown to be sufficiently problematic. Key mechanismal factors could be identified and efficacy of various treatments could be evaluated.

References

Kaplansky AS, Durnova GN, Burkovskaya TE Vorotnikova. (1991) The effect of microgravity on bone fracture healing in rats flown on Cosmos-2044. *Physiologist* 34: S196-9.

Ilyina-Kakueva IE, Burkovskaya TE. (1991) The microgravity effect on a repair process in ***M. soleus*** of the rats flown on Cosmos-2044. *Physiologist* 34: S141-2.

Davidson JM, Aquino AM, Woodward SC, Wilfinger WW. (1998) Sustained microgravity reduces intrinsic wound healing ond growth factor responses in the rat. *FASEB J* 13: 325-329.

Provenzano PP, Martinez DA, Gindeland RE, Dwyer KW, Turner J, Vailas AC, Vanderby R. (2003) Hindlimb unloading alters ligament healing. *J Appl Physiol* 94: 314-324.

Turner RT. (2000) What do we know about the effects of spaceflight on bone?", *J Appl Physiol* 89: 840-7.

Morey-Holton ER, Globus RK. (2002) Hindlimb unloading rodent model: technical aspects", *J Appl Physiol* 92: 1367-77.

Animal Models for Biomedical Research

Dr. Charles E. Wade

Life Sciences Division, NASA Ames Research Center, Moffett Field, CA

Guiding Principles For the Use of Animal Models

Animal experiments are undertaken only with the purpose of advancing knowledge for the improvement of human or animal health and the good of society. Consideration should be given to the appropriateness of experimental procedures, species of animals used, and number of animals required when conducting research.

Animals used in laboratory research shall be lawfully acquired, and their retention and use shall be in every case in compliance with federal, state and local laws and regulations, and in accordance with the Institute for Laboratory Animal Research (ILAR) Guide for the Care and Use of Laboratory Animals*.

Animals used in research must receive every consideration for their health and comfort. They must be properly housed and fed, and their surroundings kept in sanitary conditions. Appropriate anesthetics must be used to eliminate sensibility to pain during any surgical procedures. The care and use of animals shall be conducted such that pain and discomfort is minimized. All measures to minimize pain and distress that would not compromise experimental results must be employed. If the study requires the termination of an animal, the most humane euthanasia method consistent with the study must be used.

Justification/Validation of the Animal Model

Historical reference: There is an extensive body of literature on the use of specific experimental animal models. These historical data provide the basis for all subsequent use of the animals, facilitating refinement of experiments, reduction in the use of experimental animals, and possible replacement with alternative methods. This approach optimizes the science return on any single experiment.

Model Specimens: Recent efforts have led researchers to focus on a limited number of animal species for use in experimentation. These species were selected initially as research animals because they had common attributes as well as unique biological characteristics. Furthermore, model specimens are easy to acquire and maintain. Due to extensive use of various model specimens, identification of their genomes has proceeded at a rapid pace, further expanding their utility. NASA has considered a similar approach of using model specimens in the flight program due to limited resources (flight opportunities, crew support, etc.) and engineering constraints. Such limitations during spaceflight must be considered in the formulation and development of a space research program and in the use of animals in space research. However, the animal model selected must be appropriate for the question being investigated.

Relationship to human condition: As the NASA Biomedical research program became focused on the health and well-being of the crew during long-term exploration, critical questions have been asked regarding the use of animal models. For example, if medical care necessitating a surgical procedure is to be evaluated, the selection of a non-mammalian species may not be appropriate. Furthermore, the basis for which non-human species are selected should be well defined to mimic the human condition to be evaluated. In many cases, this is not valid in the selection of model specimens. To appropriately

evaluate the utility of an animal, the human condition must also be defined. Prevalence and etiology, as examples, should be defined to facilitate selection of the animal model.

Role of Animal Models

The safety and well-being of the human subjects is paramount. This applies under conditions in which the use of humans entails ethical issues, especially when there is a risk of morbidity or mortality. For this reason, many regulatory agencies require evaluation in animal models, or cellular models derived from prior work with animals, before allowing products or procedures to be distributed.

The use of animals in research is often as a surrogate for humans. As surrogates, animals can also be used to set limits for humans. In studies on the exposure of astronauts to a sustained elevation of ambient carbon dioxide during spaceflight, limits were taken from earlier work in submarines. The carbon dioxide limits on submarines were derived from long-term exposure in animals that were subsequently verified in humans. The use of animals to derive human applications should be followed by experimental evaluation in humans under controlled conditions with appropriate follow-up during operational applications.

Animals also serve as sentinels in the exposure to humans to novel environments or experimental conditions. In the conduct of experimental investigations, observations made in animals can lead to a better understanding of subsequent risk to humans. For example, the short life span of lower organism often elucidates long-term effects that can aid in the protection of humans.

Summary

The use of animals to understand responses to novel conditions, such as space flight, and to evaluate the efficacy of interventions and therapeutics is essential to the health and safety of humans

Reference
*Institute for Laboratory Animal Research (ILAR). Guide for the Care and Use of Laboratory Animals. Washington, D.C.: National Academy Press, 1996.

This meeting and report are products of the Center for Advanced Studies in the Space Life Sciences (CASSLS) at the Marine Biological Laboratory in Woods Hole, MA. CASSLS is funded under NASA Cooperative Agreement NCC-1266 and administered through NASA Ames Research Center.

For more information on CASSLS and its activities, please contact Diana E. Jennings, Ph.D. at 508-289-7535 or djennings@mbl.edu, or visit CASSLS on the world wide web at www.mbl.edu/CASSLS.