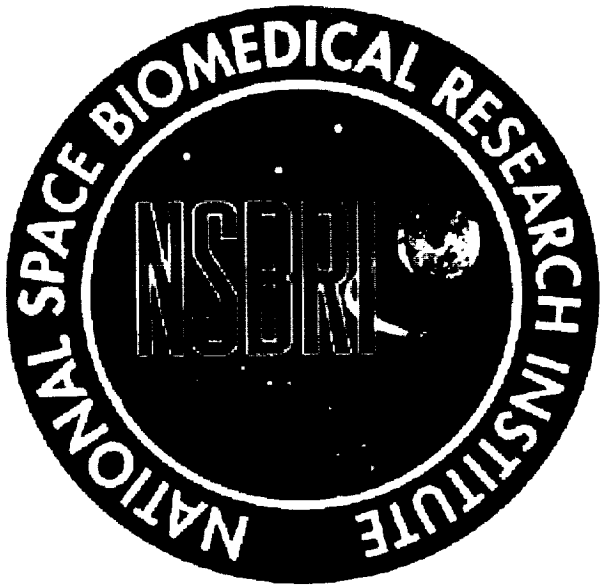
NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

Annual Report

October 1, 2000 – September 30, 2001



Cooperative Agreement NCC 9-58

with the

National Aeronautics and Space Administration
Lyndon B. Johnson Space Center
Houston, Texas

September 30, 2001

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

ANNUAL REPORT
OCTOBER 1, 2000 – SEPTEMBER 30, 2001
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1.0 INTRODUCTION

This report outlines National Space Biomedical Research Institute (NSBRI) activities during FY 2001, the fourth year of the NSBRI's programs. It is prepared in accordance with Cooperative Agreement NCC 9-58 between NASA's Lyndon B. Johnson Space Center and Baylor College of Medicine (NSBRI).

2.0 BACKGROUND

The NSBRI is responsible for the development of countermeasures against the deleterious effects of long-duration space flight and performs fundamental and applied space biomedical research directed toward this goal. Its mission is to lead a world-class, national effort in integrated, critical path space biomedical research that supports NASA's Human Exploration and Development of Space (HEDS) Strategic Plan by focusing on the enabling of long-term human presence in, development of, and exploration of space. This is accomplished by:

- designing, testing and validating effective countermeasures to address the biological and environmental impediments to long-term human space flight;
- defining the molecular, cellular, organ-level, integrated responses and mechanistic relationships that ultimately determine these impediments, where such activity fosters the development of novel countermeasures;
- establishing biomedical support technologies to maximize human performance in space, reduce biomedical hazards to an acceptable level and deliver quality medical care;
- transferring and disseminating the biomedical advances in knowledge and technology acquired through living and working in space to the general benefit of mankind, including the treatment of patients suffering from gravity- and radiation-related conditions on Earth; and
- ensuring open involvement of the scientific community, industry and the public in the Institute's activities and fostering a robust collaboration with NASA, particularly through NASA's Lyndon B. Johnson Space Center.

NASA established the NSBRI in April 1997 following competitive selection. Primary support for the NSBRI's activities is furnished by NASA through a cooperative agreement although funds to support Institute activities also come from several sources, including the institutions involved in carrying out the NSBRI's programs. The cooperative agreement award is for a five and one-half year base period and three, five-year optional extensions. The first optional extension, lasting until September 30, 2007, will be exercised. Initial annual base funding for the Institute's first two years of operation (FY 1998 and FY 1999) was approximately $10 million. In FY 2000, base annual funding was increased to approximately $14 million to develop the infrastructure needed to support planned program growth in FY 2001. In support of the

expanded research and education program, the FY 2001 base annual funding was increased to approximately $25 million.

The NSBRI is governed by a consortium of twelve institutions – Baylor College of Medicine, Brookhaven National Laboratory, Harvard Medical School, The Johns Hopkins University School of Medicine and Applied Physics Laboratory, Massachusetts Institute of Technology, Morehouse School of Medicine, Mount Sinai School of Medicine, Rice University, Texas A&M University, the University of Arkansas for Medical Sciences, the University of Pennsylvania Health System and the University of Washington. The Institute's headquarters are located in Houston at Baylor College of Medicine.

The initial Institute research program consisted of eight research teams carrying out 37, three-year projects and four, one-year "synergy" projects designed to bridge between discipline research team activities and create an appropriate atmosphere for future interdisciplinary research. Because of the competitive process used by NASA to select the NSBRI, most of the initial program was carried out at the seven original consortium institutions. There are, however, no restrictions concerning institutional participation in Institute activity. As a result of two research announcements in FY 2000, the institute expanded to 12 research teams and 85 research projects during its fourth year. In addition to its research program, the NSBRI has developed vital education and outreach and communications programs that take advantage of the Institute's core research activities.

The management plan for the Institute is based on the model used by the National Institutes of Health. An independent Board of Scientific Counselors is responsible for assuring excellence in the Institute's intramural program through independent external peer review, and an External Advisory Council is responsible for advising Institute management concerning programmatic effectiveness. The NSBRI also has a User Panel of former and current astronauts and flight surgeons responsible for assuring that the research program is focused on astronaut health and safety. An Industry Forum of representatives of aerospace, biomedical and technology industries assists in developing industry participation in NSBRI and in timely technology transfer.

3.0 RESEARCH PLAN

The NSBRI's strategic research agenda involves 12 teams of scientists focused on:

- *Bone Loss* – Addressing the loss and weakening of bone during space flight with the inherent fracture risks;
- *Cardiovascular Alterations* – Addressing inflight increase of cardiac dysrhythmias and postflight impairment of the cardiovascular response to orthostatic and exercise stress;
- *Human Performance* – Investigating maintenance of high cognitive performance and vigilance despite environmental stress and sleep disturbances;
- *Immunology, Infection and Hematology* – Addressing immune system impairment and altered susceptibility to infection, increased allergic response, decreased blood volume and postflight anemia;
- *Integrated Human Function* – Developing an overall understanding of the human body's response to space flight;
- *Muscle Alterations and Atrophy* – Focusing on the loss of skeletal muscle mass, strength and endurance that accompanies space flight;
- *Neurobehavioral and Psychosocial Factors* – Investigating methods and tools crews can utilize to cope with stress, isolation and compatibility;

- *Neurovestibular Adaptation* – Addressing the problems of space motion sickness and disorientation during flight and the postflight problems of balance and gaze disorders;
- *Nutrition, Physical Fitness and Rehabilitation* – Developing methods to maintain health and fitness before, during and after space flights;
- *Radiation Effects* – Addressing the problem of increased cancer risk caused by the natural space radiation environment;
- *Smart Medical Systems* – Developing new methods of remote medical diagnosis and treatment; and
- *Technology Development* – Developing instrumentation that will enhance the research of the other teams and transferring the technology to industry for the benefit of society.

Each research team consists of investigator groups working on complementary projects focused on a common theme. Team management and coordination is the responsibility of a program director called a Team Leader while overall scientific direction is the responsibility of the Institute Director and Associate Director. For FY 2001, the total intramural research program, including all 12 research areas, involved 85 projects. These projects were selected from the 281 proposals submitted through NSBRI's two FY 2000 research announcements. The average annual funding per project was approximately $280,000 (Direct + Indirect Costs).

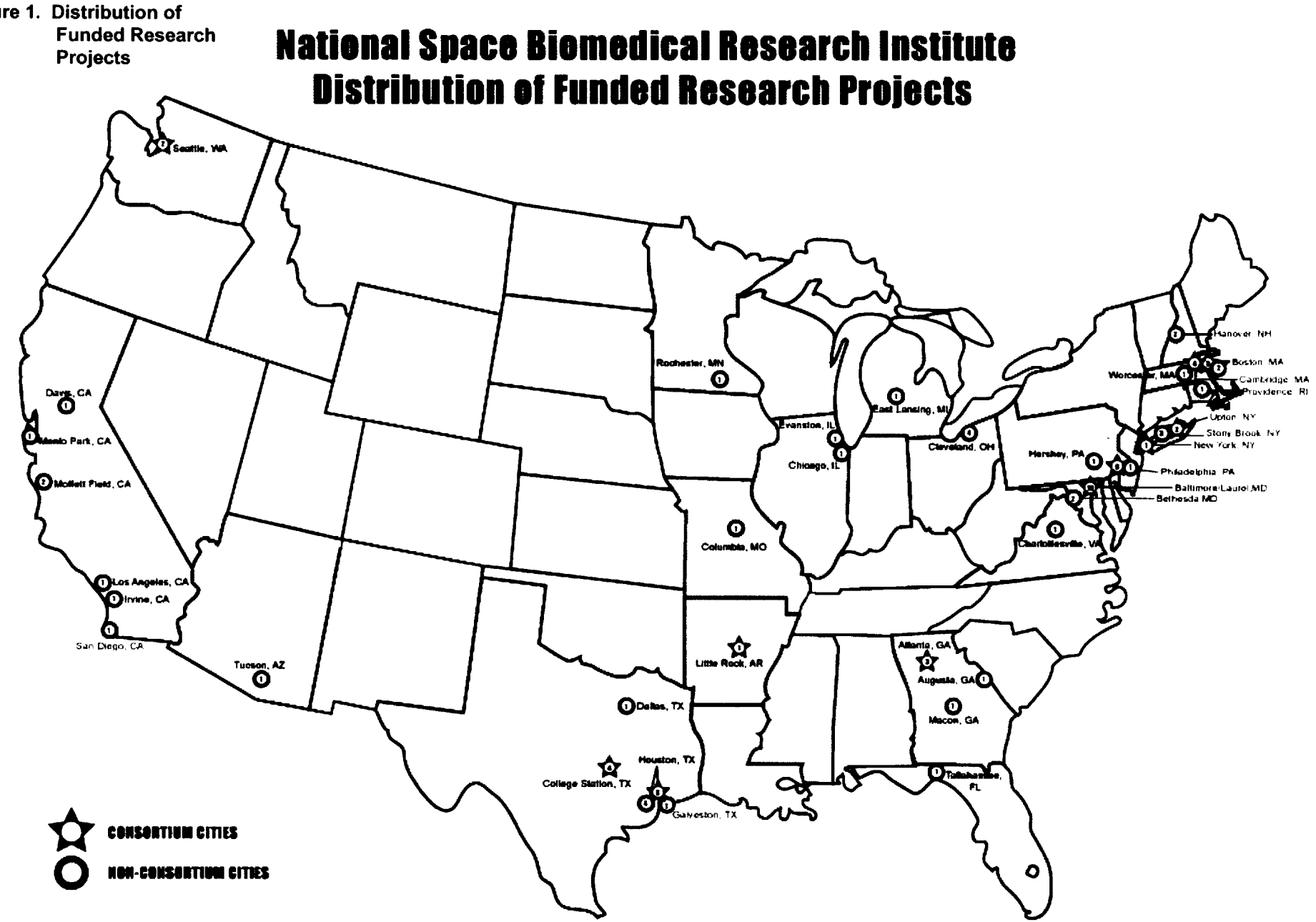
At the start of FY 2001, NSBRI had not received a firm budget from NASA. As a result, the Institute funded only continuing projects and new projects associated with Team Leaders or Associate Team Leaders. The additional project proposals, ranked through peer review as available for selection, were placed in a holding status until the FY 2001 budget was available. In January, the NSBRI received its budget from NASA and funded additional projects on February 1, bringing the project total to 85. The February-funded projects were allowed to stagger their start-up date from March to July. In addition, five projects were selected for further definition as space-flight experiments. These projects are currently undergoing feasibility review by NASA.

It should be noted that the NSBRI research announcements succeeded in drawing researchers from outside the consortium with a total of 47 projects funded at consortium institutions and 38 projects funded at non-consortium institutions. Seventy-two institutions have either a Principal Investigator or Co-Investigator working on an NSBRI project. Figure 1 shows the distribution of projects across the United States. A summary of each project is provided in Appendix A. Further details concerning the research program are provided on the NSBRI Web site, www.nsbri.org, and in Appendix B, containing the program reports for each research team. *(Note that the pre-publication results presented in this Appendix are intended for NASA internal use only as these results are privileged.)*

In FY 2001, NSBRI's continuing investigators attended the NASA Bioastronautics Meeting in Galveston. In May and June, two teams, Integrated Human Function and Nutrition, Physical Fitness and Rehabilitation, benefited from specialized working groups composed of experts who provided counsel on ways to strengthen and focus each team. Reports from each workshop are provided in Appendices C and D. A number of research results were published during FY 2001. Due to staggered start dates for the 85 projects, the list in Appendix E covers primarily the activities of the NSBRI projects funded at the start of FY 2001. The document lists papers, reports, abstracts and presentations resulting from full or partial NSBRI support.

In addition to the core intramural research program, the NSBRI has developed a joint program with the National Institute on Deafness and Other Communication Disorders (NIDCD) that

Figure 1. Distribution of Funded Research Projects



jointly funds six competitively awarded extramural grants related to the dynamic adaptation of central vestibular function, an area of common interest. Appendix F gives funding information for this five-year joint program initiated in FY 1999. NIDCD contributed more than $1.1 million to this program in FY 2001, while the NSBRI contributed $218,423 from private sources.

Table 1 presents the summary of major NSBRI activities taking place in FY 2001. The activities ranged from the NASA three-year review and team workshops to management-related meetings of the Council and Board designed to provide guidance and oversight to the Institute's programs.

Table 1. MAJOR NSBRI ACTIVITIES
October 1, 2000 – September 30, 2001

DATE	ACTIVITY	LOCATION
November 9-10	NASA Three-Year Review Rehearsal	Houston
November 13-15	Peer Review of Education and Outreach Proposals	Houston
Nov. 29 – Dec. 1	NASA Three-Year Review	Houston
January 17-19	NASA Bioastronautics Meeting	Galveston
February 1	Research Award Announcement	N/A
Feb. 28 – March 1	External Advisory Council Meeting	Houston
March 22	Board of Director's Meeting	Houston
April 24-25	NSBRI Education and Public Outreach Team Workshop	Houston
May 2-3	Integrated Human Function Working Group	Houston
May 15-17	NASA/NSBRI Space Medicine Workshop	Clear Lake
June 21-22	Nutrition, Physical Fitness and Rehabilitation Working Group	Conroe
July 31-August 2	Standardized Flight Data Collection Working Group	Houston

3.1 Space Medicine/Operational Issues

Two joint NASA/NSBRI activities were held in FY 2001 addressing current space medicine issues.

NASA/NSBRI Space Medicine Workshop
The first NASA/NSBRI Space Medicine Workshop was held May 15-17. The workshop focused on Resuscitation, Stabilization and Critical Care for the Space Shuttle and International Space Station. The group was charged with developing an evidence-based practice of space medicine and with using current information to develop an educational program for space medical officers. After initial discussion, the committee decided to rewrite the current protocols for care in space based on space needs and space physiology and to develop a training program based on this knowledge. To achieve this process a list of all potential problems that could occur in space was

developed addressing neuropsychological problems; general medical, cardiac and respiratory problems; and trauma problems. The report from the first workshop is provided as Appendix G. This effort is an ongoing activity that will continue into FY 2002.

NSBRI/NASA Standardized Flight Data Collection Working Group
NSBRI coordinated a working group on this topic. Its first meeting was held July 31-August 2. Over time, the panel will develop a proposal for the collection of a standardized, integrated physiological/medical data set on all persons who fly in space as part of the U.S. space program. The data set will be named the Clinical Status Evaluation Data Set (CSE). The initial focus of the group will be to develop the CSE for entry, landing and the immediate postflight period. The working group report is included in Appendix H. This working group is an ongoing activity.

3.2 Research Announcement Plans

In preparation for a possible joint research announcement with NASA in FY 2002, NSBRI Team Leaders provided management with draft language for their section of the announcement. Each Team Leader presented focused research questions that were mindful of the team's current gaps and consistent with the critical path risks and questions.

4.0 EDUCATION AND PUBLIC OUTREACH PLAN

The Education and Public Outreach Team supports the NSBRI's mission by ensuring open involvement in the Institute's activities by the scientific community, industry and the public, and by ensuring a robust exchange with NASA. Activities target multiple and diverse populations and aim to:

- inform a large community about NSBRI activities;
- attract young people to careers in science, engineering and medicine;
- promote excellence and innovation in America's science education system;
- increase scientific literacy among teachers, students, their families and the public; and
- create public awareness and appreciation of the opportunities and benefits of NSBRI's space biomedical research.

Through a variety of innovative programs, space research activities and discoveries are transferred to teachers at levels K-undergraduate, students and the general public.

The National Research Council's Committee on Undergraduate Education has challenged the scientific community and institutions of higher learning to provide opportunities for professional collaborations that create innovative inquiry-based, multidisciplinary courses and instructional materials; to use the most sophisticated multimedia capabilities to disseminate new materials; and to develop a seamless pipeline of minority-group science students. The NSBRI has embedded this challenge in its education and public outreach mission.

Previously, education and public outreach activities were led by teams at three consortium institutions: Morehouse School of Medicine, Texas A&M University and Baylor College of Medicine.

In response to a Special Program Announcement for NSBRI Consortium Institutions (NSBRI 00-02), issued in FY 2000, 18 proposals to expand the education and public outreach activities of the Institute were received. The Announcement specified that proposals must be based at one of the 12 consortium institutions, but encouraged non-consortium schools to collaborate with

consortium institutions. A specialized panel of peers reviewed the proposals on November 13-15 in Houston. A list of the panel is provided in Appendix I. Following peer review, six projects were selected for funding. The six projects received funds in early February. Another five proposals were placed in a special hold category of proposals that scored well but did not receive funds due to budgetary reasons. In August 2001, funds were allocated to add one project from the hold category to the program.

The NSBRI Education and Public Outreach Team held a retreat on April 24-25, 2001, in Houston. The retreat allowed team members to learn the details of each project and examine possible collaborative efforts. Investigators presented information about their projects. The team also shared information on teacher professional development, dissemination models, products and results, technology applications, high school and undergraduate student programs, and research topics and journal publication opportunities.

The team's program report is included as Appendix J, and Appendix K provides an executive summary of each project. A brief outline of each project is presented below.

Baylor College of Medicine
Baylor College of Medicine is producing and disseminating a series of teacher activity guides, *From Outerspace to Innerspace*, that make NSBRI research areas relevant to young students and allow students to investigate these topics. The guides are designed for grades 4-6.

Massachusetts Institute of Technology
The Massachusetts Institute of Technology is developing and evaluating two graduate-level curricula and adapting these materials for undergraduate-level courses, to educate a generation of scholars in space life sciences.

Morehouse School of Medicine
Morehouse School of Medicine is providing teacher professional development and student educational opportunities by writing secondary-level problem-based cases, conducting a summer research program and maintaining an NSBRI film archive.

Mount Sinai School of Medicine
Mount Sinai School of Medicine investigators are working with teachers and students in the New York City area to develop a stand-alone curriculum that will use space biomedical research as a theme to teach math and science.

Rice University (in collaboration with The University of Texas Medical Branch)
Led by researchers and educators at Rice University and The University of Texas Medical Branch, high-school students spend a summer working in research laboratories and exploring the life of a research scientist. The project also features a professional development opportunity for secondary-school teachers that enhances their knowledge of space biomedicine.

Texas A&M University
Through the Teacher Academy, Texas A&M University is establishing a group of highly-trained teachers recruited from across the nation. These Master Teachers will take what they learn about implementing space-based science curriculum and pass their skills and knowledge on to peers in their own schools and regions.

University of Washington
Students in the University of Washington's Technical Communications program will have the opportunity to interview NSBRI researchers as part of a science writing course. Articles appear in the magazine, *Northwest Science & Technology*.

5.0 MANAGEMENT PLAN

The original management plan described in the proposal to establish the NSBRI has continued to serve the Institute's needs during the Institute's fourth full year of operation and has not been modified. For convenience, the management structure is shown in Figure 2, adopted from the original proposal.

Key Personnel
During FY 2001, the senior Institute management team (Bobby R. Alford, M.D., Chairman of the Board and Chief Executive Officer, Laurence R. Young, Sc.D., Director, and Ronald J. White, Ph.D., Associate Director) did not change. However, with the determination by the Board of Directors in their March 2000 meeting that it was in the best interest of the Institute to have a full-time director resident at NSBRI's Headquarters in Houston, a search has been ongoing to replace Dr. Young, who wished to remain at MIT. Dr. Young has agreed to serve until the Board can appoint his replacement. By the end of FY 2001, the Board had interviewed a number of candidates and is continuing to do so.

During FY 2001, all 12 Team Leaders began or continued to function as the research "program directors." However, the Team Leader of the Technology Development Team, Vince Pisacane, announced his retirement. He was replaced by Jeffrey Sutton, Harvard-MIT Division of Health Sciences and Technology, as acting Team Leader with assistance from Harry Charles, Jr., The Johns Hopkins University Applied Physics Laboratory, as Associate Team Leader. Paul Bottomley, Co-Investigator on the Pisacane project, is now the Principal Investigator.

One Principal Investigator changed in FY 2001. In the area of Neurobehavioral and Psychosocial Factors, Gary Ashton-Jones, a Co-Investigator on the project "Stress, Performance and Locus Coeruleus," is now the Principal Investigator, replacing James P. Druhan. The project remains based at the University of Pennsylvania School of Medicine. Two Principal Investigators relocated during FY 2001. In the area of Muscle Atrophy and Alterations, Robert W. Wiseman moved from the University of Washington to Michigan State University. In the area of Integrated Human Function, P. Bryant Chase relocated from the University of Washington to Florida State University.

Board of Directors
The current membership of the NSBRI Board of Directors is shown in Table 2. The Board met in Houston once during FY 2001. One member of the Board changed during FY 2001. Alan L. Schiller, Mount Sinai School of Medicine, replaced Robert Berne, New York University. The second Board of Directors meeting, originally scheduled for September 25, was postponed due to the delay of the External Advisory Council meeting. The meeting was rescheduled for November 2001.

Figure 2. Originally Proposed NSBRI Structure.

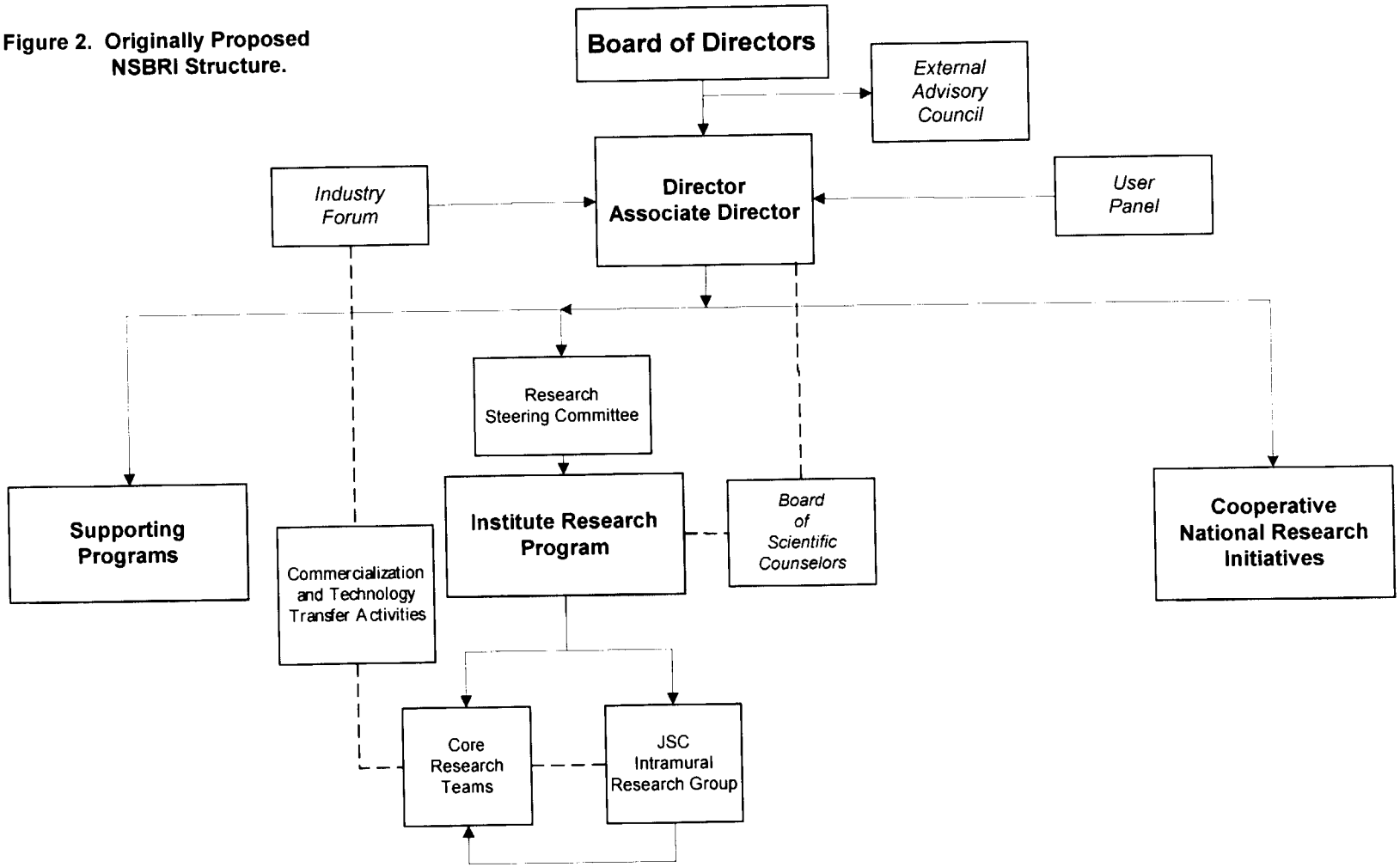


Table 2. **NSBRI BOARD OF DIRECTORS**

Bobby R. Alford, M.D. (Chairman) Executive Vice President and Dean of Medicine Baylor College of Medicine	**William L. Allen** Editor National Geographic Magazine	**Carl W. Anderson, Ph.D.** Chairman Department of Biology Brookhaven National Laboratory
Thomas E. Andreoli, M.D. Professor and Chairman Department of Internal Medicine University of Arkansas College of Medicine	**Joseph V. Bonventre, M.D., Ph.D.** Co-Director Harvard-MIT Division of Health Sciences and Technology Harvard Medical School	**James F. Buchli** Space Station Program Manager United Space Alliance
Aaron Cohen Zachry Professor of Mechanical Engineering Texas A&M University	**Michael E. DeBakey, M.D.** Chancellor Emeritus Baylor College of Medicine	**Richard E. Ewing, Ph.D.** Vice President for Research Texas A&M University
Martin J. Fettman, D.V.M., Ph.D. *(ex officio)* Associate Dean for the Professional Veterinary Medical Program Colorado State University	**Alfred P. Fishman, M.D.** Senior Associate Dean Office of Program Development University of Pennsylvania Health System	**Glen N. Gaulton, Ph.D.** Vice Dean for Research and Research Training University of Pennsylvania School of Medicine
Martha L. Gray, Ph.D. Co-Director MIT-Harvard Division of Health Sciences and Technology Massachusetts Institute of Technology	**E. Nigel Harris, M.D.** Dean and Senior Vice President for Academic Affairs Morehouse School of Medicine	**Richard J. Johns, M.D.** Distinguished Service Professor of Biomedical Engineering The Johns Hopkins University School of Medicine
Dennis Kasper, M.D. Executive Dean of Academic Programs Harvard Medical School	**Joseph P. Kerwin, M.D.** Senior Vice President Wyle Laboratories	**Steven Knapp, Ph.D.** Provost and Vice President for Academic Affairs The Johns Hopkins University
Jordan Konisky, Ph.D. Vice Provost for Research and Graduate Studies Rice University	**Alvin L. Kwiram, Ph.D.** Vice Provost for Research University of Washington	**J. David Litster, Ph.D.** Vice President for Research and Dean of Graduate Education Massachusetts Institute of Technology
Larry McIntire, Ph.D. E.D. Butcher Professor of Chemical Engineering Rice University	**Francis D. Moore, M.D.** (Emeritus) Moseley Professor of Surgery Emeritus Harvard Medical School	**James W. Patrick, Ph.D.** Vice President and Dean of Research Baylor College of Medicine
Peter Paul, Ph.D. Deputy Director Science and Technology Brookhaven National Laboratory	**Mary R. Rifkin, Ph.D.** Dean for Academic Affairs Mount Sinai School of Medicine	**Alan L. Schiller, M.D.** Chairman Department of Pathology Mount Sinai School of Medicine
Walter W. Sullivan, Ph.D. Vice President of Operations and Planning Morehouse School of Medicine	**W. Dalton Tomlin** (Secretary/Treasurer) Senior Vice President and General Counsel Baylor College of Medicine	**Robert L. Van Citters, M.D.** Professor and Dean Emeritus University of Washington School of Medicine
Arnold N. Weinberg, M.D. (Emeritus) Medical Director Massachusetts Institute of Technology	**Torsten N. Wiesel, M.D.** President Emeritus Rockefeller University	**I. Dodd Wilson, M.D.** Executive Vice Chancellor Dean, College of Medicine University of Arkansas for Medical Sciences
Laurence R. Young, Sc.D. (*ex officio*) Institute Director		

External Advisory Council
The current membership of the NSBRI External Advisory Council is shown in Table 3. This Council met once in Houston during FY 2001. One member of the Council stepped down. Victor Wilson, Rockefeller University, resigned his position. Changes to the format of Team Leader presentations have allowed more discussion between Team Leaders and Council members. The second External Advisory Council meeting, originally scheduled for September 13-14 in Boston, was postponed due to travel restrictions. The meeting was rescheduled for October 2001.

Board of Scientific Counselors
NSBRI's Board of Scientific Counselors did not change in FY 2001. Membership rotation will occur in FY 2002. Nominations have been solicited by NSBRI Management to replace outgoing counselors. A specialized panel was recruited in November 2000 to peer review the Education and Public Outreach proposals received in response to NSBRI Research Announcement 00-02: An Opportunity to Participate in the NSBRI Education and Public Outreach Program.

User Panel
Membership in the User Panel was stable in FY 2001. The panel did not meet as a group because countermeasure ideas had not yet developed to the point where User Panel review was appropriate. Two members of the User Panel were involved with the joint NSBRI/NASA Standardized Flight Data Collection Working Group, which met July 31 through August 2.

5.1 NASA Three-Year Site Visit and Review of NSBRI

The NASA Three-Year Site Visit and Review of NSBRI occurred November 29-December 1, 2000, in Houston. The Review Team recommended that the Institute continue for its second, five-year funding period, from October 1, 2002 to September 30, 2007. The report also commended the NSBRI on providing added value. Appendix L contains correspondence related to the NSBRI's response to the findings and recommendations of the site visit review report.

6.0 SUPPORTING PROGRAMS

Three programs, Industry Forum, Data Archive, and Communications and Outreach, support the research and educational activities of the Institute. A summary of each program's activities in FY 2001 follows.

Industry Forum
During FY 2001, the Industry Forum membership (Table 4) increased with the addition of Raytheon Technical Services Company. The Forum continued to express an interest in educating the Institute's investigators in the management and protection of intellectual property. The group is actively seeking additional representation from the biotechnology and pharmaceutical industries. InDyne, Inc., continued to support the nsbri.com Web site containing the Industry Forum Web pages, and The Boeing Company supported the Institute by providing graphic design for NSBRI public service ads. These ads will appear in *Northwest Science & Technology* magazine in 2002 and 2003.

Table 3. NSBRI EXTERNAL ADVISORY COUNCIL

Martin J. Fettman, D.V.M., Ph.D. (Chairman) Associate Dean for the Professional Veterinary Medical Program Colorado State University	**Leon Alkalai, Ph.D.** Director Center for Integrated Space Microsystems Jet Propulsion Laboratory	**J. A. Anderson, Ph.D.** Professor of Cognitive and Linguistic Sciences Brown University
Ruth Benca, M.D., Ph.D. Professor and Associate Chair Department of Psychiatry University of Wisconsin	**Hal E. Broxmeyer, Ph.D.** Chairman and Professor Walther Oncology Center Indiana University School of Medicine	**Thomas F. Budinger, M.D., Ph.D.** Professor and Chair Department of Bioengineering Lawrence Berkeley National Laboratory
Dennis S. Charney, M.D. Chief of Mood and Anxiety Disorders Research Program National Institute of Mental Health	**Victor A. Convertino, Ph.D.** Research Physiologist U.S. Army Institute of Surgical Research	**Thomas A. Fleisher, M.D.** Chief, Department of Laboratory Medicine National Institutes of Health
Michael N. Gould, Ph.D. Professor of Human Oncology University of Wisconsin	**Amy Kronenberg, Sc.D.** Group Leader Radiation Biology and Environmental Toxicology Lawrence Berkeley National Laboratory	**Robert Y. Moore, M.D., Ph.D.** Professor and Chairman of Neurology University of Pittsburgh
Charles B. Nemeroff, M.D., Ph.D. Professor and Chairman of Psychiatry Emory University	**Lawrence A. Palinkas, Ph.D.** Professor Family and Preventative Medicine University of California, San Diego	**Danny A. Riley, Ph.D.** Professor of Cell Biology and Anatomy Medical College of Wisconsin
Irwin H. Rosenberg, M.D. Professor of Medicine and Nutrition Tufts University	**M. Rhea Seddon, M.D.** Assistant Chief Medical Officer Vanderbilt University Medical Center	**Warren K. Sinclair, Ph.D.** President Emeritus National Council on Radiation Protection and Measurement
Ronald J. White, Ph.D. (*ex officio*) Institute Associate Director	**Thomas J. Wronski, Ph.D.** Professor of Physiological Sciences University of Florida	**Bill J. Yates, Ph.D.** Assistant Professor of Otolaryngology and Neuroscience University of Pittsburgh
F. Eugene Yates, M.D. Professor of Medicine University of California, Los Angeles	**Laurence R. Young, Sc.D.** (*ex officio*) Institute Director	

Table 4. **NSBRI INDUSTRY FORUM MEMBERSHIP**

The Boeing Company
The Charles Stark Draper Laboratory
InDyne, Inc.
Lockheed Martin Astronautics
MBI International
Payload Systems, Inc.
Raytheon Technical Services Company
Roche Laboratories, Inc.
SGI
Southwestern Bell
United Space Alliance
Veridian
Wyle Laboratories

NSBRI Data Archive

Established in FY 1998, the goal of the Web-based Data Archive system is to maintain an appropriate, accessible archive of the data collected through NSBRI research projects. During FY 2001, the Data Archive program also completed work on the NSBRI's Electronic Proposal Submission System. This system will be utilized for all future NSBRI research opportunities.

Communications and Outreach

The NSBRI Communications and Outreach Office develops and implements diverse communications and outreach initiatives contributing to the successful accomplishment of the NSBRI mission. The program identifies and targets messages to the NSBRI's key publics – the general public, the scientific community, industry, consortium members and NASA. Key activities in FY 2001 included the development of a research-based news release program, the collaboration with public affairs offices at 46 funded institutions to maximize news outreach related to the FY 2001 NSBRI research program, meetings with NASA-JSC public affairs to increase awareness of NSBRI activities, and an increase in media inquiries and news clippings related to NSBRI's space-related research. In FY 2001, the Institute received 94 media inquiries compared to 69 inquiries received in FY 2000. Similarly, 121 newspaper, magazine or on-line articles mentioned NSBRI, up from 46 the previous year. Since the NSBRI does not utilize a news clipping service, the clipping figure is based on material received from reporters and other sources.

7.0 INSTITUTE DIVERSITY AND SCIENTIFIC COMMUNITY OUTREACH

Seed money was provided in FY 2000 to Morehouse School of Medicine to enable several of their investigators to develop pilot data for future projects. As a result, three young investigators at Morehouse proposed to the Institute's research announcements. Two of these projects, "Possible Countermeasures to Post-Suspension Hypotension in the Head-Down Tilt Rat Model" (M. Bayorh) and "Long-Term Exposure to Dim Light Desynchronizes the Circadian System of Rats" (G. Tosini), were included in the NSBRI's FY 2001 research program.

In addition, efforts are being made to provide future research announcements to a wide and diverse group of potential investigators. Interested researchers can register on-line at www.nsbri.org for NSBRI E-News announcements. NSBRI also continues to maintain a copy of the mailing list that NASA uses for announcements related to the life sciences. This list is the backbone of all postcard mailings used to inform the community of NSBRI research announcements.

This year, as part of its outreach program, five minority undergraduate students participated in the NSBRI Summer Research Program held at Morehouse School of Medicine. They were selected from a national pool of 62 applicants. Participants came from five different institutions: Cornell University, Morehouse, Oakwood College, Wesleyan University and Xavier University.

Education and Public Outreach summer programs at Mount Sinai School of Medicine, Rice University and The University of Texas Medical Branch provided research experiences for underrepresented minority high school students. While these programs do not exclude non-minority applicants, the programs draw applicants from school districts that have large African-American, Hispanic and Puerto Rican populations.

Institute investigators, team leaders and management continued to reach out to the scientific community through presentations made at symposia and meetings. A partial list of these presentations is provided in Appendix E. A sampling of meetings involving NSBRI participants include the International Astronautical Congress, the Biomedical Engineering Society, the Society for Neuroscience, the American Association for the Advancement of Science and the Aerospace Medical Association. Preliminary discussions between NASA, NSBRI and the Australian biomedical research community were held in Canberra, Australia in August 2001. Participants discussed possible areas of cooperation in research relevant to the International Space Station through Life Sciences research and development.

8.0 SPECIAL PROJECTS

The *Cooperative Agreement Management Plan* between NASA and the NSBRI enables the partners to undertake special projects outside of the core-funding envelope of the NSBRI. During FY 2001, six new projects were initiated, one previous project was completed and three were continued.

Project 97-3, *National Space Biomedical Research Institute Visiting Scientist/Research Associate Program*, continued to enable young and established university-based researchers an opportunity to work side-by-side with government employees in JSC laboratories. Table 5 provides a list of the participants in this program; and other projects relating to Bioastronautics positions at NASA-JSC.

Project 00-4, *Risk Assessment & Management*, examines the issues related to the risks associated with human space flight using new risk models to be developed. This project was carried out by Baylor College of Medicine's Risk Management Department working with a consultant team from Marsh, Inc., and the Actuarial Research Group. Initial work was completed and a report submitted in FY 2000. Two additional risk areas (*Decompression Sickness Incidence During EVAs and the Evaluation of Alternative Pre-Breathe Protocols* and *Cancer Incidence in the Astronaut Population*) were identified by NASA. Marsh, Inc., and the Actuarial Research Group completed the additional work and provided reports in FY 2001.

Project 00-5, *Acoustics Specialists, Operational Habitability Project (OHP)*, establishes NSBRI Visiting Scientist positions for technically, academically qualified and experienced acoustics experts to work at or consult with NASA-JSC. Applied acoustics is important for the International Space Station and will be important in designing an exploration mission of the future. These experts will guide the major acoustics work necessary to support the Operational Habitability effort including: mission planning and implementation support, data collection and analysis, lessons learned identification, assessments, interface and contact maintenance, and project management. In FY 2001, NSBRI hired Jim Warnix and Ferdinand Grosveld as additional consultants on the project.

Project 00-7, *Leadership of Stowage and Housekeeping Research & Analysis Element of the Space Human Factors and Habitability Office*, is designed to enable the NSBRI to develop special strength in this area and to attract a qualified and experienced crew systems engineer and technical leader to the Bioastronautics Office at Johnson Space Center. This person must develop an expert's knowledge of stowage and housekeeping facets of spacecraft habitability management and provide technical leadership in this area. To carry out this project, the NSBRI hired Laura Duvall in FY 2000. In FY 2001, a higher need for Laura's expertise was identified. It was agreed upon by all parties that she transition to Project 01-3, leaving Project 00-7 currently vacant.

Project 01-1, *Discipline Coordinating Scientist, Space Human Factors and Habitability Office*, establishes this NSBRI Visiting Scientist position for an academically qualified and experienced human factors expert to work at or consult with NASA-JSC. Critical Space Human Factors questions must be answered through ground-based and in-flight research, which are being accomplished through in-house applied research, NASA-funded extramural basic research and collaboration with other government agencies, academia and industry. A process of methodically bridging the gap between basic research and applied human factors analysis for flight programs was identified. This person must coordinate the various research groups, acting as the lead Space Human Factors engineer for the office, providing advice, consultation and expert recommendations. The position also facilitates the application of the latest technologies into operational space flight programs. To carry out this project, the NSBRI hired John Brian Peacock in FY 2001.

Table 5. VISITING SCIENTIST/RESEARCH ASSOCIATE PROGRAM – FY 2001

Name	Current Position	JSC Sponsor	Period
Matt Alexander	Research Associate	Clarence Sams *(see Project 97-3)*	2/01/01 – 3/24/01
Tatiana Christian	Senior Engineer	Jerry Goodman *(see Project 00-5)*	5/22/00 -
Johnny Conkin, Ph.D.	Assistant Professor	John Stanford	6/1/98 -
Dominick D'Aunno, M.D.	Assistant Professor	Jan Meck	11/1/97 -
Laura E. Duvall	Senior Engineer	Thomas Rathjen *(see Project 00-7 and 01-03)*	9/25/00 -
John N. Evanoff, Ph.D.	Project Manager	William Paloski *(see Project 01-04)*	7/23/01-
Philip Foster, M.D.	Assistant Professor	John Stanford	10/19/98 -
Todd Hellner	Lead Engineer	Thomas Rathjen *(see Project 01-3)*	9/24/01
Meena Husein	Project Manager	Jim Logan *(see Project 01-2)*	6/1/01 -
Ralph Krog	Project Manager	Jim Logan *(see Project 01-5)*	9/17/01 -
Lawrence H. Kuznetz, Ph.D.	Assistant Professor	William Paloski *(see Project 02-01)*	8/20/01
Giles Maule, Ph.D.	Research Associate	Clarence Sams *(see Project 97-3)*	1/24/00 -
Ajit K. Mulavara, Ph.D.	Assistant Professor	Jacob Bloomberg *(see Project 97-3)*	8/20/01 -
Jennifer Novak, Ph.D.	Assistant Professor	Dane Russo	1/4/00 -
John B. Peacock, Ph.D.	Associate Professor	Thomas Rathjen *(see Project 01-1)*	10/30/00 -
Michele Perchonok, Ph.D.	Assistant Professor	Dane Russo	9/5/00 -
Sudhakar Rajulu, Ph.D.	Assistant Professor	Dane Russo	4/17/00 -
Lawrence Spector	Senior Engineer	Thomas Rathjen	9/25/00 -
Wendy Waters, Ph.D.	Assistant Professor	Jan Meck	11/24/97 -

Project 01-2, *Manager, Information Systems Projects*, provides a highly qualified manager to the Medical Informatics and Health Care Systems Office of the Medical Sciences Division at NASA-JSC. This person will be responsible for managing information systems projects as a DBS/MIS manager in an ISO 9000 compliant environment. To carry out this project, the NSBRI hired Meena Husein in FY 2001.

Project 01-3, *Manager, ISS Internal Volume Configuration Working Group*, is designed to attract an academically qualified and experienced Internal Volume Configuration Working Group project lead to the Bioastronautics Office at NASA-JSC. This person will be responsible for researching and analyzing systems engineering, human/machine interaction, configuration management and SHF/habitability control requirements and techniques and their effects on habitability. To carry out this project, the NSBRI, in cooperation with NASA-JSC SHFHO management, moved Laura Duvall from the position referenced in Project 00-7 to take over this position. Since she will leave the Institute in early FY 2002, the NSBRI hired Todd Hellner to replace her.

Project 01-4, *Project Manager, Countermeasures Evaluation and Validation Project*, provides a qualified and experienced project manager for the Human Adaptation and Countermeasures Office at NASA-JSC. The person will provide motivational leadership and advocacy for operational/clinical research projects and funding and implementation of this research with the Biomedical Research Laboratories, Space Medicine Office, Mission Project Management Office, Space and Life Sciences Directorate, NSBRI, NASA Headquarters, Astronaut Office, International Space Station (ISS) International Partners, and ISS and Shuttle Programs. This position will provide the focal point for managing all aspects of the project. To carry out this project, the NSBRI hired John Evanoff in FY 2001.

Project 01-5, *Project Manager, Senior Software Systems Architect*, provides a highly qualified senior software systems architect for the Medical Informatics and Health Care Systems Office at NASA-JSC. This person will be responsible for the design and implementation of new or refractored software to support current and/or anticipated Medical Informatics and Health Care Systems products, projects, programs or equipment. This position includes development, management, administration and oversight of the software systems. To carry out this project, the NSBRI hired Ralph Krog in FY 2001.

Project 02-1, *Project Manager, Flight Research Program*, provides a qualified and experienced science manager for the Human Adaptation and Countermeasures Office at NASA-JSC. The person will guide all major activities necessary to plan and implement the Human Adaptation and Countermeasure Office flight research program. Duties includes preparing science budgets for research and countermeasures, coordination of manifesting flight and analog programs, management of the life sciences mission science team, serving as mission scientist for shuttle missions, providing advocacy for ISS flight investigations and overseeing science hardware development. To carry out this project, the NSBRI hired Lawrence Kuznetz in FY 2001.

9.0 FUTURE DIRECTIONS

The NSBRI is developing an updated strategic plan that will provide a clear roadmap to guide our future development. This new plan will contain an overall Institute strategy and specific strategies for the research and education teams. During FY 2001, Team Leaders for each area provided input for this strategic plan and the External Advisory Council reviewed these inputs and provided general and specific advice concerning the strategic plan. In FY 2002, the NSBRI Strategic Plan will be submitted to the Board of Directors.

Appendix A

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

CORE RESEARCH PROGRAM
YEAR 4 - FY 2001

August 29, 2001

National Space Biomedical Research Institute
Core Research Program – Year 4
FY 2001
May 30, 2001

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NSBRI RESEARCH PROGRAM
BONE LOSS

Team Leader:	**Shapiro, J. R.**		**Uniformed Services University of the Health Sciences (USUHS)**	
Associate Team Leaders:	**Bloomfield, S. A.**		**Texas A&M**	
	Schaffler, M. B.		**Mount Sinai**	
Bloomfield, S. A.	**PI**	**Texas A&M**	**Bone and Muscle Recovery from Simulated Microgravity**	**4**
Hogan, H. A.	CO-I	Texas A&M		
Smith, C. L.	CO-I	Baylor		
Bolander, M. E.	**PI**	**Mayo Clinic**	**Effect of Microgravity on Fracture Healing: Ultrasound as a Possible Countermeasure**	**5**
Turner, R. T.	CO-I	Mayo Clinic		
Greenleaf, J. F.	CO-I	Mayo Clinic		
Isales, C. M.	**PI**	**MCG Research**	**Therapeutic Modulation of Systemic Glucose-Dependent Insulintropic Peptide Levels to Counteract Microgravity-Induced Bone Loss**	**6**
Bollag, R. J.	CO-I	MCG		
Karsenty, G.	**PI**	**Baylor**	**Leptin as a Regulator of Bone Formation in Microgravity**	**7**
Rubin, C. T.	**PI**	**SUNY**	**A Biomechanical Countermeasure for Disuse Osteopenia**	**8**
Hadjiargyrou, M.	CO-I	SUNY		
Schaffler, M. B.	**PI**	**Mount Sinai**	**Resorption Suppression and Bone Health in Disuse**	**9**
Jepsen, K. J.	CO-I	Mount Sinai		

Shapiro, J. R.	**PI**	**USUHS**	**Defining and Preventing Bone Loss: A Microgravity Model**	**10**
Toerge, J. D.	CO-I	Nat. Rehab. Hosp.		
Baldwin, K. M.	CO-I	UC, Irvine		
Ruff, C. B.	CO-I	Hopkins/SOM		
Beck, T. J.	CO-I	Hopkins/SOM		
Oden, Z. M.	CO-I	UT-Houston		
Potember, R. S.	CO-I	Hopkins/APL		
Burman, K. D.	CO-I	Wash. Hosp. Ctr.		
Ballard, P. H.	CO-I	Nat. Rehab. Hosp.		
Smith, C. L.	**PI**	**Baylor**	**Receptor Countermeasures to Bone Loss in Microgravity**	**11**
Weigel, N. L.	CO-I	Baylor		
Bloomfield, S. A.	CO-I	Texas A&M		
Zerwekh, J. E.	**PI**	**UT-SW**	**Prevention of Microgravity-Induced Stone Risk by KMgCitrate**	**12**
Pak, C. Y. C.	CO-I	UT-SW		
Antich, P. P.	CO-I	UT-SW		
Wuermser, L.-A.	CO-I	UT-SW		

RESEARCH AREA:	**Bone Loss**
PRINCIPAL INVESTIGATOR:	**Susan Bloomfield, Ph.D.**
ORGANIZATION:	**Texas A&M University**
PROJECT:	**Bone and Muscle Recovery from Simulated Microgravity**

Project Executive Summary

Dramatic losses of bone mineral density (BMD) and muscle strength are two of the best-documented changes observed in humans after prolonged exposure to microgravity. Recovery of muscle upon return to a 1-G environment is well studied, however, far less is known about the rate and completeness of BMD recovery to pre-flight values. Using the mature tail-suspended adult rat model, this proposal will focus on the temporal course of recovery in tibial bone following a 28-d period of skeletal unloading. Through the study of bone density and muscle strength in the same animal, time-points during recovery from simulated microgravity will be identified when bone is at an elevated risk for fracture. These will occur due to the rapid recovery of muscle strength coupled with a slower recovery of bone, producing a significant mismatch in functional strength of these two tissues. Once the time-point of maximal mismatch is defined, various mechanical and pharmacological interventions will be tested at and around this time-point in attempt to minimize the functional difference of bone and muscle. The outcomes of this research will have high relevance for optimizing the rehabilitation of astronauts upon return to Earth, as well as upon landing on the Martian surface before assuming arduous physical tasks. Further, it will impact significantly on rehabilitation issues common to patients experiencing long periods of limb immobilization or bed rest.

RESEARCH AREA:	**Bone Loss**
PRINCIPAL INVESTIGATOR:	**Mark E. Bolander, M.D.**
ORGANIZATION:	**Mayo Clinic Rochester - Rochester**
PROJECT TITLE:	**Effect of Microgravity on Fracture Healing: Ultrasound as a Possible Countermeasure**

Project Executive Summary

The NSBRI Conference that was convened in Clear Lake, Texas, on November 16-17, 2000, identified fracture healing during space flight as an area where further information would be required to appropriately prepare for long-term space missions, and developing countermeasures to restore normal fracture healing was identified as a priority for current research. The RFA dated February 22, 2000, (NSBRI II 00-01) requested studies evaluating the effect of space flight on fracture healing and developing countermeasures. This application is submitted in response to that RFA.

Our current understanding of bone physiology suggests that fracture healing will be abnormal in the microgravity environment. This hypothesis is supported by two published studies, the first an abstract reporting abnormal healing in rats undergoing hindlimb unloading, the second a manuscript (in Russian) that describes abnormal fracture healing in five rats with fibula fractures flown on Cosmos-2044. This latter study reports that abnormalities seen in fracture healing after space flight were duplicated in the hindlimb-unloading model.

The goals of the experiments proposed in this application are 1) to confirm the previous reports that microgravity adversely affects fracture healing, and 2) to determine if ultrasound treatment, which has been shown to accelerate fracture healing in clinical studies, will reverse the impaired cellular events in fracture healing that are related to microgravity. If ultrasound does not act as an effective countermeasure we will undertake detailed evaluation of our histologic samples to identify potential targets for other countermeasures.

RESEARCH AREA:	**Bone Loss**
PRINCIPAL INVESTIGATOR:	**Carlos M. Isales, M.D.**
ORGANIZATION:	**MCG Research Institute, Inc.**
PROJECT TITLE:	**Therapeutic Modulation of Systemic Glucose-Dependent Insulintropic Peptide Levels to Counteract Microgravity-induced Bone Loss**

Project Executive Summary

Glucose-dependent Insulinotropic Peptide (GIP) is a 42 amino acid peptide synthesized and secreted largely from endocrine cells in the small intestine. We propose that GIP modulates bone formation and resorption in response to nutrient uptake. The documented actions of GIP imply an important role in coupling food intake and absorption in the intestine with metabolic events in a number of tissues. Our observations support a role for GIP in modulating bone cell function, since GIP receptors localize to osteoblasts, osteoblast-like cells, osteoclasts, and osteocytes. Furthermore, GIP dose-dependently increases intracellular cAMP and calcium contents in the osteoblast-like cell line SaOS, and stimulates alkaline phosphatase activity, collagen synthesis, and inhibits osteoclast-induced bone resorption. To evaluate further the hypothesis that GIP is anabolic for bone, we generated a genetic model of GIP overexpression in transgenic mice, and a rat model for hypogonadal osteoporosis, in both of these models higher GIP levels were associated with increases in bone density. Based on these findings, we propose that GIP may act as a link between the nutritional state of the organism and the balance between bone formation and resorption. We hypothesize that the effect of diminished gravitational load during space flight can be overcome by exploiting the hormonal cues received by bone. We propose that therapeutically elevated GIP levels coupled with strict dietary control could supersede the impact of decreased load bearing in space, and thereby mitigate the negative effects of microgravity on bone mass. To address this thesis, we propose to define the effects of GIP on bone mass and bone turnover *in vivo*. Towards this end, we have generated transgenic mice expressing the murine GIP cDNA under control of the metallothionein gene regulatory sequences. Our first aim is to characterize the bone phenotype in these mice to evaluate whether GIP can serve an anabolic and anti-resorptive function. To further address the potential osteoprotective potential of GIP in microgravity-induced bone loss, transgenic mice will be exposed to a simulated microgravity environment, hindlimb unloading by tail suspension. By evaluating the effects of GIP in bone physiology, we hope to establish that GIP is an important hormonal link between dietary intake of nutrients and bone metabolism. As such, GIP would serve as an attractive hormonal intervention to alleviate microgravity-induced bone loss during space flight.

RESEARCH AREA:	**Bone Loss**
PRINCIPAL INVESTIGATOR:	**Gerard Karsenty, M.D., Ph.D.**
ORGANIZATION:	**Baylor College of Medicine**
PROJECT TITLE:	**Leptin as a Regulator of Bone Formation in Microgravity**

Project Executive Summary

The biochemical bases controlling bone formation in physiologic situations and disease states are not known. This is a biochemical question of critical importance since osteoporosis, a low bone mass disease characterized by a relative decrease of bone formation, is the most prevalent disease in developed countries. Importantly bone loss during prolonged stay in space is a major health problem in extended duration flights. We have recently demonstrated that leptin is a powerful inhibitor of bone formation whose absence leads to a high bone mass phenotype even in ovariectomized animals. This latter result indicates that leptin is the most powerful regulator of bone formation identified to date as it is the only regulating pathway that can overcome the deleterious consequences of hypogonadism on bone mass. Lastly, we have shown that leptin must bind to its hypothalamic receptor, not to the osteoblast, to exert its regulatory role on bone formation, thus uncovering that bone formation is a central function. We intend in this application to explore the molecular bases of this leptin action on bone formation. We believe that this project may lead to the design of novel therapeutics enhancing bone formation for osteoporosis. The specific aims of this application are:

1. To determine whether leptin controls bone mass by releasing a humoral substance following its binding to its hypothalamic receptor.
2. To determine whether the sympathetic nervous system is involved in mediating leptin control of bone formation.
3. To determine whether a naturally occurring soluble form of the leptin receptor can prevent leptin inhibitory action on bone formation.

RESEARCH AREA:	**Bone Loss**
PRINCIPAL INVESTIGATOR:	**Clinton Rubin, Ph.D.**
ORGANIZATION:	**State University of New York – Stony Brook**
PROJECT TITLE:	**A Biomechanical Countermeasure for Disuse Osteopenia**

Project Executive Summary

Osteoporosis, the progressive loss of bone density and strength which cripples tens of millions on our planet, distinguishes itself as perhaps the greatest physiologic obstacle to an extended human presence in space. The principal objectives of this proposal are to establish the efficacy of a unique, biomechanical countermeasure to inhibit bone loss in an animal model of disuse osteoporosis, and correlate this regulatory influence to the expression patterns of several genes critical to bone formation and resorption. Using a ground based model of microgravity, the tail-suspended rat, we have shown that brief exposure (10 minutes) to extremely low magnitude (0.25g, engendering < 5 microstrain), high frequency (30-90 Hz) mechanical signals will inhibit the bone loss which typically parallels disuse, even though 10 minutes of full weight bearing failed to curb this loss. Longer-term experiments in sheep have shown this stimulus to be strongly anabolic, increasing bone mineral density, trabecular number and connectivity, and improving bone strength. In a series of four specific aims, we will use several morphometric assays on the mouse model of tail-suspension to rigorously establish the efficacy of a specific mechanical signal (10 minutes at 30Hz, 0.3g; parameters being used in clinical trials to inhibit bone loss in the elderly) to inhibit and/or reverse 28 days of disuse osteopenia. In an effort to understand the mechanisms by which this signal is anabolic, we will also monitor the temporal and spatial expression of nine genes, each indicative of a specific process of bone formation or resorption. The use of the mouse will facilitate many aspects of the protocol, including comprehensive genomic profiling and expedited access to space flight. Considering that many flight opportunities are brief and thus do not permit long term morphologic adaptations in bone to occur, combining the molecular with the tissue level strategies will facilitate establishing countermeasure efficacy even following short term exposure to microgravity. In essence, this work represents a critical step in establishing a physiologically based, non-pharmacologic, non-invasive treatment for osteoporosis, for use on Earth or in space.

RESEARCH AREA:	**Bone Loss**
PRINCIPAL INVESTIGATOR:	**Mitchell B. Schaffler, Ph.D.**
ORGANIZATION:	**Mount Sinai School of Medicine**
PROJECT TITLE:	**Resorption Suppression and Bone Health in Disuse**

Project Executive Summary

Bone loss in microgravity, and the resulting bone fragility that ensues, have been identified by NASA and NSBRI as key barriers to successful long-term space flight and the recovery of normal function in astronauts upon returning to Earth's gravity. Overcoming these problems will require safe and effective countermeasures not only to prevent bone loss, but also to maintain the functional-mechanical integrity of the tissue during prolonged space flight. *A necessary prerequisite for development of those countermeasures is the identification of appropriate cellular targets for both processes. Those cellular targets have not yet been fully characterized.* We posit that 1) preventing bone loss and 2) maintaining bone health during long-duration in the absence of normal loading involve different cellular mechanisms and so will require different countermeasures. 1) Osteoclasts are clearly the agents driving bone loss due to unloading. Thus, they present an obvious countermeasure target for modulating bone loss in space flight and in other hypodynamic loading situations. Available pharmacological strategies to inhibit osteoclastic resorption have a high likelihood of success, though *definitive long-term data for remodeling suppression after unloading do not exist.* 2) Changes in osteocyte integrity result from long-term loss of normal mechanical loading. If the normal remodeling response of bone to impaired osteocytes is suppressed, as would be the case with treatment using an anti-resorptive agent, then regions of osteocytes can die, leading to the accumulation of devitalized bone. Devitalized bone becomes mechanically fragile, raising the fundamental question of whether bone loss resulting from withdrawal of normal mechanical usage can be safely prevented for the long-term, without paradoxically impairing osteocyte function, and thereby bone's ability to function mechanically in a normal load-bearing environment. The proposed experiments test the hypotheses that 1) Long-term suppression of bone remodeling in disuse will successfully maintain bone mass, microarchitecture, stiffness, and strength, but will result in compromised fracture resistance properties; and 2) Decreased mechanical usage in the presence of an antiresorptive agent results in loss of osteocyte integrity and accumulation of bone with impaired viability.

To test these hypotheses, we will undertake a series of long-term immobilization experiments in a canine model, with biphosphonate treatment to prevent bone loss. Bone health will be assessed from conservation of tissue mechanical properties and from in situ assessments of osteocyte viability. We will determine whether suppression of bone resorption superimposed in unloading leads to impaired osteocyte viability and increased brittleness of bone, and whether the extent of such alterations can be sufficient to cause significant bone fragility.

RESEARCH AREA:	**Bone Loss**
PRINCIPAL INVESTIGATOR:	**Jay R. Shapiro, M.D.**
ORGANIZATION:	**Medstar Research Institute**
PROJECT TITLE:	**Defining and Preventing Bone Loss: A Microgravity Model**

Project Executive Summary

Muscle atrophy and bone loss are major complications of spinal cord injury (SCI), chronic bed rest and exposure to microgravity. Space medicine research has amply documented the extent to which muscle and bone loss may impair strength and increase fracture risk. We propose that the SCI patient can serve as a surrogate for studying microgravity exposure. A primary objective of this research program is to limit the extent of bone loss in SCI patients by treating with a potent intravenous bisphosphonate, zoledronate for a period of one year. The zoledronate effects on bone will be measured using bone density values and femur scan structural analysis as the indicators of bone integrity. We will determine the effects of zoledronate on biomarkers of bone resorption and formation and on serum calcitropic hormone levels. To study the process of muscle atrophy when weightless, we will determine the relationships between changes in thigh muscle cross-sectional area measured by CT scan, muscle biopsy immunohistochemistry, muscle protein translation markers and markers for protein synthesis activation and protein degradation. To further understand mechanisms involved in bone loss we will determine sequential changes in femur bone geometry and structural parameters obtained from DEXA scans by established 2-D curved beam analysis methods. Using femur CT images we will measure changes in femur bone dimensions and will apply 3-D finite element analysis to estimate fracture risk. The new time-of-flight mass spectrometer will permit measuring the excretion of zoledronate in urine and plasma levels. We will compare these to radiologic measurements and bone biomarkers. The objectives of this research are: 1) to develop a regimen for minimizing bone loss in SCI subjects that may be appropriate for astronauts during extended microgravity exposure, and 2) to investigate mechanisms related to muscle and bone loss during weightlessness, and 3) to explore the SCI patient as a surrogate for the investigation of microgravity induced musculoskeletal atrophy.

RESEARCH AREA:	**Bone Loss**
PRINCIPAL INVESTIGATOR:	**Carolyn L. Smith, Ph.D.**
ORGANIZATION:	**Baylor College of Medicine**
PROJECT TITLE:	**Receptor Countermeasures to Bone Loss in Microgravity**

Project Executive Summary

The prevention of bone loss due to skeletal unloading is a complex problem and the reasons for this loss have not been elucidated. The overall goal of the bone team of NSBRI is to develop countermeasures that will not only prevent quantitative loss of bone, but also maintain bone strength. Measures that simply prevent resorption may maintain mass, but may block the necessary remodeling that ensures adequate bone strength. Studies to date suggest that good nutrition and exercise regimes will be insufficient to achieve this goal so *pharmacological alternatives must be considered.* The biological actions mediated by the estrogen receptor (ER) and vitamin D receptor (VDR) play key roles in the normal control of bone growth and skeletal turnover that are necessary for skeletal health. These receptors act by controlling the differentiation and/or function of osteoblasts and osteoclasts, and other cell types within the bone and bone marrow microenvironment as well as playing a role in calcium absorption (VDR). *We hypothesize that the appropriate combination of an agent that will improve calcium absorption and encourage bone formation (VDR agonist) and an agent that will reduce bone resorption (selective estrogen receptor modulators [SERM]) will achieve the goal of maintaining bone mass and bone strength.* To test this we will 1: Assess the ability of novel receptor agonists of the ER and VDR, alone or in combination, to modulate osteoblastogenesis, mature osteoblast function and osteoclastogenesis *in vitro* and *in vivo*. 2. Assess the ability of novel receptor agonists of the ER and VDR, alone or in combination, to prevent bone loss in the hindlimb suspension model of skeletal unloading. Effects of unloading and the countermeasures will be assessed by: 1. Measuring changes in bone mineral density, histomorphometry, mechanical strength testing and biochemical markers of bone metabolism, 2. Determining the effects of these treatments on osteoblastogenesis and osteoclastogenesis and function, and 3. Characterizing gene expression profiles in bone resulting from skeletal unloading and administration of the countermeasures. Collectively, these studies will lead to a better understanding of the changes associated with skeletal unloading and will test the utility of VDR agonists and SERMS as countermeasures.

RESEARCH AREA:	**Bone Loss**
PRINCIPAL INVESTIGATOR:	**Joseph E. Zerwekh, Ph.D.**
ORGANIZATION:	**UT Southwestern Medical Center at Dallas**
PROJECT TITLE:	**Prevention of Microgravity-Induced Stone Risk by KMgCitrate**

Project Executive Summary

The broad long-term objective of this proposal is to evaluate the effectiveness of a countermeasure in the prevention of increased propensity for renal stone formation and skeletal mineral loss sustained during space flight. Ground-based studies, as well as a limited number of space flight studies, have clearly demonstrated an increased risk for kidney stone formation as determined from the composition of the urinary environment. Increased bone resorption raises urinary calcium and the urinary state of saturation with respect to the calcium salts, calcium oxalate and brushite. However, documented changes in other urinary components such as citrate, pH, and magnesium appear to also raise the risk for the formation of not only calcium oxalate and calcium phosphate stones but also uric acid stones as well. Nutritional modifications to counter the tendency toward stone formation might include increased fluid consumption and supplementation with an appropriate nutriceutical that would decrease the risk of stone formation by increasing urinary pH and inhibitor concentrations. The hypothesis to be tested in this project is that potassium magnesium citrate supplementation will attenuate the increased risk for stone formation and diminish microgravity-induced bone loss. This hypothesis will be tested during five weeks of bed rest in normal volunteers through three specific aims: 1. Assess the efficacy of supplementation with potassium magnesium citrate (KMgCit) in preventing microgravity-induced increased risk of renal stone formation. 2. Evaluate the effect of KMgCit supplementation in averting diminished muscle magnesium and potassium concentrations that may occur during microgravity-induced muscle atrophy and 3. Assess the efficacy of supplementation with KMgCit in reducing microgravity-induced increases in bone resorption and urinary calcium losses.

NSBRI RESEARCH PROGRAM
CARDIOVASCULAR ALTERATIONS

Team Leader:	**Cohen, R. J.**		**MIT**	
Associate Team Leader:	**Shoukas, A. A.**		**Hopkins/SOM**	
Bayorh, M. A.	**PI**	**Morehouse**	**Possible Countermeasures to Post-Suspension Hypotension in the Head-Down Tilt Rat Model**	**15**
Cassone, V. M.	**PI**	**Texas A&M**	**Microgravity and Circadian Cardiovascular Function**	**16**
Cohen, R. J.	**PI**	**MIT**	**Cardiovascular Effects of Simulated Microgravity in Man**	**17**
Williams, G. H.	CO-I	Harvard		
Sheynberg, N.	CO-I	Harvard		
Cohen, R. J.	**PI**	**MIT**	**Effects of Space Flight on Cardiovascular Stability (Flight Study)**	**18**
Meck, J. M.	CO-I	NASA JSC		
Delp, M. D.	**PI**	**Texas A&M**	**Circulatory Remodeling with Simulated Microgravity**	**19**
Wilson, E.	CO-I	Texas A&M		
Zawieja, D. C.	CO-I	Texas A&M		
Meck, J.	**PI**	**NASA JSC**	**Mechanisms of Post-Space Flight Orthostatic Intolerance (Flight Study)**	**20**
Ziegler, M. G.	CO-I	UC, San Diego		
Mills, P.	CO-I	UC, San Diego		
D'Aunno, D. S.	CO-I	Baylor		
Waters, W. W.	CO-I	Baylor		
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RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Mohamed A. Bayorh, Ph.D.**
ORGANIZATION:	**Morehouse School of Medicine**
PROJECT TITLE:	**Possible Countermeasures to Post-Suspension Hypotension in the Head-Down Tilt Rat Model**

Project Executive Summary

Exposure to microgravity in humans causes cardiovascular deconditioning with orthostatic hypotension and tachycardia. Post-flight orthostatic intolerance is a dramatic physiological consequence of human adaptation to microgravity made inappropriate by a sudden return to normal gravity. Loss of appropriate cardiovascular reflexes contributes to the cardiovascular deconditioning, but the specific mechanisms remain unclear. The hypothesis of the proposed studies is that post-suspension hypotension in rats following simulated microgravity is due to overproduction of nitric oxide (NO) and prostacyclin (PGI_2) and that specific inhibitors of these vasodilatory factors can attenuate the reduction in blood pressure post-suspension. To test this hypothesis, we propose to use the 30° tail-suspended (hindlimb-unloaded) rat model to evaluate the roles of a prostacyclin synthase inhibitor (U-51605), a non-selective nitric oxide synthase inhibitor (L-NAME) and a selective nitric oxide synthase II inhibitor (2-amino-5, 6-dihydro-6-methyl-4H-1, 3-thiazine; AMT) as countermeasures against post-suspension hypotension in Sprague-Dawley rats. For each of the above agents, we will investigate the specific cardiovascular (i.e., blood pressure and heart rate-tail cuff; blood flow - Transonic flow meter; left ventricular function-sonomicrometer) and signal transduction (i.e., the role of cAMP, cGMP, IP_3, intracellular Ca^{2+}, K^+ channels, etc. - enzyme immunoassays and fluorescent dyes) mechanisms involved, including detailed studies on baroreflex responses, vascular reactivity and left ventricular function. Data derived from the proposed detailed mechanistic studies will lead to the development of more effective countermeasures.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Vincent Cassone, Ph.D.**
ORGANIZATION:	**Texas A&M University**
PROJECT TITLE:	**Microgravity and Circadian Cardiovascular Function**

Project Executive Summary

Cardiovascular function is regulated by the mammalian circadian clock in the hypothalamic suprachiasmatic nucleus (SCN) in large part via inducing rhythmic changes in the sympathetic release of norepinephrine in the heart and other peripheral tissues. However, the precise pathway by which cardiovascular function is rhythmically regulated is not yet described. This circadian variation in cardiovascular function has many important biomedical implications. Among these is the fact that many cardiovascular accidents occur during particular times of the day. Several cardiovascular anomalies have been described in astronauts and cosmonauts living in microgravity conditions along with changes in circadian clock function, and these have been implicated in astronaut performance and sleep-wake schedules. In addition, simulated microgravity conditions (head-down, bed-rest) have revealed similar changes in circadian cardiovascular function. However, the mechanisms for these changes are unknown.

The present proposal seeks funding to:

1) Determine the central pathway(s) by which the SCN influences circadian changes in heart rate, blood pressure and regional blood flow.
2) Determine the effects of microgravity, employing an accepted method for simulation of microgravity in rodents, on circadian changes in these cardiovascular parameters.
3) Determine the pathways by which these changes are mediated, to better design countermeasures for future human uses.

The data obtained in this project will integrate circadian physiology with cardiovascular medicine, providing benefits for ground-based research and future long-term presence in space.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Richard J. Cohen, M.D., Ph.D.**
ORGANIZATION:	**Massachusetts Institute of Technology**
PROJECT TITLE:	**Cardiovascular Effects of Simulated Microgravity in Man**

Project Executive Summary

Many astronauts after being weightless in space become hypotensive and presyncopal upon assuming an upright position. This phenomenon, known as orthostatic intolerance, may interfere with astronaut function during reentry and following space flight, and may limit the ability of an astronaut to exit a landed spacecraft unaided during an emergency. Orthostatic intolerance is more pronounced following long-term space flight and is a major concern with respect to the extended flights expected aboard the International Space Station and for interplanetary exploration class missions, such as a human mission to Mars. This problem has also been observed to be more pronounced among women than among men. In addition to the problem of post-flight orthostatic intolerance, a variety of heart rhythm disturbances have been observed in astronauts during and after space flight. The potential lethal arrhythmic risk for astronauts is sustained ventricular tachycardia or ventricular fibrillation, while non-sustained ventricular tachycardia could cause syncope. Older individuals, particularly men, may be most susceptible to ventricular arrhythmias during flight.

In previous ground based bed-rest studies sponsored by NSBRI we have applied two new techniques that we have developed to study the effects of simulated microgravity on the cardiovascular system. Cardiovascular system identification (CSI) has been used as a non-invasive means of measuring alterations in closed-loop cardiovascular regulation and the measurement of microvolt level T wave alternans (TWA) has been used as a non-invasive measure of susceptibility to ventricular arrhythmias. We have also successfully tested the alpha-1 sympathetic agonist midodrine as a countermeasure to the development of orthostatic intolerance. We have found that 16 days of bed-rest results in altered cardiovascular regulation in particular alterations in baroreceptor sensitivity, altered electrical stability of the heart, and that midodrine is an effective countermeasure to the development of orthostatic hypotension.

In this proposal we plan to apply the same measurement techniques of CSI and TWA to two groups of subjects before and after 16 days of bed rest. Premenopausal women and men over age 55. The women will be randomized to placebo or midodrine to see if this countermeasure is effective in these subjects who are more susceptible to orthostatic hypotension than men of the same age. The group of older men will be randomized to placebo or spironolactone, an aldosterone-blocking agent. Spironolactone will be evaluated as a countermeasure to the pro-arrhythmic action of aldosterone, which is elevated in these subjects during bed rest.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Richard J. Cohen, M.D., Ph.D.**
ORGANIZATION:	**Massachusetts Institute of Technology**
PROJECT TITLE:	**Effects of Space Flight on Cardiovascular Stability**

Project Executive Summary

Many astronauts after being weightless in space become hypotensive and presyncopal upon assuming an upright position. This phenomenon, known as orthostatic intolerance, may interfere with astronaut function during reentry and following space flight, and may limit the ability of an astronaut to exit a landed spacecraft unaided during an emergency. Orthostatic intolerance is more pronounced following long-term space flight and is a major concern with respect to the extended flights expected aboard the International Space Station and for interplanetary exploration class missions, such as a human mission to Mars. This problem has also been observed to be more pronounced among women than among men. In addition to the problem of post-flight orthostatic intolerance, a variety of heart rhythm disturbances have been observed in astronauts during and after space flight. The potential lethal arrhythmic risk for astronauts is sustained ventricular tachycardia or ventricular fibrillation, while non-sustained ventricular tachycardia could cause syncope.

In previous ground based bed rest studies sponsored by NSBRI we have applied two new techniques that we have developed to study the effects of simulated microgravity on the cardiovascular system. Cardiovascular system identification (CSI) has been used a non-invasive means of measuring alterations in closed-loop cardiovascular regulation and the measurement of microvolt level T wave alternans (TWA) has been used as a non-invasive measure of susceptibility to ventricular arrhythmias. We have also successfully tested the alpha-1 sympathetic agonist midodrine as a countermeasure to the development of orthostatic intolerance. We have found that 16 days of bed rest results in altered cardiovascular regulation. In particular, we have demonstrated alterations in baroreceptor sensitivity, altered electrical stability of the heart, and that midodrine is an effective countermeasure to the development of orthostatic intolerance.

In this proposal we plan to apply the same measurement techniques of CSI and TWA to astronauts pre- and post-flight and to test midodrine as a countermeasure to the development of orthostatic intolerance. This study will allow us to determine if the changes in cardiovascular regulation and cardiac electrical stability measured in a ground-based model also occur during actual space flight. In addition we will test for the first time a potentially highly effective countermeasure for the development of post-flight orthostatic intolerance.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Michael D. Delp, Ph.D.**
ORGANIZATION:	**Texas A&M University**
PROJECT TITLE:	**Circulatory Remodeling with Simulated Microgravity**

Project Executive Summary

The human body is exquisitely adapted for maintaining an upright posture on Earth. However, when the force of gravity is removed during space flight, there is a cephalic fluid shift and an elimination of the head-to-foot hydrostatic pressure. This change in the fluid pressure distribution has been hypothesized to trigger adaptations within the cardiovascular system that are subsequently rendered inappropriate upon return to the Earth's gravitational environment. One of the most profound consequences of microgravity on the cardiovascular system is orthostatic intolerance. It is now becoming increasingly evident that the etiology of post-flight orthostatic intolerance is multifactorial, resulting from such factors as hypovolemia and altered regulation of the peripheral vasculature. According to Watenpaugh and Hargens (Handbook of Physiology, 1996) the primary cardiovascular adaptations that contribute to orthostatic intolerance involve the arterial, venous and lymphatic portions of the circulatory system. In order to study these phenomena on Earth, the hindlimb-unloaded (HU) rat has been used to simulate the effects of microgravity. This model induces the cephalic fluid shifts, and these animals manifest many of the adaptations that are characteristic of exposure to microgravity, such as hypovolemia, a diminished capacity to elevate peripheral vascular resistance, and orthostatic hypotension. In addition, previous work with conduit and resistance arteries indicates that hindlimb unloading alters both function and structure of the arterial circulation. Therefore, using this animal model, the general aim of this proposal is to determine the effects of simulated-microgravity on 1) the molecular mechanisms mediating structural remodeling of the arterial resistance vasculature, and 2) the functional ability of the lymphatics to generate and modulate lymph flow. More specifically, we propose to: identify early regulatory events leading to hypertrophic remodeling of cerebral arteries in response to hindlimb unloading (Aim 1); characterize signaling events leading to atrophy of resistance arteries in the soleus and gastrocnemius muscle in response to hindlimb unloading (Aim 2); and evaluate the effects of hind limb unloading on the ability of the lymphatics from different regions of the body to generate and modulate lymph flow, and thus, regulate overall body fluid homeostasis (Aim 3). Furthermore, we propose to determine the effectiveness of a lower-body negative pressure countermeasure to attenuate adaptations of the arterial and lymphatic circulation (Aim 4). These studies will provide new and important functional and mechanistic information about the etiology of microgravity-induced orthostatic intolerance.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Janice Meck, Ph.D.**
ORGANIZATION:	**NASA-Johnson Space Center**
PROJECT TITLE:	**Mechanisms of Post-Space Flight Orthostatic Intolerance**

Project Executive Summary

There is still a significant number of astronauts at Johnson Space Center who suffer from post-flight orthostatic hypotension and presyncope. The mandatory use of fluid loading with salt tablets and water, anti-gravity suits, and the liquid cooling garment has not eliminated the problem. A growing body of evidence suggests that there are major physiological systems that become dysfunctional as a result of space flight. The degree of dysfunction varies from minimal to severe. Several studies have provided evidence that autonomic function is impaired during and after space flight. Additional factors such as local factors, could also be involved. An area that has not been studied in humans is the effects of space flight on nitric oxide physiology and its modulation of blood pressure. The study proposed in this application will continue the pursuit of mechanisms of autonomic dysfunction in presyncopal astronauts. In addition, it will begin to elucidate changes in nitric oxide production and the resulting effects on the cardiovascular system. This study will not have in-flight measurements. All procedures will be performed before launch, on landing day and three days after landing. The study has two specific aims: 1) to compare pre-flight to post-flight changes in responses of veins to adrenergic agonists between presyncopal and nonpresyncopal astronauts; 2) to compare pre-flight to post-flight changes in nitric oxide levels, inducible nitric oxide synthase messenger RNA and protein, cell adhesion molecules associated with endothelial activation, responses to acetylcholine with and without nitric oxide synthase inhibition, and reactive hyperemia responses in the brachial artery, the arm and the popliteal artery in the leg, between presyncopal and nonpresyncopal astronauts. Presyncopal and nonpresyncopal astronauts will be defined by their ability to complete a 10-minute upright tilt test on landing day.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Beverly H. Lorell, M.D.**
ORGANIZATION:	**Harvard – Beth Israel Deaconess Medical Center**
PROJECT TITLE:	**Cardiac Unloading: Biologic Mechanisms and Countermeasures for Cardiac Atrophy**

Project Executive Summary

The objective is to determine the cellular and molecular mechanisms of cardiac atrophy caused by microgravity (already demonstrated in space-flown rats and long-duration space flight in humans), determine the functional consequences on cardiac contractile reserve, and identify specific countermeasures. We will study a rodent model of cardiac unloading (heterotopic transplantation of the heart to the abdomen). Work in the current grant period showed that this model affords assessment of progressive degrees and duration of unloading as a test-bed for genes, gene products, and human-applicable hormonal interventions with the potential to protect against atrophy. Functional consequences of cardiac unloading and countermeasures will be studied by echocardiography and hemodynamic measurements, analysis of isolated myocyte contraction and intracellular ions using fluorescence microscopy, and microscopy of cell morphology. Activity of key endogenous growth regulators will be monitored by measurements of gene expression and immunohistochemical localization. We will address four Specific Aims: (1) Are ventricular myocyte contractile function and intracellular ion regulation (Ca^{++} and Na^{+}/H^{+} exchange) modified in cardiac unloading and atrophy? (2) Does cardiac unloading stimulate myocyte cell death (apoptosis), in addition to the remodeling of cardiac geometry and muscle cell size? (3) Do hormonal and genetic countermeasures with direct trophic effects on muscle cell growth and contractility effectively blunt atrophy of the unloaded heart *in vivo*, or block the functional impairments? (4) High-throughput profiling of gene expression (by microarray studies and subtractive hybridization) will be done to identify changes in gene expression, which are unique to cardiac unloading. These studies of integrated cardiac physiology and molecular biology are an outgrowth of work in the current grant period that confirmed our hypothesis that the remodeling of the heart upon cardiac unloading *in vivo* is associated with reinduction of the fetal-hypertrophic cardiac gene program. In this project, countermeasures will focus on a-1-adrenergic pharmacological interventions. The rationale is that this approach has synergy with efforts elsewhere in the Cardiovascular Alterations Team, using the a-1-adrenergic pathway for treatment of orthostatic intolerance in humans. In addition, we have established that the pathway of RNA polymerase II signaling, which regulates the transcription machinery of the cell, is engaged by a-1-adrenergic signaling, a canonical trigger to increase cardiac mass. These studies will elucidate molecular and physiologic mechanisms of cardiac unloading which will lead to specific hypotheses and countermeasures which have the potential to be rapidly tested in the near future in human space flight missions.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Roger G. Mark, M.D., Ph.D.**
ORGANIZATION:	**Massachusetts Institute of Technology**
PROJECT TITLE:	**Computational Models of the Cardiovascular System and its Response to Microgravity and Disease**

Project Executive Summary

One of the highest priority problems in the current manned space program is orthostatic intolerance (OI) experienced by astronauts upon their return to the normal gravitational environment. The problem has been well known since the earliest days of manned space flight, and has been intensely investigated during in-flight studies and many Earth-based (bed-rest) studies. A number or countermeasures have been proposed and evaluated, but no effective and practical countermeasure has been developed.

Computational models of the cardiovascular system can help. They represent in a quantitative manner the current state of physiological understanding, and can be designed at a level of complexity appropriate to the problem under consideration. Models are powerful adjuncts to experiments, and permit investigators to quantitatively examine whether experimental observations are consistent with a particular hypothesis. Models also permit the evaluation of potential countermeasures.

Computational models of the cardiovascular system may also play a powerful role in clinical medicine. They can play a major role in improving the organization and interpretation of multiparameter physiologic data in intensive care units, and in tracking patient status over time. The model being developed in this research, although aimed primarily at the operational problem of microgravity-induced orthostatic intolerance, has important potential clinical applications. This project will develop a general, modular model of the cardiovascular system that contains the essential features associated with the effects of gravity, and will use this model to examine the short-term hemodynamic response of the cardiovascular system to abrupt orthostatic transitions. The model will facilitate the understanding of the physiology and treatment (prevention) or OI in post-flight astronauts. We will extend the progress already made over the past 2.5 years, with the following specific aims:

1. Enhance the current version of our cardiovascular simulation to better represent the short-term effects of abrupt orthostatic stress. Specifically we will: a) add rapidly acting vasoactive hormone loops to the control system; b) increase the sophistication of the control system by adding threshold/saturation characteristics, latencies, and dynamics to the individual effector limbs, adding a term to the baroreceptor proportional to rate of change of pressure, and explicitly accounting for the aortic baroreceptors; c) add atria to the cardiac model to enhance stroke volume at high heart rates.
2. Verify the model and use it to investigate and evaluate various hypotheses for OI, and to predict the effects of countermeasures. This objective will require extensive collection and archiving of experimental data from collaborators.
3. Complete, document, and disseminate to other investigators a form of the model implemented in JAVA.
4. Apply the cardiovascular model to the clinical problem of intelligent patient monitoring both in the context of intensive care and in tracking chronic cardiovascular disease.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Chester A. Ray, Ph.D.**
ORGANIZATION:	**Pennsylvania State University**
PROJECT TITLE:	**Effect of Simulated Microgravity on the Vestibulosympathetic Reflex in Humans**

Project Executive Summary

Despite the long recognized problem of post-space-flight orthostatic intolerance (OI), the physiological mechanism(s) responsible for this condition remains unresolved. Impaired sympathetic activation is a possible factor for post-space flight OI. One possible mechanism that may be responsible for impaired sympathetic nerve activity after space flight is the vestibulosympathetic reflex. Microgravity has been demonstrated to elicit marked morphological and physiological changes to the vestibular system. Despite this information, no studies to date have examined if the vestibulosympathetic reflex is altered after space flight or its ground-based model for studying autonomic and cardiovascular function, head-down tilt bed rest. The specific aims and hypotheses of this research project are: 1) To determine muscle sympathetic nerve activity (MSNA) responses to head-down neck flexion (HDNF) before and after 1 and 7 days of 6° head-down tilt bed rest (HDBR). HDNF has been used in our laboratory to activate the vestibular system (i.e., otolith organs) in humans and has been shown to increase MSNA. We hypothesize that MSNA responses to HDNF will be attenuated after HDBR and that the attenuation of MSNA will increase as a function of HDBR duration. If this hypothesis is true, this would be the first evidence that the vestibular system may participate in regulating MSNA after HDBR and possibly space flight; and 2) To determine MSNA responses to HDNF during lower-body negative pressure before and after HDBR. We have shown that MSNA is augmented by HDNF during lower-body negative pressure. Thus in healthy adults, the vestibulosympathetic reflex can help defend against orthostatic challenges by increasing MSNA. We hypothesize that the increase in MSNA by HDNF during lower-body negative pressure will be attenuated after HDBR. Therefore, after HDBR the vestibulosympathetic reflex will be impaired and will not be able to help defend against an orthostatic challenge by increasing MSNA. This finding would give credence to the concept that alterations in the vestibulosympathetic reflex may participate importantly in post-space flight OI. These findings should have important implications in understanding the cause of OI following space flight. Moreover, these studies should provide a solid rationale for developing countermeasures involving stimulation of the vestibular system during space flights in order to minimize post-space-flight OI.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Artin A. Shoukas, Ph.D.**
ORGANIZATION:	**Johns Hopkins University School of Medicine**
PROJECT TITLE:	**Mechanics of Cardiovascular Deconditioning**

Project Executive Summary

Changes in cardiac output result from the altered myocardial contractility or through changes in venous filling pressure via the Frank Starling mechanism. Our laboratory has previously shown the importance of veno-regulation by the carotid sinus baroreceptor reflex system on overall circulatory homeostasis, and in particular the regulation of cardiac output. Decreases in SV responses to an orthostatic challenge are the seminal pathophysiologic observation after space flight. This proposal aims to test our overall hypothesis that alterations in venous capacitance function by the carotid sinus baroreceptor reflex system is an important determinant of the cardiac output response seen in astronauts after returning to Earth from long term exposure to microgravity. We will use the hind limb unweighted rat model to simulate the patho-physiological effects as they relate to cardiovascular deconditioning in microgravity. To determine mechanisms of impaired stroke volume responses integrated cardiovascular function *(in vivo)*, and contractile reserve will be tested using miniaturized conductance micro-manometry catheters. The role of cardiac atrophy in cardiovascular deconditioning will be tested using magnetic resonance imaging to noninvasively measure cardiac mass. Since venous capacitance function and arterial resistance determine ventricular preload and afterload respectively, mechanisms of impaired contractile responses in both arterial venous and pulmonary vascular beds will be studied. Molecular mechanisms of endothelial dependent (e.g., nitric oxide), and independent (Ca2+ homeostasis and vascular smooth muscle myofilament Ca2+sensitivity) vascular hyporesponsiveness to sympathetic stimulation will be studied, using vascular contractility bioassays (*in vitro*), pressure-dimension analysis both (*in vivo* and *in vitro*), and intracellular Ca2+ measurement (fluorescence spectrophotometry). We plan to test novel countermeasures based on mechanisms that impair both cardiac output responses and vascular hypo responsiveness in our rat model. These studies will provide important new data concerning normal capacitance vessel function in compensating for postural blood volume redistribution, test our novel hypothesis regarding the pathogenesis of orthostatic intolerance following micro-gravity exposure, and provide insights into potential countermeasures and therapies to prevent problematic postural hypotension on reentry. Our laboratory currently performs experiments from chronic instrumented animals to the cellular and molecular mechanisms involved in cardiovascular regulation and control. It is fully equipped to independently perform all of the necessary experiments outlined in this proposal.

RESEARCH AREA:	**Cardiovascular Alterations**
PRINCIPAL INVESTIGATOR:	**Gordon H. Williams, M.D.**
ORGANIZATION:	**Harvard – Brigham and Women's Hospital**
PROJECT TITLE:	**Influence of Gender and Age on Renal and Cardio-Endocrine Responses to Simulated Microgravity**

Project Executive Summary

Orthostatic intolerance remains an operational problem following space flight, and has been observed to be more pronounced among women than among men. In addition, there is growing evidence that cardiac dysrhythmias may pose a threat to the health of space travelers. In our previous studies we observed that different degrees of sodium balance response on a constant high dietary sodium intake during simulated weightlessness, correlated with orthostatic tolerance and subjects age. Our previous findings also are consistent with an increased basal tone of the RAAS in many subjects. These findings are even more intriguing since several previous subjects demonstrated changes in electrical stability of the myocardium following microgravity simulation. We propose to conduct a detailed investigation of effects of simulated weightlessness on the renal-endocrine system in menstruating women, and in a population of men over the age of 50, applying many of the same methodologies we previously applied to the study of predominantly younger men. This proposal is closely related to a companion study "Influence of Gender and Age on Cardiovascular Responses to Simulated Microgravity" by Richard J. Cohen, M.D., Ph.D., Principal Investigator. His proposal will investigate whether there appear to be any correlative factors between perturbations of the RAAS and effects on myocardial electrical stability.

This work has implications for the treatment and prevention of maladaptive hemodynamic responses experienced by astronauts in flight and on return to Earth. It will increase our understanding of the mechanisms by which weightlessness changes volume and sodium homeostasis, and possibly cardiac electrical stability, thereby, providing entree to develop appropriate countermeasures. Perhaps most importantly, it will broaden our database to include older individuals and women, two groups who are well represented among our population of current and future space travelers. Finally, the results of these studies may further our understanding of the pathophysiology of alterations in volume homeostatic mechanisms in cardiovascular diseases such as congestive heart failure.

NSBRI RESEARCH PROGRAM
HUMAN PERFORMANCE FACTORS

Team Leader: **Czeisler, C. A.** **Harvard**

Associate Team Leader: **Jewett, M. E.** **Harvard**

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RESEARCH AREA:	**Human Performance Factors, Sleep and Chronobiology**
PRINCIPAL INVESTIGATOR:	**George C. Brainard, Ph.D.**
ORGANIZATION:	**Jefferson Medical College of Thomas Jefferson University**
PROJECT TITLE:	**Optimizing Light Spectrum for Long Duration Space Flight**

Project Executive Summary

Risk factors for the health and safety of astronauts and NASA ground control workers disturbed circadian rhythms and altered sleep-wake patterns. These physiological changes can result in decrements in alertness, concentration, and performance, all of which threaten the safety of personnel and the objectives of space missions. In studies of astronauts and NASA ground control workers, light treatment has been used as an effective countermeasure to provide entrainment of circadian rhythms and of sleep-wake patterns. For civilians, light treatment is being tested for improving circadian entrainment and enhancing both performance and alertness in shift workers. A recent Congressional report estimates that 20 million full-time workers in the U.S. are shift workers and that they have increased health problems including higher risk to cardiovascular disease, gastrointestinal distress, as well as cognitive and emotional problems. We need to optimize light as a countermeasure for circadian and sleep disruption in space flight missions. The long term goal of our research is to determine the best wavelengths of light for use as a countermeasure during long-duration space flight, as well as for adjusting circadian and sleep disruption in civilians.

To achieve this aim, an action spectrum (the relative effectiveness of different wavelengths for eliciting a biological response) will be established to help identify the photoreceptor system for light regulation of melatonin in humans. This action spectrum may then be used as a tool for investigating the action spectrum and related photoreceptor system involved in circadian entrainment and phase shifting. One specific aim of this research is to extend an action spectrum for light-induced plasma melatonin suppression using monochromatic wavelengths below 440 nm and above 600 nm. These wavelengths are relevant to astronauts who have to adapt to extraterrestrial environments that have spectral characteristics different from those found on Earth. For example, Martian skylight has an abundance of long wavelengths above 600 nm. Data from the proposed studies can be used to optimize the lighting environment of astronauts on long-term missions. Specifically, these data can be used to 1) improve light treatment as a countermeasure for circadian and sleep-wake disruption in NASA space flight missions, 2) identify the best spectral transmission for space suit visors and the windows used in space vehicles and habitats, and 3) engineer the ideal spectral distribution for illumination of general living quarters during space exploration.

RESEARCH AREA:	**Human Performance Factors, Sleep and Chronobiology**
PRINCIPAL INVESTIGATOR:	**Charles A. Czeisler, Ph.D., M.D.**
ORGANIZATION:	**Harvard – Brigham and Women's Hospital**
PROJECT TITLE:	**Circadian Entrainment, Sleep-Wake Regulation and Performance During Space Flight**

Project Executive Summary

NASA's planned exploration class mission to Mars, currently scheduled for 2018, of astronauts to sustain a high level of performance during an exploration class mission to Mars will be critically dependent upon the ability of the human circadian pacemaker to adapt to the 24.65-h day length. Preliminary data from our laboratory reveals that the entrainment limits of the human circadian pacemaker to synchronizing stimuli of weakened strength, (i.e., a dim light-dark cycle similar to that aboard the mid-deck of the space shuttle), are very near 24 h, and that the 24.65-h day lies outside the range of entrainment to this weak synchronizer. Given that the intrinsic period of the human circadian pacemaker is closer to 24 (~24.14 ± 0.16 h) than previously recognized, with ~25% of humans exhibiting periods ≤ 24.0 h, these results demonstrate the need to develop effective countermeasures to adapt the human circadian pacemaker of all crewmembers to the ~24.65 day.

We propose to test a countermeasure of intermittent bright light to entrain the human circadian pacemaker to a longer-than-24-hour day. Specifically, we propose to test the following hypotheses: i) that synchronization of the human circadian pacemaker to a sleep-wake and light-dark schedule with an imposed period ~4% longer than the pacemakers intrinsic circadian period will be disturbed; ii) that this disturbed circadian synchronization will result in the secretion of the sleep-promoting hormone melatonin during the waking day, disturbed sleep, reduced growth hormone secretion, and impaired performance and daytime alertness; iii) that two relatively brief (45 minute) daily exposures to evening bright light (~10,000 lux) will establish a normal entrained circadian phase, in those subjects, resulting in improved sleep consolidation, undiminished endogenous growth hormone and cortisol secretion and enhanced daytime alertness and performance as compared to subjects on the same schedule with out the evening bright light exposure. We have proposed a randomized 64-day clinical trial to test these hypotheses. The results of the proposed studies will answer fundamental questions on the mechanisms underlying circadian entrainment in humans and lead to the development of space flight countermeasures to produce robust circadian entrainment to the Mars day.

RESEARCH AREA:	**Human Performance Factors, Sleep and Chronobiology**
PRINCIPAL INVESTIGATOR:	**David F. Dinges, Ph.D.**
ORGANIZATION:	**University of Pennsylvania School of Medicine**
PROJECT TITLE:	**Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss**

Project Executive Summary

This project seeks to prevent neurobehavioral deficits and fatigue due to inadequate sleep in astronauts during prolonged space flight, where sleep is chronically restricted to a mean of 6hr/day, which has been shown to result in cumulative sleepiness and performance deficits. Using a response surface experimental paradigm (RSM), we will identify the most effective ways to obtain sleep by investigating how variations in sleep duration and its circadian placement relate to the return of performance per time invested in sleep. In the past 3 years we have been testing in a dose-response manner across chronic sleep restriction, 18 combinations of nocturnal anchor sleep and daytime naps in order to develop an RSM for diurnal functioning. Preliminary analyses of this diurnal RSM reveal that total sleep time per 24hr in the range experienced by astronauts' results in cumulative neurobehavioral deficits, and that shorter nocturnal sleep periods combined with longer daytime naps may partially mitigate this effect. We propose to repeat this effort on an additional n = 90 healthy men and women undergoing a 14-day ground-based laboratory protocol involving the same 18 sleep-dosage cells, but placed at the opposite circadian phase from the first experiment (i.e., simulated night shift operations), to develop an RSM for nocturnal functioning. The sleep conditions will involve 4 anchor sleep durations (4.2, 5.2, 6.2, 8.2hr) and 6 nap sleep durations (0.4, 0.8, 1.2, 1.6, 2.0, 2.4hr) crossed to yield a total of 4 anchor-sleep-only conditions, and 14 anchor+nap sleep conditions, thereby spanning a dynamic range of cumulative sleep debts (i.e., from 0 to 40hr in a 10-day period). During the protocol we will monitor neurobehavioral performance and mood, waking EEG, core body temperature, plasma levels of hormones (melatonin, cortisol, growth hormone), behavioral motility, and sleep PSG, while subjects remain in a laboratory that simulates the low light, tight quarters, and lack of social contact with the outside world that characterize long-duration space flight. The resulting RSM for nocturnal functioning will provide information on the fundamental relationship between sleep duration and waking neurobehavioral capability when night operations are required. Comparison of the RSM for nocturnal functioning to the RSM for diurnal functioning will help establish the contribution of circadian placement of sleep and waking to the development of cumulative neurobehavioral deficits. Together these nocturnal and diurnal RSMs will provide a comprehensive view of the extent which sleep can be safely reduced during space flight.

RESEARCH AREA:	**Human Performance Factors, Sleep and Chronobiology**
PRINCIPAL INVESTIGATOR:	**Charles A. Fuller, Ph.D.**
ORGANIZATION:	**University of California, Davis**
PROJECT TITLE:	**Primate Circadian Rhythms in the Martian Environment**

Project Executive Summary

To maintain health and homeostasis, an organism must regulate each of its physiological systems in concert with all of the others and with the external environment. The Circadian Timing System (CTS) has evolved to allow coordination of an organism's physiology and behavior both internally and with the external 24.0 hr terrestrial day. The mammalian CTS is adapted to the lighting environment found on Earth. As we move toward exploration-class space missions, we will be exposing astronauts to non-Earth environments for increasing lengths of time. Changes may include altered gravity and spectral, intensity and day-length differences. This raises the concern of whether or not humans will be able to synchronize to such an alien environment. For example, a Mars-type exploration would entail stays on Mars of one to two years. Compared with the Earth, the Martian environment has a photic spectrum shifted to the red, low illumination level, a periodicity of 24.62 hr, and a 0.38 G gravitational field. The mammalian CTS is most sensitive to light of the blue-green wavelengths and adapted to synchronize to a 24.0 hr day. In addition, light must be relatively bright to affect the CTS of primates, especially humans. Further, altered CTS function including rhythm amplitude and wave form, sensitivity to light, and CTS period, have been reported in both the microgravity environment of space flight and in hyperdynamic fields on the Earth. This program will examine the ability of primates (male and female rhesus monkeys) CTS to cope with the Martian environment. The first three experiments will examine responses to the Martian day, while the last experiment will examine the effects of G on the period of the circadian pacemaker. Experiment 1 will examine the ability of the CTS to synchronize to the Martian photic (spectrum and period) environment. We will examine long-term (4-month) physiological and behavioral responses. Experiment 2 will similarly examine long-term responses to a photic environment composed of a Martian day and Earth light spectrum. Experiment 3 will use the primate model to initiate the development of countermeasures to assure optimum entrainment of the CTS. This experiment will examine the effects of timed bright light pulses on CTS entrainment. Using the forced desynchrony protocol, experiment 4 will examine the effects of 1.0, 1.5 and 2.0 G on the period of the circadian pacemaker. We will develop a G vs. period model to predict the effect of the 0.38 G Martian environment on the period of the circadian pacemaker. This model will be used to develop countermeasure requirements to be tested in experiment 3. Thus, this program will develop a primate model to evaluate physiological and behavioral consequences of long-term exposure of male and female subjects to altered lighting and gravitational environments.

RESEARCH AREA:	**Human Performance Factors, Sleep and Chronobiology**
PRINCIPAL INVESTIGATOR:	**Megan E. Jewett, Ph.D.**
ORGANIZATION:	**Harvard – Brigham and Women's Hospital**
PROJECT TITLE:	**Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure**

Project Executive Summary

Due to the unusual light/dark patterns and sleep/wake schedules to which they are exposed, astronauts may frequently experience circadian misalignment, during which their circadian rhythms are not appropriately synchronized with their work schedules so that their performance and ability to sleep can be severely compromised. Carefully scheduled light exposure can realign astronauts' circadian rhythms to match their sleep/wake schedules thereby improving their performance levels and sleep quality. Therefore, it is imperative that NASA have the capacity to easily design and modify lighting schedules to improve circadian alignment throughout long duration space flights.

To reach this goal, this proposal addresses four specific aims: 1) to further develop and refine our mathematical *Dynamic Stimulus Processing* model so that it can accurately predict the phase and amplitude of the human circadian system under any lighting conditions, especially those which occur in space. To do this, data will be simulated from four completed studies on the effects of light on the human circadian system; 2) to validate this refined *Dynamic Stimulus Processing* model using data from four other studies on the effects of brief and extended bright light pulses and very dim light on circadian phase and entrainment; 3) to incorporate this refined and validated *Dynamic Stimulus Processing* model into our mathematical *Neurobehavioral Performance* model, which will then be validated against performance data collected under all eight of the studies analyzed in specific aims 1 and 2; 4) to develop a user-friendly *Performance Simulation Software* program that can be used to specify appropriate light schedules as a countermeasure to the poor performance and sleep quality associated with circadian misalignment in space.

Potential applications of this model and software include the specification of lighting requirements aboard spacecraft necessary to insure proper entrainment of astronauts while in space, and the design of shift schedules to allow astronauts to receive available bright light at appropriate times for proper circadian alignment with their sleep/wake schedules.

RESEARCH AREA:	**Human Performance Factors, Sleep and Chronobiology**
PRINCIPAL INVESTIGATOR:	**Michael Menaker, Ph.D.**
ORGANIZATION:	**University of Virginia**
PROJECT TITLE:	**A Model of Circadian Disruption in the Space Environment**

Project Executive Summary

We have developed a transgenic rat model that enables us to measure circadian rhythms of transcription of a "clock gene" (Per1) in the brain and in peripheral tissues. Using this model we have shown that Per transcription is circadian *in vitro* preparations of suprachiasmatic nucleus (SCN), several other brain areas, liver, lung, muscle, and other peripheral organs. This demonstrates that each of these areas and organs contain circadian oscillators – those in the SCN are self-sustained and persist for more than a month *in vitro*, while those in other areas damp out within one to six cycles once tissues containing them have been removed from the animal. These data support a hierarchical model of mammalian circadian organization, with the SCN as the dominant synchronizer of rhythms in other brain areas and in the periphery. In addition, our new rat model provides unique opportunities to test the effects of "abnormal" environments, such as those that will be encountered in space travel, on organization of the mammalian circadian system.

We propose to evaluate the effects of "constant" conditions and of shift-work schedules on both the maintenance of circadian rhythmicity in central and peripheral structures, and on temporal synchrony among them (specific aims 1 and 2). Our preliminary data indicate that under these conditions, some circadian rhythms will be abolished, others will be abnormally phased (i.e., will peak at the "wrong" time relative to the light cycle and/or rhythms in other tissues), while yet others (in particular, the SCN) may be unaffected. We have called the resulting abnormal circadian organization "dysphasia," and hypothesize that it is responsible for the malaise and performance decrements seen in jet lag and shift-work syndrome. We believe that to the degree possible, it should be avoided in space travel.

In a final set of experiments (specific aims 3 and 4), we will attempt to ameliorate or prevent dysphasia by manipulating meal timing, melatonin administration, forced exercise, and short pulses of complete darkness. We have focused on these interventions because they could be applied with relative ease and safety to humans in space, and there is good reason to expect that they would be effective.

RESEARCH AREA:	**Human Performance Factors, Sleep and Chronobiology**
PRINCIPAL INVESTIGATOR:	**Lawrence P. Morin, Ph.D.**
ORGANIZATION:	**State University of New York – Stony Brook**
PROJECT TITLE:	**Circadian and Vestibular System Relationships**

Project Executive Summary

In order to adequately understand the circadian rhythm system, it is important to recognize at the outset that it is a system functioning in the context of a variety of influences mediated by the rest of the brain. A primary concern of the circadian rhythm research field has been with photic time giving stimuli. However, it is readily apparent that other, non-photic stimuli gain access to the circadian system and can modulate rhythm phase. The present proposal is derived from our recent and continuing anatomical description of the extensive interconnections between the intergeniculate leaflet (IGL) of the circadian rhythm system and other nuclei of the subcortical visual system. Preliminary data are described in the application, which demonstrate neurons projecting from the vestibular nuclear complex to the IGL. This novel pathway has significantly modified our thinking about the route by which a correlate of the non-photic stimulus, locomotion, might gain access to the circadian rhythm system and shift rhythm phase. It has also opened the possibility that the vestibular system is a specific route by which sensory information related to head movement might gain access to the circadian system. The proposal consists of three specific aims: (1) The efferent and afferent connections between the vestibular nuclei and the IGL will be fully identified. A part of this aim will be to demonstrate that vestibular neurons projecting to the IGL actually connect with neurons projecting to the circadian clock in the suprachiasmatic nucleus. (2) The visual system will be activated with patterned moving light (an optokinetic stimulus) and shown to functionally activate neurons in the IGL as indicated by immunoreactive FOS protein induction and by circadian rhythm phase responses of the neuropeptide y type. (3) The vestibular system will be activated using a three-dimensional motion stimulus and shown to also induce FOS protein in the IGL. The stimulus will be tested for its ability to induce circadian rhythm phase response of the neuropeptide y type. Joint application of optokinetic and vestibular stimuli will also be tested for FOS protein induction and phase shifting capability. The anatomical tract tracing methods will include antero- and retrograde tracing using the tracers, Phaseolus lectin leucoagglutinin and cholera toxin B fragment, respectively. We will also implement transsynaptic viral tract tracing methods to determine that vestibular neurons indeed connect to IGL neurons which, in turn, project to the circadian clock. The results will enable a better comprehension of the relationship between the visual and vestibular systems and may contribute significantly to the understanding of motion sickness. The proposed research will be the first to link these issues with the circadian rhythm system.

RESEARCH AREA:	**Human Performance Factors, Sleep and Chronobiology**
PRINCIPAL INVESTIGATOR:	**Gianluca Tosini, Ph.D.**
ORGANIZATION:	**Morehouse School of Medicine**
PROJECT TITLE:	**Long-Term Exposure to Dim Lighting Desynchronizes the Circadian System of Rats**

Project Executive Summary

Many biochemical, physiological and behavioral parameters exhibited by organisms show daily fluctuations and most of these daily rhythms persist in constant conditions, thus, demonstrating that they are driven by endogenous oscillators. The rhythms that persist in constant conditions with a period close to 24 hours are called circadian rhythms. One the most important aspects of space flight is the absence of geophysical 24-hour cycles, which, of course, affects the overall temporal organization of the organisms. In the case of long-duration manned space flight, it is crucial to understand how the whole circadian system would react and behave in such circumstances.

We discovered that exposing rats to constant dim light for 60 days significantly affected the phase-relationship among circadian outputs in the SCN, retina and pineal, demonstrating that in these animals internal desynchronization of the circadian rhythms is occurring. We also observed that the circadian rhythm in arylalkylamine N-acetyltransferase (the enzyme that is responsible for the circadian rhythm in melatonin synthesis) was abolished in both the retina and in the pineal gland. Our data also indicated that locomotor activity rhythm might be an unsatisfactory marker to assess the circadian status of the whole organism. Internal desynchronization has profound effects on the capability of the organisms to perform (mentally and physically) and to remain healthy. In this research proposal, we have designed a series of experiments to: a) understand the mechanisms responsible for the observed desynchronization; b) investigate the effect that internal desynchronization has on the immune response and motor and cognitive performances; and c) evaluate if melatonin can be used as a pharmacological agent to counteract desynchronization of the circadian rhythms. We believe that the model we have generated will be useful in to foreseeing and preventing dysfunction of the circadian system that may arise after long periods in the space environment where the normal cycle has been altered.

RESEARCH AREA:	**Human Performance Factors, Sleep and Chronobiology**
PRINCIPAL INVESTIGATOR:	**Fred W. Turek, Ph.D.**
ORGANIZATION:	**Northwestern University**
PROJECT TITLE:	**Animal Model for Sleep Loss and Circadian Disruption**

Project Executive Summary

Under normal conditions, there is a stable phase relationship between circadian rhythmicity, sleep and the light-dark cycle in essentially all-animal species. However, humans routinely disrupt the normal synchronization between the circadian clock and the sleep-wake and light-dark cycles either for short periods of time (i.e., following rapid travel across time zones, commonly referred to as "jet lag"), or for long periods of time (i.e., as occurs in shift- or night workers). This disruption of the circadian system often results in chronic partial sleep loss. Chronic sleep loss and circadian disruption are expected to occur during extended-duration missions in space as well as in support ground personnel due to the demands of the mission. Despite the fact that millions of individuals are affected by chronic misalignment of the sleep and circadian clock systems, few practical countermeasures to circadian disruption and sleep loss have been developed in which rigorous scientific evidence attests to their effectiveness. A major impediment to the development of such countermeasures is the lack of adequate animal models that could be used to study the human equivalent of disrupting the normal synchronization of circadian rhythmicity, sleep and the light-dark cycle from one another on a chronic basis. The proposed studies are designed to further develop and to exploit a unique animal model to determine the effects on circadian rhythmicity and the sleep-wake cycle of the chronic misalignment of the activity-rest cycle with the entraining light-dark cycle and with normal circadian time. We will also determine if this chronic misalignment and any resultant circadian disruption or sleep loss causes a decrement in neurobehavioral or motor performance. This animal model will also be used to determine if specific pharmacological (i.e., melatonin) or behavioral (i.e., exercise) interventions can be used to reduce the effects of circadian disruption and sleep loss.

The mouse will be used as the animal model in the proposed studies because it is in this species where the discovery of the function of most mammalian genes is expected to be made. Ultimately it is expected that it will be possible to integrate the function of known, and yet to be discovered, circadian clock and sleep genes in the development of new strategies for alleviating the adverse effects of chronic sleep loss and circadian disruption on human physiology and behavior. The use of an animal model for the study of circadian disruption and sleep loss is expected to lead to new insights into the interactions between the sleep and circadian clock systems, and should provide important information about countermeasures that could prove useful in alleviating the adverse effects associated with work at different times of day in humans on the ground and in the space environment.

NSBRI RESEARCH PROGRAM
IMMUNOLOGY, INFECTION AND HEMATOLOGY

Team Leader: **Shearer, W. T.** — **Baylor**

Associate Team Leaders: **Butel, J. S.** — **Baylor**; **Sonnenfeld, G.** — **Morehouse**

Butel, J. S.	**PI**	**Baylor**	**Viral Infections and Mucosal Immunity**	**38**
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Gridley, D. S.	CO-I	Loma Linda		
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Ochs, H. D.	CO-I	Washington		
Reuben, J. M.	CO-I	UT-MDACC		
Rosenblatt, H. M.	CO-I	Baylor		
Smith, E. O.	CO-I	Baylor		
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RESEARCH AREA:	**Immunology, Infection and Hematology**
PRINCIPAL INVESTIGATOR:	**Janet S. Butel, Ph.D.**
ORGANIZATION:	**Baylor College of Medicine**
PROJECT TITLE:	**Viral Infections and Mucosal Immunity**

Project Executive Summary

Space flight conditions are known to effect changes in the systemic immune system and the unanswered question is whether infectious diseases will pose a major risk to the success of long-duration missions. We seek to continue our studies of possible deleterious effects of space flight on viral infections and the mucosal immune system, an important defense against microbial infections. We are testing the hypothesis that long-duration space flight conditions will alter immune responses, lead to reactivation of latent viruses, increased viral infections and disease, and possible development of virus-associated cancers. We are focusing the virus studies on herpesviruses and polyomaviruses and are using the antiorthostatic suspended *(AOS)* mouse model for mucosal immunity studies. We have established normal baselines for viral shedding from healthy volunteers and have preliminary data from on-going collaborations of human space flight analogs of increased viral reactivation and shedding and from the *AOS* model that the mucosal immune system shows alterations. We propose to continue and extend our comparative studies of virus reactivation and infection, combined with immunological measures, through collaborations on several ground-based models. These models include Antarctica isolation, closed chambers, sleep disruption, and HIV infections, plus samples from astronauts. We plan to develop mouse models of acute and latent infections with murine herpesvirus and polyomavirus for collaborative proton irradiation studies. We propose to apply new gene array technology to characterize global changes in mucosal immune responses in *AOS* mice before and after virus infections. We expect the animal studies to generate hypotheses that can be tested in the future in human ground-based models. Through linkages to other NSBRI applications, involving sleep disruption, space immunodeficiency, proton irradiation, and the stress-related HPA axis, our team is poised to provide comprehensive insights into the possible effects and underlying mechanisms of long-duration space flight on infectious disease processes and mucosal immunity and then to plan and develop specific countermeasures.

RESEARCH AREA:	**Immunology, Infection and Hematology**
PRINCIPAL INVESTIGATOR:	**George E. Fox, Ph.D.**
ORGANIZATION:	**University of Houston**
PROJECT TITLE:	**Microorganisms in the Spacecraft Environment**

Project Executive Summary

NASA's Critical Path Roadmap document and the NSBRI Immunology, Infection and Hematology team priorities identify microbial infection as a significant factor affecting human space flight. Herein, we proposed to continue and expand our studies on microorganisms in the space environment. We will continue our efforts to develop assays for determining the microbial content of the air and water systems on spacecraft. These assays will be useful for studies of the microbial ecology of the space environment and as monitoring tools on eventual long duration missions. Improved space-flight-compatible assay formats will be tested, and results validated against classical methods. These methods, which will be useful throughout the work, include novel sample preparation chemistries and the use of molecular beacons and beacon arrays for low-labor multiplex detection. Project expansions include the use of whole-genome hybridization array technology to identify changes in microbial gene expression under modeled microgravity. If such effects occur they may affect an organism's pathogenicity or ability to evolve unexpected phenotypic properties, e.g. novel mechanisms of pathogenicity. Finally, a novel, very general spacecraft compatible approach to identifying the genetic affinity of any problematic bacterium will be developed. This general identification system is being developed as a safeguard against the appearance of a novel pathogen, or pathogenic traits in organisms previously not expected to have the potential for pathogenicity.

RESEARCH AREA:	**Immunology, Infection and Hematology**
PRINCIPAL INVESTIGATOR:	**Alan M. Gewirtz, M.D.**
ORGANIZATION:	**University of Pennsylvania School of Medicine**
PROJECT TITLE:	**Effect of Deep Space Radiation on Human Hematopoietic Stem Cells**

Project Executive Summary

Astronauts on long-term missions in deep space will be placed at risk from a variety of hazards. Some of these are known while others may be anticipated. Damage to hematopoietic stem cells as a result of radiation exposure is as an example of the latter. Our long-term goal is to identify and quantitate the risks of deep space radiation to the human hematopoietic system, with particular emphasis on the hematopoietic stem cell. Stem cells are the ultimate source of both the blood and immune systems and damage to these cells could have grave immediate and long-term consequences. At the same time, because these cells can be readily removed from the body, manipulated, and stored, they are also unique candidates for countermeasures that might obviate, or totally negate, damage incurred to them. Accordingly, this project will have three specific aims that support our long-term goals. These are to: 1) *Investigate the cellular consequences of exposing human hematopoietic stem cells to an environment which simulates the radiation environment of deep space.* While much is known about the effects of "conventional" radiation on hematopoietic cells, virtually nothing is known about the effects of deep space, high LET radiation on human hematopoietic stem (HSC) and progenitor (HPC) cells. 2) *Examine the molecular consequences of exposing human hematopoietic stem cells to an environment which simulates the radiation environment of deep space.* This aim has two purposes. If radiation leads to degradation of hematopoietic cell function it will clearly be of interest to look for the molecular lesions potentially responsible for such damage. Alternatively, more long-term, but initially occult damage may also be induced. The consequences of such damage could lead either to a complete failure of hematopoiesis (aplastic anemia) or the development of hematological malignancies. Identification of such damage is therefore important. 3) *Design potential countermeasures to obviate or negate cellular and molecular damage discerned during the course of carrying out Aims* 1 *and* 2. We propose both simple and more complex solutions to problems that might be identified during the course of this study. We suggest that prophylactic (pre-flight) harvest and storage of astronaut stem cells might be a safe, effective, and relatively inexpensive mechanism for countering long-term damage to cells of the hematopoietic systems. Countermeasures that might prove effective in combating damage encountered during flight will also be developed and explored for their utility.

RESEARCH AREA:	**Immunology, Infection, and Hematology**
PRINCIPAL INVESTIGATOR:	**William T. Shearer, M.D.**
ORGANIZATION:	**Baylor College of Medicine**
PROJECT TITLE:	**Space Flight Immunodeficiency**

Project Executive Summary

Using ground-based models, the investigators of this grant application ("Space Flight Immunodeficiency") wish to determine the possibility that long-term space travel will weaken human immune responses and render astronauts susceptible to reactivated viral infection and cancer. The two ground-based models that will be used are: 1) humans exposed to the harsh conditions of the Antarctic winter-over and 2) mice exposed to proton and y-ray radiation and infected with polyomavirus and gamma-herpesvirus. The human Antarctic-exposure model will mimic the space travel conditions of stress, isolation, containment, and microbial contamination, and the animal radiation model will mimic the solar radiation that astronauts will experience. In preliminary human studies in collaboration with Dr. Desmond Lugg and the Australian National Antarctic Research Expedition, we have preserved over 1,500 plasma and peripheral blood mononuclear cells of volunteer subjects for analysis of the effects of the Antarctic winter-over experience on lymphocyte subset expression, lymphocyte proliferation to specific antigens, and cytokine production. With collaborator Dr. Janet Butel, who will be evaluating saliva, urine, and white blood cells of Antarctic subjects for evidence of reactivation of viral infection by virus culture and DNA quantitation, we will be able to assess lymphocyte reactivity to latent viral antigens (e.g., EBV) and determine the degree of immunosuppression. Our collaborators at Loma Linda University Medical Center, Dr. Daila Gridley and Dr. Gregory Nelson, have very recently published exciting radiation studies in mice that demonstrate an acute reduction in critical lymphocyte subsets and loss of specific immune responses. These studies serve as preliminary studies for our animal model, in which we will attempt to show that viral infection in irradiated mice will lead to a permanent state of acquired immunodeficiency. Thus, by exposing mice to doses of proton irradiation and latent viruses, we will attempt to show an effect of solar radiation (80% proton irradiation) upon latent viruses in humans in long-term space travel. If our experiments demonstrate that astronauts are likely to develop chronic viral infection and tumors, it would be appropriate to develop countermeasures, such as radio-protective compounds that could shield astronauts from solar radiation. Thus, support of this research program would enable our research team to quantitatively assess the importance of ground-based space model conditions on human immune function, chronic infection, and cancer.

RESEARCH AREA:	**Immunology, Infection and Hematology**
PRINCIPAL INVESTIGATOR:	**Gerald Sonnenfeld, Ph.D.**
ORGANIZATION:	**Morehouse School of Medicine**
PROJECT TITLE:	**Suspension, the HPA Axis and Resistance to Infection**

Project Executive Summary

Space flight has been shown to result in changes in immune responses of humans and other animals, but the effects of space flight on resistance to infection remains to be established. Using a murine model, we now propose to explore potential effects of space-flight-like conditions on resistance to infection and novel mechanisms for regulating resistance to infection. The hypothesis to be tested is: antiorthostatic (AOH or hindlimb) suspension of mice, a model for some of the effects of space flight on the immune system, results in altered resistance to infection with pathogens. Testing of this hypothesis will provide data to allow development of future studies to determine if space flight affects resistance to infection and if countermeasures can be developed to prevent any detrimental effects.

The specific aims of the proposed study are (1) To expand the range of infections altered by AOH suspension. We have already shown that resistance to some infections that are not likely to be risks during space flight has been altered by AOH suspension and we now wish to determine if infections that could be a risk during space flight that are affected by the suspension model; (2) To determine the mechanism of alteration of resistance to infection induced by AOH suspension. Although previous studies have shown that immune responses are altered by space flight, we now wish to extend these studies to determine the role of the neuroendocrine system in regulating infections. This will be carried out using two approaches. The data obtained from experiments using both approaches will be integrated to allow for development of a model for the mechanism(s) of the effects of hindlimb suspension on resistance to infections: A) The first approach will involve an evaluation of AOH suspension-induced alterations of host immune and neuroendocrine responses; B) The second approach will involve an evaluation of direct effects of the host neuroendocrine response on the pathogen. We have preliminary data to indicate that catecholamines can affect bacterial pathogen growth and production of virulence factors. We will explore the mechanism of the effects of the neuroendocrine system on bacterial infections and, together with Dr. Butel, begin exploration of effects of the neuroendocrine system on viral infections.

Understanding of these mechanisms could aid the design of future space flight experiments and allow for the future development of countermeasures to prevent induction of these difficulties by space flight.

NSBRI RESEARCH PROGRAM
INTEGRATED HUMAN FUNCTION

Team Leader: **Kushmerick, M. J.** **Washington**

Associate Team Leader: **Coolahan, J. E.** **Hopkins/APL**

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RESEARCH AREA:	**Integrated Human Function**
PRINCIPAL INVESTIGATOR:	**Donald M. Bers, Ph.D.**
ORGANIZATION:	**Loyola University Chicago**
PROJECT TITLE:	**Integrative Cardiac Myocyte Model: Ion Channels, Ca and Contraction**

Project Executive Summary

Our long-term objective is to build a detailed quantitative model of cardiac muscle, which will include electrophysiological properties, cellular Ca-regulation and contractile activation and relaxation. Aims of the project are: 1) To develop a more up-to-date electrophysiological model of cardiac myocyte dynamics; 2) to incorporate new Ca-transport data on SR Ca-uptake, release and Na/Ca exchange; 3) To extend the model to include cooperative Ca-dependent myofilaments activation, contraction and relaxation; and 4) Implement this model on highly accessible formats (one user-friendly and one computer-friendly for interfacing with other models). The rationale for our proposal stems from a lack of integration of information from the level of ion channels to Ca transients to myofilament force and shortening. We plan to develop a comprehensive quantitative model that incorporates up-to-date information on ion channels, modulation of Ca-cycling processes and myofilament activation. The approach involves a team of investigators with long-standing laboratory and modeling experience in each of these processes. Drs. Bers and Puglisi of Loyola University have extensive experience in modeling and quantitative experimental studies of regulation of Ca-cycling and membrane currents in the cardiac myocyte. Similarly, Drs. De Tombe and Solaro of the University of Illinois at Chicago have extensive experimental and modeling experience that focuses on the modulation of myocyte response to Ca. The approach involves the generation of computer models constrained by dynamic data generated in these and other laboratories. A preliminary working model LabHEART4 shows feasibility and utility of the proposed modeling format. This endeavor will provide new insights into normal physiological regulation of myocyte activity as well as providing a baseline from which altered function induced by alterations in hemodynamic loading can be readily considered. A fully integrated model of cardiac myocyte activity and regulation of myocyte activity developed here will have a broad application to acute changes in the environment as occurs in space travel and re-entry. This understanding will also have a broad significance to the understanding the effects of altered gene and protein expression associated with long-term changes in cardiac loading that occur during space travel and return to Earth.

RESEARCH AREA:	**Integrated Human Function**
PRINCIPAL INVESTIGATOR:	**Marco E. Cabrera, Ph.D.**
ORGANIZATION:	**Case Western Reserve University**
PROJECT TITLE:	**Metabolic Adaptations of Skeletal Muscle to Training/Detraining: A Systems Model**

Project Executive Summary

Space travel (detraining) has detrimental effects on skeletal muscle structure, metabolism, and function, including reductions in muscle size, strength, and endurance. Exercise (training) in space can counteract some of these deleterious effects. Even with the relatively abundant information gathered from studies in space and from ground-based simulated microgravity of humans and rodents, no physiologically based mathematical/computational models of human function are available that integrate cellular and whole body data. Such models are essential to provide a framework for quantitative understanding of the skeletal muscle metabolic adaptations to periods of training and detraining. Thus, the long term objectives of this proposal are to: (a) quantitatively evaluate the interactions among morphological, molecular, and biochemical variables that alter the metabolic/functional attributes of muscle fibers during periods of training/detraining, and (b) to perform quantitative predictions of work capacity after periods of training/detraining. Accordingly, mathematical models of skeletal muscle intermediary metabolism will be developed. The models will integrate cellular, tissue, and whole body data, with emphasis on cellular energetics and substrate utilization and regulation in skeletal muscle. These models will describe metabolite and enzyme concentrations altered during acute and chronic adaptations to training and detraining. The models will incorporate parameters that quantify the adaptations, as well as parameters that relate to muscle mass, enzymatic activity, mitochondrial content, and fiber-type distribution. The specific aims of this project are:

1) To identify the metabolic adaptations to training/detraining in order to develop databases containing (a) information on the structural, biochemical, physiological, and functional adaptations of skeletal muscle to microgravity and exercise training in rats and humans and (b) the underlying molecular and biochemical mechanisms mediating these adaptations.
2) To develop mathematical models of intermediary metabolism in skeletal muscle that account for the effects of training and detraining.
3) To investigate the relative significance to work capacity and muscle efficiency of metabolism-related parameters that are affected by training or detraining.
4) To simulate the short- and long-term effects on skeletal muscle intermediary metabolism and energetics of space flight and exercise in space, to quantitatively test the hypotheses that after a period of space travel or exercise training, the observed changes in the rates of carbohydrate and fatty acid oxidation in skeletal muscle are a result of (a) a partial conversion of slow-twitch to fast-twitch fibers and (b) alterations in glycolytic and oxidative enzymes.

RESEARCH AREA:	**Integrated Human Function**
PRINCIPAL INVESTIGATOR:	**P. Bryant Chase, Ph.D.**
ORGANIZATION:	**Florida State University**
PROJECT TITLE:	**Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle**

Project Executive Summary

This proposal is targeted to the National Space Biomedical Research Institute's Integrated Human Function Team (primary) with a secondary target as the existing Muscle Team. The overall goal of this proposal is to produce a muscle cell model (digital cell) that will: explain biomechanical adaptations that occur with alterations in muscle protein isoforms due to changes in activity level; predict bioenegetic changes associated with changes in activity level; and be integrated into computational models of human limb and heart. The essential molecular and subcellular components of the model will be identified and algorithms constructed based on experimental data obtained in a controlled environment. The cell model will be tested against published biomechanical and bioenergetic data obtained under a broad spectrum of environmental conditions. Our muscle cell model will be one of the main building blocks for constructing a model of integrated human function because the cell is the basic unit of physiological organization; the musculoskeletal system is ~80% of body mass and thus is a major determinant of energy consumption, as well as being responsible for movement and cardiovascular function. To accomplish our goal of constructing a digital muscle cell, we will: (1) identify contractile protein composition of skeletal and cardiac muscles from high- and low-activity rats; (2) characterize contractile properties (phenotype) of selected muscles containing unique mixtures of protein isoforms, as identified in Aim 1; and (3) develop the "digital" cell biomechanical model.

RESEARCH AREA:	**Integrated Human Function**
PRINCIPAL INVESTIGATOR:	**James E. Coolahan, Ph.D.**
ORGANIZATION:	**Johns Hopkins University Applied Physics Laboratory**
PROJECT TITLE:	**Distributed Simulation of Integrated Human Function**

Project Executive Summary

The long-term objective of the *Distributed Simulation of Integrated Human Function* project for the National Space Biomedical Research Institute (NSBRI) is to demonstrate the ability to simulate Integrated Human Function (IHF) over time by providing a technical framework to permit simulations of different human physiological functions, executing in separate locations, to interact to produce synergistic results. The hypothesis of this research is that interoperable simulations of human physiological functions applicable to the space flight environment executing interactively can produce integrated results that cannot be produced by these simulations executing independently. The specific aims of this research are: to develop, at the Johns Hopkins University (JHU), a computational model of the human ventricular myocyte and a finite element model of the geometry and fiber structure of the human heart; to develop a distributed simulation of human cardiac function, incorporating the simulation of the human cardiac ventricular cell resident at JHU, and a simulation of coupled mechanical and electrical function resident at the University of California, San Diego (UCSD); and, working with the NSBRI IHF team, to select other appropriate models that can be represented over time using simulations, and integrate them into (a) a distributed simulation of cardiovascular function; and (b) a multi-function distributed simulation of cardiovascular, bone, and muscle systems representative of the IHF simulations that will be needed for long-term space flight. The research will leverage the significant body of research in constructing distributed interoperable simulations that has been performed in connection with the development of the High Level Architecture (HLA) for simulations developed by the U. S. Department of Defense. The initial distributed simulation of human cardiac function will be constructed by developing a Federation Object Model (FOM) describing the objects, attributes, and interactions of the respective JHU and UCSD simulations, and incorporating a structured Federation Development and Execution Process (FEDEP) to ensure proper systems integration. An HLA Runtime Infrastructure (RTI) will provide the means for distributed execution across the coast-to-coast network implementation. The FOM developed for this initial application will be extended in subsequent years to incorporate simulations of other aspects of cardiovascular system function, and ultimately, related elements of bone and muscle systems, so that a multi-system distributed simulation will be executed in the final year of the project.

RESEARCH AREA:	**Integrated Human Function**
PRINCIPAL INVESTIGATOR:	**Martin J. Kushmerick, M.D., Ph.D.**
ORGANIZATION:	**University of Washington**
PROJECT TITLE:	**Integrating Human Muscle Energetics and Mechanics**

Project Executive Summary

We propose a novel combination of ^{31}P NMR spectroscopies, ultrasound functional images, biomechanical analyses and multi-level modeling for analysis leading to an integration of human limb muscle function. We will integrate macroscopic properties in terms of molecular mechanisms. Human limb muscle will provide an exemplar for the integrated human function team: the analysis and modeling of different cell types and tissues in the limb as a functional organ will provide enabling concepts and technology for larger scale modeling of the "digital human" and guide strategies for database and global computer system development. We believe the current best strategy to develop milestones and to make progress on the ambitious goal of the "digital human" is to commence work on one body part that includes important pieces of NASA's critical path analysis. An understanding of limb muscle function is crucial to the planning of training exercises and to selecting personnel for the most strenuous activities with optimal efficiency and minimal risk. The science of this proposal evaluates the mechanisms responsible for transient and steady state performance of limb muscle. This analysis requires the specification of: 1) the mechanical power output by specific muscles during limb functions; 2) the analysis of the properties of different muscles in the same individual and of the same muscles in different individuals; 3) the quantification of energy demand by mechanical output; 4) the division of energy supply between glycolytic and oxidative processes and analysis of their inter-related controls; and 5) analysis of the response of these components with integrated models of the system. The information obtained through these experimental approaches is crucial to develop a model-based approach to the study of *in vivo* muscle energy balance in humans for two reasons: 1) the relevant data is not available; and 2) more importantly, the conceptual basis for integrating the component mechanisms can only be evolved from these new observations. We will show that the tissues in the limb have ideal properties and components that render hierarchical modeling feasible. Many properties of these processes are known and characterized *in vitro* but *in vivo* they form an integrated system, the characteristics and regulation of which is largely unknown. We will expand a mathematical model for intracellular energetics to include mechanics and blood flow. The goal is a hierarchical and mechanistic model of these crucial components of limb muscle function which can be extended to include additional metabolic and cellular features developed by other investigators, bone mechanical properties and, eventually, cardiovascular and respiratory analyses currently under development in other teams. We expect the intermediate milestone of the limb functional model will be a powerful tool for analyzing altered physiological responses to space environment and for testing efficacy of countermeasures.

RESEARCH AREA:	**Integrated Human Function**
PRINCIPAL INVESTIGATOR:	**Andrew D. McCulloch, Ph.D.**
ORGANIZATION:	**University of California, San Diego**
PROJECT TITLE:	**Integrated Modeling of Cardiac Mechanical and Electrical Function**

Project Executive Summary

Many of the most critical health risks of exposure to space environments involve cardiac mechanical and electrical function, especially impaired responses to orthostatic and exercise stress, ventricular atrophy and remodeling, and cardiac dysrhythmias. The objective of the proposed research is to develop computational models of human cardiac electrical and mechanical responses to weightlessness and low G that integrate (i) from cell to organ; (ii) with other functional subsystems of the heart; and (iii) with other organ systems. Since the initial insult, microgravity, is fundamentally mechanical and because models must be predictive to be useful for countermeasure development and assessment, the core of the models will be physically-based anatomically detailed three-dimensional continuum models developed by the applicants over the past 15 years. These organ models will provide a framework for bridging cellular models developed by ourselves and other project groups of myocyte metabolism, ionic currents, excitation-contraction coupling, crossbridge dynamics and signal transduction with systems models of hemodynamics and cardiovascular control. Model developments will focus on including mechanisms at the cell, tissue, organ and organ system level that are most relevant to the risks of cardiac deconditioning, dysrhythmia, atrophy and remodeling. The models will be implemented using modular, object-oriented software engineering techniques that allow component models developed by other projects in the integrative human function team to be readily integrated through standard broker architectures for software interoperability. The project will result in software for anatomically and physically based computational models that bridge kinetic models of cellular physiology to systems models of whole body physiology. Since the models are based on validated finite element methods for biomechanics and electro-physiology, the software will also have applications to other vital aspects of integrated human function in space such as musculoskeletal performance.

NSBRI RESEARCH PROGRAM
MUSCLE ALTERATIONS AND ATROPHY

Team Leader: **Baldwin, K. M.** **UC, Irvine**

Associate Team Leader: **Goldberg, A. L.** **Harvard**

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RESEARCH AREA:	**Muscle Alterations and Atrophy**
PRINCIPAL INVESTIGATOR:	**Parker B. Antin, Ph.D.**
ORGANIZATION:	**University of Arizona**
PROJECT TITLE:	**Calpains in Simulated Microgravity-Induced Muscle Atrophy**

Project Executive Summary

The overall goal of this proposal is to test the hypothesis that inhibition of calpain activity in skeletal muscles can reduce myofibril degradation and muscle atrophy. Muscle wasting is an important impediment to extended space travel, and studies have shown that muscle size is regulated by the balance between myofibrillar protein synthesis and degradation. Calpain is the major calcium activated protease in animal cells and plays a primary role in regulating the rate of muscle protein accumulation. Considerable evidence suggests that increasing the levels of calpastatin, a protein inhibitor of calpains, enhances muscle protein accumulation. Inhibition of calpain activity, either by increasing calpastatin levels or by expression of dominant negative forms of calpain, may therefore reduce or inhibit muscle atrophy. Research in this proposal will explore these possibilities and has the following specific aims: 1) investigate whether targeted over expression of calpastatin will reduce skeletal muscle atrophy in transgenic mice using the hindlimb unweighting model; and 2) investigate the use of dominant negative forms of calpains to inhibit calpain activity and reduce skeletal muscle protein degradation and atrophy. Studies will use either the muscle creatine kinase promoter or a fully characterized tetracycline inducible system to express calpastatin or mutated calpains in muscles of transgenic mice or in cultured L8 muscle cells. Muscles will be analyzed for changes in overall size, nucleus/cytoplasm ratio, fiber type, total protein accumulation and degradation rates, and accumulation of individual myofibrillar proteins. Information gained is expected to broaden our understanding of muscle growth and may suggest approaches for alleviating muscle atrophy in space and on Earth.

RESEARCH AREA:	**Muscle Alterations and Atrophy**
PRINCIPAL INVESTIGATOR:	**Kenneth M. Baldwin, Ph.D.**
ORGANIZATION:	**University of California, Irvine**
PROJECT TITLE:	**Role of Muscle Loading on Mechanisms of Protein Translation and the Impact on Unloading-Induced Atrophy**

Project Executive Summary

Previous studies on both humans and other species (e.g., rats) clearly show that chronic unloading of skeletal muscle, as occurs during exposure to microgravity and/or equivalent ground-based models (hindlimb/lower extremity suspension; bed rest), can cause 1) a marked degree of atrophy in those muscles used primarily for ground support and locomotion; and 2) corresponding losses in proteins comprising the contractile machinery, the chief of which involves net losses in slow type I myosin heavy chain (MHC) coupled to increases in expression of fast(er) MHCs. While the mechanisms responsible for muscle wasting and transformation in contractile phenotype are poorly understood, studies suggest that one factor involves an imbalance in protein synthesis activity relative to protein degradation capacity. Therefore, the main goals of the proposal are 1) to determine how changes in mechanical loading impact muscle derived IGF-1 gene expression coupled to its signaling pathway activation for modulating regulatory processes that control protein translation efficiency in the context of skeletal muscle growth (hypertrophy) and atrophy; and 2) to systematically develop a rodent resistance training program designed to attenuate the atrophy process and blunt slow to fast transitions in contractile phenotype. These goals will be accomplished by identifying acute loading paradigms that maintain the target muscle in a positive protein balance state, as defined by the functional status (phosphorylation state and activity) of key proteins known to impact rate-limiting steps in protein translation (initiation and elongation steps) and degradation (disassembly of myofilaments and their degradation via the ubiquitin proteasome complex) processes. While the focus of the present proposal is aimed at an animal model in order to expedite the attainment of scientific information of a mechanistic nature, we feel that outcomes of such research can be easily translated to studies involving human subjects for subsequent implementation into a viable countermeasure program, e.g., one that is predicated on fundamental scientific principles governing key subcellular processes impacting the protein balance of gravity sensitive skeletal muscle.

RESEARCH AREA:	**Muscle Alterations and Atrophy**
PRINCIPAL INVESTIGATOR:	**Alfred L. Goldberg, Ph.D.**
ORGANIZATION:	**Harvard Medical School**
PROJECT TITLE:	**The Activation of Protein Breakdown in Muscle Upon Unloading and Possible Countermeasures**

Project Executive Summary

The marked loss of muscle mass that occurs in astronauts in space due to muscle unloading and also in many systemic diseases results primarily from accelerated degradation of muscle proteins, especially myofibrillar components. We have shown that the enhancement of protein breakdown in these various types of muscle atrophy results mainly from activation of the ubiquitin-proteasome system and that one pair of ubiquitination enzymes ($E2_{14K}$ and $E3\alpha$), which comprise the "N-end rule" pathway, plays a particularly important role in atrophying muscles. Our major goals now are to clarify the basis for the activation of this ubiquitination pathway in the unloaded muscles and to develop pharmacological inhibitors of the ubiquitin-proteasome pathway that may retard muscle wasting. We shall attempt to identify more potent, selective inhibitors of $E3\alpha$ and to use genetic models to examine the consequences of blocking the "N-end rule" pathway *in vivo*. Proteasome inhibitors are promising new agents for treating human disease, and based on recent findings about proteasome function, we hope to synthesize new types of inhibitors that act allosterically to reduce proteolysis partially and thus should be safer and more appropriate for use against muscle wasting.

To obtain a more complete picture of the changes in gene expression leading to the loss of muscle mass and functional capacity, we are also undertaking a gene microarray analysis to identify systematically the transcriptional changes that occur during atrophy of rat muscles induced by hind-limb suspension or glucocorticoid treatment, as well as in human muscle biopsies obtained during prolonged bed rest. This approach should help to identify novel targets for inhibitor development and useful markers to monitor muscle wasting and efficacy of countermeasures.

To explore how atrophy may be prevented by nonpharmacological approaches, we also plan to analyze animal muscles from certain unusual physiological states, black bears during winter and rats upon dietary protein restriction, both of which suppress proteolysis and preserve muscle mass, despite reduced caloric intake and disuse.

RESEARCH AREA:	**Muscle Alterations and Atrophy**
PRINCIPAL INVESTIGATOR:	**Marc T. Hamilton, Ph.D.**
ORGANIZATION:	**University of Missouri**
PROJECT TITLE:	**Genomics of Human Skeletal Muscle During Bed Rest and Exercise**

Project Executive Summary

Reduced use of weight-bearing skeletal muscles during microgravity and sedentary life on Earth causes unhealthy and potentially dangerous consequences. For example, leg muscles atrophy, and also have a profound reduction of lipoprotein lipase activity (an enzyme in the blood vessels of muscles with a protective effect against lipoprotein risk factors for coronary heart disease). It is likely that an unbiased determination of the global expression pattern of the human genome with microarrays will reveal many muscle mRNAs increasing and decreasing, including mRNAs that heretofore have never even been hypothesized to contribute to the "microgravity or sedentary phenotype." Additionally, large scale genomic studies are likely to begin to reveal clusters of related mRNAs that provide clues as to the sets of genes orchestrating some of the cellular signaling, transcriptional changes, cellular growth, and metabolism. This project will build upon recent experience established from microarray studies of hindlimb suspension, endurance exercise, and muscle fiber type that support the statements described above. The effects of bed rest and one-leg exercise (as a countermeasure to attenuate the effects of inactivity) on the soleus muscle of 6 men and 6 women will be studied. Using state-of-the-art microarray methodologies, this project will measure the expression of ~12,000 full-length sequence verified mRNAs and ~3,000 of the most abundant muscle ESTs. This project is being proposed by a laboratory already using microarrays in the study of muscle physiology, in collaboration with a bioinformatics laboratory, a physical therapy laboratory focused on muscle function, a physician-scientist studying muscle diseases, and a core laboratory for microarray development. This study is likely to discover novel candidate genes and clusters of related genes potentially responsible for the unhealthy responses to reduced muscle use during physical inactivity.

RESEARCH AREA:	**Muscle Alterations and Atrophy**
PRINCIPAL INVESTIGATOR:	**Susan C. Kandarian, Ph.D.**
ORGANIZATION:	**Boston University**
PROJECT TITLE:	**Gene Expression Profiling of Unloaded Skeletal Muscle**

Project Executive Summary

Prolonged periods of biomechanical unloading due to space travel or physical inactivity are marked by significant decreases in the size and functional capacity of skeletal muscle. The overall aim of the proposed work is to delineate cellular mechanisms involved in unloading-induced skeletal muscle atrophy. In the face of overall muscle wasting, some subsets of genes are actually upregulated indicating the complexity of the biological processes underlying atrophy and the need for characterization at a molecular level. As a first step in elucidating these mechanisms we will analyze global gene expression patterns in rat soleus muscles using Affymetrix GeneChips at 1, 4, 7, 14 days of biomechanical unloading. Temporal analysis of the parallel expression of ~7,000 full-length genes and several thousand expressed sequence tags will serve as a window into the signaling networks that underlie the atrophy process. Microarray data analysis software will be used to segregate gene expression changes based on involvement in known functional groups, regulatory and signaling pathways. Clustering algorithms will be used to elucidate sets of genes with known or unknown functions that have similar temporal expression patterns. These types of analyses will be used to illuminate gene associations and candidate players in pathways that may be involved in the progression of muscle atrophy. Further investigation of candidate players will be performed using more quantitative and conventional techniques such as northern blot, western blot, activity assays, and immunolocalization studies. Sequence alignment algorithms such as AlignACE will be used to identify regulatory sequence conserved among genes that are co-regulated. This type of analysis will expedite attempts to determine unloading-sensitive regulatory sequences and will facilitate a better understanding of how activity patterns regulate transcriptional machinery. Protein-DNA binding assays using these conserved regulatory motifs will complement the computational analysis. By characterizing atrophy at this level we will be in a better position to design effective countermeasures to mitigate the deleterious changes in muscle function, which not only has applications for life in space but also for the quality of life on this planet.

RESEARCH AREA:	**Muscle Alterations and Atrophy**
PRINCIPAL INVESTIGATOR:	**Michael B. Reid, Ph.D.**
ORGANIZATION:	**Baylor College of Medicine**
PROJECT TITLE:	**Redox Modulation of Muscle Function in Microgravity**

Project Executive Summary

Prolonged space flight predisposes skeletal muscle to premature fatigue and is associated with loss of muscle mass. Both fatigue and atrophy of muscle are partially mediated by reactive oxygen species (ROS). Ionizing radiation stimulates ROS production in muscle during space flight and therefore is likely to exaggerate both fatigue and atrophy. This project will assess the importance of ROS signaling and radiation exposure on these two processes and will assess antioxidant supplementation as a possible countermeasure. We have four specific aims: Aim 1. To determine if oxidative stress contributes to muscle fatigue during handgrip exercise. Fatigue of hand and forearm muscles may limit task performance by crewmembers during extravehicular activities. N-acetylcysteine (NAC) is a reduced thiol donor with antioxidant properties that inhibits muscle fatigue in humans. We will address this Aim by measuring muscle fatigue and indices of oxidative stress in humans during endurance handgrip exercise performed with and without NAC. Aim 2. To determine whether ionizing radiation accelerates ROS production and fatigue in skeletal muscle. The radiation absorbed during EVA is predicted to increase tissue ROS levels and accelerate fatigue. Antioxidant supplementation should blunt these effects. We will test both postulates by measuring ROS production and fatigue characteristics of rodent limb muscles in a proton radiation field. Aim 3. To evaluate oxidative stress as a mediator of muscle atrophy caused by gravitational unloading. In other experimental conditions, atrophy is partially mediated by oxidative stress and can be ameliorated by antioxidants. We will evaluate oxidative stress as a cause of microgravity-induced atrophy by studying mice conditioned by hindlimb suspension, either with or without antioxidant supplementation. Aim 4. To determine if radiation stimulates atrophic signaling in muscle. ROS accelerate protein loss in differentiated muscle cells by activating nuclear factor-κB (NF-κB), a redox-sensitive transcription factor that influences expression of regulatory proteins in the ubiquitin/proteasome pathway. We postulate that radiation-derived ROS stimulate this pathway, accelerating protein loss. We will test this hypothesis by measuring changes in the activity of this pathway following exposure of cultured myotubes and muscles of experimental animals to proton radiation.

RESEARCH AREA:	**Muscle Alterations & Atrophy**
PRINCIPAL INVESTIGATOR:	**Shantanu Sinha, Ph.D.**
ORGANIZATION:	**University of California, Los Angeles**
PROJECT TITLE:	**In-Vivo Stress-Strain Dynamics in Human Muscle**

Project Executive Summary

Muscle atrophy is a complication of prolonged exposure to microgravity and likely involves an alteration in the strain properties of the muscle-tendon unit. Such alterations in biomechanical properties are likely to predispose muscle to strain injury as well as create errors in motor function, both in the microgravity environment and upon return to Earth-based activities. Quantifying the magnitude and distribution of the stress-strain properties of muscle during both the atrophic and recovering state is a specific aim of this project. Preliminary evidence shows that the strain distribution within muscle is highly heterogeneous but closely linked to the anatomical architecture of the muscle. Reduced levels of mechanical load likely plays a significant role in the development of muscle atrophy through alteration of the strain characteristics of muscle, (magnitude and the strain distribution). In our experimental design, a group of subjects (n=12) will undergo unilateral lower limb suspension (ULLS) for 6 weeks to induce muscle atrophy of the triceps surae muscle complex (TSMC) in one extremity. Strain magnitude and distribution within the muscle will be measured by velocity encoded cine-phase contrast MRI during an *in vivo* isometric contraction of the TSMC with maximal effort. Static muscle volumes of individual muscles of the TSMC, peak muscle velocity, and torque at that point of time will also be quantified. A custom designed force transducer apparatus will measure torque. The scanning and testing sessions for the ULLS group will be at 2 weeks and 1 day prior to suspension, last day of a 6-week suspension period and 2, 4, and 6 weeks post suspension. The muscle stress-strain dynamics will demonstrate the regions of muscle most affected by visualization and quantification of atrophy and the corresponding susceptibility to strain injury. Development of the MRI technology used in this study should be useful in future studies to test the efficacy of a wide range of exercise countermeasures by providing an objective measure of changes in stress-strain properties and recovery of these parameters following muscle atrophy.

RESEARCH AREA:	**Muscle Alterations and Atrophy**
PRINCIPAL INVESTIGATOR:	**Robert W. Wiseman, Ph.D.**
ORGANIZATION:	**Michigan State University**
PROJECT TITLE:	**Ca^{+2} Homeostatis and Muscle Phenotype: Role of Cellular Energetics**

Project Executive Summary

Exposure of skeletal muscle to space flight results in a significant loss of mass and a shift in the phenotype from slow to fast muscle isoforms. To a limited extent, astronauts are able to ameliorate this remodeling of muscle tissue through exercise. If the mechanistic link between physiologic function and phenotype were better understood, design of countermeasures using combinations of exercise protocols and pharmaceuticals could be employed to increase the efficacy of training while on space missions. We propose that altered physiologic function signals the initiation of the remodeling process through Ca^{+2} sensitive transcription factors (CSTFs) which are activated through changes in two homeostatic processes; mitochondrial ATP synthesis and sarcoplasmic reticulum (SR) ATPase Ca^{+2} handling. It is our assertion that alterations in phenotype in response to changes in load bearing or any other metabolic stress involves processing information from the physiology in the form of feedback from these two homeostatic processes. We use an integrative approach to study this problem in isolated superfused skeletal muscles using a combination of non-invasive techniques (^{31}P-NMR and fluorescence spectroscopy and mechanics) and molecular techniques. In the first Aim we determine the sensitivity of cytosolic Ca^{+2} handling to metabolic loads induced by electrical pacing and metabolic inhibitors. In the second aim we test the response of CSTFs to alterations in Ca^{+2} homeostasis using ionophores, SR ATPase inhibitors as well as the metabolic stresses we develop in Aim 1. We believe once the mechanistic link is established that we may be able to design countermeasures to mask the loss of mechanical loading by direct manipulation of cytosolic Ca^{+2} and more effectively stave off the changes occurring in limb musculature.

NSBRI RESEARCH PROGRAM
NEUROBEHAVIORAL AND PSYCHOSOCIAL FACTORS

Team Leader: **Dinges, D. F.** **Penn**

Associate Team Leader: **Wood, J.** **Baylor**

Ashton-Jones, G.	**PI**	**Penn**	**Stress, Performance and Locus Coeruleus**	**62**
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RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**Gary Ashton-Jones, Ph.D.**
ORGANIZATION:	**University of Pennsylvania School of Medicine**
PROJECT TITLE:	**Stress, Performance and Locus Coeruleus**

Project Executive Summary

Future space missions will often require astronauts to work in space for prolonged periods of time and perform highly technical tasks while persistently enduring extreme environmental, cognitive, and physiological stresses. To ensure the success of such missions, it will be necessary to understand the impact of stress on neurobehavioral function and cognitive performance so that appropriate countermeasures can be developed to sustain high levels of functioning during extended stays in space.

The ability to sustain attention is critical to the successful performance of complex tasks over extended periods of time. Recent studies by the Co-PI indicate that optimal attentional performance in non-human primates is associated with moderate basal firing rates of locus coeruleus (LC) noradrenergic (NA) neurons, and strong phasic activation of these cells in response to reward-related stimuli. In contrast, poor attentional performance is associated with unusually high or low basal LC activity, and an absence of phasic activation by reward-related stimuli. Initial studies proposed in this application would confirm these observations in the rat, and determine whether manipulations of LC activity alter behavioral performance in a sustained attention task. Subsequent experiments will determine whether the disruptive effects of stress on attentional performance are related to alterations in the tonic and phasic activity of LC neurons, and whether animals become more susceptible to the disruptive effects of stress on LC activity and behavioral performance after prolonged or repeated exposure to stressful stimuli. A final set of experiments will determine whether drugs that inhibit stress-related inputs to LC prevent the disruptive effects of stressors on LC function and behavioral performance. These studies will be directly relevant to the goals of the new Neurobehavioral and Psychosocial Factors research team established by the NRSBI, and will address several research objectives of this team, including: i) Risk Assessment, ii) Mechanisms and Process, and iii) Countermeasures. Our studies will be most relevant to the specific research questions in the NSBRI RFA regarding the effects of acute and chronic space-related stressors on the nervous system and related neurobehavioral processes, and the effects of space-related stressors on the neurobehavioral functions underlying performance capability.

RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**Joseph Brady, Ph.D.**
ORGANIZATION:	**Johns Hopkins University School of Medicine**
PROJECT TITLE:	**Psychosocial Performance Factors in Space Dwelling Groups**

Project Executive Summary

Despite uncertainties regarding the performance requirements of projected space laboratories, work stations, interplanetary vehicles and settlements beyond the Earth's atmosphere, a common feature of space flight endeavors over the next half-century will be extended stays by human groups in extraterrestrial habitats. The imperatives and opportunities associated with configuring effective psychosocial performance models in support of such space dwelling groups are best served by research approaches that are both heuristic and innovative. The development of functional human and ecological models for such space dwelling groups must, in the first instance, be based upon sound scientific principles with research objectives focused upon the management of semi-permanent as well as permanent groups involved in both operational and space science missions.

The research methodology will involve development of a distributed interactive multi-person simulation in computer generated environments as an experimental test bed for modeling psychosocial performances within and between space-dwelling and Earth-based groups. The simulation approach provides an automated means of setting the context for the analysis of performance in space-dwelling groups and monitoring electronically the effects of varying experimental conditions that alter psychosocial interactions.

Distributed interactive simulation experiments will characterize the effects of variations in the structure and function of communication channels within and between space-dwelling and Earth-based groups as well as the effects of stressful environmental and behavioral interactions upon psychosocial performance effectiveness. Simulation experiments will also determine the effects of variations in the appetitive/aversive characteristics of incentive control systems as well as the effects of selection, training, and experience within and between space-dwelling and Earth-based groups. Communication modes, frequencies, durations, and content will be recorded and analyzed with performance effectiveness evaluations based upon assigned group task outcome measures. Conceptual and methodological advances that effectively promote psychosocial and ecological stability will ultimately benefit larger societal units, including those that remain Earth-bound, by enhancing an educational and training technology that assures communication of an expanded generalizeable knowledge base.

RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**Lane J. Brunner, Ph.D.**
ORGANIZATION:	**The University of Texas at Austin**
PROJECT TITLE:	**Effect of Space Flight on the Pharmacokinetics of Psychotherapeutic Agents**

Project Executive Summary

The physiological changes that have been shown to occur during space flight may potentially alter the pharmacokinetics and pharmacodynamics of drugs administered to crewmembers. The rate and extent of drug absorption from the gastrointestinal tract following oral administration depend upon gastric emptying, gastrointestinal fluid volume, interaction with components of the gastrointestinal tract and mixing. The extent to which a drug distributes proteins, red blood cells or other blood components (dependent upon both drug and component concentration), binding to tissue proteins, and the actual perfusion of tissues by the blood. Drug elimination is dependent upon organ perfusion, primarily the kidney and liver, as well as binding to various blood components. While these pharmacokinetic characteristics of drugs have been well studied under normal conditions and the influence of disease states, age, sex, etc., investigated, the effects of space flight and zero gravity on such parameters are unknown. Since the therapeutic effect of a drug is related to its absorption, distribution and elimination, knowledge of the effects of space flight on these parameters is essential for rational use of therapeutic agents during space flight. This issue will be of particular importance during long-term space flights where the likelihood of medication use for such illnesses as depression, anxiety, and other psychogenic disorders will greatly increase.

A long-term objective of these studies is to determine the effect of space flight on drug pharmacokinetics and pharmacodynamics and the underlying physiologic processes. The present proposal will investigate the effect of space flight on gastric motility and drug absorption through the examination of the absorption profile of the anti-anxiety drug, lorazepam before, during, and after space flight to estimate alterations in gastric emptying rate. In addition, lorazepam pharmacokinetics will be examined following both oral and intravenous dosing. The absorption, distribution, metabolism, and elimination profile of the anti-depressant, venlafaxine, before, during, and after space flight will also be studied to determine the effect of zero gravity on the pharmacokinetics of this model compound. These data together will address the effect of space flight on the disposition of these therapeutic agents and how future dosing regimens of these and other agents should be determined during actual space flight to maximize effectiveness and minimize toxicity.

RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**James Carter, Ph.D.**
ORGANIZATION:	**Dartmouth College**
PROJECT TITLE:	**Designing a Smart Medical System for Psychosocial Support**

Project Executive Summary

Psychosocial problems in the Russian and American space programs have led to poor productivity, interpersonal tension and even mission termination. Both depression and interpersonal conflicts have been significant problems in isolated settings in space. Diagnosing, treating, and preventing these problems is challenging, since psychological services are unavailable on most space missions. Additionally, acknowledging psychosocial problems can stigmatize the crew and confidentiality is difficult to maintain. Recent work has demonstrated that computer-based systems can be used for self-diagnosis and treatment of psychosocial problems. Recent studies show that mental health patients respond more openly to questions posed by a computer system than by mental health professionals and that, for minor depression and anxiety, computer-based treatment can have efficacy comparable to live treatment. We propose to develop a Smart Medical System for Psychosocial Support (SMS-PS) prototype, including the systems infrastructure and basic functions of 3 modules, including self-diagnosis of psychological problems, treatment of depression, and conflict management. The SMS-PS could subsequently be expanded to include numerous additional modules for diagnosis, treatment, patient management, and prevention of any possible psychosocial problems that might arise on space missions. The prototype would apply IML's Virtual Practicum model, creating an immersive, welcoming environment in which to seek assistance for psychosocial problems. The interface of the system would be flexible, depending on crew's needs. For crewmembers seeking assistance, the Interactive Media Lab's (IML's) "Virtual Practicum" model could be applied, presenting a realistic, immersive environment, such as a "Virtual Space Station," with a warm, "human" feel crewmembers seeking rapid access to guidance or information the SMS-PS could take the form of an easily-searchable, cataloged database. Evaluation of the program will guide the expansion and complete development of the SMS-PS.

RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**David Dinges, Ph.D.**
ORGANIZATION:	**University of Pennsylvania School of Medicine**
PROJECT TITLE:	**Optical Computer Recognition of Behavioral Stress**

Project Executive Summary

Manned space flights of increasingly longer durations (including inter-planetary missions) are being planned. There is evidence from both U.S. and Russian missions that astronauts involved in long-duration space flight will be exposed to stressors that can adversely affect subjective well being, physiology, and operational performance capability. In order to identify and provide countermeasures for stressor-induced impairments in astronauts, objective, unobtrusive measures of the presence of stress reactions are needed. This project involves collaboration between established laboratories at the University of Pennsylvania with demonstrated expertise in optical computer recognition of human subjects' subtle anatomical and motoric changes in facial expressions and gestures (Prof. D. Metaxas), and neurobehavioral performance under stressful and non-stressful conditions (Prof. D. Dinges). The goal is to develop and test an optically based computer recognition algorithm of the face to reliably detect the presence of stress during performance demands. The proposal is in response to Research Announcement 99-02 from the NSBRI. It specifically addresses critical path questions in the Neurobehavioral and Psychosocial Factors research area, by seeking to identify an unobtrusive, objective technique for use during long-duration space flight to effectively detect emotional distress, neurocognitive difficulties, and neuroendocrine responses to behavioral stressors. Video input to the system will be provided from an experiment in which n = 60 healthy adult subjects (males and females of different ages and ethnic backgrounds) will be exposed initially to both a control (no stress) and a standard/validated behavioral stressor (i.e., Trier Social Stress Test) for algorithm development. The developed optical computer recognition algorithms will then be prospectively tested for their accuracy in predicting both the presence and absence of stress reactions in the same 60 subjects exposed to three different types of behavioral stressors: (1) performance in the face of a physiological deficit (i.e., sleep-inertia challenge); (2) performance of very difficult cognitive tasks (i.e., workload difficulty challenge); and (3) performance in the face of an aversive expectation (i.e., venipuncture anticipation challenge). The importance to algorithm accuracy of age, gender, and ethnic background, as well as psychological (i.e., stress ratings, mood states), behavioral (i.e., cognitive performance), and physiological (i.e., cortisol secretion, heart rate variability) responses to the behavioral stressors will be explored. Development and validation of an optically based computer recognition algorithm will provide a critically needed method for detecting the development of stress responses in astronauts, and it will form a key component in the prevention and countermeasure strategies for stress.

RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**Nick Kanas, M.D.**
ORGANIZATION:	**Northern California Institute for Research and Education**
PROJECT TITLE:	**Psychosocial Education (PSE) Training for ISS Missions**

Project Executive Summary

Previous studies suggest that changes occur in the interpersonal environments of crewmembers and mission control personnel during long-duration space missions that influence performance and well being. The objective of this proposal will be to evaluate the effectiveness of our new Psychosocial Education (PSE) training program, which is aimed at optimizing crew and ground safety, well being, and performance. It will consist of two parts: a 5-hour PSE pre-launch training session and two 30-minute PSE Post-launch training sessions.

Five ISS crews and their associated mission control personnel will receive the PSE training. Following their missions, these subjects are expected to exhibit: 1) less overall tension, 2) more overall cohesion, 3) more leader support, 4) less displacement of group tension and dysphoria to outside personnel, 5) less negative interpersonal impact from cultural effects, 6) less tendency toward 2nd half increases in tension and decreases in cohesion and leadership support, and 7) more expressiveness and personal growth relative to the subject responses from our previous Shuttle/Mir and ISS investigations.

All subjects will complete standard mood and interpersonal group climate questionnaires, a critical incident log, and a culture and language questionnaire. The culture and language questionnaire will be administered once before each mission. The other measures will be completed on a weekly basis before, during, and after each mission; will take 15-20 minutes to fill out; and will already be in use onboard the ISS. In addition, the subjects will be given a semi-structured interview within a month of their return evaluating the usefulness and effectiveness of the PSE training. The effectiveness of the PSE training also will be evaluated descriptively by comparing the interpersonal environment of these crews with the group climate reported in our previous Shuttle/Mir and current ISS studies.

RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**Stephen Kosslyn, Ph.D.**
ORGANIZATION:	**Harvard College**
PROJECT TITLE:	**Quick Assessment of Basic Cognitive Function: Blood Pressure Cuffs for the Mind**

Project Executive Summary

It is unclear what effects prolonged microgravity will have on human cognitive function. Moreover, it is unclear how such effects will interact with other variables that affect our cognitive abilities, such as the lack of sleep. The present proposal is aimed at developing a new set of tools, which are analogous to "blood pressure cuffs" for the mind. The general goal is to develop tasks that can be self-administered very quickly to obtain an objective assessment of the state of basic cognitive processes. The specific goal is to develop two kinds of tasks. The first will be computerized versions of 12 "standard" tasks from cognitive psychology, which tap a range of basic cognitive abilities. The second will be very short versions or variants of these tasks. Our aim is to design short, easy-to-administer variants that best capture the processing differences indicated by the scores on the standard tasks.

We will administer both sets of tasks to 50 subjects. After this is accomplished, we will correlate performance on the two versions, and redesign all short version tasks that correlate less than r=.80 with the standard version. A new group of 50 subjects will be tested on the redesigned short versions and corresponding standard versions. This process will be repeated until criterion is reached for all tasks. We will then perform factor analysis, multidimensional scaling, and hierarchical cluster analysis on the data from both versions separately; the purpose of these analyses is to validate that the tasks do in fact tap different underlying processes, and that the short versions tap the same underlying processing as the long versions.

RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**Philip Lieberman, Ph.D.**
ORGANIZATION:	**Brown University**
PROJECT TITLE:	**Speech Monitoring Cognitive and Personality Alterations**

Project Executive Summary

We propose to develop a system to monitor cognitive and linguistic deficits and personality alterations during long space missions using automated acoustic measures of speech. Our previous studies demonstrate that language comprehension, cognition, and decision-making can be remotely monitored by means of acoustic measures of a person's speech. Advances in neuroscience show that damage to some of the neural structures that regulate speech motor control also affects cognition, sentence comprehension, and can cause personality alterations such as apathy, irritability, and disinhibition. The system that we propose would monitor the flow of normal conversation, using speech recognition systems to segment speech sounds for analysis using auto-mated computer-implemented procedures that we will develop and verify by studying the speech and behavior of individuals in a "space-analog," and patients having neurodegenerative diseases that affect neural processes similar to those that may be compromised in space.

RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**Judith Orasanu, Ph.D.**
ORGANIZATION:	**NASA-Ames Research Center**
PROJECT TITLE:	**Distributed Team Decision Making in Exploration Missions**

Project Executive Summary

Successful space exploration missions will depend on the ability of flight crews to solve problems and make decisions under highly stressful conditions. Consequences of poor judgment can be catastrophic. In some cases the crew will not be able to rely on ground support for assistance, or communication with ground may be delayed. Crews will be culturally diverse and made up of individuals with varied skills and backgrounds. Our proposed work will (a) examine factors that affect the nature and quality of team interaction and decision making strategies under a variety of stressful conditions, and (b) assess a technology for detecting when crew interactions are degrading, so that appropriate interventions (countermeasures) can be introduced to prevent further deterioration of crew performance. Five specific questions will be addressed: (1) How do various stressors influence interaction and decision strategies of small teams? (2) How do gender and cultural background influence interaction and decision making strategies in small teams? (3) How does communication medium influence distributed team decision making and interactional processes? (4) Is physiological arousal associated with particular interactional patterns while making decisions? (5) Do patterns of physiological arousal differ across gender and culture groups engaged in decision making? Earth-based research will provide baseline data for comparison with space-based performance, and will develop methodologies for use in space. Two primary dimensions will be examined: Team structure and communication medium. Team structure will be manipulated in a variety of ways: homogenous vs. heterogeneous groups (varied by gender and ethnic/cultural background), assigned role (mission leader vs. member), and original vs. new crew member. The communication medium will be either face-to-face, audio only, or email. Stressors will be embedded in several realistic and challenging team tasks (time pressure, risk level and information accuracy/completeness). Study participants will include advanced students in technical areas (e.g. engineering or biology) similar to mission specialists, pilots and ground support (dispatchers/maintenance personnel), and astronauts. Males and females from various cultural backgrounds will be included. Dependent measures will include task performance, decision strategies, team interaction patterns, and physiological arousal levels. Findings will contribute to selection, structuring, and training of crews for exploration missions, and to development of portable, minimally invasive sensors to monitor arousal and team function levels which can serve to trigger countermeasures.

RESEARCH AREA:	**Neurobehavioral and Psychosocial Factors**
PRINCIPAL INVESTIGATOR:	**JoAnna Wood, Ph.D.**
ORGANIZATION:	**Baylor College of Medicine**
PROJECT TITLE:	**Individuals and Cultures in Social Isolation**

Project Executive Summary

The proposed research is designed to study the roles of personality, culture, and group influences on behavior, performance, and health outcomes in winter-over Antarctic research stations. These remote and isolated habitations provide an environment analogous to long duration space missions, such as those planned for the International Space Station and eventually a piloted expedition to the planet Mars. The ultimate objectives of this project are to:

1. Increase our understanding of the effects of personality, culture, and group characteristics on both individual and group performance in extreme environments.
2. Identify those elements of leadership that maximize crew functioning in extreme environments.
3. To understand how individual and group factors affect physical health under prolonged stress.

We will examine changes in weekly self-assessment of individual and group adaptation, monthly levels of several neuropeptides, and other health outcomes, as a function of individual (personality, demographic, personal history) and group characteristics (leader traits, culture mix, group tensions) and local events. This study will use Hierarchical Linear Modeling to partition variance in our dependent variables among relevant individual, group, and time factors.

NSBRI RESEARCH PROGRAM
NEUROVESTIBULAR ADAPTATION

Team Leader: **Oman, C. M.** **MIT**

Associate Team Leaders: **Cohen, B.** **Mount Sinai**
Wall, C. C. **Harvard**

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RESEARCH AREA:	**Neurovestibular Adaptation**
PRINCIPAL INVESTIGATOR:	**Jacob J. Bloomberg, Ph.D.**
ORGANIZATION:	**NASA-Johnson Space Center**
PROJECT TITLE:	**Understanding Full-Body Gaze Control During Locomotion**

Project Executive Summary

Locomotion is a complex task, demanding coordination of the eye-head, head-trunk and the lower limb locomotor apparatus. During locomotion the performer must satisfy two performance criteria: maintain stable forward translation and stabilize gaze (the direction of the eye with respect to space). Fulfilling both criteria places substantial demands on multiple sensorimotor subsystems for precise coordination. After space flight astronauts experience locomotor dysfunction because these multiple subsystems are altered. Traditionally, gaze stabilization has been studied almost exclusively as a problem of eye-head or eye-head-trunk coordination. However, coordination among the eye, head and trunk may not be the only mechanism aiding gaze stabilization during locomotion. Another important factor is the regulation of energy flow or shock wave transmission through the body at high impact phases with the support surface like that which occurs at heel-strike. Allowing these excessive transmissions of energy to the head may compromise gaze stability, leading to oscillopsia and decreased dynamic visual acuity. Specific coordinated actions at the lower limb may contribute to attenuation of the shock wave to the head. Thus, stabilized gaze during natural body movement results from full-body coordination of the eye-head and head-trunk systems combined with the lower limb apparatus. From this point of view, the whole body is an *integrated* gaze stabilization system, with several subsystems contributing to gaze stabilization during body motion. For this investigation, we propose to systematically alter each gaze stabilization subsystem (eye-head, head-trunk and lower limb apparatus) to determine how they interact to preserve visual acuity during locomotion. In this manner, we can observe the emergent full-body coordination patterns resulting in appropriate gaze stabilization during locomotion.

Therefore, the goal of this study is to determine how the multiple, interdependent full-body sensorimotor subsystems aiding gaze stabilization during locomotion are functionally coordinated. Subjects will perform a gaze stabilization task during treadmill locomotion under the following conditions: 1) before and after exposure to minifying and magnifying lenses to adaptively modify vestibulo-ocular reflex gain; 2) while wearing a neck brace that will induce a head-trunk "strapped down" strategy, and 3) while wearing a knee brace that will result in an increase in the shock-wave transmitted to the head during the high impact phases of the gait cycle.

This information will enable a better understanding of the different aspects of full-body coordination which function to preserve gaze stabilization during locomotion. This information will lead to improved tests of post flight locomotion dysfunction that will enable the effective evaluation of the efficacy of sensorimotor countermeasures used to mitigate the deleterious effects of space flight on locomotor control.

RESEARCH AREA:	**Neurovestibular Adaptation**
PRINCIPAL INVESTIGATOR:	**John L. Dornhoffer, M.D.**
ORGANIZATION:	**University of Arkansas for Medical Sciences**
PROJECT TITLE:	**Pharmacological Countermeasures for Space Motion Sickness**

Project Executive Summary

To establish effectiveness and quantify side effects for potential drug countermeasures for Space Motion Sickness (SMS), we need to standardize measures of oculomotor function, postural stability, and cognitive performance. We plan to rely on our extensive clinical experience in treating vertigo as a correlate of SMS to examine drug countermeasures that leave cognitive ability intact, using the rotary chair and an extensive operant test battery to standardize our findings and a unique unilateral otolith testing system to correlate our results with the unloading of otoliths that occurs at 0 G. SMS is generally believed to be due to a neural mismatch caused by unweighting of the otolithic organs whereas vertigo is due to under- or over-stimulation of the semicircular canals. We hypothesize that the vestibular dysfunction due to over-stimulation of the semicircular canals by the rotary chair can serve as a paradigm for SMS, thus enabling us to effectively test drug countermeasures. This study addresses one of the main exploration-mission risk areas set forth by NASA in the Critical Path Roadmap and represents a countermeasure readiness level of 6. Specific Aim 1 will determine the effects of drug countermeasures currently used to treat the vertiginous patient (lorazepam, meclizine, promethazine, scopolamine), in alleviating motion sickness induced by vestibular stimulation with a rotary chair. Specific Aim 2 will determine the effects of these countermeasures on cognitive performance using an Operant Test Battery to assess time perception, short-term memory, and learning, and measurement of the P50 potential to assess any deficits in sensory gating. Specific Aim 3 will use 3-D oculography and unilateral otolith testing to determine the extent of correlation between vestibular dysfunction induced by the rotary chair and unloading of otolithic organs due to 0 G. Our proposed study is crucial for addressing one of the key impediments to human space flight, namely, the need to retain cognitive function while treating SMS. Our results should validate appropriate drug countermeasures while standardizing measures of SMS and cognition, providing countermeasures that allow astronauts to avoid SMS while allowing them to meet the cognitive and physical requirements of space flight.

RESEARCH AREA:	**Neurovestibular Adaptation**
PRINCIPAL INVESTIGATOR:	**Charles M. Oman, Ph.D.**
ORGANIZATION:	**Massachusetts Institute of Technology**
PROJECT TITLE:	**Visual Orientation and Spatial Memory: Mechanisms and Countermeasures**

Project Executive Summary

We propose a 3-year extension of a multi-institutional, multi-investigator NSBRI neurovestibular research project designed to develop countermeasures for orientation, navigation, and spatial memory problems described by Skylab, Shuttle, MIR and ISS crewmembers. Our goal is to develop strong scientific hypotheses, and perform critical experiments leading to a program of pre-flight visual orientation training of astronauts, as well as in-flight and post-flight countermeasures, including human factors standards. Our specific aims are to study:

Human visual orientation. To better understand static and dynamic visual orientation illusions in 0-G by quantifying them in 1-G. To determine how visual frame, polarity, motion and gravireceptor cues influence the direction of the subjective vertical, the response of the oculomotor and motor control systems, stability of the visual world (oscillopsia), and how viewing one's own body, environmental brightness and color cues determine the subjective vertical. (I. Howard, et al, York University)

Three-dimensional spatial memory and learning. To understand why astronauts have difficulty making spatial judgements between modules with different visual verticals, by quantifying how humans use visual cues in 1-G to establish "spatial frameworks" within and between adjacent visual environments. To develop a computerized technique for teaching generic 3-D spatial orientation and memory skills. To investigate and evaluate ISS allocentric coordinate marking systems, and to develop a "virtual porthole" display so trainees can learn to visualize the spatial relationships of ISS modules and potential escape routes in three dimensions. (C. Oman, et al, MIT/W. Shebilske, et al, Wright State)

Neural coding of spatial orientation in an animal model. To define how the preferred direction of limbic system head direction cell depends on visual, vestibular, gravireceptive, proprioceptive and motoric cues in a rat animal model during three-dimensional locomotion. To understand how the vestibular system contributes to these head direction cell responses. Ultimately, to develop a neurophysiological understanding of visual reorientation illusions and spatial cognition in astronauts. (J. Taube, et al, Dartmouth)

Our research also pertains to human health on Earth, particularly to the treatment of aging, vestibular, and Alzheimer's patients.

RESEARCH AREA:	**Neurovestibular Adaptation**
PRINCIPAL INVESTIGATOR:	**Millard F. Reschke, Ph.D.**
ORGANIZATION:	**NASA-Johnson Space Center**
PROJECT TITLE:	**Modification of Eccentric Gaze-Holding**

Project Executive Summary

Clear vision is a prerequisite for reliable performance of motor tasks. Space flight confronts the crewmember with a stimulus rearrangement that requires adaptation to function effectively with the new requirements of altered spatial orientation and motor coordination. Adaptation and motor learning driven by the effects of cerebellar disorders may share some of the same demands that face our astronauts. One measure of spatial localization shared by the astronauts and those suffering from cerebellar disorders that is easily quantified, and for which a neurobiological substrate has been identified, is the control of the angle of gaze (the "line of sight"). The disturbances of gaze control that have been documented to occur in astronauts, both in-flight and post-flight, can be directly related to changes in the extrinsic gravitational environment and intrinsic proprioceptive mechanisms thus, lending themselves to description by mathematical models. The basic models can be formulated using normal, non-astronaut test subjects and subsequently extended using centrifugation techniques to alter the gravitational and proprioceptive environment of these subjects. Further tests and extensions of the models can be made by studying abnormalities of gaze control in patients with cerebellar disease. Finally, tests of astronaut subjects during and after exposure to space flight, in association with the corresponding sensory-motor adaptations, will allow us to evaluate and extend our developed understanding of adaptation in the control of eccentric gaze-holding. The specific aims of this study are: (1) To investigate the mechanisms of gaze-holding in normal, non-astronaut subjects, with the head held in various orientations with respect to gravity and the head held in various orientations relative to both gravity and the trunk. This will involve characterizing the *time constant of centripetal gaze drift*, the rate in which the eyes naturally drift back toward the null position following an eccentric eye movement. (2) To investigate the mechanisms that adaptively compensate for gaze-holding failure, especially the "rebound nystagmus" phenomenon, which decreases the rate of centripetal drift of the eyes. We will study the time course of rebound nystagmus in normal, non-astronaut subjects. (3) To investigate the stimulus rearrangement and adaptation resulting from exposure to gravitoinertial environments *greater* than 1 G using prolonged exposure to centrifugation. (4) To study mechanisms that adaptively compensate for gaze-holding failure in patients with vestibular cerebellar disease who show impaired gaze-holding ability. We will compare gaze-holding defects and rebound nystagmus in patients with that obtained in our normal subjects. (5) To compare the gaze-holding abilities of astronaut subjects prior to, during, and immediately following space flight with specific predictions made as a consequence of the ground-based research. Tests similar to those performed upon normal, non-astronaut subjects will be conducted to quantify changes in the time constant of centripetal drift of the eyes in relation to changes in the gaze-holding induced as a result of the stimulus rearrangement of space flight. (6) To measure the stability of gaze, during all phases of flight, with the eye at the central position in astronauts to investigate the occurrence of saccadic intrusions known as "square wave jerks" (SWJ), and to relate SWJ mechanisms common to the failure of gaze-holding.

RESEARCH AREA:	**Neurovestibular Adaptation**
PRINCIPAL INVESTIGATOR:	**Mark J. Shelhamer, Sc.D.**
ORGANIZATION:	**Johns Hopkins University School of Medicine**
PROJECT TITLE:	**Context-Specificity and Other Approaches to Neurovestibular Adaptation**

Project Executive Summary

The major goal of this project is to develop and assess countermeasures to some of the debilitating effects of space flight on the neurovestibular system. Such effects include motion sickness, perceptual illusions, gaze instability, and postural and locomotor disturbances upon return to planetary gravity. Some of these effects can be long lasting, and many are of operational concern as NASA prepares for long-term human presence in space, including anticipated missions to Mars.

Our approach to this problem centers on context-specific adaptation (CSA), by which we mean the ability of an organism to maintain, simultaneously, two different sensorimotor calibrations, each appropriate for a particular context (such as gravity level), and to switch between these two calibrations immediately upon transitions between contexts (rather than undergoing de-adaptation and re-adaptation each time). An obvious application of this strategy is during the g-level transitions inherent in space flight.

We will investigate several aspects of neurovestibular adaptation, with a set of eight specific aims distributed over five primary investigators at three different institutions (Johns Hopkins, Washington University, University of Mississippi). Our experiments include the study of: a) adaptation of saccades and of torsional alignment to counteract dysmetria due to altered torsion in 0g [aims 1, 3]; b) consolidation and cue augmentation as means of enhancing the acquisition of CSA [aims 2 and 8b]; c) adaptation of the translational linear VOR (LVOR) and smooth pursuit, their interaction, and the role of the cerebellum, in humans and monkeys [aims 4, 5, 6, 9]; d) transfer of adaptation between eye and limb movements [aim 7]; e) characteristics and effective strategies for adaptation of the LVOR [aim 8].

RESEARCH AREA:	**Neurovestibular Adaptation**
PRINCIPAL INVESTIGATOR:	**Conrad Wall III, Ph.D.**
ORGANIZATION:	**Harvard – Massachusetts Eye and Ear Infirmary**
PROJECT TITLE:	**Advanced Techniques to Assess and Counter Gait Ataxia**

Project Executive Summary

The overall goals of this project are to develop "countermeasure assessment criteria" to evaluate recovery from perturbations, turning, circular walking and ascending and descending stairs. We will also consider countermeasures using a balance prosthesis and dynamic exercises designed to challenge and increase subjects' balance. We will determine the sensitivity of the countermeasure assessment criteria in evaluating effects the prosthesis and the exercises on postural stability and locomotion. Using human subjects, the specific aims of this proposal are to: 1. Study body and head movements during precise perturbations of gait during continuous straight locomotion. 2. Study body, head and eye movements during continuous straight or circular locomotion on a circular treadmill. 3. Study body, head and eye movements during ascending and descending a staircase. 4. Study body, head and eye movements during standing, linear walking and treadmill walking with a balance prosthesis designed as a countermeasure for vestibular adaptation. 5. Study the effect of dynamic balance exercises for vestibulopathic subjects upon their ability to stand quietly and to recover from mild perturbations. It is important to note that these proposed studies are based upon research questions raised in the National Space Biomedical Research Institute Research Announcement 00-01. We have two countermeasures with proof-of-concept data, which we propose to develop into Countermeasure Readiness Level 6 to demonstrate efficacy for improving balance problems demonstrated by returning astronauts, with future application for use in microgravity. The two countermeasures (aims 4 and 5) are "leveraged" from already funded projects. In keeping with the Neurovestibular Adaptation Team strategy of forming synergistic links between its projects, some of the aims of this project will be coordinated with the aims of two other proposed projects.

RESEARCH AREA:	**Neurovestibular Adaptation**
PRINCIPAL INVESTIGATOR:	**Laurence R. Young, Sc.D.**
ORGANIZATION:	**Massachusetts Institute of Technology**
PROJECT TITLE:	**Neuro-Vestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation**

Project Executive Summary

Artificial gravity (AG) is a candidate general countermeasure against the adverse physiological effects of extended weightlessness. Intermittent short-radius centrifugation (SRC) is particularly attractive for practical reasons. However, it brings with it numerous neurovestibular side effects (e. g. nausea, illusory body tilt sensations) that must be better understood and eventually overcome. Unfortunately, the rotation rate required for SRC is much higher than the 6 - 10 rpm previously thought to limit adaptation to Coriolis cross-coupled accelerations. Existing research on neurovestibular side effects focused on slow rotation rates. For the first time, we have established that human subjects can adapt to head movements in the rotating environment of a horizontal SRC rotating at 23 rpm, which produces a 1-g centripetal acceleration at the feet of a 168 cm subject. Inappropriate eye movements, motion sickness and illusory body tilt are all reduced after several adaptation periods of head movements in the light. Numerous questions must be answered before we can proceed with SRC as a countermeasure. The questions addressed in the proposal fall into two broad categories: basic and applied, each comprising several specific aims. The *basic* questions address the issue of how an adaptive state is best learned and recognized: What triggers the change to the correct adaptive state once astronauts are adapted to several gravito-inertial environments? What role do vestibular and other sensory signals play? How do head movements differ from limb movements in this regard? What is the *context* for context-specific adaptation? The *applied* aims relate to *optimizing the conditions for adaptation.* Is visual information needed at all for vestibular adaptation? What are the optimal duty cycles and inter-session intervals? Is incremental adaptation a viable solution? Can astronauts be pre-adapted to unusual gravito-inertial environments? What is the minimal radius that allows full adaptation while maintaining at least 1g at the feet? Do proven anti-motion-sickness drugs interfere with adaptation?

Practical countermeasure development for SRC and larger AG devices requires exploration over a large range of radii, subject orientations, g-forces and exposure durations for animals as well as humans. Existing centrifuge facilities at five institutions will be used for this purpose in an integrated research program.

NSBRI RESEARCH PROGRAM
NUTRITION, PHYSICAL FITNESS AND REHABILITATION

Team Leader: Lupton, J. R. Texas A&M

Lupton, J. R.	**PI**	**Texas A&M**	**Nutritional Countermeasures to Radiation Exposure**	**83**
Turner, N. D.	CO-I	Texas A&M		
Chapkin, R. S.	CO-I	Texas A&M		
Mallick, B. K.	CO-I	Texas A&M		
Braby, L. A.	CO-I	Texas A&M		
Ford, J. R.	CO-I	Texas A&M		
Schneider, S. M.	**PI**	**NASA JSC**	**Treadmill Exercise as a Countermeasure for Microgravity Deconditioning (Flight Study)**	**84**
Cavanagh, P.	CO-I	Penn State		
Smith, S.	CO-I	NASA JSC		
Greenisen, M.	CO-I	NASA JSC		
Tobin, B. W.	**PI**	**Mercer**	**Nutritional Modulation of Pancreatic Endocrine Function in Microgravity**	**85**
Leeper-Woodford, S.	CO-I	Mercer		
Uchakin, P. N.	CO-I	Mercer		
Rothenberg, M. R.	CO-I	Mercer Southern		
Smith, S. M.	CO-I	NASA-JSC		
Lakey, J. R. T.	Co-I	U of Alberta		
Wolfe, R. R.	**PI**	**UTMB**	**Skeletal Muscle Response to Bed Rest and Cortisol-Induced Stress**	**86**
Ferrando, A. A.	CO-I	UTMB		
Urban, R. J.	CO-I	UTMB		

RESEARCH AREA:	**Nutrition, Physical Fitness & Rehabilitation**
PRINCIPAL INVESTIGATOR:	**Joanne Lupton, Ph.D.**
ORGANIZATION:	**Texas A&M University**
PROJECT TITLE:	**Nutritional Countermeasures to Radiation Exposure**

Project Executive Summary

Long-term space flight necessarily involves astronaut exposure to cosmic radiation. It is improbable that shielding will protect them from potentially cancer-causing radiation. Therefore, countermeasure development is important. Diet offers a route through which the potential for DNA damage may be reduced. We have shown that dietary n-3 fatty acids (in fish oil) reduce DNA damage and increase targeted apoptosis. In addition, we show a synergistic protective effect of fish oil and pectin (a highly fermentable fiber) on both tumor incidence and enhancement of spontaneous apoptosis. The overall goal of this project is to design diets that protect against radiation-induced carcinogenesis and reduce subsequent carcinogen-induced carcinogenesis. Our hypotheses are that: 1) intervention diets will decrease tumorigenesis and/or intermediate markers of tumorigenesis when an individual is exposed to radiation and chemical carcinogens, and that the early effects are predictive of tumor incidence; and 2) mRNA from fecal material will provide a noninvasive means for detection of radiation exposure and response to that exposure. To test these hypotheses we have three specific aims: 1) determine *in vivo* if defined diets protect against the development of colon cancer with or without radiation exposure by reducing DNA damage, preneoplastic lesion development, tumor development, and establish the relationship between diet effects on intermediate markers and eventual tumor incidence; and 2) if mRNA isolated from fecal material of rats exposed to radiation and a chemical carcinogen are predictive for DNA damage by oxidation and/or methylation, response to that damage, and/or tumor development. Collectively, the experiments will determine if an intervention diet reduces the potential danger to astronauts of radiation- and chemically-induced cancer of the colon. At the end of these experiments we will know if diet can ameliorate radiation- and carcinogen-induced colon cancer, if short term studies are predictive of subsequent tumor development, and if a noninvasive technique can be used to predict for cancer-causing radiation exposure. The consequences of this research will be improved safety for astronauts, possible mechanisms of in-flight detection of damage from radiation and chemicals that may lead to cancer, and justification for shorter-term studies to reduce the time for discovery of other means of protection against cosmic radiation.

RESEARCH AREA:	**Nutrition, Physical Fitness & Rehabilitation**
PRINCIPAL INVESTIGATOR:	**Suzanne Schneider, Ph.D.**
ORGANIZATION:	**NASA-Johnson Space Center**
PROJECT TITLE:	**Treadmill Exercise as a Countermeasure for Microgravity Deconditioning**

Project Executive Summary

Treadmill exercise is the primary countermeasure during space flight to maintain aerobic, cardiovascular fitness. It also may provide some protection against the atrophy in bone and muscle. Yet little is known about the effect of microgravity and the bungee-harness system that must be used to provide footward loading on the physiological responses to treadmill exercise. The purpose of this proposal is two-fold: 1) to obtain cardiovascular (heart rate), metabolic (oxygen consumption), and biomechanical data (peak forces, foot pressure, gait analyses) during treadmill exercise at several treadmill speeds in microgravity, and 2) to evaluate the effectiveness of two treadmill countermeasures to maintain aerobic capacity, leg muscle strength, and to prevent increases in bone resorption and muscle atrophy markers. We hypothesize that daily, moderate treadmill exercise for 30 minutes each day will maintain aerobic capacity, leg strength, and reduce the increase in bone and muscle markers. Ten Shuttle crewmembers will exercise either daily (n=5) as a simulation of the level of aerobic exercise currently planned for ISS or every third day (n=5) as a simulation of the minimum level of aerobic exercise currently required for crewmembers during Shuttle missions > 10 days. The effectiveness of these countermeasures to maintain post-flight aerobic fitness will be assessed using a graded treadmill test; to maintain leg strength and muscle performance will be assessed using isotonic and isokinetic tests, respectively; and, to prevent degradation of muscle and bone will be assessed by measuring changes in catabolic markers for muscle (3-methyl-histidine) and bone (collagen cross-links) from 24 hr urine pools. Simultaneous video analysis with heart rate and metabolic responses in 1-G and 0-G environments will allow us to understand the effect of the bungee-harness system and microgravity to alter the cardiovascular and metabolic responses to the treadmill. The information from these tests are required to determine the most appropriate treadmill prescriptions during long duration stays on ISS and during exploratory missions.

RESEARCH AREA:	**Nutrition, Physical Fitness & Rehabilitation**
PRINCIPAL INVESTIGATOR:	**Brian Tobin, Ph.D.**
ORGANIZATION:	**Mercer University School of Medicine**
PROJECT TITLE:	**Nutritional Modulation of Pancreatic Endocrine Function in Microgravity**

Project Executive Summary

Ground-based and in-flight investigations illustrate changes in insulin, glucose, and amino acid metabolism in space flight. These observations may relate to altered pancreatic endocrine function, which is insufficient to meet the needs of microgravity-induced insulin resistance, and altered amino acid metabolism. The changes observed include decreased glucose tolerance, increased circulating insulin, and increased reliance upon glucose in muscles. The metabolic milieu resembles an insulin resistant syndrome, accompanied by a compensatory increase in pancreatic insulin secretion. However, the increase in insulin secretion is insufficient to ameliorate muscle atrophy. The increased insulin secretion is well correlated to muscle atrophy in space flight. The influence of these changes upon the loss of muscle mass and general endocrine metabolic state are not well established, however. Countermeasures which could modulate insulin and glucagon secretion in a compensatory manner to overcome insulin resistance and promote amino acid uptake by peripheral musculature might decrease muscle atrophy and reduce injury following re-adaptation to unit gravity.

We hypothesize that human pancreatic islets of Langerhans have an increased requirement for amino acids in microgravity. We further hypothesize, that supplementation with specific additional amino acids will augment, enhance and normalize insulin secretion, when space flight paradigm stressors known to decrease insulin secretion, are applied. Our specific aims in this study are to: 1) assess the effect of a microgravity model cell culture on basal amino acid requirements and endocrine secretory function in human islets of Langerhans, and 2) determine human islet endocrine function while testing amino acid countermeasures in the microgravity model.

It is anticipated that these studies will further refine our understanding of human pancreatic amino acid requirements and endocrine regulation: phenomenon which may be limiting to extended-duration space flight missions. These studies will test countermeasures to augment pancreatic endocrine function, while considering both insulin and glucagon production in a way that will involve supplementation of diet with additional amino acids. These measures are ultimately aimed at improving space flight induced muscle atrophy, and ameliorating current re-adaptation constraints.

RESEARCH AREA:	**Nutrition, Physical Fitness & Rehabilitation**
PRINCIPAL INVESTIGATOR:	**Robert Wolfe, Ph.D.**
ORGANIZATION:	**The University of Texas Medical Branch - Galveston**
PROJECT TITLE:	**Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress**

Project Executive Summary

This project is part of a coordinated effort between our institution (UTMB) and the University of Arkansas Medical School, Little Rock, to investigate mechanisms responsible for muscle loss with prolonged inactivity (i.e., space flight), and to develop appropriate countermeasures. The primary focus of this proposal will be to develop nutritional countermeasures to ameliorate muscle wasting.

Prolonged space flight causes a loss of muscle mass that is detrimental to physical function, and amelioration of this response is essential for successful prolonged missions. Based on our earlier work, we anticipate a mixture of essential amino acids (15g) and carbohydrates (30g) given as a supplement twice per day will limit the loss of muscle, and in turn muscle function, during our model of space flight, which is prolonged bed rest + hypercortisolemia. In addition to making measures of muscle mass and function, we will quantify muscle amino acid and protein kinetics at the beginning and the end of bed rest in order to gain insight into the mechanisms responsible for the loss of muscle mass in untreated subjects, as well as into the mechanisms by which supplementation serves to decrease muscle catabolism. Specifically, we will determine muscle amino acid and protein kinetics over 24-hour periods before and at the end of bed rest in order to address the following hypotheses:

1. The normal anabolic response of muscle to a meal diminishes with prolonged inactivity and stress.
2. An amino acid/carbohydrate supplement will stimulate net muscle protein synthesis over the one-hour immediately following ingestion.
3. The normal anabolic effect of meals will not be affected by prior ingestion of a supplement.
4. The post-absorptive nadir in net muscle protein synthesis will be no greater in subjects receiving supplementation than in control subjects.

Studies to quantify rates of muscle protein synthesis, breakdown and transmembrane amino acid transport will utilize femoral arterial and venous catheters, muscle biopsies, and stable isotope tracer methodology. Studies will be performed in both women and men, so that, when combined with the results from the University of Arkansas, we will be able to assess the affect of gender on the response. We anticipate that the amino acid/carbohydrate supplement will be equally effective in both men and women.

NSBRI RESEARCH PROGRAM
RADIATION EFFECTS

Team Leader: **Dicello, J. F.** **Hopkins/SOM**

Associate Team Leaders: **Kennedy, A. R.** **Penn**
Vazquez, M. E. **Brookhaven**

Chang, P. Y.	**PI**	**SRI Int'l**	**Charged Particle Radiation-Induced Genetic Damage in Transgenic Mice**	**88**
Dicello, J. F.	**PI**	**Hopkins/SOM**	**Radiation Effects Core Project: In Vivo and In Vitro Studies**	**89**
Huso, D. L.	CO-I	Hopkins/SOM		
Williams, J. R.	CO-I	Hopkins/SOM		
Huso, D. L.	**PI**	**Hopkins/SOM**	**Chemoprevention and Radiation-Induced Neoplasms**	**90**
Dicello, J. F.	CO-I	Hopkins/SOM		
Kennedy, A. R.	**PI**	**Penn**	**Countermeasures for Space Radiation Biological Effects**	**91**
Biaglow, J. E.	CO-I	Penn		
Dicello, J. F.	CO-I	Hopkins/SOM		
Wan, X. S.	CO-I	Penn		
Vazquez, M. E.	**PI**	**Brookhaven**	**Risk Assessment and Chemoprevention of HZE-Induced CNS Damage**	**92**
Pena, L. A.	CO-I	Brookhaven		
Anderson, C. W.	CO-I	Brookhaven		

RESEARCH AREA:	**Radiation Effects**
PRINCIPAL INVESTIGATOR:	**Polly Chang, Ph.D.**
ORGANIZATION:	**SRI International**
PROJECT TITLE:	**Charged Particle Radiation-Induced Genetic Damage in Transgenic Mice**

Project Executive Summary

The outer space atmosphere comprises particle radiation in a wide spectrum of energies and charges, and radiation exposure is a serious potential health hazard to humans in long-term, manned space exploration. Evaluation of risks involving alterations in the genome using whole animal systems are therefore essential to missions in space. The *lacZ* transgenic mouse model is the only system available, to date, for the assessment of alterations in the genome in every tissue of the animal. In this model system, every cell of the animal contains multiple copies of an integrated but inert target transgene. Radiation-induced mutations can be measured and specific genetic alterations characterized using established protocols. Genetic alterations in tissues that are of high priority in NASA's Strategic Program Plans but are not accessible using conventional techniques, e.g., the central nervous system, can be evaluated using this model system. In addition, early (1-2 days) and late (up to 16 weeks) radiation responses *in vivo* can be examined in parallel by enumerating micronuclei in peripheral blood, evaluating chromosomal damage by using fluorescence *in situ* hybridization techniques, assessing *hprt* mutations in splenic T-lymphocytes and induced gene expressions by using RT-PCR.

Variation in genetic background has been shown to impact an individual's radiation sensitivities. The tumor suppressor *p53* gene function has been shown to be radiation responsive and very important in the regulation of cell growth, proliferation, differentiation, and apoptotic signaling pathways in many tissues. *LacZ* transgenic mice with different *p53* genotypes will be used to assess tissue-specific *p53*-dependent (or -independent) molecular and genetic mechanisms in radiation-induced damage resulting from exposure to particle beams in the energy range corresponding to space radiation.

The cytokine IL-1 is known to be a biomolecule that is radiation responsive and has been shown to be an effective countermeasure that protects animals from low LET radiation toxicity. We will test the hypothesis that IL-1 protects against radiation lethality by enhancing repair and reducing adverse long-term consequences such as mutagenesis *in vivo* and therefore, can be considered as a biological countermeasure for particle radiation. The countermeasure readiness of this concept is at basic research level 2.

RESEARCH AREA:	**Radiation Effects**
PRINCIPAL INVESTIGATOR:	**John Dicello, Ph.D.**
ORGANIZATION:	**Johns Hopkins University School of Medicine**
PROJECT TITLE:	**Radiation Effects Core Project: In Vivo and In Vitro Studies**

Project Executive Summary

This is a continuation of the "Core Project" of the NSBRI Radiation Effects Team begun two and a half years ago. Our main objective has been to determine the risks of human diseases arising from exposures to galactic and solar radiations during interplanetary missions and to test the hypothesis that pharmaceuticals could be used to reduce the risk of relevant diseases, particularly carcinogenesis. There has been a close relation between this project and notably the Chemoprevention and Cytogenetic, but also the Technology and Immunology Teams, the Johnson Space Center, and several other universities. We have designed and constructed experimental systems and developed procedures and methods for transporting large numbers of animals and related cells safely to and from the heavy-ion accelerator at the Brookhaven National Laboratory, the proton facility of the Loma Linda University Medical Center (LLUMC), and Johns Hopkins University (JHU). We are accumulating medical and biological data for a group of more than 3000 rats and innumerable cells exposed to 1-GeV iron ions, 250-MeV protons, or cobalt-60 or cesium-137 gamma rays. At the recommendation of the External Review Council of the National Space Biomedical Research Institute, the research will examine a mouse model for a tumor site having minimal hormone stimulation, and will use that same model to evaluate the possibility of damage to the central nervous system. We have chosen the Min (multiple intestinal neoplasia) mouse and intestinal tumors as this major endpoint. Colon cancer was chosen because it is one of the major tissues contributing to the effective doses to be received by astronauts in space. Moreover, recent advances with this model at the University of Pennsylvania have resulted in relatively non-specific, non-toxic pharmaceuticals, such as Bowman-Birk Inhibitor, that reduce the risk of multiple types of late cancers. Further, these drugs work primarily by reducing oxidative stresses, suppressing the initiation stage of carcinogenesis while retaining the desirable characteristic of working as well in the promotion and progression stages. Such drugs have the potential for reducing the risks for cancer in astronauts because they appear to be relatively nontoxic, broadly effective in a variety of tumors and tumor types, and can be used effectively as diet supplements after the exposures. The hypothesis to be examined to this end is that there exist non-specific, non-toxic drugs that would effectively reduce the risks of multiple types of cancers or CNS damage.

RESEARCH AREA:	**Radiation Effects**
PRINCIPAL INVESTIGATOR:	**David Huso, Ph.D.**
ORGANIZATION:	**Johns Hopkins University School of Medicine**
PROJECT TITLE:	**Chemoprevention and Radiation-Induced Neoplasms**

Project Executive Summary

The major long-term risk associated with the types of low-dose radiation exposure that could possibly be encountered during prolonged space missions is radiation-induced cancers and they probably wouldn't appear until later in life. Proton and heavy ions as well as their secondaries are the major types of radiation likely to occur in the space environment. Chemopreventatives offer potential pharmaceutical countermeasures to reduce the increased cancer risks; however, no studies have tested chemopreventatives in studies relevant to proton or heavy ion-induced cancer. Surging scientific and public interest in applying chemoprevention strategies to people in the general population that have been identified to carry even slight increases in the genetic risk of developing cancer is fueling the identification of exciting new chemopreventive agents. Appropriate animal models provide a powerful tool for examining the effects of proton and heavy ion radiation at doses relevant to space as well as for directly evaluating the effectiveness of particularly promising chemopreventatives against cancers that occur following such exposures.

During the middle of the previous funding period, Dr. Huso, with NSBRI approval, assumed responsibility as the principal investigator for chemoprevention studies within the radiation effects team. By working closely with Dr. Dicello, the PIs were able to start the type of large-scale rodent studies necessary to begin to answer important questions surrounding the carcinogenic effects of proton and heavy ion radiation. In addition, the focus was on a radiation-induced breast cancer model to test tamoxifen as a potential chemopreventative for breast cancer induced by heavy ions and protons. After these studies had begun, the first large-scale trial demonstrating that tamoxifen was effective in chemoprevention of human breast cancer was reported. Prior to this study, tamoxifen had long been used in chemotherapy to treat breast cancer, but its effectiveness for chemoprevention had not been demonstrated. This has provided an additional strong rationale for continuing and completing the lifetime studies on tamoxifen chemoprevention of mammary tumors induced by protons and heavy ions that started during the previous project.

In this renewal, the synergistic collaboration between Dr. Dicello and Dr. Huso that resulted in excellent progress on the radiation effects studies and chemoprevention studies will be continued. There is now a large group of tamoxifen-treated and untreated female rats that have already received whole body radiation with iron ions and protons and are the focus of two of the specific aims of this renewal proposal. In addition, we plan to begin new studies that focus on chemoprevention of proton and heavy ion-induced colorectal cancer using a mouse model. The specific aims are: 1) to establish a model for proton and iron ion-induced colorectal cancer in a whole body radiation mouse model for both sexes, 2) to determine the effectiveness of sulindac in preventing proton and iron ion-induced GI tumors in the model 3) to determine the long-term effects of proton, photon, and heavy ion radiation by completing our radiation effects studies using our rat mammary gland model 4) to determine the long-term effectiveness of tamoxifen in rats exposed to iron ions and protons as young adults and then receiving tamoxifen chemoprevention for life.

RESEARCH AREA:	**Radiation Effects**
PRINCIPAL INVESTIGATOR:	**Ann Kennedy, Ph.D.**
ORGANIZATION:	**University of Pennsylvania School of Medicine**
PROJECT TITLE:	**Countermeasures for Space Radiation Biological Effects**

Project Executive Summary

The hypothesis to be tested in this research program is that control of radiation induced oxidative stress will reduce the risk of cancer development. The overall objective of the proposed investigations for the initial three-year grant period is to determine the types of dietary supplement agents or agent combinations that will be the most effective at reducing the level of oxidative stress and the cancer risks associated with exposure to ionizing radiation in space. The efficacy of the dietary supplement agents will first be evaluated in cultured human breast epithelial cells, in which the effects of the dietary supplement agents on the baseline levels of oxidative stress and radiation induced oxidative stress will be determined. The agents or agent combinations that are effective in reducing oxidative stress in the *in vitro* assays will then be evaluated in irradiated Min mice to assess the efficacy of these agents or agent combinations in reducing radiation induced oxidative stress in animals. The levels of oxidative stress will also be measured in Min mice that will be irradiated with a dose of radiation from 1-GeV iron ions or protons; colon tumor formation in these animals will be evaluated as part of the grant proposal submitted by Dr. John Dicello. If both of these grant proposals are funded, the levels of oxidative stress measured in these irradiated mice will be compared with the colon cancer rates in these animals to determine whether the oxidative stress levels predict the cancer incidence rates. In addition, the effect of the dietary supplement agents on the levels of oxidative stress will be compared to the effect of these agents on colon cancer development to determine whether these two effects are correlated. Assuming that there is a reduced cancer rate in the animals receiving the dietary supplement, the dietary supplement studies will be extended to human trials in the future.

RESEARCH AREA:	**Radiation Effects**
PRINCIPAL INVESTIGATOR:	**Marcelo Vazquez, M. D., Ph.D.**
ORGANIZATION:	**Brookhaven National Laboratory**
PROJECT TITLE:	**Risk Assessment and Chemoprevention of HZE Induced CNS Damage**

Project Executive Summary

Because successful operations in space depend on the performance capabilities of astronauts, radiation-induced neurological damage, could jeopardize the successful completion of mission requirements, as well as have long-term consequences on the health of astronauts. It is therefore necessary to understand the nature of this risk in order to assess its seriousness and to develop countermeasures. Compared to the large literature associated with radiation therapy, knowledge is limited about the cellular and molecular responses of cells to high-LET HZE radiation in general, and very limited about the central nervous system (CNS) specifically. Therefore, we propose to compare the effects of charged particle (Fe, Si), protons, gamma and x-ray radiation on the cells of the CNS, namely neurons and glial (astrocytes and oligodendrocytes). Cell cultures of CNS cells, both cycling and post-mitotic differentiated cells, will be utilized as model systems. We will test the hypothesis that exposure to low fluences/doses of heavy ions and protons can induce cell death in neural CNS neural cells and that increasingly dense ionizing radiation will be increasingly toxic. The activation of two separate stress signal transduction pathways will be examined (p53 and ceramide) for their role in causing cell death or other deleterious changes caused by irradiation. And with respect to p53, we will determine which of the post-translational modifications in regulating p53 function are relevant for charged particle induced cell death. Finally, we will test the hypothesis that modulating the stress signal transduction pathways will modify the radiation response of brain cells exposed to heavy ions and protons, and test the efficacy several compounds as potential countermeasures for HZE radiation toxicity.

NSBRI RESEARCH PROGRAM
SMART MEDICAL SYSTEMS

Team Leader: **Sutton, J. P.** **Harvard**

Associate Team Leader: **Crum, L. A.** **Washington**

Crum, L. A.	**PI**	**Washington**	**Guided High Intensity Focused Ultrasound (HIFU) for Mission-Critical Care**	**95**
Carter, S. J.	CO-I	Washington		
Bailey, M. R.	CO-I	Washington		
Kaczkowski, P. J.	CO-I	Washington		
Vaezy, S.	CO-I	Washington		
Davies, P. F.	**PI**	**Penn**	**Vascular Genomics in Gravitational Transitions**	**96**
Stoeckert, C.	CO-I	Penn		
Klempner, M. S.	**PI**	**Boston Univ.**	**Smart Medical System for Detection of Microorganisms**	**97**
Cunningham, B. T.	CO-I	SRU Biosystems		
Pepper, J. R. W.	CO-I	SRU Biosystems		
Putcha, L.	**PI**	**NASA JSC**	**Microcapsule Gel Formulation of Promethazine Hydrochloride for Intranasal Administration**	**98**
Soller, B. R.	**PI**	**UMass**	**Noninvasive Measurement of Blood and Tissue Chemistry**	**99**
Heard, S. O.	CO-I	UMass		
Puyana, J. C.	CO-I	UPittsburgh Medical Center		
Sutton, J. P.	**PI**	**Harvard/MIT**	**Near Infrared Brain Imaging for Space Medicine**	**100**
Boas, D. A.	CO-I	Harvard		
Koroshetz, W. A.	CO-I	Harvard		
Rosen, B. R.	CO-I	Harvard/MIT		
Strangman, G. E.	CO-I	Harvard		

Thomas, J. D.	**PI**	**Cleveland Clinic**	**Diagnostic Three Dimensional Ultrasonography: Development of Novel Compression, Segmentation and Registration Techniques for Manned Space Flight Applications**	**101**
Greenberg, N. L.	CO-I	Cleveland Clinic		
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Hale, J. C.	CO-I	Cleveland Clinic		
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Garcia, M. J.	CO-I	Cleveland Clinic		
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Morehead, A. J.	CO-I	Cleveland Clinic		

RESEARCH AREA:	**Smart Medical Systems**
PRINCIPAL INVESTIGATOR:	**Lawrence Crum, Ph.D.**
ORGANIZATION:	**University of Washington**
PROJECT TITLE:	**Guided High Intensity Focused Ultrasound (HIFU) for Mission-Critical Care**

Project Executive Summary

One of the most exciting new frontiers for the Manned Space Program is a long-term flight, perhaps to Mars. For such a mission, all efforts must be made to ensure that mission-critical failures do not occur. One of the most difficult medical conditions to treat, especially when operating facilities are not available, is that of blunt abdominal trauma. Indeed, it is known from studies of combat casualty care that exsanguination (uncontrolled bleeding) is the principal cause of battlefield mortality, and for those combatants who do not receive immediate hospital care, the mortality rate has not significantly improved since the civil war. Although a variety of drugs are becoming available that are intended to stop internal bleeding, they have not yet met with acceptable success. To treat a number of mission-critical medical conditions that might arise during long term space flight, we envision a lightweight, portable, smart medical device that can adequately control internal bleeding, as well as address a number of other medical conditions that require surgery. This device will use diagnostic ultrasound for guidance and High Intensity Focused Ultrasound (HIFU) for therapy. Specifically, we proposed to build an image-guided transcutaneous device for acoustic hemostasis and bloodless surgery. Because the scope of this NASA program is limited, we do not propose to deliver the flight-ready device at the conclusion of this proposed study; rather, we propose to develop an integrated ultrasound guidance and therapy engineering prototype that will be tested on large animals. Under DoD support, we are currently developing a similar instrument for use in the forward echelons of the battlefield and our experience in this area can be directly applied to the goals and objectives of this effort.

RESEARCH AREA:	**Smart Medical Systems**
PRINCIPAL INVESTIGATOR:	**Peter Davies, Ph.D.**
ORGANIZATION:	**University of Pennsylvania**
PROJECT TITLE:	**Vascular Genomics in Gravitational Transitions**

Project Executive Summary

Orthostatic intolerance, an undesirable consequence of cardiovascular-adaptations to microgravity, frequently occurs upon the return of astronauts from prolonged space flight and requires medical management. During prolonged missions to other planets, countermeasures must be developed to prevent incapacity upon entry into remote gravity environments such as the NASA exploratory missions to Mars (approx. 3-G atmospheric entry, 0.3-G at the surface). The cardiovascular system is able to adapt to altered mechanical conditions, including gravitational changes. Reorganization of blood vessel structure and function during prolonged microgravity results in attainment of a new equilibrium state, the abrupt disturbance of which (upon re-entry) predisposes the system to orthostatic intolerance. Vascular adaptation is largely orchestrated through gene transcription. This ground-based project will address *at the level of gene expression the structural and regulatory changes in vascular tissues associated with (i) exposure to simulated microgravity, (ii) return to normal posture, and (iii) prolonged exposure to hypergravity, and its acute reversal.* Hypergravity experiments will be performed at the NASA/Ames animal centrifuge facility. We propose that the underlying mechanisms of adaptive tolerance/intolerance to gravitational shifts be studied at a fundamental but comprehensive level in blood vessels by following changes in thousands of genes in small amounts of tissue and in small numbers of vascular cells obtained from gravitationally-relevant locations in a mouse model. Linear amplification of very small amounts of vascular RNA for analysis on microarrays enables "spatio-temporal transcription profiling" of the vasculature. Changes in regions of the vascular tree known to be (a) of particular relevance to human orthostatic intolerance, and (b) of critical importance in normal blood vessel regulation, will be investigated. In addition to the use of available commercial mouse genomic arrays, customized cardiovascular microarrays and mouse-specific array building will be performed. Gene expression arrays will be constructed from clones representing both genes of known cardiovascular importance and genes of vascular cell "transcriptomes" empirically derived from subtracted, normalized libraries. In addition to regional vascular tissues, small groups of cells will be harvested from the vasculature by laser capture microscopy or mechanical microdissection, and RNA will be amplified. Hybridized arrays will be subjected to rigorous and sophisticated bioinformatics analysis and the output will be archived as raw images and as curated and annotated data. These will be made available to the space research community to provide well-defined site-specific vascular phenotypes responsive to gravitational change. The use of a murine model will later facilitate follow-up flight studies.

RESEARCH AREA:	**Smart Medical Systems**
PRINCIPAL INVESTIGATOR:	**Mark S. Klempner, M.D.**
ORGANIZATION:	**Boston University**
PROJECT TITLE:	**Smart Medical System for Detection of Microorganisms**

Project Executive Summary

The goal of this program is to develop a revolutionary, non-culture based microbial detection, identification and quantification system that can be used as part of a Smart Medical System for exploratory space travel. Rapid detection and identification of microorganisms are critical to many military and civilian applications ranging from food and water safety monitoring, biological warfare agent detection and to diagnostic microbiology of human and other biological specimens. For long-term exploratory space travel there will be a critical need for a smart medical system to monitor the air and water supply for microbial contaminants, as well as an intermittent need for assessment of biological specimens from symptomatic astronauts.

Current microbial identification systems are based on the gold standard of *in vitro* culture or DNA/RNA fingerprinting. Both require considerable sample manipulation, delay in readout, are semiquantitative and subject to interfering substances and contamination, and require additional processing to resolve complex mixtures of microorganisms. This proposal involves the development of a novel smart medical system to detect and identify bacteria through the use of microsensors and includes three steps: 1) Development of "fingerprinting" phage display libraries which can detect, identify, quantify and discriminate bacterial species in environmental and biological specimens; 2) Application of phage displayed peptides and antibody fragments in a microarray to the surface of a microsensor to demonstrate the microarray microbial fingerprint response to selected bacterial species using optical readout and electronic MEMS resonator arrays and to characterize the sensitivity and specificity for detecting and discriminating between bacterial species using surface "fingerprints;" and 3) Development of algorithms from the microarray response for the real time identification and discrimination of bacterial species.

RESEARCH AREA:	**Smart Medical Systems**
PRINCIPAL INVESTIGATOR:	**Lakshmi Putcha, Ph.D.**
ORGANIZATION:	**NASA-Johnson Space Center**
PROJECT TITLE:	**Microcapsule Gel Formulation of Promethazine Hydrochloride for Intranasal Administration**

Project Executive Summary

A continuing challenge for space medical operations at NASA is the management of pathology associated with neurovestibular adaptation during space flight. A primary manifestation of this problem, particularly in the first few flight days of shuttle missions, is space motion sickness (SMS). The current treatment of choice for symptoms associated with SMS is promethazine (PMZ). Although oral tablets and rectal suppositories have been used during space flights, the intramuscular route appears to be most effective. On the other hand, intramuscular administration of drugs is an invasive procedure and PMZ causes irritation at the site of injection. A key research topic in the Smart Medical Systems area of the NSBRI 99-02 research announcement is development of novel therapeutic modalities for remote site medical operations such as space missions. In response to this initiative, the goal of the proposed research is to develop an intranasal dosage formulation of PMZ that will provide crewmembers with a non-invasive means of self-administering SMS medications. Accordingly, the following three aims will be addressed: 1) Develop a microencapsulated, pH-balanced gel dosage formulation and a combination form with a corticosteroid for intranasal administration of PMZ; 2) Establish the release kinetics and shelf life of the optimized dosage forms; and 3) Assess bioavailability, nasal mucosal irritability and toxicity of the selected dosage forms in rats.

The proposed formulation development will focus on tailoring the release characteristics of the dosage form to optimize therapeutic index and minimize irritability at the site of administration. Once the optimal dosage form has been identified based on release kinetics and stability characteristics, bioavailability, nasal irritability and toxicity after single and multiple dose administration will be assessed in an animal model. Development of an intranasal drug delivery system for motion sickness treatment will benefit pharmacotherapeutics in space as well as on Earth.

RESEARCH AREA:	**Smart Medical Systems**
PRINCIPAL INVESTIGATOR:	**Babs R. Soller, Ph.D.**
ORGANIZATION:	**University of Massachusetts Medical School**
PROJECT TITLE:	**Noninvasive Measurement of Blood and Tissue Chemistry**

Project Executive Summary

Medical monitoring and diagnosis of acute and chronic conditions during long-duration space flight is critical to the success of these missions and must be able to be carried out by personnel with limited medical training and equipment. The most successful technologies will be those that allow noninvasive measurement of multiple parameters that can be combined for algorithm-driven decision making. Near infrared spectroscopy (NIR) has been successfully used to noninvasively assess blood and tissue for the measurement of oxygenation, pH, glucose and hematocrit and the diagnosis of cancer because NIR light can penetrate through skin and bones. Currently, this technology is limited in its ability to accurately measure these parameters for people with dark skin color and significant fat content. The hypothesis of this proposal is that NIR, in combination with unique statistical methods, can be used to noninvasively measure blood and tissue chemistry for any human subject. This project will develop new statistical methods which will enhance the processing of NIR spectral data so that medical parameters can be accurately measured on all humans, irrespective of skin color and gender. This new approach will be demonstrated by developing techniques to noninvasively measure blood hematocrit and muscle pH and oxygenation on human surgical and ICU patients. These parameters are important in diagnosing and treating hypoxia and trauma that may arise from exposure to radiation, toxic chemicals and blunt or sharp injury. They may also be useful in evaluating exercise as a countermeasure for extended weightlessness. The measured patient data will then be used to develop algorithms to diagnose shock and hypovolemia and guide resuscitative therapies. Finally, optical specifications will be developed to build a miniaturized system to collect NIR data. This system will serve as a platform for NIR measurement of multiple parameters and the development of computerized algorithms to assist in the diagnosis and treatment of several medical conditions. The specific system demonstrated in this proposal is intended to evolve into a medical monitoring system for use during extended space flight, but will also find immediate application in terrestrial hospitals, emergency vehicles and emergency rooms.

RESEARCH AREA:	**Smart Medical Systems**
PRINCIPAL INVESTIGATOR:	**Jeffrey Sutton, M.D. Ph.D.**
ORGANIZATION:	**Harvard – Massachusetts General Hospital**
PROJECT TITLE:	**Near Infrared Brain Imaging for Space Medicine**

Project Executive Summary

This application is to the Smart Medical Systems team of the National Space Biomedical Research Institute (NSBRI). NASA's cross-risk prioritization for long duration space missions identifies four medical problems at the highest level, including (1) human performance failure because of poor psychosocial adaptation and (2) trauma and acute surgical problems. The brain is the organ central to human performance and psychosocial adaptation, and alterations in the nervous system, including those induced by trauma, can have deleterious effects to a space mission. Almost no countermeasures exist to address the possible biological and environmental impediments affecting the brain on long term human space flight. The development and implementation of a non-invasive, low power, portable, functional imaging technology for monitoring brain activity in microgravity is therefore an important advance for astronaut care. An instrument of this type could be used for ongoing monitoring, early identification of alterations, diagnosis, procedures and evaluation of countermeasures. When coupled to models of individual astronauts and a smart informatics system, it could assess brain function, and aid in countermeasure readiness, with unprecedented sophistication and autonomy.

In this proposal, the investigators plan to utilize the resources of the Massachusetts General Hospital (MGH) to engineer and apply a new non-invasive, portable imaging device capable of performing diffuse optical tomography (DOT) for space medicine. The technology performs spectroscopy with near infrared light to monitor oxyhemoglobin and deoxyhemoglobin concentrations in the brain, and is easily adapted for use in microgravity. The research builds on collaborative work among the project's investigators, and allows for validation of the instrument using other technologies, namely functional magnetic resonance imaging (fMRI) and optical coherence tomography (OCT). Specifically, DOT will be validated by imaging healthy subjects using the simultaneous, and non-interacting, methods of DOT and fMRI. Subjects will perform motor tasks of varying complexity under normal and sleep deprived conditions to assess cortical function during simulated flight tasks. DOT will also, along with OCT, be used to assess patients with altered intracranial pressure (ICP). Changes in ICP are associated with fluid shifts, headache and performance failure, and they are amenable to countermeasures. All functional imaging data will be used to help refine a system for automated assessment, warning, and countermeasure evaluation. It is anticipated that the technologies developed in this proposal will have direct, applications for health care on Earth.

RESEARCH AREA:	**Smart Medical Systems**
PRINCIPAL INVESTIGATOR:	**James Thomas, M.D.**
ORGANIZATION:	**The Cleveland Clinic Foundation**
PROJECT TITLE:	**Diagnostic Three Dimensional Ultrasonography: Development of Novel Compression, Segmentation and Registration Techniques for Manned Space Flight Applications**

Project Executive Summary

The NSBRI has identified that the efficient and automated delivery of health care in space is a key research arena for the future. Specifically, they propose to develop a "Smart Medical System" that will be able to monitor crew health, identify deviations from ground-based norms, and allow timely intervention by crew members who may have only a moderate amount of training in medicine. For the last three years, the principal investigator and colleagues have worked closely with NASA scientists, flight surgeons, and engineers to optimize research and diagnostic ultrasound aboard the International Space Station (ISS) and thus are well positioned to develop the necessary tools and techniques to integrate ultrasound into the Smart Medical System. A principal limitation of ultrasound technology is its extreme dependence on the expertise of both the acquiring examiner and the interpreting physician. This is particularly true of two-dimension ultrasound, where the examiner is required to obtain precisely oriented anatomical sections of the organ of interest.

Three-dimensional ultrasound has the advantage of acquiring a large anatomic volume from a single ultrasonic window, and thus may be less dependent upon the expertise of the examiner. Furthermore, this large volume may contain sufficient anatomic landmarks to allow unambiguous registration with previously obtained three-dimension data from either ultrasound or other modalities such as magnetic resonance imaging (MRI) or computed tomography (CT). One could thus envision a system by which whole organs or even the entire body would be imaged in three-dimensions prior to launch; data which could be used to compare with subsequently obtained three-dimensional data sets using in-flight ultrasonography. The overall purpose of this grant is therefore to perform ground-based research, development, and validation aimed at optimizing diagnostic ultrasound in manned space flight, with the following general hypothesis:

Unifying hypothesis: Serial three-dimensional ultrasound examinations will enhance diagnostic capabilities in manned space flight.

The technical aspects of this program will be pursued with the following specific aims:

1. Optimize the acquisition methods for three-dimensional sonography, utilizing reconstruction and real-time techniques.
2. Develop techniques for registering anatomical images from two- and three-dimensional ultrasound with those obtained from prior ultrasound examination and from magnetic resonance and computed tomographic imaging, considered "gold standards" for non-invasive anatomical imaging.
3. Develop tools for abstracting, in an automated fashion, anatomical changes from serial three-dimension and two-dimension ultrasound studies.

4. Develop algorithms for the optimal compression of three-dimensional ultrasound images and refine current two-dimensional compression algorithms.
5. Assess the ability of novice examiners to obtain three-dimensional sonographic data sets following minimal training.

These objectives will be pursued using data from a variety of *in vitro*, animal and clinical models. In particular, we will take advantage of a well-established collaboration with the National Institutes of Health, which permits highly sophisticated chronic animal models to be examined with a minimum of additional resources. Although the tools developed here should be applicable to any organ of the body, we will focus our efforts on the kidneys and the heart.

At the conclusion of this project, we anticipate delivering to the NSBRI and its Smart Medical System a set of algorithms and software for the non-rigid morphological registration and comparison of serial two- and three-dimensional ultrasound data sets and validated algorithms for optimal compression of four-dimensional ultrasound data. In addition to these technical deliverables, our validation work on nephrolithiasis will provide important diagnostic clues for assessing this condition in manned space flight. Similarly, the work on cardiac mass regression following unloading will be invaluable to the NASA research and medical operations community in assessing the impact of long-term space flight on cardiac atrophy and utility of prophylactic countermeasures.

RESEARCH AREA:	**Smart Medical Systems**
PRINCIPAL INVESTIGATOR:	**James Thomas, M.D.**
ORGANIZATION:	**The Cleveland Clinic Foundation**
PROJECT TITLE:	**Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity**

Project Executive Summary

The cardiovascular system undergoes significant changes in microgravity, including an early cephalad shift of lower extremity blood volume, loss of plasma volume over 24 to 48 hours, and long-term reduction in ventricular chamber volume and mass. In the weightless environment, these alterations generally are well tolerated, but upon return to Earth, astronauts often suffer from serious orthostatic intolerance and reduced exercise capacity, changes that may limit the long-term presence of man in space. It is essential that the mechanisms for these alterations be understood so that reliable countermeasures can be tested and implemented. Hypovolemia, cardiac atrophy, and autonomic dysfunction have each been hypothesized to contribute to this post-flight debility, but their relative importance is unclear. Furthermore, it is unknown whether actual abnormalities in the myocardium itself develop with long-term space flight. Therefore, reliable portable noninvasive methods will be needed in order to detect and quantify these changes.

Alone among such imaging modalities of radiography, magnetic resonance imaging and computerized tomography, echocardiography has the unique ability to characterize cardiovascular anatomy and physiology in ground-based models, pre- and post-flight, and most importantly during flight. Indeed, the Science Working Group (SWG) for the International Space Station (ISS) Human Research Facility (HRF) has recognized the primacy of ultrasound for medical diagnosis and physiology research, with plans to launch a specially modified commercial ultrasound instrument to the ISS in 2001. Echocardiography is similarly being used before and after shuttle flights and in a variety of bed-rest studies sponsored by NSBRI and NASA. Unfortunately, while ultrasound has the potential for high spatial and temporal resolution imaging of the heart, in the past it has been severely limited by operator inexperience and inconsistency in its subjective interpretation. Needed are new methodologies for assessing the load-independent function of the heart and consistent, objective quantification of a wide range of NASA echo studies, whether obtained on the ground, in flight or in experimental models. We propose to provide such a facility while validating novel methods for the load independent assessment of myocardial function. Our central hypothesis is that:

Microgravity affects cardiovascular function not only through changes in chamber volume and mass but also through changes in myocardial properties.

A definitive test of this hypothesis is at least several years away when dedicated life science missions are possible aboard the ISS. However, within the scope of this grant, we propose several specific aims that will be critical to the ultimate comprehensive study of the cardiovascular system in space. Key issues: 1) Validation of non-invasive Doppler echocardiographic indices for the assessment of left ventricular contractility and relaxation including color M-mode Doppler derived diastolic intraventricular pressure gradients (IVPG) and tissue Doppler derived myocardial systolic and diastolic strain rates (e's, e'd); 2) Validation of Doppler derived exercise cardiac output and contractile reserve and their potential utility for the

early detection of myocardial dysfunction during prolonged space flight. Additional deliverables to NSBRI: 3) Development and distribution of stand-alone software and algorithms for implementing the quantitative analysis of Doppler echocardiographic data, as described above, so they may be applied to ultrasound data obtained from remote sources; 4) Establishment of an Echocardiographic Core Facility to the NASA research and clinical community, capable of applying standard and novel analysis techniques in a rigorous fashion to echocardiographic data obtained from selected ground-based experimental models, pre- and post-flight examinations, and eventually from in-flight acquisitions.

If successfully implemented, these aims will allow the cardiovascular sequellae of space flight to be studied much more rigorously, while providing consistent, objective echocardiographic interpretation to the entire NASA community.

NSBRI RESEARCH PROGRAM
TECHNOLOGY DEVELOPMENT

Acting Team Leader: **Sutton, J. P.** **Harvard**

Acting Associate Team Leader: **Charles, H. K. Jr.** **Hopkins/APL**

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Kinnison, J. D.	CO-I	Hopkins/APL		
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RESEARCH AREA:	**Technology Development**
PRINCIPAL INVESTIGATOR:	**Paul A. Bottomley, Ph.D.**
ORGANIZATION:	**Johns Hopkins University School of Medicine**
PROJECT TITLE:	**Development of a Space Qualifiable MRI System**

Project Executive Summary

This proposal is to develop a proof-of-concept engineering model of a space qualified Magnetic Resonance Imaging (MRI) system for small animals and astronaut limbs with mass of < 130 kg and average power when on but not scanning < 1 kW and when scanning < 1.2 kW, not including the processor. An onboard processor or a high-performance PC can be adapted. MRI provides high-resolution, high-quality anatomical information without ionizing radiation so it can be safely used repeatedly to track changes without deleterious effects. As a result, the study of physiological alterations in space and the development, verification, and maintenance of countermeasures will be significantly enhanced. Mice and small rat models are useful surrogates to carry out in-orbit physiological studies. Measuring alterations in the limbs of astronauts, especially the lower limbs, will provide partial confirmation of the effectiveness of proposed countermeasures and the utility of Earth-based animal models. In-flight MR imaging of mice and rats will especially benefit the countermeasure developments of several of the NSBRI research teams. The proposed concept is based on traditional MRI principles and uses advanced technology and advanced engineering techniques to reduce mass and power to acceptable levels. The system consists of a 1 to 1.5 Tesla cryogen-free high temperature superconducting magnet subsystem and advanced electronics that will have magnetic field inhomogeneities ≤ 8 ppm over a spherical imaging volume of 10 cm diameter and ≤ 10 ppm out to 15 cm diameter. The magnet cryocooler subsystem will be designed using high temperature superconducting materials to significantly reduce the mass and power of the cryocooler. The highest resolution mode gives a resolution of 117 microns for small animals over a spherical imaging volume of 6 cm diameter and a resolution of 352 microns for human limbs over a spherical imaging volume of 18 cm diameter. The standard resolution mode will provide a resolution of 234 microns and 703 microns, respectively. The pulse sequence scenarios used will be those traditionally used in MR imaging to achieve images that are proton-density, T1 or T2 weighted so that a significant amount of structural information will be available. Because of budget limitations, only selected electronics will be reengineered to demonstrate the minimum mass and power that can be achieved. We ask that the panel consider a supplemental budget request that allows redesign and fabrication of all of the electronics to minimize mass and power. The team is composed of individuals and organizations with a unique combination of expertise including: MRI systems development at the General Electric Research and Development Center, advanced MRI development and small animal experimentation at the Johns Hopkins School of Medicine, and the development of reliable medical and low-mass, low-power systems for space applications at the Johns Hopkins University Applied Physics Laboratory.

RESEARCH AREA:	**Technology Development**
PRINCIPAL INVESTIGATOR:	**Jay C. Buckey, M.D.**
ORGANIZATION:	**Dartmouth Hitchcock Medical Center**
PROJECT TITLE:	**Improved Bubble Detection for EVA**

Project Executive Summary

Assembly of the International Space Station (ISS) requires extensive and unprecedented extra-vehicular activity. Because spacesuits operate at low internal pressure, astronauts are highly susceptible to decompression sickness (DCS), and a range of pre-breathe strategies are employed to mitigate this risk. During ISS construction, in-suit Doppler bubble monitoring will be provided to monitor for DCS risk. Doppler bubble detection is effective, but (1) it is motion sensitive, (2) detects only moving bubbles, and (3) only detects bubbles that are approximately 80 μm greater in size. Our goal is to build on the successful development of two novel transcutaneous ultrasonic bubble detection and sizing instruments developed under NASA funding that exploit bubble resonance (not Doppler), making the instruments capable of sizing bubbles as well as detecting stationary bubbles. One instrument is optimized for intravascular bubbles in the 30 to 200 μm size range, and the other is optimized for extravascular bubbles in the 1 to 10 μm size range. The intravascular bubble instrument has been demonstrated in extensive *in-vitro* trials and preliminary *in-vivo* trials to detect and size intravascular bubbles down to 30 μm in size. The extravascular bubble instrument is currently under development and is intended to detect stationary bubbles in tissue. The ability to detect these small tissue bubbles may be highly advantageous in terms of assessing DCS risk and developing efficient pre-breathe strategies. The extravascular bubble-sizing instrument has been demonstrated *in vitro* down to bubble sizes of approximately 1 μm, and it is presently being tested with tissue phantoms to demonstrate *in-vitro* transcutaneous operation. This project will combine the Creare development team with the hypo- and hyperbaric facilities at Dartmouth-Hitchcock to validate the application of these instruments for *in-vivo*, transcutaneous bubble detection. Testing and application of these instruments in research and practical field applications may lead to (1) better understanding of DCS and (2) improved monitoring and prevention techniques for DCS.

RESEARCH AREA:	**Technology Development**
PRINCIPAL INVESTIGATOR:	**Harry K. Charles, Jr., Ph.D.**
ORGANIZATION:	**Johns Hopkins University Applied Physics Laboratory**
PROJECT TITLE:	**AMPDXA Scanner for Precision Bone and Muscle Loss Measurements During Long-Term Space Flight**

Project Executive Summary

Under the National Space Biomedical Research Institute (NSBRI) support, the concept and working test beds (laboratory and clinical) for a full-body Advanced Multiple Projection Dual-Energy X-ray Absorptiometer (AMPDXA) have been developed by The Johns Hopkins University Applied Physics Laboratory (JHU/APL) and have demonstrated that it is possible to build a flight-qualified, compact, lightweight instrument of about 46 kg that uses multiple, angularly spaced projections to determine bone density with accuracy and precision. The instrument is significantly superior to conventional, single-projection dual-energy X-ray absorptiometry (DXA) and provides geometric details (bone geometry) unavailable from current DXAs. Bone density and geometry (e.g., section moduli) are important in evaluating and monitoring the effectiveness of countermeasures to bone loss and determining the risk of fracture of astronauts rather than relying on statistical correlation for risk assessment based on population means. This project expands on the previous AMPDXA development work by addressing the significant remaining technological issues necessary to lighten and miniaturize the AMPDXA equipment to meet flight qualification requirements including a mass of 46 kg (100 lb.). On completion of this project, the AMPDXA will be in a compact proto-flight form, capable of being used to evaluate astronauts during pre- and post-flight missions as well as being easily upgraded to be able to fly on the Shuttle, Space Station, or a mission to Mars. This study consists of nine tasks. Task 1 is to continue to calibrate, validate, and optimize the current AMPDXA ground-based systems (laboratory and clinical test beds) using calibration specimens and animal and cadaver parts. Task 2 is to develop a series of algorithms based on Task 1 that optimizes bone mineral density (BMD), bone structure extraction, and risk of fracture estimation while minimizing X-ray pulse duration, power, and angular repetition rates. Task 3 is to perform specific pre-clinical testing to ensure safety prior to human testing. Task 4 consists of tests on humans to verify the exact instrument requirements necessary to produce highly accurate BMD and structural information. Task 5 addresses the proto-flight development process, including lightweight X-ray tube, lightweight compact power supply, and techniques for embedded fiber thermal management coupled with a strong rigid mechanical structure. Task 6 looks at complete automation of the testing process so that the resulting proto-flight instrument is not only compact but easy to use. Task 7 produces a proto-flight demonstration unit (key elements fully working) and a complete specification package necessary for flight unit procurement. Tasks 8 and 9 are to provide periodic reports to the NSBRI and publish papers, respectively.

RESEARCH AREA:	**Technology Development**
PRINCIPAL INVESTIGATOR:	**Brian L. Davis, Ph.D.**
ORGANIZATION:	**The Cleveland Clinic Foundation**
PROJECT TITLE:	**Design and Validation of a Dynamic Exercise Countermeasure Device**

Project Executive Summary

Bone demineralization is a well-documented physiologic effect of space flight. In 1-G, animal experiments have clearly indicated that (i) certain bone strains and strain rates do stimulate bone deposition, and (ii) repetitive loading of the lower extremity can increase osteonal bone formation even as proximally as the vertebral column. Such studies have also indicated that a relatively small number of appropriate loading cycles may lead to bone deposition. Based on prior research that we have performed with foot loading experiments, we propose the development of a dynamic exercise countermeasure device (DECD) that utilizes jumping as the mode of exercise for astronauts. This project falls under the technology development designation of the NSBRI program.

Our project will be divided into three phases. In year one we will collaborate with Foster Miller Inc., a company that has expertise in the design of both lightweight structures and vibration isolation methodology. The goal of this phase is to construct a device that permits dynamic jumping exercise in microgravity and that is suitable for the International Space Station. A key design component of this apparatus will be its ability to prevent vibrations and/or unbalanced forces from being transmitted to the surrounding environment. In year two we will test the system using our zero-gravity simulator that has been developed under NASA NAGW-5006. Specifically, we will verify that muscle activation patterns and limb loading data are similar to the results we have obtained thus far for tethered jumping in microgravity. In year three we will confirm the efficacy of the DECD in true microgravity through KC-135 experiments.

RESEARCH AREA:	**Technology Development**
PRINCIPAL INVESTIGATOR:	**Richard H. Maurer, Ph.D.**
ORGANIZATION:	**Johns Hopkins University Applied Physics Laboratory**
PROJECT TITLE:	**Neutron Energy Spectrometer Flight Experiments**

Project Executive Summary

We propose to develop a Neutron Energy Spectrometer to monitor the flight radiation environment on the International Space Station (ISS). This project would continue the efforts of our original NSBRI grant that developed an engineering prototype instrument for aircraft flights. We would extend and adapt the fabricated engineering prototype and Martian Neutron Energy Spectrometer (MANES) proposal concept for the ISS opportunity. In addition to the flight instrument development, we seek to continue the aircraft flights for operating experience and data from a multi-energy neutron environment similar to that expected on the ISS. The work done on the FY 1998-2000 NSBRI grant has advanced the instrument development from Countermeasure Readiness Level 2 (preliminary studies to demonstrate feasibility) to Level 5 (proof of concept). We now seek to advance from Level 5 to Level 8 (validation in actual space flight) with the funding of this proposal. We present results from the activities of the preceding NSBRI grant and describe the concept for the ISS instrument, including cross-sectional and block diagrams. The research plan contains costs for both the purchase of flight parts during the first year of funding and extensive accelerator calibration during the third year. The second year involves design, drawing, and fabrication of the flight instrument at APL. We have included NASA Form C for proposed flight experiments as requested. No animal or human subject testing is involved in this effort because the NSBRI Neutron Energy Spectrometer only monitors the radiation environment due to neutrons inside the ISS where astronauts will live and work.

RESEARCH AREA:	**Technology Development**
PRINCIPAL INVESTIGATOR:	**Richard S. Potember, Ph.D.**
ORGANIZATION:	**Johns Hopkins University Applied Physics Laboratory**
PROJECT TITLE:	**Real-Time Analysis of Biomarkers and Countermeasures Using a Miniature Time-of-Flight Mass Spectrometer**

Project Executive Summary

The long-term objective of this project is to lay the scientific and engineering foundations to design, build and launch a flight qualified "Miniature Time-of-Flight Mass spectrometer" (TOFMS). This instrument will be used on space platforms such as the Space Shuttle, the International Space Station (ISS) and a human mission to Mars. The TOFMS is small, lightweight, requires little power, and a rugged instrument that can be used continuously with advanced signal processing diagnostics. TOFMS has demonstrated mass capability resolution from under 100 to beyond 10,000 atomic mass units in a very small, low power prototype for biological analysis. The development of "Miniature Time-of-Flight (TOF) Mass Spectrometer" will provide the NSBRI/NASA with a complete medical diagnostic system to monitor human physiological functions routinely and non-invasively. The specific aims are to (1) develop sampling and sample preparation techniques that enable the TOF mass spectrometer system to reliably detect, identify and quantify extremely low levels of chemical and biological substances in complex body fluids (urine, blood, breath) with very low error rates. This will be achieved by developing and testing a fast, portable (GC-MS) system for human space flight applications. The miniature TOF system will also be used to detect and quantify circulating muscle atrophy biomarkers that appear in serum during space flight. These biomarkers are a measure of muscle damage. Detection and quantification of these markers using the miniature TOF technology will allow real-time monitoring of muscle damage on-orbit and the mass spectrometer can also be used to study the effectiveness of resistive exercise countermeasures in space flight. Several metabolic bone markers and the bone regulating hormones will be studied in conjunction with the NSBRI Bone Team. Melatonin and cortisol will also be studied in breath samples. Currently, the assessment of melatonin and cortisol through established laboratory techniques requires days to obtain a result. The development of online methods for monitoring and assessing the status of circadian organization is listed as one of the five primary themes for the Human Performance, Sleep and Chronobiology Team.

RESEARCH AREA:	**Technology Development**
PRINCIPAL INVESTIGATOR:	**Yi-Xian Qin, Ph.D.**
ORGANIZATION:	**State University of New York – Stony Brook**
PROJECT TITLE:	**A Non-Invasive Scanning Confocol Ultrasonic Diagnostic System for Bone Quality**

Project Executive Summary

Musculoskeletal complications induced in extended space mission represent a key astronaut health problem. In normal gravity, early diagnosis of progressive bone loss or poor bone quality indicates prompt treatment and thus will dramatically reduce the risk of bone fracture. The principal diagnostic methods for osteoporosis and microgravity induced osteopenia is dual-energy X-ray absorptiometry (DEXA), which provides only an index of bone mineral density and/or content, and not bone's physical properties. More recently, advents in ultrasonic techniques provide an intriguing method for characterizing the material properties of bone in a manner that is non-invasive, non-destructive, repeatable, safe and relatively accurate. Limitations with this approach, however, leave non-invasive ultrasound – in its current configuration – as a first order screening tool, rather than a highly accurate diagnostic.

Our principal goal is to continue development and evaluation of a scanning confocal acoustic diagnostic (SCAD) system capable of generating non-invasive, high-resolution ultrasonic attenuation and velocity maps of trabecular and cortical bones. This system, relevant not only for ground-based determination of bone's physical properties, can effectively be used in the space environment for determining even subtle changes in density and strength during extended flights. In addition to the development of this device, we will validate the structure and density information detected by SCAD using μCT and mechanical testing methods in both *in vivo* and *ex vivo* animal models, including human subject. These data will provide a database for further testing in the space environment. Importantly, the SCAD system is small and lightweight.

The proposed study will focus on a series of four areas: 1) *Develop a SCAD system for non-invasively mapping of wave velocity and attenuation in bone;* 2) *Correlate SCAD determined velocity and attenuation to micro-CT identified BMD and architecture;* 3) *Predict the risk of trabecular bone failure associated with osteoporotic status in the animal model; and* 4) *Map and monitor strength of bone to predict BMD and structural modulus in osteoporotic and normal humans using the SCAD, and correlate these measurements to DEXA.*

RESEARCH AREA:	**Technology Development**
PRINCIPAL INVESTIGATOR:	**Veljko Radeka, Ph.D.**
ORGANIZATION:	**Brookhaven National Laboratory**
PROJECT TITLE:	**Heavy Ion Microbeam and Micron Resolution Detector**

Project Executive Summary

The ability to place discrete numbers of particles in defined cellular and extracellular locations is now possible by using microbeam irradiation facilities. Such a facility permits heavy-ion radiobiology to address specifically the impact of signal transduction between cellular compartments as well as issues related to intercellular communication at limiting low fluences where not all the cells in a population have been traversed by even a single particle. Moreover, a high-energy, heavy-ion microbeam will permit to address an important unanswered question: whether neurons that survive traversal by HZE particles develop changes as a late consequence of the damage they incurred. Therefore, these low-fluence studies promise to aid in our understanding of the consequences of exposure to high-LET radiation such as encountered in the space radiation environment.

The purpose of the proposed project is to make possible such studies by developing the following tools:

1. A microbeam of heavy ions (e.g., iron) at energies higher than at existing microbe am facilities (3GeV/nucleon). The microbe am would have a sufficiently small diameter (about 10 micrometers) to localize the ions to a single cell.
2. An electronic position-sensitive detector for heavy ions with a position resolution better than 1 micrometer, to localize the position of ion impact within a particular region of the cell. These developments will advance significantly the state-of-the art of high-energy, heavy ion microbeams and of high-resolution heavy-ion detectors. For the cell studies employing these tools, the necessary infrastructure will include a micropositioning stage with a microscope and auxilliary detectors.

Appendix B

NATIONAL
SPACE BIOMEDICAL
RESEARCH INSTITUTE

RESEARCH PROGRAM REPORTS
YEAR 4 - FY 2001

National Space Biomedical Research Institute
Research Program Reports
Year 4 - FY 2001

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BONE LOSS

CARDIOVASCULAR ALTERATIONS

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MUSCLE ALTERATIONS & ATROPHY

NEUROBEHAVIORAL AND PSYCHOSOCIAL FACTORS

NEUROVESTIBULAR ADAPTATION

NUTRITION, PHYSICAL FITNESS AND REHABILITATION

RADIATION EFFECTS

SMART MEDICAL SYSTEMS

TECHNOLOGY DEVELOPMENT

Bone Team: Annual Program Report

Team Leader Jay R. Shapiro, M.D., Uniformed Services University
Associate Team Leader, Susan Bloomfield, Ph.D., Texas A & M University
Associate Team Leader: Mitchell Schaffler, Ph.D., Mt. Sinai School of Medicine

I. Executive Summary

1) The Research Problem: Microgravity-related bone loss represents a major health and operational hazard for America's Astronauts. Pre- and post-flight bone mineral density readings characterize bone loss during 6 month flight as follows: 1) there is wide inter individual differences in the rates of loss, 2) bone loss averages 1-2% per month during a 6 month flight but may range from no loss to 20% loss, and 3) the processs driving bone loss is likely to be continuous as long as mechanical strain on bone is absent. There is net increase in bone resorption compared to a decrease in bone formation. Furthermore, the failure of bone mass to return to normal for prolonged periods after exposes Astronauts to increased fracture risk during a period of musculoskeletal reconditioning. Current methods for maintaining muscle mass and bone mass during extended flight have not been successful. Thus the primary research problem is the development of safe and effective methods for the maintenance of bone mass in the face of forces altering normal bone remodeling in favor of bone loss over bone formation. The development of effective countermeasures to bone loss in turn depends on greater understanding of the basic mechanisms driving bone loss during microgravity exposure and factors limiting bone formation following return to earth's gravity.

2) Bone Team Program Strengths; key findings and discoveries: The Bone Team program combines basic and clinical studies related to countermeasure development. Each investigator has moved to develop a fruitful research theme. It should be noted that the majority of projects received initial funding during Feb./March 2001. The Zerwekh program was started in June 2001.

a) Isales (Glucose Dependent Inhibitory Peptide (GIP) modulation of osteoblast function) : using Gene Chip technology we have found that GIP activates a number of growth factors in bone including transforming growth factor beta (TGF Beta) and others currently under investigation.

b) Karsenty (Leptin Modulation of Bone Resorption) We show here that unlike body weight bone formation cannot be affected by modulating plasma leptin levels. In contrast, when delivered centrally, minimal amount of leptin that do not affect body weight decreases bone mass by acting on a distinct group of hypothalamic neurons forming the central center of bone formation (CCBF). Genetic experiments demonstrate that the sympathetic nervous system mediates CCBF function. Thus, leptin regulation of bone formation does not follow the hormonal paradigm that applies for body weight control.

c) Smith (Estrogen and Vitamin Receptor Agonists): Using the hindlimb suspended rat these investigators show that estrogen or raloxifene reduces markers of bone tunover in this microgravity model. Estraediol and raloxifene prevent loss of bone mineral density in mechanically unloaded rats as measured by pQCT. Hormone treatment selectively increased bone mineral content in distal femur and proximal humerus. Site specific differences are important as these agents protected trabecular bone in the femur and the humerus, and the tibia where cortical bone was protected. No effect compared to placebo was seen on cortical bone in tibia, humerus and femur.

d) Bloomfield (Bone and Muscle Recovery from Microgravity): The animals testing system is operational. Preliminary data indicate a 26% reduction in peak isometric muscle force after 28-d hindlimb unloading, which is substantially recovered after 14 d cage activity. In groups with enough numbers, there is a reduction in peak isometric force at lower velocities, and reduced power at most velocities. Studies are in progress to measure parallel changes in muscle and bone after return to weightbearing.

e) Rubin (Biomechanical Countermeasure to Disuse Osteoporosis): The differential response of trabecular bone tissue to two distinct stimuli, one anabolic (low level mechanical vibration for 10min/d) and the other catabolic (hind-limb suspension), were evaluated in three strains of adult mice (C57BL/6J - low bone density; Balb/cByJ - mid-density; C3H/HeJ-high density). Trabecular bone from two strains of mice is differentially mechanosensitive at the tissue and molecular level. In BALB/cByJ mice, the low level mechanical signal increased bone formation rates in the proximal tibia by 34% as compared to long-term control ($p<0.02$), while disuse decreased bone formation rates by 48% ($p<0.02$). In contrast, neither anabolic nor catabolic signals influenced any index of bone turnover in C3H/HeJ mice. Together, these data indicate not only a genetic basis for bone architecture, but also that the sensitivity of the tissue to both anabolic and catabolic stimuli is influenced by the genome.

f) Schaffler (Disuse Osteoporosis and Osteocyte Integrity): Current studies confirm preliminary observations, that there is a significant loss of osteocyte integrity in areas of bone undergoing resorption. Osteocytes integrity was unchanged in non-resorbing areas of bone. These observations are consistent with the recent observation that osteocyte apoptosis occurs in strong association with osteoclastic activity indicating that resorption serves as a mechanism for maintaining the integrity of the tissue, by removing nonviable osteocytes.

g) Bolander (Fracture Healing): The hindlimb suspended rat is used as a non-weight bearing model in which to study fracture healing and its modification by ultrasound. Initial stuidies indicate that fracture callus formation is abnormal histologically and on biomechanical testing in non-weightbearing femurs of hindlimb suspended rats.

h) Shapiro (Spinal Cord Injury as a Microgravity Model): Structural and geometric changes in the femur following spinal cord injury (SCI) measured by DEXA mimic those reported in Mir Cosmonauts (Beck, Ruff) This confirms the value of this model as a surrogate for studying bone loss and muscle during space flight. The patterns of bone loss in SCI patients indicate that loss of cortical bone, BMD and bone strength may not be

compensated for by an increase in periosteal bone width which occurs in normals. This focuses on the periosteum as playing a role in mechanical strain transmission to cells in the bone matrix. Treatment with zoledronate/placebo has been initiated (double blind, placebo-controlled study).

i) Zerwekh: (KMgCit as a Countermeasure to Renal Stone formation) Since the initiation of funding in June 2001, we have recruited two subjects for the aforementioned protocol. The first subject is now beginning his 3rd week of bed rest. We anticipate recruiting all subjects within the next two years. If the results disclose that KmgCitrate is an effective countermeasure for renal stones, the future direction would be to evaluate the medication during space flight.

3. Gaps in the team's program: The NASA Critical Risks summary includes 4 major health hazards related to bone and soft connective tissues: 1) accelerated bone loss, 2) fracture risk and fracture healing, 3) connective tissue injuries and 4) renal calculus formation. The current bone research program does not include projects related to connective tissue injuries sustained during and after flight and it includes one project related to fracture healing. These areas require additional studies and this has been requested in the recent (Oct. 31, 2001) NASA/NSBRI request for proposals. The current bone research program includes a study examining rates of muscle/bone return after non-weightbearing in the hindlimb suspended rat (Bloomfield et al). These studies should be extended to human studies.

4. Implications of key findings for future research: To date, no countermeasure, including various exercise regimens, diet, and nutritional supplements including calcium and vitamin D, has limited bone loss during 6 months of space flight. The findings noted above (Key Findings) during the first year of these projects have direct bearing on preventive measure useful to other subjects in non-weightbearing circumstances such as those disabled due to injury of advanced age.

a) The testing of proposed countermeasures during flight is critical: no countermeasure can be adequately tested in the absence of microgravity conditions. Current bed rest research in normal volunteers suggests that oral bisphosphonates (alednronate: LeBlanc, and Shakelford) may effectively reduce bone loss. However, a more suitable formulation (e.g. intravenously administered long-acting bisphosphonate under study in spinal cord injured subjects (Shapiro et al.), may be appropriate for flight testing within the next 2 years. The utilization of vibrational mechanical strain as a means to increase bone mass is well recognized, and is investigated in the research program of Rubin et al. A proposal to test a prototype instrument during flight has been submitted to NASA by Rubin et al. Research on normal volunteers at chronic bed rest on the effect of oral KMgCit as a countermeasure to renal stone formation are in progress. However, NASA has committed to flight studies using oral K citrate supplements. Collaboration on this research is indicated. The use of the magnesium-containing formulation may have additional salutory effects on skeletal muscle and on the arrhythmic abnormities expected during flight. As noted above, the failure of bone mass to return following microgravity exposure is of importance to Astronaut health. Confirmation of a "mismatch" between the return of muscle strength and bone mass would

indicate the need to develop a rigorous examination of reconditioning programs following space flight.

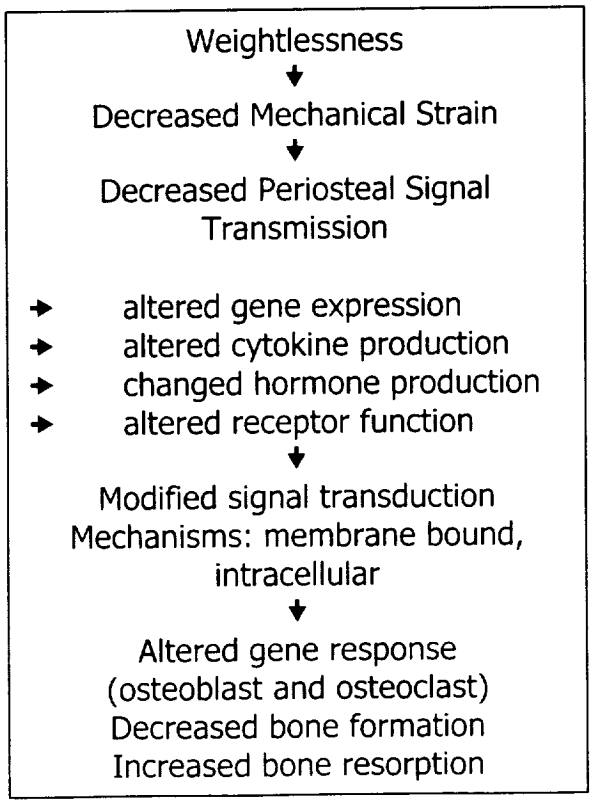
II. Introduction

Bone density measurements in both Astronauts and Cosmonauts indicate that bone loss approximates 1-2% per month in the majority of individuals. However, as illustrated in the data of Vico et al. (Lancet, 2000) obtained from Mir Cosmonauts, bone loss in the tibia during 6 months of weightlessness may vary from 0% in certain subjects to 23% in others. Furthermore, although the fracture rate among crew during flight is not public information, it is acknowledged that stress fractures have occurred in crew after return to earth's gravity and during the fitness re-conditioning process. Renal calculi are another complication of excessive mobilization of calcium from bone and dehydration, and this has also been a problem during and after space flight. Back pain, in part due to soft tissue injury is a common complaint during, and after flight, however, its etiology with regard to soft tissue changes is not defined.

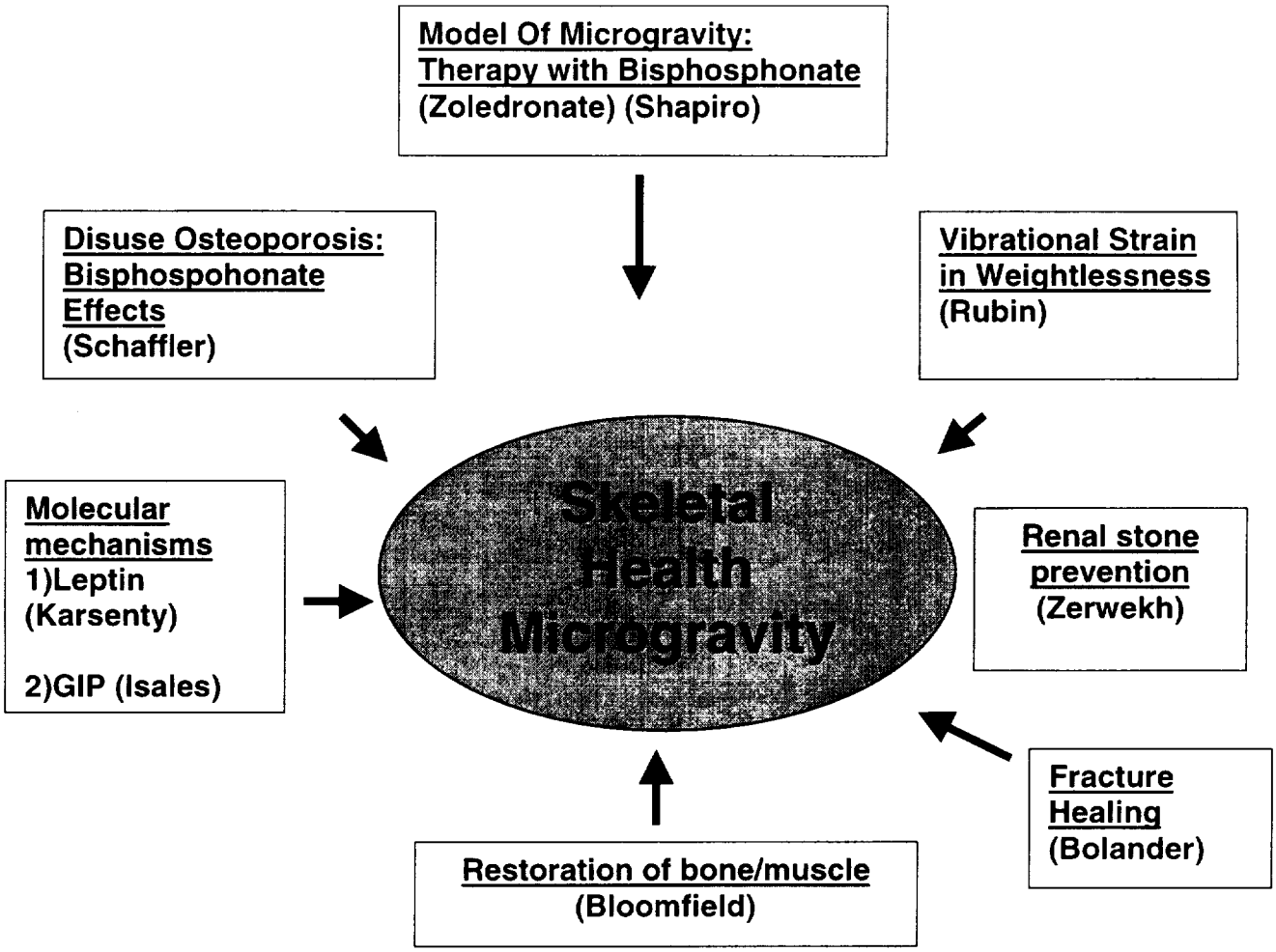
Accelerated bone loss, fracture healing and fracture risk, soft tissue injury and renal stone formation are the critical elements in the NASA Critical Risk formulation. Added to these is the problem of delayed return to pre-flight bone mass and as a corollary, the potential increase in fracture risk after return to earth's gravity that may continue for the life of the crew member.

Understanding the multiple factors promoting bone loss during non-weight bearing is critical to the development of countermeasures that will effectively and safely maintain bone mass during extended exposure to microgravity. It is understood that that the loss of normal mechanical strain exerted by muscle on bone initiates the process of bone loss. Altered interstitial fluid dynamics and altered hormonal/receptor interactions contribute to impaired bone cell function. The response of bone is a change in the balance of bone remodeling due to an increase in bone resorption and a decrease in bone formation which leads to net bone loss. Furthermore, after return to earth's gravity muscle mass returns within a period of several weeks, while bone mass remains diminished for more than a year: It may not return to pre-flight values for a longer period of time.

Contributing mechanisms to microgravity induced bone loss: The potential importance of the periosteum in modulating the effect of mechanical strain on bone is illustrated by DEXA analysis of femur shaft indicating inadequate response of periosteal bone to endocortical bone loss following microgravity exposure.



III. Research Program Structure and Design



Project List-1

Basic Studies (Countermeasure Readiness Levels: 1-3)

1. Leptin as a Regulator of Bone Formation in Microgravity Bone Loss: Karsenty, Elefteriou, Dacquin, (Baylor)

Leptin inhibits bone formation through unknown mechanisms following binding to hypothalamic receptors. We show here that unlike body weight bone formation cannot be affected by modulating plasma leptin levels. In contrast, when delivered centrally, minimal amount of leptin that do not affect body weight decreases bone mass by acting on a distinct group of hypothalamic neurons forming the central center of bone formation (CCBF). Genetic experiments demonstrate that the sympathetic nervous system mediates CCBF function. Thus, leptin regulation of bone formation does not follow the hormonal paradigm that applies for body weight control.

To elucidate the mechanisms by which leptin regulates bone formation we relied initially on the postulate that leptin acts as a hormone for this action as it does in the control of body weight. However, no relationships between leptin plasma level and bone mass could ever be established. In contrast several lines of evidence indicate that leptin, which is produced by hypothalamic neurons, acts locally with great efficiency to regulate bone formation. Chemical lesioning and the use of various mutant mouse strains identified a group of hypothalamic neurons that forms the central center of bone formation (CCBF) whose function is regulated by leptin. Finally we show that the efferent signal of the CCBF is also of neuronal origin. These results propose a novel bone formation-enhancing treatment for osteoporosis.

Identification of a central center of bone formation (CCBF).

To elucidate leptin mode of action on bone formation one must first identify hypothalamic neurons that control bone formation and that are a leptin target. Two hypothalamic nuclei are particularly rich in neurons expressing ObRb, the signal-transducing form of the leptin receptor, they are the arcuate nucleus and the ventromedial hypothalamic (VMH) nucleus. We used chemical lesioning to study their role in the regulation of bone formation.

Leptin plasma levels do not correlate with bone mass: We showed previously that ob/+ mice have a HBM phenotype. In the conceptual framework of a hormonal mode of action of leptin this observation was puzzling as these mice have a nearly normal leptin plasma level of the ob/+ mice, it raised the hypothesis that leptin may not use humoral means to control bone formation. To address this question thoroughly we created three different transgenic mouse models. In the first model we ectopically expressed *leptin cDNA* in osteoblasts under the control of the osteoblast-specific fragment of the *α1(I) collagen* promoter. The rationale of this experiment was to achieve a high level of leptin in the bone microenvironment with only a marginal increase of its plasma level to determine if, in vivo, leptin could act locally on osteoblasts. As shown in Figure 3 Panel A the two lines of α1(I) collagen-leptin mice that were analyzed expressed high levels of leptin in osteoblasts but had only a moderate increase of leptin plasma level. Histologic and histomorphometric analysis of the bone of these mice at 3 and 12 months of age did not reveal any modification in bone mass or in bone formation parameters. These results along with the absence of signal transducing leptin receptor on osteoblasts (Ducy et al, 2000) indicate that, in vivo, leptin does not act locally on the osteoblasts.

Next we constructed transgenic mice expressing *Leptin* cDNA under the control of the apoliproprotein E (ApoE) promoter and its liver-specific enhancer. These regulatory elements have been used before to achieve high serum levels of other circulating molecules. In these transgenic mice as in the following one (see below) we used modification of fat mass as an indicator of modified plasma leptin levels. Two transgenic lines (ApoE-leptin 1 and 2) were obtained, leptin plasma levels were increased 10 and 100 times compared to wild-type in lines 1 and 2 respectively. Progenies from both lines were lean with no detectable fat pad. This result demonstrated to us that this high plasma leptin level had deleterious consequences on one biologic function of leptin. However, histologic analysis revealed that bone volume was identical in 3 and 6 month-old ApoE leptin and wild-type mice. Furthermore, the bone formation rate, an indicator of osteoblast function, was normal in ApoE-leptin transgenic mice (data not shown). These data demonstrated that raising plasma leptin levels did not affect bone mass or bone formation parameters as efficiently as it affected fat mass.

To decrease leptin plasma levels we used the same regulatory elements to generate transgenic mice with high plasma concentrations of the soluble form of the leptin receptor (ObRe). ObRe should bind circulating leptin and thereby decrease circulating levels of free leptin. Two transgenic lines were studied at 3 and 6 months of age; in both lines plasma free leptin levels were decreased at least 50%, this resulted in an increase in the weight of fat pads of these transgenic mice, indicating that this ObRe overexpression was effective on one major leptin function. Despite this low free leptin level ApoE-ObRe mice had bone volume and bone formation rates that were undistinguishable from the ones of wild-type littermates. Taken together these three transgenic mouse models indicate that modifying leptin peripheral concentration while it can affect fat mass and body weight was could not affect bone mass. This suggests that other models are at work to explain leptin regulation of bone mass.

Bone formation regulation by leptin is independent of hyperinsulinism: Two lines of evidence indicate that hyperinsulinism is not the cause of the HBM phenotype observed in absence of leptin signaling. First, animals that are hyperinsulinemic and hypogonadic like A^y mice did not develop a HBM phenotype. Second, MSG-treated mice that are overweight and hypogonadic had increased bone formation by histomorphometry but were not hyperinsulinemic. These results with the normal insulin levels of one month-old ob/ob mice that already have a HBM phenotype establish that the HBM phenotype observed in absence of leptin signaling is not a consequences of hyperinsulinism.

Extreme sensitivity of bone mass to leptin when delivered centrally

To demonstrate this, we performed one month-long ICV infusion of decreasing amounts of leptin in several groups of wt mice. As previously reported ICV infusion of 8 and 4 ng/h of leptin led to a significant decrease in body weight whereas lower amounts of leptin were inefficient. In contrast ICV infusion doses of leptin as low as 1 ng/h caused a significant decrease in bone mass. This extreme sensitivity of bone mass to leptin delivered centrally is also consistent with the presence of leptin transcripts in the hypothalamus. Immunocytochemical analysis of wild-type and ob/ob brain revealed the existence of leptin producing neurons in the arcuate and VMH nuclei in wild-type, this staining was absent in ob/ob mice. The expression of leptin was also examined in MSG-treated and GTG-treated mice. The extreme sensitivity of bone formation to leptin when administered centrally, the presence of leptin-producing neurons in the hypothalamus together with the absence of correlation of leptin blood levels and bone mass are consistent with the hypothesis that leptin produced locally in the hypothalamus controls bone

formation. This hypothesis would explain the discrepancy we observed in leptin efficiency on bone formation when delivered centrally or peripherally.

Neuronal nature of the efferent signals controlling bone formation
So far we demonstrated the existence of a central center of bone formation formed by GTG-and MSG-sensitive neurons. We have also shown that in the leptin-dependent regulatory loop controlling bone formation the afferent arm is not of humoral nature and that hypothalamic neurons forming the central center of bone formation are exquisitely sensitive to leptin when administered centrally.

2. Therapeutic Modulation of Systemic Glucose Dependent Insulinotropic Peptide Levels to Counteract Microgravity-induced Bone Loss:

Isales, Bollag, Mulloy (Med. College of Georgia)

For the last three decades GIP has been considered a major "incretin" hormone, that is that its major role was to potentiate nutrient-induced insulin secretion. GIP is made exclusively in the small intestine and is secreted in response to fat, carbohydrates and amino acids. Interestingly however, the receptor for GIP is widely distributed being found in brain, adrenal gland, heart and endothelium. This receptor belongs to the seven transmembrane domain G-protein coupled family of receptors. Our laboratory was the first to describe that the receptors for GIP were also found in bone, in osteoblasts and osteoclasts. These receptors are functional being dually linked to the calcium and cAMP messenger systems. In *in vitro* experiments, GIP is anabolic for osteoblasts (increasing alkaline phosphatase activity and collagen type I message and synthesis) and inhibitory for osteoclasts (inhibiting PTH-induced bone resorption). In *in vivo* experiments, GIP administered to Sprague-dawley rats by tail vein injection or Alzet pump increases bone mass. In addition we have generated a transgenic mouse overexpressing GIP and these mice have a higher bone mass than their littermate controls. We have almost completed a study in which markers for bone turnover have been measured in these transgenic mice and hope to further characterize the changes in bone by bone histomorphometry. In addition, using Gene Chip technology we have found that GIP activates a number of growth factors in bone including transforming growth factor beta (TGF-Beta) and others currently under investigation. Funding by the NSBRI began in 2/01 for studies on the potential use of GIP for prevention of bone loss under conditions of microgravity. This work so far, has resulted in a manuscript being submitted to the Journal of Bone and Mineral Research and is the subject of a presentation in the Fall meeting of the ASBMR (Phoenix, AZ), acknowledging NSBRI support:

Manuscript:
(1) Zhong Q, Ding K-H, Bollag R, Isales CM. Glucose-dependent Insulinotropic Peptide-induced Elevations in Transforming Growth Factor Modulate Proliferation in Osteoblastic-like Cells, submitted, J Bone Miner Res., 2001

Abstract:
(1) Zhong Q, Ding K, Mulloy AL., Bollag RJ, Isales CM; Glucose-dependent insulinotropic peptide stimulates proliferation and TGF-beta synthesis in osteoblastic-like cells. American Society for Bone and Mineral Research, Phoenix, AZ 2001

Applied Studies **(Countermeasure Readiness Level-3-6)**
3. Receptor Countermeasures to Bone Loss in Microgravity
(Smith, Weigel, Bloomfield, Narayanan, Suva) (Baylor)

Mechanical unloading results in loss of bone mineral density (BMD) and is associated with an increased risk of fracture. Raloxifene is a selective estrogen receptor modulator (SERM) which inhibits bone loss associated with reductions in circulating sex steroids. To determine if raloxifene could alleviate unloading-induced bone loss, we examined the ability of raloxifene, in comparison to estradiol or placebo, to maintain BMD in 5-month old, ovariectomized (OVX) virgin female Sprague Dawley rats subjected to 28 days of hindlimb suspension (HLS). After OVX, animals were allowed to recover for ~four weeks and then randomized into raloxifene (R; 535 μg/day; n=10), 17β-estradiol (E2; 12 μg/day; n=9) or placebo (n=8) treatment groups. Hormones were administered via slow release pellets implanted immediately prior to the initiation of HLS. The tibia proximal metaphyses (total slice BMD, and cortical and cancellous compartment BMD) were assessed in vivo by peripheral quantitative computed tomography (pQCT; Stratec Research-M) prior to OVX, prior to HLS and post-HLS. At the conclusion of the unloading period, serum, urine and tissues were collected for analyses. Uterine wet weights in E2-treated rats were 4-fold greater than in placebo controls confirming appropriate hormone administration; raloxifene treatment was without effect. Urinary pyridinium cross-links and serum osteocalcin values were 50.4% and 39.3% lower in R and 31.0% and 30.0% lower in E2 treatment groups, respectively, in comparison to placebo indicating a reduction in bone turnover. Animals lost an average of 13.0% of total BMD at the proximal tibia as a result of OVX prior to HLS. Subsequent treatment with either R or E2 maintained total BMD over the period of HLS, while total BMD loss of 12% occurred in control animals. Interestingly, trabecular BMD loss was detected for all groups (placebo -30.4%, R -24.3% and E2 -19.6%), while cortical BMD was increased in R (+3.2%) and E2 (+3.5%) treated, but not placebo (-0.7%) groups. Ex vivo pQCT measurements of excised femur distal metaphysis also indicated that total BMD was greater in R (+16.5%) and E2 (+31.0%) groups than in placebo controls although this was due largely to an increase in trabecular and not cortical BMD. These results indicate that treatment of ovariectomized, unloaded female rats with raloxifene or estradiol alleviates loss of BMD associated with mechanical unloading, and suggests that estrogen receptor based therapies may have site-specific effects on the cortical or trabecular compartments depending on the skeletal site examined.

Significant decreases in 25(OH) and 1,25(OH)2 D occur during space flight. The reasons for this are not defined, however, it is appreciated that vitamin supplementation may not reverse these changes. Studies have been conducted using 1.25(OH)2 vitamin D and a selective vitamin D receptor agonist EB 89 (Weigel) .Both bone cell differentiation and bone mass are examined. Although the active form of vitamin D appears more effective than EB 89 in maintaining bone mass in the suspended rat, this occurs with the risk of hypercalcemia and hypercalciuria. Studies with selective D agonists will evaluate the risk/benefit ratio of various receptor agonist compounds.

Presentation: American Society for Bone and Mineral Research, Phoenix, Oct 2001.

4.Bone and Muscle Recovery From Simulated Microgravity: Bloomfield, Hogan, Smith, Warren, Schultheis) (Texas A & M, Baylor, Medstar Foundation, Georgia State University)

This series of investigations are designed to determine how mechanical interactions between bone and skeletal muscle contribute to the recovery of bone mass and bone strength after exposure to prolonged microgravity.

a) Acquiring the equipment and expertise to perform *in vivo* muscle stimulation studies

The first six months of this project focused on building an *in vivo* muscle stimulation system for adult rats and training of our laboratory staff by one of this project's consultants, Dr. Gordon Warren of Georgia State University. This system is a replica of that routinely used by Dr. Warren for studies of mouse muscle function, except scaled up in size for the adult rat that is being utilized in these studies. To successfully complete these studies, it is important the PI's laboratory be able to perform in-house measurements of muscle contractile properties in a highly reproducible fashion. We have learned how to utilize percutaneous needle stimulation of the posterior crural (ankle extensor) muscles in order to measure peak torque generation in isometric and concentric contraction, force-velocity curves, and fatigue curves, among other parameter (see Figure 1 below). Two visits by Dr. Warren to the PI's lab in College Station, TX were required to build the system from all its component parts, adjust the mechanics on the footplate and successfully calibrate force measurements, and to train Ms. Jan Stafinsky , Senior Research Associate in the PI's lab, in its use. Further troubleshooting of the system took another month or two.

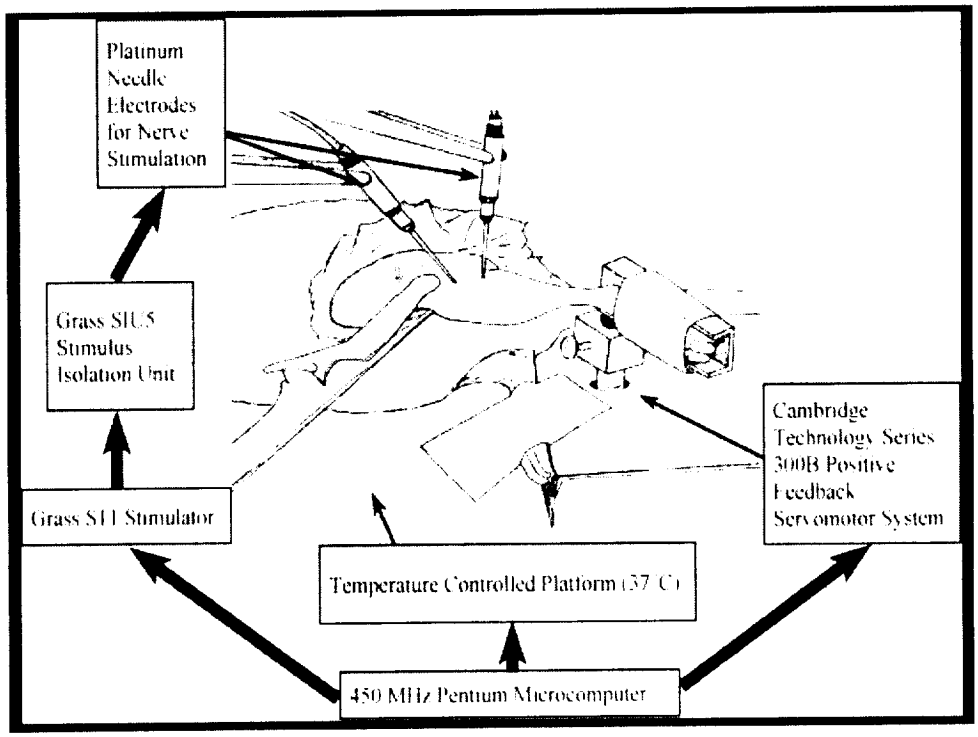


Figure 1
Percutaneous needle stimulation of ankle extensor muscles in the anesthetized rat. Force production at the ankle is measured by the servomotor controlling the foot plate.

For the first time course experiment of this project, these muscle function measurements are made just before sacrifice of the experimental animal. In later experiments, we anticipate using this system to "train" the posterior crural muscles during recovery, which will require the implantation of an indwelling nerve cuff around the tibial nerve.

b) Completion of 32 hindlimb unloading and recovery experiments, with *in vivo* measurements of muscle function and bone parameters by pQCT.

Time course experiments began in June of 2001; we are working with batches of 12-18 animals, including many aging (non-suspended) controls, within each batch. To date we have preliminary *in vivo* data on 32 animals and report herein some of those results. Dr. Smith's laboratory at Baylor College of Medicine has been performing osteoprogenitor cell studies on bone marrow harvested from femurs of these animals, but data are not yet available from those cell cultures.

We have been able to verify in these mature adult male rats that indeed peak muscle torque declines after 28 days of unloading to a similar magnitude as observed in young, growing rats. Figure 2 below illustrates the 26% reduction in peak isometric muscle force after 28-d HU, which appears to recover to baseline values after 14 days of normal cage activity.

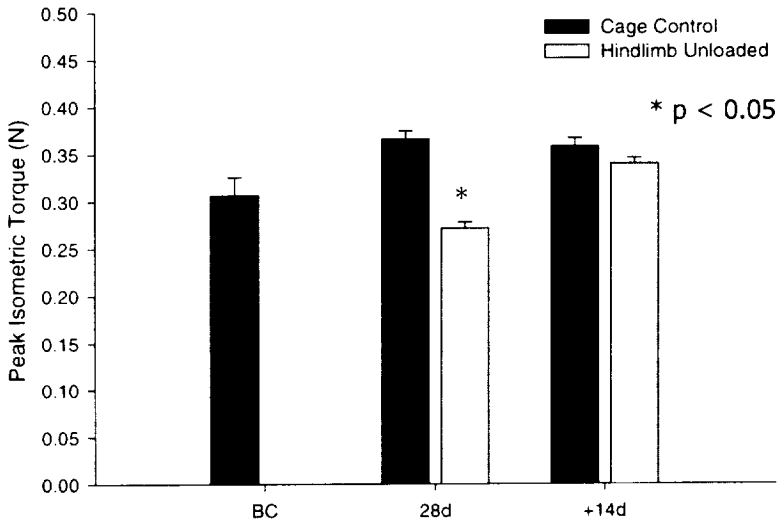
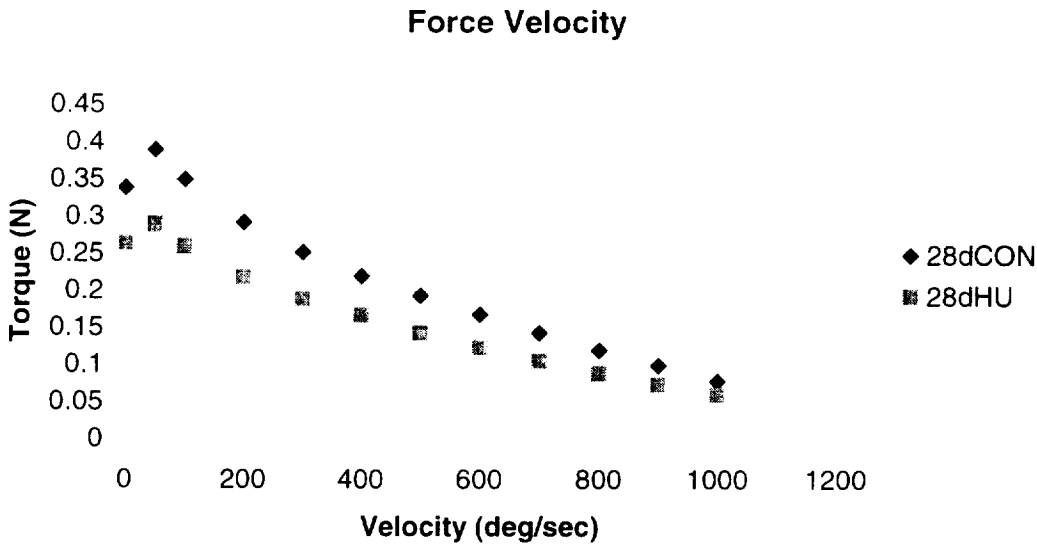


Figure 2. Changes in peak isometric torque with 28 d hindlimb unloading and 14 d recovery (normal cage activity) in mature adult male rats.

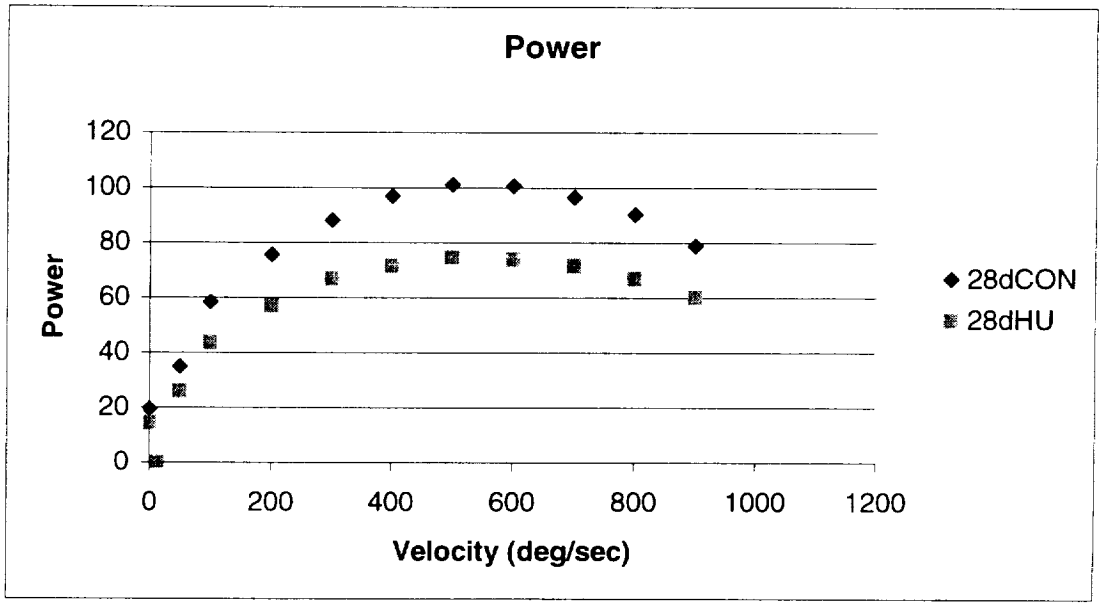
Additional measurements of muscle function performed include force velocity and power velocity curves, samples of which are illustrated in Figure 3 (following page). These are preliminary data, with no more than 5 animals per group [the eventual n/group should be 10 animals]. They illustrate a reduction in peak torques at the slower muscle velocities (< 600 deg/sec), but a reduction in power (rate of force production) at all but the slowest velocities. Data for fatigue curves are also being generated but are not yet tabulated; observation during testing verifies that the posterior crural muscles fatigue significantly faster after 28 d hindlimb unloading. These data are not unique nor new. What these studies will contribute to the literature is the simultaneous measurement of these muscle function parameters and bone parameters in the same animal so that scaling of the two interdependent tissues' function can be studied.

Figure 3.

A. Peak force generated during concentric contractions over a range of ankle extension velocities in control and 28-d hindlimb unloaded adult rats.



B. Power production by ankle extensor muscles over a range of velocities in control and 28-d hindlimb unloaded adult rats.



With the assistance of our consultant and accomplished muscle physiologist Dr. Gordon Warren, we will be analysing the muscle-focused data in much more detail over the next few months.

We are collecting *in vivo* pQCT data on these rats at the initiation of hindlimb unloading, after 28 d unloading, and (as applicable) after an assigned period of recovery. Preliminary data indicate modest bone loss of cancellous bone mineral density (-4.5 to 6%) at the proximal tibia after 28 days of unloading that in some cases worsens over the next 7 days of recovery.

Due to the large size of 6-month-old Sprague-Dawley male rats, and muscle torques that occasionally outstrip the capacity of our servomotor, we are presently exploring the feasibility of performing these experiments on Fischer rats.

Other outcome measures that are presently being generated on these animals:

Cell culture studies: number and viability of osteoblast progenitor populations
Histomorphometry of cortical and cancellous bone sites, to assess bone formation rates
Mechanical testing of cortical and cancellous bone sites

Publications:

Colleran, P.N., M.K. Wilkerson, S.A. Bloomfield, L.J. Suva, R.T. Turner, and M.D. Delp. Alterations in skeletal perfusion with simulated microgravity: a possible mechanism for bone remodeling. *J. Appl. Physiol.* 89: 1046-1054, 2000.

Bloomfield, S.A., H.A. Hogan, and M.D. Delp. Decreases in bone blood flow and bone material properties in aging Fischer-344 rats. *Clin. Orthop. Rel. Res.* (In press)

Bloomfield, S.A., M.R. Allen, H.A. Hogan, and M.D. Delp. Site- and compartment-specific changes in bone with hindlimb unloading in mature adult rats. *Bone* (In review)

Presentations:

Allen, M.R. and S.A. Bloomfield. (2001, August) *Periosteal resorption at the proximal tibiametaphysis due to mechanical unloading: evidence from longitudinal pQCT measurements.* Poster presentation: University of Utah Hard Tissue Workshop, Sun Valley, ID.

Bloomfield, S.A. and M.R. Allen. (2001, August). *Mature rat skeletal changes in response to simulated microgravity: a gender comparison.* Poster presentation: University of Utah Hard Tissue Workshop, Sun Valley, ID.

Allen, M.R. and S.A. Bloomfield. (2001, June). *Mature rat skeletal changes in response to simulated microgravity: a gender comparison.* Poster presented at the Annual Meeting of the American College of Sports Medicine, Baltimore, MD.

Bloomfield, S.A., M.R. Allen, M. Zhang, and R. Turner. (2001, April). *Greater deficit of bone formation in the mature adult rat with 28-d hindlimb unloading.* Poster presentation: Experimental Biology, Orlando, FL.

Allen, M.R., S.E. Gordon, B. Davis, M.L. Fiorotto, R.J. Schwartz, F.W. Booth, S.A.Bloomfield. (2000, September). *Skeletal muscle IGF-I overexpression alters bone development and prevents short-term hindlimb unloading bone loss in adult mouse tibia.* Poster presentation: Annual Meeting of the American Society of Bone and Mineral Research, Toronto, Canada.

Bloomfield, S.A., M.R. Allen, H.A. Hogan. (2000, September). *Site-specific changes in bone strength and mineral density with 28-d hindlimb unloading in the mature adult rat.* Poster presentation: Integrative Biology of Exercise, American Physiological Society, Portland, ME.

5. A Biomedical Countermeasure for Disuse Osteopenia

Rubin, Hadjiargyrou, Zhi, Judex, Dowd, Donahue (State University of New York at Stony Brook and Brookhaven National Labs).

The structure of the adult skeleton is determined, in large part, by the genome. Whether genetic variations may influence the effectiveness of interventions to combat skeletal diseases such as osteoporosis, remains unknown. The differential response of trabecular bone tissue to two distinct stimuli, one anabolic (low level mechanical vibration for 10min/d) and the other catabolic (hind-limb suspension), were evaluated in three strains of adult mice (C57BL/6J - low bone density; Balb/cByJ - mid-density; C3H/HeJ - high density). Protocols were applied for up to three weeks. In BALB/cByJ mice, the low level mechanical signal increased bone formation rates in the proximal tibia by 34% as compared to long-term control ($p<0.02$), while disuse decreased bone formation rates by 48% ($p<0.02$). In contrast, neither anabolic nor catabolic signals influenced any index of bone turnover in C3H/HeJ mice. Together, these data indicate not only a genetic basis for bone architecture, but also that the sensitivity of the tissue to both anabolic and catabolic stimuli is influenced by the genome. Extrapolated to humans, these results may in part explain why prophylaxes for osteoporosis are not universally effective, yet also indicate that there may be a genotypic indication of people who are at reduced risk of ever suffering from the disease.

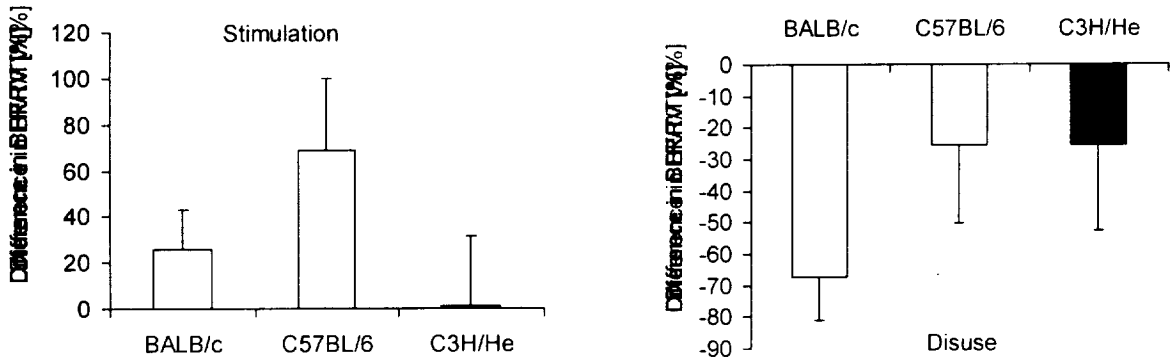


Fig. 1. *Percent difference in bone formation rates (BFR/TV) between* ***(a.)*** *mechanically stimulated and age matched control mice and* ***(b.)*** *disuse and age matched control mice in the three genetically distinct strains of mice (mean±SD of the difference). Labels here refer to BALB/cByJ, C57BL/6J, and C3H/HeJ. It is clear that the genetic makeup of the animals helps define the extent to which they respond to anabolic and/or catabolic stimuli.*

Papers/Abstracts published, in press, submitted:

Judex, S., Zhi, J., Xu, G., Hadjiargyrou, M., Rubin, J., Rubin, C. (2001) Osteoclast differentiation factor mRNA expression and bone formation rates related to disuse and mechanical stimulation are inversely proportional. *Transactions of the Orthopaedic Research Society* 47, San Francisco, CA, p. 39.

Judex, S., Xu, G., Donahue, L.R, Hadjiargyrou, M., Rubin, C. (2001) Changes in trabecular bone formation induced by mechanical stimulation and disuse are accompanied by differential gene expression in a mouse model. *Transactions of the Orthopaedic Research Society* 47, San Francisco, CA, p.533.

Judex, S., Zhi, J., Hadjiargyrou, M., Rubin, C. (2001) Inhibition of disuse osteopenia by low level mechanical stimulation is paralleled by alterations in gene expression. *Transactions of the NASA Bioastronautics Investigators' Workshop*. Galveston, TX, pp. 85-86

Rubin, C.T, Sommerfeldt, D.W., Judex, S., Qin, Y.X. (2001) Inhibition of osteopenia by low magnitude, high frequency mechanical stimuli. *Drug Discovery Today* 6(16), 848-858.

Rubin, C.T., Xu, G., Judex, S. (2001) The anabolic activity of bone tissue, suppressed by disuse, is normalized by brief exposure to extremely low magnitude mechanical stimuli. *The FASEB Journal* 15, 2225-2229.

Rubin, C.T., Judex, S., McLeod, K.J., Qin, Y.X. (2001) Inhibition of Osteopenia by Biophysical Intervention. In: *Osteoporosis*. Marcus, R., Feldman, D., Kelsey, J. (eds.) 2nd edition,Academic Press, San Diego, CA.

Judex, S., Lombardo, F., Donahue, L.R., Hadjiargyrou, M., Rubin, C. (2001) Changes in transcriptional activity induced by altered mechanical demand of the skeleton. *Annals of Biomedical Engineering* 29(S1), p. S37.

Judex, S., Hadjiargyrou, M., Donahue, L.R., Rubin, C. (2001) Trabecular bone from two strains of mice is differentially mechanosensitive at the tissue and molecular level. *Journal of Bone and Mineral Research* 16(S1), p. S151.

Judex, S., Rubin, C.T. (in press) Mechanical influences on bone mass and morphology – investigating how exercise may regulate adaptation in the skeleton. In: *Osteoporosis: Scientific Principles and Clinical Practice*. Orwoll, E.S., Bliziotes, M. (eds.). The Humana Press Inc, NJ.

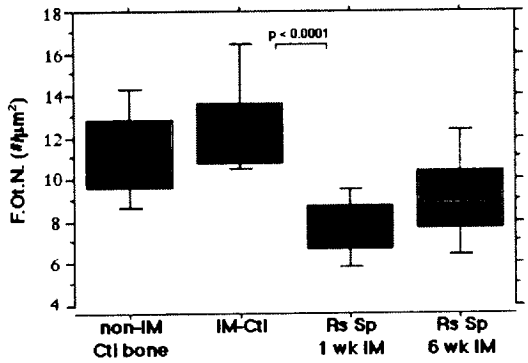
Judex, S., Donahue, L.R., Rubin, C.T. (submitted) Genotypic predisposition to osteoporosis is paralleled by an enhanced sensitivity to signals anabolic to the skeleton. *PNAS*.

6. Resorption Suppression and Bone Health in Disuse Bone Loss:
Schaffler , Jepsen (Mt. Sinai School of Medicine)

This protocol tests the hypothesis that long-term suppression of bone remodeling with bisphosphonate in a disuse situation will result in preserved bone mass and architecture but reduced resistance to fracture because of decreased osteocyte viability and integrity. Using a canine single limb immobilization (IM) model to produce a long-term disuse osteoporosis, animals receive treatment with risedronte or placebo control. At the end of one year of IM/treatment, cortical and cancellous bone will be assessed using micro CT to measure tissue architectural changes. Confocal microscopy will be used to assess osteocyte integrity. Whole bone and tissue-level biomechanical testing will be used to determine whether bisphosphonate-treated tissue retains normal mechanical properties, with particular emphasis on assessing changes in the fracture toughness (brittleness) in bone, which has been shown to be a very sensitive marker of alterations in bone quality.

Project update: (Active project period: 2/1/01 – present). This is a new project, with funding initiated in February, 2001. At the start of the project, we experienced a significant delay (4+ months) in initiating work on this project because of physical plant problems at Mount Sinai. These necessitated our finding alternative housing for our animals. This situation was resolved satisfactorily by arranging to house our dogs at the Bronx VA Medical Center (a part of the Mount Sinai program). However, this alternative arrangement introduced additional delays into our research schedule, as modification of the dog pen flooring at the VA was needed to accommodate our protocol. This modification took approximately 4 months to complete. Since June, 2001, we have been conditioning animals and entering them into the study, we now have all 28 experimental animals in the protocol. Acquisition of bones and tissues for biomechanical, compositional and microstructural analyses will begin in June 2002 and will be completed by August 2002.

During this start-up period, we have initiated studies of osteocyte integrity in immobilized canine bones that were archived as part of our previously funded NASA project. These studies confirm our preliminary observations, presented first in our NSBRI grant, that there is a significant loss of osteocyte integrity in areas of bone undergoing resorption. Osteocytes integrity was unchanged in non-resorbing areas of immobilized bone (IM-ctl). These observations are consistent with the recent observation that osteocyte apoptosis occurs in strong association with osteoclastic activity (Verborgt et al, JBMR 15:60, 2000), indicating that resorption serves as a mechanism for maintaining the integrity of the tissue, by removing nonviable osteocytes. These data have been submitted for presentation at the upcoming meeting of the Orthopaedic Research Society.



7. The Effect of Microgravity on Fracture Healing: Ultrasound as a Possible Countermeasure: Bolander, Turner, Greenleaf (Mayo Clinic)

The objective of this research is a determination of the effects of weightlessness on fracture healing, and the utility of ultrasound as a countermeasure to promote fracture healing.

Experimental Model:

- Tail suspended Lewis rat
- Males; 6 months of age at fracture; 15/gp
- Bonnarens Einhorn fracture model

Experimental Design:

- Evaluate Mechanical strength of callus 7, 5, and 3 weeks after fracture
- Determine time required for complete healing, i.e. equal in strength to non-fractured femur
- Identify cellular events by histology evaluation

Summary of Mechanical Data

We have evaluated Mechanical strength testing 5 and 7 weeks after fracture and histology in 3, 5 and 7 week groups. At 7 weeks a comparison of the mechanical characteristics of the fracture callus in tail suspension and weight bearing animals shows decreased rotation and energy absorption in callus from tail suspension animals. Stiffness is greater in the callus from the tail suspension animal, suggesting that the fracture calluses in tail suspension animals are more 'brittle". At 7 weeks neither weight bearing or tail suspension femur has mechanical properties equal to the non-fractured femur, indicating that neither has completely healed.

Summary of Histology

Histology from the 3, 5, and 7 week animals shows a similar pattern of repair in both the tail suspended and weight bearing animals. In both groups from 3 weeks to 5 weeks there is the progression of intramembranous bone formation, cartilage formation, and endochondral bone formation. Intramembranous bone formation and chondrogenesis are prematurely terminated in the calluses from tail suspended animals, however, resulting in a smaller fracture callus. Decreased cartilage in the fracture callus from tail suspended animals leads to earlier completion of endochondral ossification, but as shown by mechanical testing, this is associated with decreased energy resorption in these calluses.

Preliminary interpretation

We interpret these findings as indicating that in the tail suspended environment there is premature termination of the cellular events that are critically responsible for forming the fracture callus, including cartilage and bone formation. This results in a small fracture callus, with decreased energy resorption, and the formation of more brittle bone bridging the fracture site.

Experiments have not yet allowed us to determine the effect of tail suspension on the time required to complete fracture healing. Studies addressing that question are currently underway.

8. Defining and Preventing Bone Loss: A Microgravity Model:
Shapiro, Toerge, Ballard, Baldwin, Beck, Ruff, Burman, Mustapha: (Unformed Services University, Johns Hopkins University, National Rehabilitation Hospital)

Bed rest during 15-17 weeks using normal volunteers is an accepted method for simulating bone loss during weightlessness. This project: 1) employs the spinal cord injured subject (SCI), tetraplegic or paraplegic, as a model for the bone and muscle lost during exposure to weightlessness, and 2) employs zoledronate, a potent third generation bisphosphonate administered intravenously over 15 minutes, as a potential countermeasure to bone loss when exposed to weightlessness during space flight. The advantage of the SCI subject compared to normal volunteers at bed rest includes the ability to study subjects over longer duration and the fact that the pattern of bone loss and rates of bone loss from the lower torso is similar to that during space flight. In addition to ease of administration, an advantage of zoledronate is that its duration of action may extend to one year after a single injection of 5 milligrams, making it suitable for protection during extended duration flight. This is a double-blind, placebo controlled study lasting one year for each subject. The evaluation of research subjects includes geometric and structural analysis of bone density scans (DEXA) and 3-D finite element analysis of femur shaft for evaluation of fracture risk. Muscle biopsies are obtained at time 0 and 6 months from the vastus lateralis muscle and sent to Dr. Ken Baldwin's laboratory at U. C. Irvine for biochemical analysis.

Recruitment of quadriplegic or paraplegic subjects to this study has been slower than projected due to: 1) prospective SCI subjects not meeting study criteria, and 2) reluctance of injured subjects to enter an investigational study. Nevertheless, 6 subjects have entered the program to date. Additional information about rates of bone loss in SCI subjects and patterns of bone loss compared to that observed in Mir astronauts has been obtained from SCI subjects who have not met criteria for entry the therapy protocol but in whom bone loss has occurred due to weightlessness.

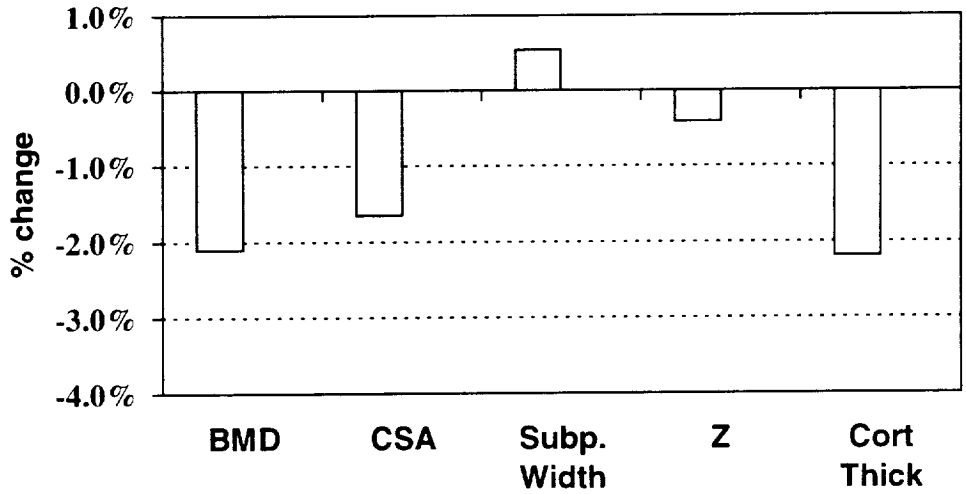


Figure 1: Change in Femur Shaft BMD Compared to Baseline: 46 Year Old Tetraplegic Woman

BMD= bone mineral density, CSA=cros sectional area, Sub.Width=subperiosteal width
Z=Z score, Cort Thick= Cortical thickness.

The rate of bone loss is in 3 months comparable to that reported following space flight. Measurement of sctional modulus (not shown) indicates a loss of bone strength during this period. In addition, attention is focused on the change in subperiosteal width which differs in microgravity or weightlessness compared to the increase in subperiosteal bone recorded in bone loss associated with normal aging. The results suggest that SCI is an appropriate model in which to evaluate therapeutic countermeasures to bone loss.

9. Prevention of Microgravity-Induced Stone Risk by KMg Citrate:
Zerwekh, Wuermeser, Pak, Antich, (UT Southwestern Medical Center at Dallas)

The formation of a renal stone during space flight may have serious negative effects on the health of the crewmembers and the success of the mission. Ground-based studies, as well as a limited number of space flight studies, have clearly demonstrated an increased risk for kidney stone formation as determined from the composition of the urinary environment. Increased bone resorption raises urinary calcium and the urinary state of saturation with respect to the calcium salts, calcium oxalate and brushite. However, documented changes in other urinary components such as citrate, pH, and magnesium appear to also raise the risk for the formation of not only calcium oxalate and calcium phosphate stones but also uric acid stones as well. Nutritional modifications to counter the tendency toward stone formation might include increased fluid consumption and supplementation with an appropriate nutriceutical that would decrease the risk of stone formation by increasing urinary pH and inhibitor concentrations. The hypothesis to be tested in this project is that potassium magnesium citrate supplementation will attenuate the increased risk for stone formation and diminish microgravity-induced bone loss. This hypothesis will be tested during five weeks of bed rest in normal volunteers through three specific aims: 1; assess the efficacy of supplementation with potassium magnesium citrate (KMgCit) in preventing microgravity-induced increased risk of renal stone formation. 2; evaluate the effect of KMgCit supplementation in averting diminished muscle magnesium and potassium concentrations that may occur during microgravity-induced muscle atrophy and 3; assess the efficacy of supplementation with KMgCit in reducing microgravity-induced increases in bone resorption and urinary calcium losses.

Research Program Structure and Design:
Subject data: Participating in the study will be 20 normal men and women between the ages of 18 and 50, of any ethnicity.
General study protocol: All subjects will undergo 8 weeks of study, comprised in succeeding order of 3 phases (A-C): **A**-Ambulatory (wk 1), **B**-Bed rest (wk 2-6), and **C**-Reambulation (wk 7, 8) (Table 1). The entire study will be conducted at the GCRC, University of Texas Southwestern Medical Center at Dallas. During weeks 2 through 6, the subjects will remain at complete bed rest. Subjects in the treatment group will receive potassium magnesium citrate (Relyte®), 3 tablets with breakfast and 3 tablets with dinner (42 mEq potassium, 21 mEq magnesium, and 63 mEq citrate) while the placebo-treated group will undergo an identical dosing regimen with placebo medication. This dose of KMgCit has previously been shown to significantly raise urinary potassium, magnesium, citrate, and pH.

Procedures: Specific aim 1: Assess the efficacy of supplementation with potassium magnesium citrate (KMgCit) in preventing microgravity-induced increased risk of stone formation. Metabolic evaluations will be performed each week of the study. During each metabolic evaluation, subjects will be maintained on a constant metabolic diet with a daily composition of 800 mg calcium, 1200 mg phosphorus, 100 mEq sodium, and constant fluids (3L) for the last 4 days of each week. During the last 2 days of the 4-day metabolic diet, urine will be collected in 24-hour pools under oil for stone-forming risk factors. Parameters to be quantitated include calcium, oxalate, uric acid, citrate, ammonium, pH, total volume, sodium, potassium, sulfate, phosphate, magnesium, chloride, creatinine, urinary saturation (relative saturation ratio of calcium oxalate, brushite, monosodium urate and undissociated uric acid), and deoxypyridinoline. Serum will be obtained in the fasting state for SMA-20, PTH (IRMA), 1,25-$(OH)_2D$, osteocalcin, and bone specific alkaline phosphatase on one of the last two days of each metabolic evaluation. In addition to measuring the urinary parameters as key indicators of the relative risk for kidney stone formation, we will also monitor changes in acid-base status via determination of net renal acid excretion and net gastrointestinal absorption of alkali.

Specific aim 2: Evaluate the effect of KMgCit supplementation in averting diminished muscle magnesium and potassium concentrations that may occur during microgravity-induced muscle atrophy Subjects will undergo two muscle biopsies during this study. The first will be obtained during week 1 of ambulation and the second (on the contralateral side) during week 6 following 5 weeks of bed rest.

Specific aim 3: Assess the efficacy of supplementation with KMgCit in reducing microgravity-induced increases in bone resorption and urinary calcium. This specific aim will be addressed principally from an examination of urinary calcium, phosphate, and the change in the biochemical markers of bone turnover. Ultrasound velocity of cancellous and cortical bone will be measured by critical angle reflectometry, in the calcaneus, tibia, trochanter, radius, ulna and frontal bone.

Research Program Accomplishments: Since the initiation of funding in June 2001, we have recruited two subjects for the aforementioned protocol. The first subject is now beginning his 3rd week of bed rest. The second has decided to quit the study after 1 week of immobilization due to personal reasons.

We anticipate recruiting all subjects within the next two years. If the results disclose that KmgCitrate is an effective countermeasure for renal stones, the future direction would be to evaluate the medication during space flight.

TEAM STRATEGY FOR COUNTERMEASURE DEVELOPMENT

1. Increase collaborative efforts and information exchange between the NSBRI Bone Team and scientists at JSC and Ames: The purpose of this is to facilitate countermeasure development directed at limiting bone loss. Two examples of this effort are:

a) NSBRI sponsorship of a full day meeting between Bone Team members and their counterparts from JSC and Ames, along with JSC leadership to develop a long range plan for testing animal and clinical countermeasures durint short term (shuttle) and long term (ISS) flights. A venue for this could be the Jan. 2002 Del Lago meeting.

b) Another example of this collaboration is increased collaboration concerning the standardization and effectiveness of current procedures designed to improve recovery of bone mass following flight

2. NSBRI leadership and Bone Team members should reviews and critically evaluate proposed methods to simulate gravity or otherwise provide mechanical strain induced osteogenic signals to bone in a weightless environment.

- vibrational stimuli (Rubin)
- artificial gravity nethods (Young, Caiozzo, etc)

3. Plans should be developed to test IV bisphosphonate, either pamidronate or Zoledronate, in a flight setting – shuttle studies initially with ISS studies thereafter. Metabolic studies should be performed in each setting. It is noted that the 5 mg zoledronate dose has been FDA approved for cancer treatment.

4. It is reported that K citrate is being tested during flight as a countermeasure to renal stone formation. It is proposed that similar studies evaluate the use of KMgCit in flight setting. It is reasonable to assume that bed rest studies in normal volunteers, while providing an indication of the effectiveness of these compounds in altering renal calculus indices, it is only in a weightless environment that the true effects on renal profiles will be apparent.

ANNUAL NSBRI PROGRAM REPORT
CARDIOVASCULAR ALTERATIONS TEAM

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Cardiovascular Effects of Simulated Microgravity in Man. Richard J. Cohen, contact information same as above.

Effects of Space Flight on Cardiovascular Stability (flight proposal in definition phase). Richard J. Cohen, contact information same as above.

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Influence of Gender and Age on Renal and Cardio-Endocrine Responses to Simulated Microgravity, Gordon Williams, Program Director, GCRC, Brigham and Women's Hospital, 221 Longwood Ave. 2nd Flr., RFB2, Boston, MA 02115-5817. Telephone: (617) 732-5661, Fax: (617) 732-5764, Email: gwilliams@partners.org

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I. EXECUTIVE SUMMARY

The objectives of the NSBRI Cardiovascular Alterations Team are to:

- Characterize and quantify the adverse effects of space flight on cardiovascular structure and function
- Determine the mechanisms of these adverse effects
- Develop effective countermeasures to these adverse effects
- Develop new cardiovascular technologies for use in countermeasure development and for spin-off applications on earth

The Critical Cardiovascular Risks associated with long term space flight include:

1. Impaired Cardiovascular Response to Orthostatic Stress
2. Occurrence of Serious Cardiac Dysrhythmias
3. Diminished Cardiac Function
4. Manifestations of Previously Asymptomatic Cardiovascular Disease
5. Impaired Cardiovascular Response to Exercise Stress

The first three risks are considered to be of considerably higher priority than the last two.

The Critical Issues faced by the Cardiovascular Team in addressing the Critical Risks include:

•Development of Suitable Experimental Models
•Development of Suitable Experimental Approaches
•Development of Mathematical and Computer Models
•Development of New Cardiovascular Technologies
•Addressing the Multiple Conditions Imposed by Space Flight
•Countermeasure Development Issues
•Determinants of Individual Susceptibility to the Adverse Cadiovascular Effects of Space Flight
•Cardiovascular Rehabilitation
•Development of Spin-off Technologies to Benefit Clinical Medicine on Earth
•Development of a Space Flight Database

Research Program Structure and Design

The Cardiovascular Alterations Team is designed to allow multiple investigators to interact and attack problems from the level of cells and molecules, to the level of tissue and organs, to whole animal studies, to human studies, and use of computer simulations to integrate the data and refine hypotheses to be tested experimentally. Integral to the team's effort is the development of new diagnostic and therapeutic technologies for the benefit of the space program and with spin-off applications here on earth. The goal of the team is to develop and test mechanism based countermeasures through a rigorous understanding of the basic biology and physiology. The effort is organized around the critical risks. There are multiple interactions between projects within the team as well as interactions with other NSBRI teams, and projects funded by other agencies.

Research Program Accomplishments

The multiple research accomplishments of each of the projects are discussed in this report. One major accomplishment to date is the successful testing in human ground based studies of a pharmacologic countermeasure (the alpha-sympathetic agonist midodrine) to the development of orthostatic intolerance after exposure to microgravity. This development of this countermeasure represents the culmination of investigations conducted at the cellular, tissue, organ, whole animal and human studies levels as well as accompanying computer simulations. A flight proposal, currently under review, will evaluate this countermeasure after space flight. A second accomplishment is the successful commercialization and dissemination into clinical use of microvolt T-wave alternans testing for the prevention of sudden cardiac death from ventricular arrhythmias.

Future Program Directions

The future planning for the team's activities revolves around the cardiovascular critical risks and moving projects from investigation of basic mechanisms through the proposing and testing of countermeasures.

II. INTRODUCTION

The objectives of the NSBRI Cardiovascular Alterations Team are to:

- Characterize and quantify the adverse effects of space flight on cardiovascular structure and function
- Determine the mechanisms of these adverse effects
- Develop effective countermeasures to these adverse effects
- Develop new cardiovascular technologies for use in countermeasure development and for spin-off applications on earth

RELEVANT RISKS

The research objectives of the NSBRI Cardiovascular Alterations Team are driven by the Critical Cardiovascular Risks associated with long-duration space flight which have been identified through a joint NASA – NSBRI effort.

1. Impaired Cardiovascular Response to Orthostatic Stress

Upon reentry into the Earth's gravitational field, astronauts experience orthostatic intolerance which limits their ability to function during reentry and after landing. In many cases, the orthostatic intolerance is sufficiently severe that astronauts cannot stand erect for some time after landing. Orthostatic intolerance represents a current operational problem, which may for example interfere with the ability of astronauts to egress from the spacecraft under emergency conditions. Currently used countermeasures include oral administration of salt and water prior to reentry and application of anti-gravity suits; these countermeasures are not adequate to prevent orthostatic intolerance in particular following long-duration spaceflight.

2. Occurrence of Serious Cardiac Dysrhythmias

Relatively little data are available on the association of space flight, and in particular long-duration space flight, with the development of heart rhythm disturbances. A number of anecdotal reports, including one documented 14 beat run of ventricular tachycardia during a Mir mission, suggest that long-duration space flight might lead to an increased incidence of potentially serious heart rhythm disturbances. However, data are currently inadequate to determine whether space flight in fact predisposes the heart to rhythm disturbances. If space flight does in fact significantly decrease cardiac electrical stability the effects could be catastrophic potentially leading to sudden cardiac death. Thus in this area the overriding research question is whether space flight in fact increases susceptibility to cardiac dysrhythmias. If space flight is found to increase the risk of cardiac dysrhythmias then it will be important to determine the mechanisms in order to develop appropriate countermeasures.

3. Diminished Cardiac Function

Long-term space flight may lead to a measurable reduction in cardiac mass. It is believed that this loss of cardiac mass is associated with cardiac remodeling. It is not known whether these cardiac alterations are reversible and whether they pose a long-term health risk to astronauts. The extent to which cardiac atrophy and remodeling may affect cardiac performance during long-duration space flight is inadequately understood. Investigation is required to establish the extent, reversibility, and physiologic sequelae of cardiac atrophy; to identify stimuli and signals

that lead to loss of cardiac mass and remodeling, and to identify countermeasures that may prevent these alterations.

4. Manifestation of Previously Asymptomatic Cardiovascular Disease
Long-duration space flight may in principle exacerbate previously undetected cardiovascular disease such as coronary artery disease. Research is needed to determine what procedures should be applied to screen astronauts for asymptomatic cardiovascular disease prior to long term missions. This risk is considered as lower priority than risks 1-3.

5. Impaired Cardiovascular Response to Exercise Stress
Long-term space flight may impair cardiovascular response to exercise. However, in-flight exercise programs appear adequate to maintain aerobic exercise capacity. However, research is needed to determine the type, duration and frequency of exercise necessary to maintain the integrity of the cardiovascular system and potentially prevent cardiac atrophy, and to determine whether thermoregulation is impaired during exercise. Because adequate countermeasures are considered to be effective, this risk is considered as lower priority than risks 1-3.

CRITICAL ISSUES

Experimental Models
Because of the limited opportunity for studies in space, the great majority of experimental cardiovascular research in this area involves animal or human ground-based models of space flight. One question that arises is the extent to which these ground-based models yield results which correspond to space flight. Additional data from space flight is required to evaluate the degree of correspondence with data from ground-based models in order to help validate these ground-based models.

Experimental Approaches
Investigations are required which range from the molecular, genetic and cellular level to the organ system level to the level of the entire organism.

Mathematical and Computer Models
Space flight causes alterations in multiple interacting physiologic systems. The use of mathematical and computer models is required to elucidate mechanisms, interpret data, and formulate hypotheses to be tested experimentally. Such models include forward models that simulate integrated physiologic behavior, and inverse models used to create an individualized model of physiologic function from data recorded on a single individual.

New Cardiovascular Technologies
New cardiovascular diagnostic and therapeutic technologies based on an understanding of the underlying physiology, are required to help answer research questions, to develop countermeasures, to serve as means for monitoring astronaut cardiovascular function, for treatment of astronauts, and for applications to clinical medicine here on earth.

Conditions of Space Flight
In addition to microgravity other conditions of space flight may also adversely affect the cardiovascular system including sleep disruption, reduced physical stress, environmental factors, and psycho-social stresses.

Countermeasure Development
Development of countermeasures should be based on understanding of the underlying physiologic mechanisms. Countermeasures may include pharmacological, nutritional, and physical interventions (including artificial gravity), and modifications of behavior, activity and environment.

Individual Susceptibility
Investigation of factors that make an individual more susceptible to the adverse effects of space flight on the cardiovascular system may include age, gender, genotype, and dietary, occupational and physical conditioning history.

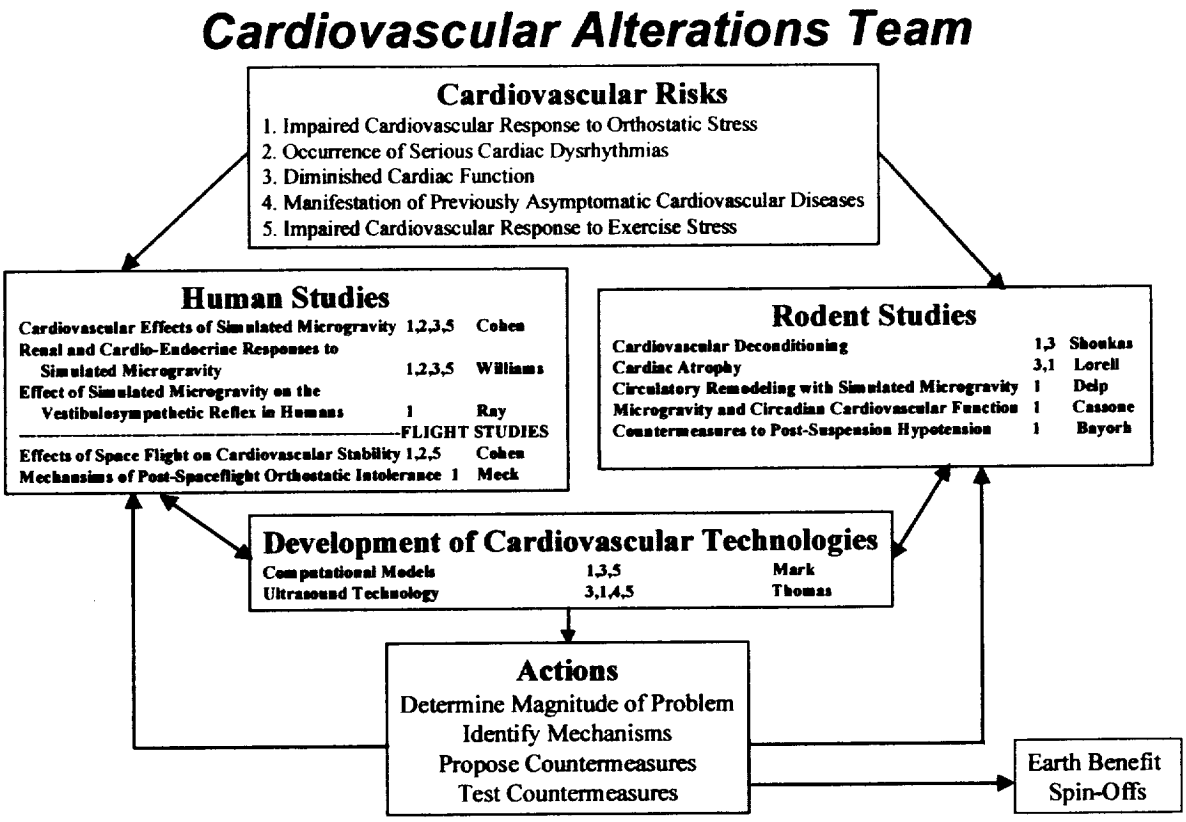
Cardiovascular Rehabilitation
The adverse effects of space flight on the cardiovascular system may persist following re-entry into a gravitational field. Identification of appropriate strategies for short-term and long-term cardiovascular rehabilitation are needed.

Earth Benefits
Studies of the adverse effects of spacef light on the cardiovascular system may also have important implications for clinical medicine issues on earth.

Cardiovascular Space Flight Database
There is an urgent need to obtain and make available to investigators a systematic database of cardiovascular data from astronauts before, during and after space flight.

III. RESEARCH PROGRAM STRUCTURE AND DESIGN

Description of Current Program
The current research program is summarized in the Figure. Many of the projects impinge on more than one critical risk. The strategy of the cardiovascular team is to have a dynamic interplay between projects focused on studies in animals, humans, and computer simulations. Part of the strategy is also to develop new technologies to be used in the studies but which also have applications for astronaut monitoring and therapy and spin-off applications on earth.



Figure

Numbers indicate risks associated with project in order of emphasis in the project

1. Impaired Cardiovascular Response to Orthostatic Stress

The primary focus of the cardiovascular alterations team has been on the problem of post-flight orthostatic hypotension. In animal and human studies we have studied mechanisms, proposed and tested countermeasures. We are examining a wide range of factors that may contribute to the development of orthostatic hypotension. These factors range from alterations in autonomic control, alterations in the vasculature and lymphatics, alterations in cardiac function, alterations in diurnal rhythms, vestibular effects, changes in endocrine and renal function. The dynamic interplay between animal, human and computer simulations has already led to the proposing of the alpha-symphathetic agonist, midodrine, as a pharmacologic countermeasure to the development of orthostatic hypotension, and the successful testing of this countermeasure in animal and ground based human studies. Flight studies of this countermeasure have been submitted and are in the definition stage.

2. Occurrence of Serious Cardiac Dysrhythmias

One current project (Cohen) deals with the issue of whether simulated microgravity alters cardiac electrical stability. Preliminary ground based data suggests that this may be the case, and flight studies have been proposed in this regard as well.

3. Diminished Cardiac Function

One project (Lorell) deals primarily with the risk of diminished cardiac function and several other projects relate to this problem as well. The focus here is to establish the development of

atrophy and remodeling in ground based models and to study molecular and genetic mechanisms and functional sequelae. We require more flight data documenting the extent of the space induced cardiac atrophy and remodeling that occurs during flight.

4. Manifestation of Previously Asymptomatic Cardiovascular Disease
There are no current projects in this area.

5. Impaired Cardiovascular Response to Exercise Stress
Although several of the projects address exercise this is not a primary focus of any of the current projects.

6. New Cardiovascular Technologies
The progress of the cardiovascular alterations team has been heavily dependent on the development of new technologies driven by the understanding of the physiology. These include computer simulation technologies, cardiovascular system identification technology for the non-invasive quantification of closed cardiovascular regulation, measurement of microvolt T-wave alternans to assess cardiac electrical stability, and ultrasound technologies for the non-invasive assessment of cardiovascular function. One of these technologies (T-wave alternans) has already been successfully commercialized for clinical use here on earth. Future progress will be dependent, in part, on the development of new diagnostic and therapeutic technologies.

Synergies
There are multiple connections between the various projects within the cardiovascular team. We have encouraged collaboration between the projects. The close collaboration between the projects has been responsible for the development of the midodrine countermeasure for orthostatic intolerance. The studies that led to the proposal for this countermeasure included *in vitro* studies of the responsiveness of cells and vascular segments to alpha sympathetic stimulation, *in vivo* animal studies, human studies and computer simulations that were used to simulate midodrine administration. Some projects are very closely allied. For example Williams and Cohen share a single bed rest study to generate the data for their projects, and these data are transferred to Mark's cardiovascular simulation project. Thomas' ultrasound technology project is responsible for overseeing the acquisition of the ultrasound data and analyzing these data in the human studies and will be similarly responsible for the ultrasound data from the flight studies. The projects involved in animal studies are collaborating on experiments and sharing of tissues (Bayorh, Cassone, Delp, Lorell, Shoukas). We are promoting interaction between investigators by holding team retreats approximately twice each year, and having regular teleconferences. We have found the team retreats to be the most useful means of exchange. We keep the formal presentations short, and focus on intensive discussions.

In addition, we have active collaborations with other NSBRI teams. Two investigators also are affiliated with other teams (Cassone – Human Performance, Thomas – Smart Medical Systems). Shoukas is working with the Technology Development Team on development of a pulsatile G-suit. Ray has a natural connection to the NeuroVestibular Team. We look forward to forming alliances as well with the Nutrition and Rehabilitation Team, Integrated Human Function, Muscle, and other teams. Furthermore, many of our investigators are involved in research

sponsored by NIH, NSF and other agencies. Their NSBRI work benefits from, and contributes to, such other research.

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

The various projects are at different stages. Some are projects that are renewed from the last grant cycle, some have just received their funding in the past few months. Some of the highlights from the various projects are listed below.

Bayorh

•Both the nitric oxide synthase inhibitor L-NAME and the prostacyclin synthase inhibitor U-51605 block the post-suspension hypotension observed in male Sprague-Dawley rats.

•Thus, post-suspension hypotension may involve increased levels of nitric oxide and/or prostacyclin.

Cassone

•The effects of deafferentation of the suprachiasmatic to sub-paraventricular pathways on the circadian variation in body temperature, activity and heart rate were determined in 12 Long Evans rats. Sham surgeries resulted in only temporary disruption of all parameters. Sub-paraventricular knife-cuts abolished or reduced the amplitude of heart rate rhythms but had no effect on activity or body temperature rhythms.

Cohen:

•Bed-rest reduces autonomic reflexes as measured by Cardiovascular System Identification.

•Reduced sympathetic reserve as measured by Cardiovascular System Identification identifies subjects with greater orthostatic intolerance.

•Alpha-sympathetic agonist midodrine is an effective countermeasure to the development of orthostatic intolerance.

•Bed-rest induces Microvolt T-Wave Alternans suggesting increased susceptibility to ventricular arrhythmias.

•Microvolt T-Wave Alternans identifies patients with heart failure at risk for sudden cardiac death.

•Microvolt T-Wave Alternans testing enters widespread clinical use.

Delp

•Hindlimb unloading does not alter eNOS mRNA content of middle cerebral arteries.

Lorell

•Isolated adult cardiac myocytes can be subjected to protocols which examine function and intracellular calcium regulation during high work states testing limits of cardiac reserve,
thus providing surrogate experimental model addressing high work states potentially affecting adult human heart during prolonged space flight (ie severe exercise or reentry).

•The heterotopic transplant model (rodent) provides a highly standardized and reproducible model to study gradations of cardiac unloading, as well as effects at molecular and functional level of interventions appropriate for immediate application in human space flight.

•Cardiac unloading, potentially simulating prolonged microgravitational unloading during human spaceflight, results in progressive changes in cardiac gene expression which modify both critical calcium-regulatory gene expression and contractile reserve (capacity to abruptly increase cardiac performance) at higher work states.

Mark

•Response to Microgravity:

- Developed computational model of cardiovascular system (including atria) capable of simulating HUT and LBNP.
- Developed automated method to match simulations to experimental recordings.
- Established a resource for data acquisition, formatting, and archiving.
- Acquired ~70 recordings of astronaut pre- and post-flight tilt/stand test data from JSC (Courtesy of Dr. Janice Meck).
- Developed JAVA version of the CV simulator.

•Response to Disease:

-Launched major data collection effort from ICU patients at BIDMC (400 patient-days/month – 40 GB/month).

- Developed database structure for hemodynamic waveforms and clinical data.
- Developed wavelet based multi-parameter algorithms to define clinically significant events.

Ray

•Completed a series of 1 day bed rest studies.

•Found that MSNA responses to vestibular otolith activation does not change with 1 day of bed rest.

•MSNA responses to otolith activation during lower body negative pressure are attenuated after 1 day of bed rest.

Shoukas

Recent finding suggest that in the HLU rat model:

•The contractile responses of the heart to sympathetic stimulation are attenuated and may contribute significantly to overall cardiovascular deconditioning and orthostatic hypotension.

•There is an increased venous capacitance and impaired contractile response of mesenteric veins and venules to sympathetic stimulation.

•There is an endothelial dependent vascular hypo responsiveness in the pulmonary vascular bed secondary to up regulation of eNOS and guanalyl cyclase.

Thomas

•Digital Echocardiographic Storage/Analysis/Database/ Capability Complete.

•Bed Rest Data Analysis– Boston NSBRI Experience.

•Novice Echocardiographic Training Evaluation - Cardiovascular Functional Assessment with Color Doppler M-mode and Strain Imaging.

Williams

•Simulated microgravity for 16 days does not change the circadian rhythm of melatonin (a surrogate marker for core body temperature), renin, cortisol, or aldosterone.

•Simulated microgravity significantly increases heart rate without changing systolic or diastolic blood pressure.

•Simulated microgravity results in a 3% reduction in bodyweight with a constant high salt intake with a recovery of one half of this loss within two days of return to ambulation.

•As a group simulated microgravity produces cumulative potassium changes sufficient to affect aldosterone secretion.

•With sleep deprivation aldosterone, cortisol, plasma renin, and blood pressure were not different from simulated microgravity alone.

•Sleep deprivation with simulated microgravity did produce significant decreases in serum postssium and sodium and less weight reduction than simulated micro-gravity alone suggesting that they're may have been more water retention with sleep deprivation.

Team

•Successfully sponsored featured symposium on the *Cardiovascular System in Space* at the 2000 annual Computers in Cardiology Meeting held in Cambridge, Massachusetts.

•Midodrine countermeasure successfully developed and tested and ready for flight studies.

V. FUTURE PROGRAM DIRECTIONS

Strategy

The objective of the team is to continue to address the critical risks and to move from basic research studies to countermeasure development and evaluation in future years. Below we discuss our plans for each of the risk areas and the area of new cardiovascular technologies. We sketch our strategy over the next five years and the following five years. Please refer to the Table as well.

1. Impaired Cardiovascular Response to Orthostatic Stress

2002-2006

We plan to continue our mechanistic studies ground based studies to further our understanding of mechanisms of post-flight orthostatic intolerance, and continue to evaluate potential mechanistically driven countermeasures which may include pharmacologic, diet, and exercise as well as artificial microgravity. We do expect to conduct two flight studies during this period, including one involving evaluation of the midodrine countermeasure. One investigator (Shoukas) has also been developing a technologically advanced G-suit which we will also consider evaluating. An important gap in our knowledge involves relating space flight data to our ground based models. We will work with a NASA-NSBRI initiative to develop a cardiovascular physiological flight database from data collected routinely during ongoing flights.

2007-2011
The focus in these years will be in testing countermeasures and increasing the proportion of flight studies.

2. Occurrence of Serious Cardiac Dysrhythmias
2002-2006
The goal during this period is initially to collect additional data from bed rest studies and the planned flight studies to establish whether in fact space flight does increase the risk of cardiac arrhythmias. An important element of this is also the collection of routine ECG data as part of developing a cardiovascular flight database. During this period we plan to be able to answer the fundamental question of whether space flight does increase risk of ventricular arrhythmias, and assuming that the answer is positive we will begin mechanistic studies to identify mechanism and identify countermeasures. Potential countermeasures include pharmacologic agents, diet, and artificial gravity.

2007-2011
Assuming that we have established in the previous period that space flight increases the risk of ventricular arrhythmias, and that we have established basic mechanisms and identified countermeasures, the focus during this period will be testing the countermeasures in ground based and flight studies.

3. Diminished Cardiac Function
2002-2006
The goal here is to establish the extent of and mechanisms of cardiac atrophy and to begin to evaluate countermeasures which are primarily hormonal (pharmacologic) and possibly exercise. The focus will be on ground based animal and human studies, and collecting pre and post flight cardiovascular database data on the extent of cardiac atrophy.

2007-2011
The focus here will be on countermeasure development in ground base and flight studies.

4. Manifestation of Previously Asymptomatic Cardiovascular Disease
2002-2006
The plan for this period is to launch at least one longitudinal ground based study the purpose of which will be to identify suitable screening techniques to identify silent cardiovascular disease that is likely to manifest itself during long duration space flight.

2007-2011
If a successful screening program is identified, testing of this procedure in potential astronauts is anticipated.

5. *Impaired Cardiovascular Response to Exercise Stress*

2002-2006

The plan for this period is to launch one study examining whether one can achieve improvement to existing countermeasures for this risk, in conjunction with the use of exercise as a countermeasure for the other risks.

2007-2012

If improved exercise countermeasures are identified, then testing of these countermeasures will be conducted in ground based and flight studies.

6. New Cardiovascular Technologies

2002-2006

The team plans to develop a training and research program for the development of new diagnostic and therapeutic cardiovascular technologies emanating from the team's research program. The goal is to develop innovative technologies to be used in the team's research program, for astronaut monitoring and treatment, and for spin-off applications here on earth.

2007-2012

We plan continued technology development as well as ground based and flight testing of the technologies previously developed.

Table
Cardiovascular Strategic Plan

	Current	2002-2006	2007-2011
Orthostatic Intolerance	Mechanistic Studies Countermeasure Studies Ground Studies Countermeasure Readiness 6	Mechanistic Studies Countermeasure Studies Ground Studies Pre and Post Flight Studies Countermeasure Readiness 7	Mechanistic Studies Countermeasure Studies Ground Studies Pre and Post Flight Studies Countermeasure Readiness 8
Dysrhythmias	Mechanistic Studies Countermeasure Studies Ground Studies Countermeasure Readiness 2	Mechanistic Studies Countermeasure Studies Ground Studies Pre and Post Flight Studies Countermeasure Readiness 3 - 5	Mechanistic Studies Countermeasure Studies Ground Studies Pre and Post Flight Studies Countermeasure Readiness 6
Impaired Cardiac Function	Mechanistic Studies Ground Studies Countermeasure Readiness 2	Mechanistic Studies Countermeasure Studies Ground Studies Countermeasure Readiness 3-4	Mechanistic Studies Countermeasure Studies Ground Studies Flight Studies Countermeasure Readiness 5-6
Manifestation of Asymptomatic CV Disease	Screening Methods Study Ground Studies Countermeasure Readiness 1	Screening Methods Study Ground Studies Countermeasure Readiness 2-3	Screening Methods Study Ground Studies Flight Studies Countermeasure Readiness 4-5
Impaired Response to Exercise Stress	Mechanistic Studies Ground Studies Countermeasure Readiness 2	Mechanistic Studies Countermeasure Studies Ground Studies Countermeasure Readiness 3-4	Mechanistic Studies Countermeasure Studies Ground Studies Flight Studies Countermeasure Readiness 4-5
New Cardiovascular Technologies	Ultrasound effort in conjunction with Smart Med Team	Establish training and research program to develop new cardiovascular technologies based on team's research	Test and apply new technologies to countermeasure development, astronaut monitoring and treatment, and spin-off applications

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

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I. PROGRAM EXECUTIVE SUMMARY

Research Problem and Basic Approach

The success of human space missions depends on each astronaut remaining alert and vigilant while operating sophisticated equipment and following complex procedures. During long-duration space flight, the space environment affects a number of physiological systems critically involved in human performance, and it is vital to mission success to understand the biological limits of human performance under space flight conditions. This team is focused on these issues and, in particular, is concerned with the following aspects of the space environment: microgravity, altered light-dark cycles, altered or reduced sleep/rest opportunities, high levels of automation, and habitation in a remote, inaccessible location. The primary thrust of this team's research program involves altered circadian organization, sleep disruption and cumulative sleep loss, and the associated neurobehavioral decrements occurring during long-duration space flight.

The goals of the Human Performance Factors, Sleep and Chronobiology Team are to: (1) Characterize and quantify the adverse effects of long-duration space flight on sleep and circadian rhythms; (2) Characterize and quantify the effect of sleep loss and/or circadian dysfunction on physical and neurobehavioral performance; (3) Understand the basic mechanisms underlying the deterioration of sleep, circadian organization and human neurobehavioral function during space flight; (4) Develop high-fidelity mathematical models of performance based on circadian organization and sleep-wake history; (5) Develop effective countermeasures to optimize sleep and facilitate circadian adaptation in the space environment and thereby maintain optimal neurobehavioral performance; (6) Develop new methods for monitoring the status of sleep, sleep homeostasis, circadian rhythmicity and neurobehavioral performance during space flight, with possible spin-off applications on Earth.

The overall team strategy and a description of each project is described in detail elsewhere (see the web site http://www.nsbri.org/Research/Sleep.html). The team research objectives are driven by the Critical Path Roadmap related to Human Performance Failure because of Sleep and Circadian Rhythm Problems. The current research program involves nine ground-based research projects. Many of the projects impinge on more than one critical risk. The strategy of the Human Performance Factors, Sleep and Chronobiology Team is to develop a synergistic interaction between research projects at the molecular, cellular, organismic, and human levels, and to integrate predictive biomathematical modeling of the sleep and circadian systems.

Within this area of research, the following five interrelated themes define the range of factors critical for optimizing human performance capability and improving crew health and safety:

A. Effects of long-duration space flight on sleep and/or circadian rhythmicity. This theme addresses the impact of the conditions of long-duration space flight (microgravity, altered light intensity, loss of geophysical cues, isolation, altered physical activity, etc.) on neurobiologic, endocrinological, and behavioral mechanisms (molecular, cellular and organismic) that control sleep and circadian systems.

B. Effects of sleep loss and/or circadian dysfunction on physical and neurobehavioral performance. The focus of this theme is to identify the range of acute and chronic adverse effects that sleep loss, sleep disruption, and/or circadian dysfunction have on critical physiologic and performance parameters during long-duration space flight (e.g., neurophysiologic function, physiological alertness, vigilance, cognitive performance, mood/morale, problem solving and communication).

C. Monitoring and assessment during space flight. This theme deals with the development of methods for monitoring the status of sleep, sleep homeostasis and circadian organization, as well as technologies that assess and update the current functional status or performance capability of the individual.

D. Predictive modeling of performance based upon circadian organization and sleep homeostasis. This theme is concerned with the development of analytical or phenomenological mathematical models that predict individual performance capability by involving multiple subsystems (e.g., circadian rhythmicity, sleep homeostasis, work-rest schedules, etc.) as an integrated unit across levels of organization, and by estimating the impact of countermeasure use designed to optimize physical and/or neurobehavioral performance.

E. Countermeasures. The research program of this team will not only define the impact of the space environment on sleep and circadian rhythmicity and the effects of the sleep loss and circadian dysfunction on performance but also will develop methods to counter the adverse physiological and behavioral events. These countermeasures may include behavioral, pharmacological, environmental or other adaptive approaches to maintain function and performance under the adverse conditions of long-duration space flight.

Description of Current Research Program

The Human Performance Factors, Sleep and Chronobiology Team is focused on developing countermeasures for the sleep loss and circadian dysfunction and associated neurobehavioral performance decrements that occur during long-duration space flight. The initial strategic research program for the Human Performance Factors, Sleep and Chronobiology Team involves nine research studies that collectively address the five thematically interrelated questions described above. The nine current ground-based experiments that comprise the NSBRI Human Performance Factors, Sleep and Chronobiology Team are summarized below:

Brainard et al.: Optimizing Light Spectrum for Long Duration Space Flight. The physiological changes caused by disturbed circadian rhythms and altered sleep-wake patterns can result in decrements in alertness, concentration, and performance. This project addresses these risk factors which threaten the safety of personnel and the objectives of space missions. ***Countermeasure goals*** include: 1. Identification of the optimum spectral stimuli for photic regulation of melatonin and the circadian pacemaker. 2. Design specifications for spectral transmission of space suit visors and the windows used in space vehicles and habitats; 3. Engineering parameters for the ideal spectral distribution for illumination of general living quarters during space exploration.

Czeisler et al.: Circadian Entrainment, Sleep-Wake Regulation & Performance in Space Flight. The goal of this project is to develop countermeasures to facilitate adaptation of the human circadian pacemaker to the 24.65-h day length of Mars, which is outside the range of entrainment of the human circadian pacemaker given the weak synchronizing stimuli within the Martian habitat. The primary ***countermeasure goal*** is to evaluate the efficacy of intermittent bright light pulses as a treatment to prevent the misalignment of circadian phase, sleep disruption, associated decrements in neurobehavioral performance and reduction in nocturnal growth hormone secretion experienced by individuals exposed to the 24.65h Martian day.

Dinges et al.: Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss. Using a response surface experimental paradigm (RSM), this project seeks to prevent neurobehavioral deficits and fatigue due to inadequate sleep in astronauts by investigating how variations in sleep duration and its circadian placement relate to the return of performance per time invested in sleep. ***Countermeasure goals*** include determination of the amount of naptime

necessary to compensate for interrupted nocturnal sleep periods for the prevention of cumulative sleepiness and performance deficits.

Fuller et al.: Primate Circadian Rhythms in the Martian Environment. This project is focused on the ability of the circadian time system to synchronize to the Martian photic (spectrum and period) by examining the effects of 1.0, 1.5 and 2.0G on the period of the circadian pacemaker. A G vs. period model will be developed to predict the effect of the 0.38 G Martian environment on the period of the circadian pacemaker. Long-term (4 months) physiological and behavioral responses will be examined. ***Countermeasure goals*** include the use of timed bright light pulses on circadian entrainment. This program will develop a primate model to evaluate physiological and behavioral consequences of long-term exposure of males and females to altered lighting and gravitational environments.

Jewett et al:Mathematical Model for Scheduled Light Exposure:Circadian/Performance Countermeasure. The goal of this project is to further develop and refine our mathematical dynamic stimulus processing model so that it can accurately predict the phase and amplitude of the human circadian system under any lighting system especially those which in space. The mathematical Neurobehavioral Performance model validated against performance data collected will result in the development of user-friendly Performance Simulation Software. ***Countermeasure goals*** include:the design of shift schedules to allow astronauts to receive available bright light at appropriate times for proper circadian alignment with their sleep/wake schedules.

Menaker et al.: A Model of Circadian Disruption in the Space Environment. This project proposes to evaluate the effects of "constant" conditions and shift work schedules on the maintenance of circadian rhythmicity when the central and peripheral structures are abnormally phased. The resulting abnormal circadian organization is "dysphasia." ***Countermeasure goals*** include an evaluation of meal timing, melatonin administration, forced exercise, and short pulses of complete darkness as a treatment to prevent circadian dysphasia.

Morin et al.: Circadian and Vestibular Relationships. This project seeks to determine the route by which a correlate of the non-photic stimulus, i.e., locomotion, might gain access to the circadian rhythm system and shift rhythm phase. It has also opened the possibility that the vestibular system is a specific route by which sensory information related to heard movement might gain access to the circadian system. ***Countermeasure goals*** include an evaluation of a non-locomotor, non-photic three-dimensional motion stimulus to activate functionally the vestibular and circadian systems, laying the groundwork for the future development of novel approaches for the treatment of space motion sickness and for resetting circadian phase.

Tosini et al.: Long-Term Exposure to Dim Light Desynchronizes the Circadian System of Rats. The goal of this project is to understand the mechanisms responsible for the desynchronization of circadian rhythm in locomotion and the enzymes responsible for the production of melatonin. Investigating the effect that internal desynchronization has on the immune response and motor and cognitive performances. *Countermeasure goals* include an evaluation the use of melatonin as a pharmacological agent to counteract desynchronization of the circadian rhythms.

Turek et al.: Animal Model for Sleep Loss and Circadian Disruption. This project will focus on determining the effects of 12 hours of imposed wakefulness on circadian rhythms, sleep-wake cycles, neurobehavioral and motor performance measures during normal active and inactive periods.

Countermeasure goals include treatment exercise and with either physiological or pharmacological dose of melatonin reduce the effects of circadian disruption and sleep loss as well as alleviate the adverse effects associated with work at different times of day.

Strengths, Key Findings and Discoveries of the Research Program

Investigators on the NSBRI HPFSC Team, through support for their research programs provided directly by the NSBRI in combination with support provided by NASA and the NIH, have made the following key findings and discoveries related to the NSBRI HPFSC Team research program:

- The average intrinsic circadian period of the human biological clock is very close to 24 h. Scheduled exposure to the current lighting conditions of space flight is sufficient to maintain circadian entrainment to the 24-h day of Earth but is not sufficient to enable circadian adaptation to the 24.65-h day of Mars.
- The human photoreceptor for regulating melatonin and providing photopic input to the circadian system is a novel non-visual receptor.
- Earth's gravity plays a key role in sleep disturbances related to upper airway obstruction. When astronauts are in space, they have fewer arousals resulting from sleep-related breathing disturbances.
- Space shuttle flight results in circadian rhythm disturbance, sleep loss, decrements in neurobehavioral performance and post-flight changes in REM sleep.
- In collaboration with the immune team, we have found that sleep deprivation increases the presence of pro-inflammatory cytokines.

Gaps in the Current Team Program

The Human Performance Factors, Sleep and Chronobiology Team has nine ground-based projects currently funded until 2003. In addition to the progress toward countermeasure development anticipated from the currently funded research projects, it is anticipated that the following four research questions which are not being addressed by the current research program will be addressed in the coming years:

Physical effects. Research is needed to determine how space flight or exposure to chronic sleep restriction and/or circadian disruption affect sleep- and/or circadian-mediated neuroendocrine, metabolic, neurologic or autonomic functions, particularly those relevant to risk mitigation (e.g., growth factors, nutrition, glucocorticoids, monoamines) during extended duration missions.

Monitoring the status of sleep, sleep homeostasis, circadian rhythmicity and/or neurobehavioral performance during space flight. Research is needed to to validate methodologies that are portable and non-intrusive in the space flight environment to assess sleep and/or circadian rhythms.

Novel countermeasure development. Research is needed to determine how recent advances in the neurobiology of sleep and/or circadian rhythms (orexin/hypocretin system, circadian photoreception, output pathways that regulate sleep or circadian rhythms) can be used to develop countermeasures to adapt to and thereby maintain optimal neurobehavioral performance during exploration class space missions.

Age, gender and inter-individual differences. Research is needed to determine how age, gender and individual biological and behavioral characteristics alter sleep- and/or circadian-mediated physiologic responses to, and risk mitigation for, prolonged space flight.

II. INTRODUCTION

The success of human space missions depends on each astronaut remaining alert and vigilant while operating sophisticated equipment and following complex procedures. During long-duration space flight, the space environment affects a number of physiological systems critically involved in human performance, and it is vital to mission success to understand the biological limits of human performance under space flight conditions. This team is focused on these issues and, in particular, is concerned with the following aspects of the space environment: microgravity, altered light-dark cycles, altered or reduced sleep/rest opportunities, high levels of automation, and habitation in a remote, inaccessible location. The primary thrust of this team's research program involves altered circadian organization, sleep disruption and cumulative sleep loss, and the associated neurobehavioral decrements occurring during long-duration space flight.

The goals of the Human Performance Factors, Sleep and Chronobiology Team are to: (1) Characterize and quantify the adverse effects of long-duration space flight on sleep and circadian rhythmicity; (2) Characterize and quantify the effect of sleep loss and/or circadian dysfunction on physical and neurobehavioral performance; (3) Understand the basic mechanisms underlying the deterioration of sleep, circadian organization and human neurobehavioral function during space flight; (4) Develop high-fidelity mathematical models of performance based on circadian organization and sleep-wake history; (5) Develop effective countermeasures to optimize sleep and facilitate circadian adaptation in the space environment and thereby maintain optimal neurobehavioral performance; (6) Develop new methods for monitoring the status of sleep, sleep homeostasis, circadian rhythmicity and neurobehavioral performance during space flight, with possible spin-off applications on Earth.

The overall team strategy and a description of each project is described in detail elsewhere (see the web site http://www.nsbri.org/Research/Sleep.html). The team research objectives are driven by the Critical Path Roadmap related to Human Performance Failure because of Sleep and Circadian Rhythm Problems. The current research program involves nine ground-based research projects. Many of the projects impinge on more than one critical risk. The strategy of the Human Performance Factors, Sleep and Chronobiology Team is to develop a synergistic interaction between research projects at the molecular, cellular, organismic, and human levels, and to integrate predictive biomathematical modeling of the sleep and circadian systems.

Within this area of research, the following five interrelated themes define the range of factors critical for optimizing human performance capability and improving crew health and safety:

A. Effects of long-duration space flight on sleep and/or circadian rhythmicity. This theme addresses the impact of the conditions of long-duration space flight (microgravity, altered light intensity, loss of geophysical cues, isolation, altered physical activity, etc.) on neurobiologic, endocrinological, and behavioral mechanisms (molecular, cellular and organismic) that control sleep and circadian systems.

B. Effects of sleep loss and/or circadian dysfunction on physical and neurobehavioral performance. The focus of this theme is to identify the range of acute and chronic adverse effects that sleep loss, sleep disruption, and/or circadian dysfunction have on critical physiologic and performance parameters during long-duration space flight (e.g., neurophysiologic function, physiological alertness, vigilance, cognitive performance, mood/morale, problem solving and communication).

C. **Monitoring and assessment during space flight.** This theme deals with the development of methods for monitoring the status of sleep, sleep homeostasis and circadian organization, as well as technologies that assess and update the current functional status or performance capability of the individual.

D. **Predictive modeling of performance based upon circadian organization and sleep homeostasis.** This theme is concerned with the development of analytical or phenomenological mathematical models that predict individual human performance capability by involving multiple subsystems (e.g., circadian rhythmicity, sleep homeostasis, work-rest schedules, etc.) as an integrated unit across levels of organization, and by estimating the impact of countermeasure use designed to optimize human physical and/or neurobehavioral performance.

Countermeasures: The research program of this team will not only define the impact of the space environment on sleep and circadian rhythmicity and the effects of the sleep loss and circadian dysfunction on performance but also will develop methods to counter the adverse physiological and behavioral events. These countermeasures may include behavioral, pharmacological, environmental or other adaptive approaches to maintain function and performance under the adverse conditions of long-duration space flight.

III. RESEARCH PROGRAM STRUCTURE AND DESIGN

The Human Performance Factors, Sleep and Chronobiology Team (HPFSC) is focused on developing countermeasures for the sleep loss and circadian dysfunction and associated neurobehavioral performance decrements that occur during long-duration space flight. The initial strategic research program for the HPFSC Team involves nine research studies that collectively address the five thematically interrelated questions described above. The nine current ground-based experiments that comprise the NSBRI Human Performance Factors, Sleep and Chronobiology Team are classified in **Table 1** relative to their respective themes, specific countermeasures and their countermeasure readiness level. The schematic of the circadian and homeostatic regulation of sleep and alertness shown in **Diagram 1** illustrates the relationships between the projects on the team, with the principal aim of each project indicated. In **Diagram 2**, both the primary and secondary aims of each project are illustrated.

Each of the individual projects is summarized below:

Project 1: Brainard et al.: Optimizing Light Spectrum for Long Duration Space Flight

The physiological changes caused by disturbed circadian rhythms and altered sleep-wake patterns can result in decrements in alertness, concentration, and performance. This project addresses these risk factors which threaten the safety of personnel and the objectives of space missions.

Countermeasure goals include:
1. Identification of the optimum spectral transmission for photic resetting of the circadian pacemaker.
2. Design specifications for space suit visors and the windows used in space vehicles and habitats;
3. Engineering parameters for the ideal spectral distribution for illumination of general living quarters during space exploration.

Project 2: Czeisler et al.: Circadian Entrainment, Sleep-Wake Regulation & Performance during Space Flight

The goal of this project is to develop countermeasures to facilitate adaptation of the human circadian pacemaker to the 24.65-h day length of Mars, which is outside the range of entrainment of the human circadian pacemaker given the weak synchronizing stimuli within the Martian habitat.

The primary ***countermeasure goal*** is to evaluate the efficacy of intermittent bright light pulses as a treatment to prevent the misalignment of circadian phase, sleep disruption, associated decrements in neurobehavioral performance and reduction in nocturnal growth hormone secretion experienced by individuals exposed to the 24.65h Martian day.

Project 3: Dinges et al.: Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss

Using a response surface experimental paradigm (RSM), this project seeks to prevent neurobehavioral deficits and fatigue due to inadequate sleep in astronauts by investigating how variations in sleep duration and its circadian placement relate to the return of performance per time invested in sleep.

Countermeasure goals include determination of the amount of naptime necessary to compensate for interrupted nocturnal sleep periods for the prevention of cumulative sleepiness and performance deficits.

Project 4: Fuller et al.: Primate Circadian Rhythms in the Martian Environment

This project is focused on the ability of the circadian time system to synchronize to the Martian photic (spectrum and period) by examining the effects of 1.0, 1.5 and 2.0G on the period of the circadian pacemaker. A G vs. period model will be developed to predict the effect of the 0.38 G Martian environment on the period of the circadian pacemaker. Long-term (4 months) physiological and behavioral responses will be examined.

Countermeasure goals include the use of timed bright light pulses on circadian entrainment. This program will develop a primate model to evaluate physiological and behavioral consequences of long-term exposure of males and females to altered lighting and gravitational environments.

Project 5: Jewett et al.: Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure

The goal of this project is to further develop and refine our mathematical dynamic stimulus processing model so that it can accurately predict the phase and amplitude of the human circadian system under any lighting system especially those which in space. The mathematical Neurobehavioral Performance model validated against performance data collected will result in the development of a user-friendly Performance Simulation Software program.

Countermeasure goals include: the design of shift schedules to allow astronauts to receive available bright light at appropriate times for proper circadian alignment with their sleep/wake schedules.

Project 6: Menaker et al.: A Model of Circadian Disruption in the Space Environment

This project proposes to evaluate the effects of "constant" conditions and shift work schedules on the maintenance of circadian rhythmicity when the central and peripheral structures are abnormally phased. The resulting abnormal circadian organization is "dysphasia."

Countermeasure goals include an evaluation of meal timing, melatonin administration, forced exercise, and short pulses of complete darkness as a treatment to prevent circadian dysphasia.

Project 7: Morin et al.: Circadian and Vestibular Relationships

This project seeks to determine the route by which a correlate of the non-photic stimulus, i.e., locomotion, might gain access to the circadian rhythm system and shift rhythm phase. It has also opened the possibility that the vestibular system is a specific route by which sensory information related to heard movement might gain access to the circadian system.

Countermeasure goals include an evaluation of a non-locomotor, non-photic three-dimensional motion stimulus to activate functionally the vestibular and circadian systems, laying the groundwork for the future development of novel approaches for the treatment of space motion sickness and for resetting circadian phase.

Project 8: Tosini et al.: Long-Term Exposure to Dim Light Desynchronizes the Circadian System of Rats

The goal of this project is to understand the mechanisms responsible for the desynchronization of circadian rhythm in locomotion and the enzymes responsible for the production of melatonin. Investigating the effect that internal desynchronization has on the immune response and motor and cognitive performances.

Countermeasure goals include an evaluation the use of melatonin as a pharmacological agent to counteract desynchronization of the circadian rhythms.

Project 9: Turek et al.: Animal Model for Sleep Loss and Circadian Disruption

This project will focus on determining the effects of 12 hours of imposed wakefulness on circadian rhythms, sleep-wake cycles, neurobehavioral and motor performance measures during normal active and inactive periods.

Countermeasure goals include treatment exercise and with either physiological or pharmacological dose of melatonin reduce the effects of circadian disruption and sleep loss as well as alleviate the adverse effects associated with work at different times of day.

Table 1. Description of Current (2001) Program for Human Performance Factors, Sleep and Chronobiology.

CRL		Sleep and Circadian								Neuro-behavioral
		Effects of space flight on biological rhythm, sleep, and performance			Counter-measures to mitigate performance deficits associated with sleep deficit	Long term effects of sleep and circadian counter-measures	Non-invasive monitoring of sleep and behavioral function	Inter-individual predictors of sleep and circadian adaptation to space	Predictive mathematical models of sleep, circadian rhythms, and performance	Long term effect to space environment on human cognition, performance, motor skills, and problem solving
		6.05			6.06	6.07	6.08	6.18	6.21	6.15
9	**CM flight tested/ready**									
8	**CM validated in space**									
7	**Operational simulation test of CM**									
6	**Lab test of CM efficacy in humans**									
5	**CM proof of concept testing**									
4	**CM concept formulation**				**Brainard** Use of spectrum to design capsule lighting & windows *Jewett*	**Jewett** Use of light and performance mathematical models in schedule design			*Jewett*	
3	**Validated problem / hypothesis**	**Czeisler** Synchronizatio n to Mar's day with two brief light pulses *Dinges*	**Menaker** The effect of meals, Melatonin, exercise, and dark pulses dysphasia		**Dinges** The use of naps to ameliorate the affects of chronic sleep restriction *Czeisler*			*Dinges*		
2	**Hypothesis formed**		**Tosini** The use of Melatonin to synchronize rhythms *Fuller*	**Turek** The use of melatonin on circadian phase.	*Turek*	*Fuller*			**Fuller** Bright light Pulses, Period vs. Gravitation *Turek*	**Morin** Three dimensional motions to stimulate the vestibular and circadian system
1	**Problem defined**									

Primary critical question designation is shown in bold. Secondary critical question designations are shown in bold italics

Diagram 1. Description of Current (2001) Program for Human Performance Factors, Sleep and Chronobiology.

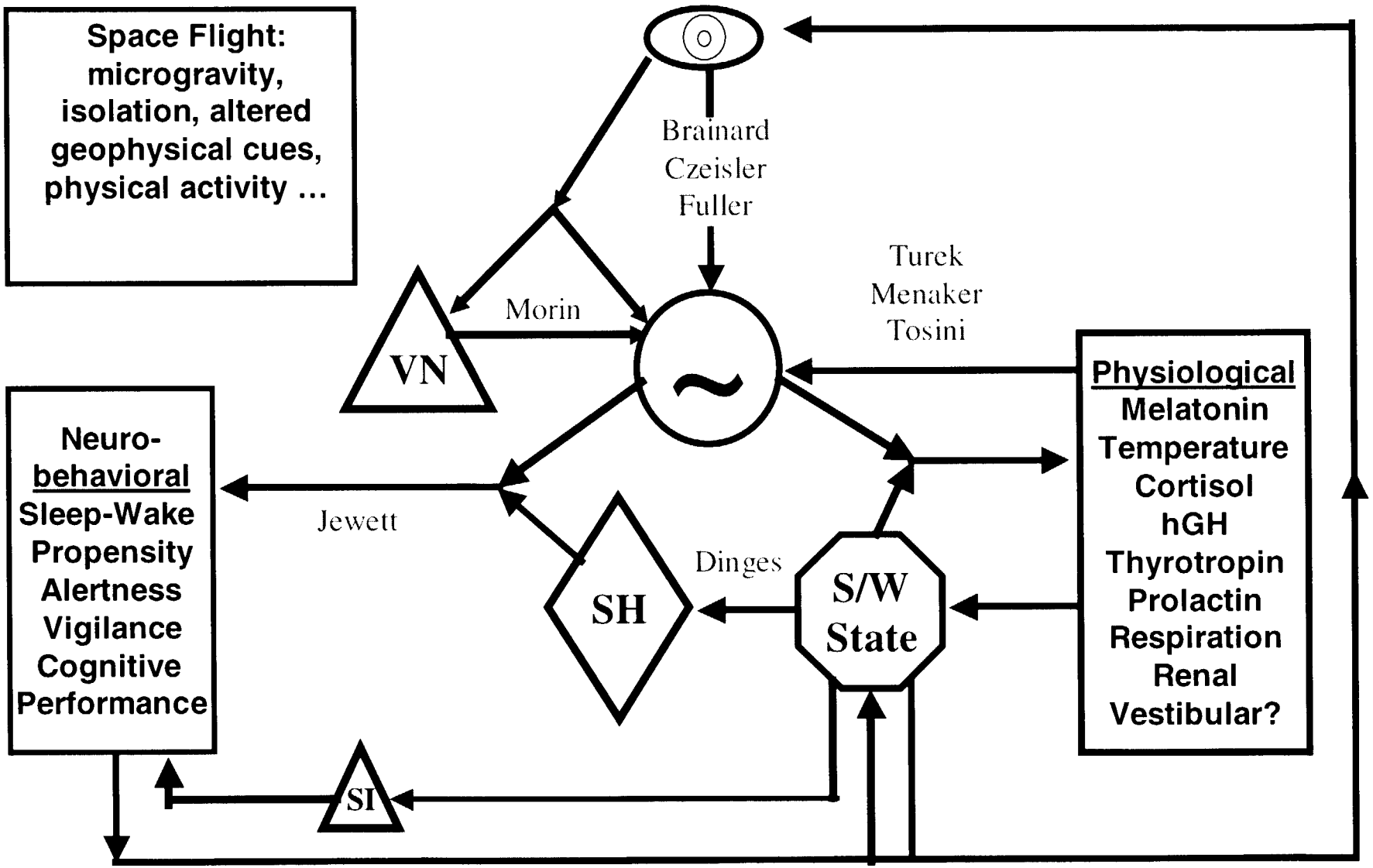
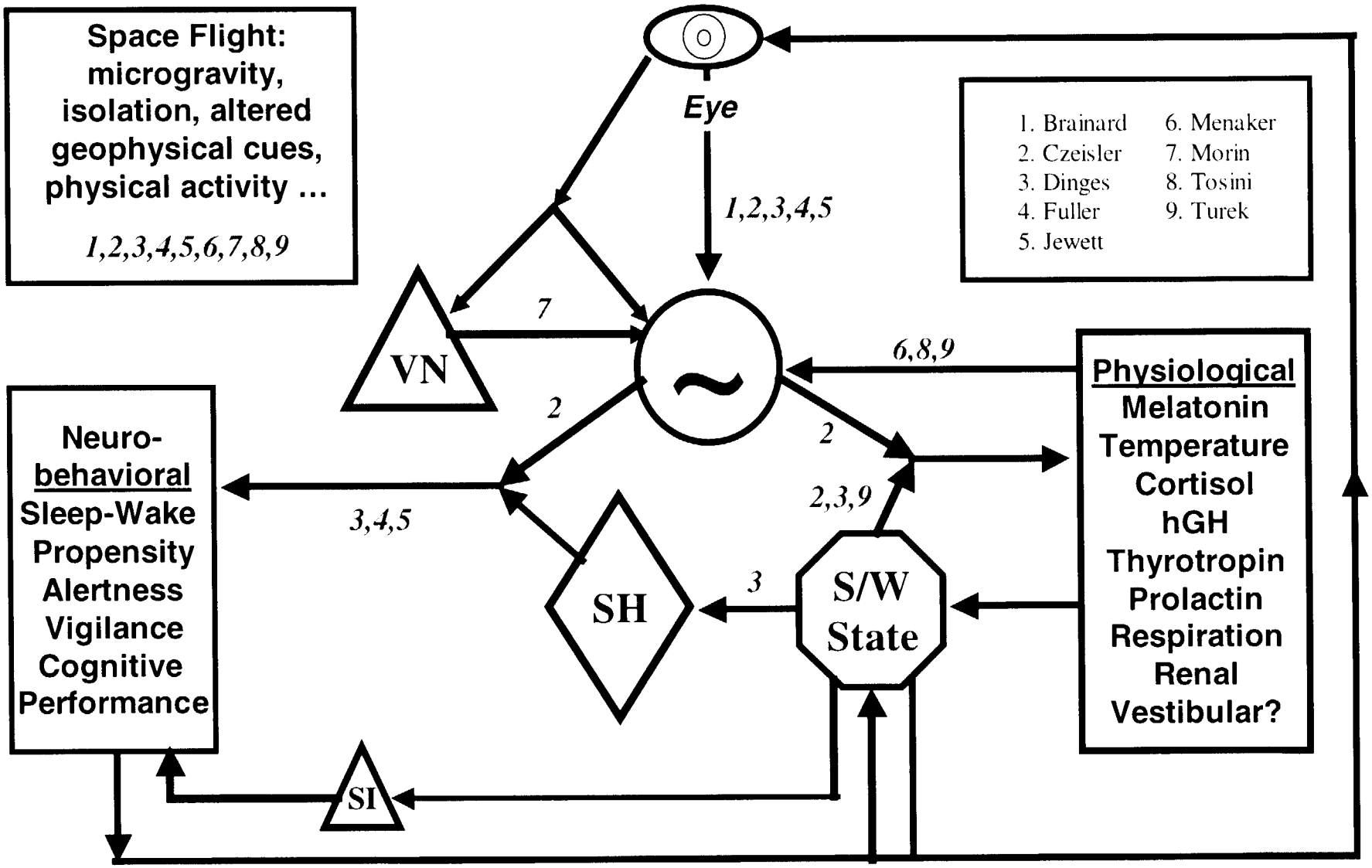


Diagram 2. Description of Current (2001) Program for Human Performance Factors, Sleep and Chronobiology.



IV. RESEARCH PROGRAM ACCOMPLISHMENTS

The program accomplishments of each of the individual projects is summarized below:

Project 1: Optimizing Light Spectrum for Long Duration Space Flight

PI: *George Brainard, Ph.D.*
Thomas Jefferson University

***Research Focus*:** Use of light spectrum and light visor design

***Specific aims*:**

Test the hypotheses that:

1. Wavelengths of light below 440 nm and above 600 nm are active in regulating melatonin secretion via measurement of fluence response curves in humans;
2. There will be a loss of sensitivity to monochromatic light when the eyes are not pharmacologically dilated during the melatonin suppression test;
3. There will be a shift in spectral sensitivity of light regulation of melatonin secretion when the eyes are not pharmacologically dilated.
4. Improve light treatment, identify optimal spectral transmission characteristics for visors and windows, and engineer the ideal spectral distribution for illumination of living quarters.

***Submissions/Publications*:**

1. Brainard GC, Hanifin JP, Greeson JM, Byrne B, Glickman G, Gerner E, Rollag MD. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J. Neuroscience* 2001; 21 16:6405-6412.
2. Brainard GC, Hanifin JP, Rollag MD, Greeson JM, Byrne B, Glickman G, Gerner E. Action spectrum for melatonin regulation in humans. *Photodermatology, Photoimmunology and Photomedicine* 2001; 17:139.
3. Brainard GC, Byrne B, Hanifin JP, Greeson JM, Gerner E, Rollag MD. Action spectrum for melatonin regulation: evidence for a novel circadian photoreceptor. 13th Annual Meeting of the Society for Light Treatment and Biological Rhythms, Stockholm, Sweden, June 24-27.
4. Brainard GC, Byrne B, Hanifin JP, Greeson JM, Rollag MD, Gerner E. Human circadian photoreception: action spectrum and ocular physiology for melatonin regulation. Melatonin and Biological Rhythms, Adelaide, Australia, August 21-23.

Grant Proposals:

1. Title: Optimizing Light Spectrum for Long Duration Space Flight
   Agency: Cooperative Agreement NCC 9-58 with NSBRI (HPF.002.08)
   Duration: Three years
   Total Direct Costs: $770,875.00
   Funding Status: *Funded*

2. Title: Ocular Control of Melatonin Regulation: Action Spectrum
   Agency: National Institute of Neurological Disorders and Stroke, National Institutes of Health
   Duration: Four years
   Total Direct Costs: $1,394,825.50
   Funding Status: *Renewed* to start Sept-Oct 2001

3. Title: The Effect of Polarized Versus Non-Polarized Light on Melatonin Regulation in Healthy Humans
   Agency: Philips Lighting B.V.
   Duration: Five years, six months
   Total Direct Costs: $44,942.00
   Funding Status: *Funded*

4. Title: Visor Efficacy and Hazard Analysis
   Agency: Bio-Brite, Inc.
   Duration: Three years
   Total Direct Costs: $19,540.00
   Funding Status: *Funded*

Presentations:

1. *International Darkskies Association Annual Symposium*, March 2001, Tucson, A.Z. "Photobiological Effects of Light at Night".
2. *Illuminating Engineering Society*, Delaware Valley Section, March 2001, Philadelphia, P.A. "The Biological, Behavioral and Therapeutic Effects of Light".

Current Progress:

1. Assembled research team and confirmed experimental aims and designs
2. Completed two Institutional Review Board documentation series to support the first study
3. Recruited eight healthy male and female volunteers
4. Completed physical health screens on all volunteers
5. Completed forty night time light-induced melatonin suppression exposures at 420 nm.

Major Findings:

The first sixteen exposures at 420 nm were highly relevant to our previous NIH-sponsored work on the melatonin action spectrum. This wavelength is very pertinent to astronaut light exposure outside of planetary atmosphere (e.g.: Earth, Mars). The data were included in our recently published paper, along with the four year study on the melatonin action spectrum (Brainard et al., 2001; see above). The data indicate that the human photoreceptor for regulating melatonin and providing photopic input to the circadian system is a novel non-visual receptor.

Project 2: Circadian Entrainment, Sleep-Wake Regulation & Performance during Spaceflight

PI: *Charles Czeisler, Ph.D, M.D.*
Brigham and Women's Hospital/Harvard Medical School

***Research Focus*:** Synchronization to Mars' day with two brief light pulses

Specific aims:
Test the hypotheses that:

1. Synchronization of the human circadian pacemaker to a sleep-wake and light-dark schedule with an imposed period ~4% longer than its intrinsic period will be disturbed.
2. This disturbed circadian synchronization will disrupt sleep, endocrine function, and impair waking alertness and performance.
3. Two relatively brief (45 minute) daily exposures to evening bright light (~10,000 lux) will establish a normal entrained circadian phase in subjects on such a schedule, resulting in improved sleep consolidation, undiminished growth hormone and cortisol secretion and enhanced daytime alertness and performance.

Submissions/Publications of general interest to NASA/NSBRI:

1. Wright, K.P., Jr, Myers, B.L., Plenzler, S.C., Drake, C.L., Badia, P. Acute effects of bright light and caffeine on nightime melatonin and temperature levels in women taking and not taking oral contraceptives. Brain Res 873: 310-317, 2000.
2. Khalsa S.B., Jewett, M.E., Duffy, J.F., Czeisler, C.A. The timing of the human circadian clock is accurately represented by the core body temperature rhythm following phase shifts to a three-cycle light stimulus near the critical zone. J Biol Rhyhtms 15:524-530, 2000.
3. Hull JT, Wright KP Jr., Czeisler CA. Circadian and Sleep-Wake Dependant Control of Urine Volume Output on a 28-h Forced Desynchrony. Sleep; 2001, 24:A90.
4. Wright KP Jr., Hull JT, Czeisler CA. Relationship Between Cognitive Performance and Core Body Temperature During a 28-hr Forced Desynchrony Protocol. Sleep; 2001, 24:A3.
5. Gronfier C, Kronauer RE, Wright KP Jr., Czeisler CA. Growth Hormone secretion during entrained and non-entrained conditions in Humans. Sleep; 2001, 24:A89.
6. Jewett ME, Wright KP Jr., Duffy JF, Rodriguez DM, Czeisler CA. Practice effects observed over a month-long 28-hour forced desynchrony protocol in a cognitive throughput task are well described by a saturating exponential function. Sleep; 2001, 24:A4.
7. Ho AH, Gronfier C, Czeisler CA. Effects of prolonged sleep deprivation on cortisol secretion in Humans. Sleep; 2001, 24:A251-A252.
8. Wright KP Jr., Hughes, RJ, Kronauer, RE, Dijk, DJ, Czeisler, CA. Intrinsic near-24-hour pacemaker period determines limits of circadian entrainment to a weak synchronizer in humans. Submitted.

Grant Proposals:

1. Title: Effects of Chronic Sleep Loss on Daytime Cognitive and Neurobehavioral Function
   Agency: Sleep Medicine Education & Research Foundation
   Duration: Two years
   Total Direct Costs: $50,000
   Funding Status: *Funded*

2. Title: Effects of Sleep Loss on Human Physiology and Behavior
   Agency: National Institutes of Health
   Duration: Four years
   Total Direct Costs: $1,016,000
   Funding Status: Submitted

3. Title: Circadian adaptation to non-24-hour sleep-wake schedules
   Agency: National Institutes of Health
   Duration: Four years
   Total Direct Costs: $1,587,500
   Funding Status: *Funded*

4. Title: Treatment of Circadian Sleep Disorders with Bright Light
   Agency: National Institutes of Health
   Duration: Five years
   Total Direct Costs: $3,522,486
   Funding Status: *Funded*

5. Title: Sleep-Wake Actigraphy and Light Exposure During Spaceflight
   Agency: NASA
   Duration: Five years
   Total Direct Costs: $783,807
   Funding Status: *Funded*

6. Title: Bright Light Treatment of Shift Rotation Insomnia
   Agency: NIH/NHLBI
   Duration: Four Years
   Total Direct Costs: $1,000,000
   Funding Status: *Funded*

7. Title: After-effects of Entrainment on Human Circadian Period
   Agency: NIH
   Duration: Four Years
   Total Direct Costs: $1,000,000
   Funding Status: *Funded*

8. Title: Effect of Extended Work Hours on ICU Patient Safety
   Agency: AHRQ/NIH
   Duration: Three Years
   Total Direct Costs: $1,180,000
   Funding Status: *Funded*

9. Title: Effect of Extended Work Hours on Intern Health and Safety
   Agency: NIOSH
   Duration: Four Years
   Total Direct Costs: $998,479
   Funding Status: *Funded*

Presentations:

1. Wright Jr., KP. Circadian Entrainment and Sleep-Wake Regulation in Humans. Sleep
2. Grand Rounds, Harvard Medical School, Boston, MA. January 2001.
3. Wright Jr., KP. Promoting Wakefulness Through Sleep Management and Circadian Rhythm Adjustment. Adaptation to the Mars Day. Mars Exploration Rover Surface Operations Human Factors Workshop. NASA Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California January 2001
4. Czeisler, CA. Physiological Basis of Fatigue at JPL During MER Surface Operations. Mars Exploration Rover Surface Operations Human Factors Workshop. NASA Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California January 2001
5. Wright Jr., KP. Regulation of Cognitive Performance by the Endogenous Circadian
6. Pacemaker and Sleep-Wake Homeostasis in Humans. Invited Lecture. Brain, Behavior, and Cognition Colloquium Series, Department of Psychology, Boston University, Boston, MA. April 2001.
7. Wright Jr., KP. Circadian Entrainment, Sleep-Wake Homeostasis and the Regulation of Neurobehavioral Performance. Invited Lecture. Department of Psychiatry, Oregon Health Sciences University, Portland, OR. April 2001.
8. Wright Jr., KP. Entrainment of the Non-24-Hour Circadian Period of the Human Pacemaker to the 24-Hour Day by a Dim Light-Dark Cy2cle. Invited Lecture, 6th Symposium of the Biologic Effects of Light, Boston, MA. June 2001.
9. Czeisler, CA. Exquisite Sensitivity of the Human Circadian Pacemaker to Photic Resetting: Implications for Assessment of Intrinsic Circadian Period. Invited Lecture, 6th Symposium of the Biologic Effects of Light, Boston, MA. June 2001.

Current Progress:

1. Obtained Institutional Review Board approval for study
2. Recruited seven healthy male volunteers
3. Completed physical and psychological screens on all volunteers recruited
4. Completed five 65-day studies
5. Currently two 65-day study ongoing

Major Findings:
Among, the first five studies completed, 2 subjects were exposed to brief bright light exposures as a countermeasure to circadian misalignment associated to living in a non-24-h day. Three subjects were not exposed to bright light exposures. The data are under analysis.

Project 3: Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss

PI: *David F. Dinges, Ph.D.*
University of Pennsylvania

***Research Focus*:** The use of naps to ameliorate the affects of chronic sleep restriction

***Specific aims*:**
1. Establish Response Surface Map to determine how to best use anchor and nap sleeps to promote neurobehavioral performance and alertness at an adverse circadian phase for waking;
2. Identify the optimal diurnal anchor sleep and nocturnal nap schedule to maintain neurobehavioral function when work is initiated with abrupt circadian displacement;
3. Determine how diurnal anchor sleep times and nocturnal nap sleep affect sleep physiology and circadian adjustment across a chronic schedule of simulated night operations.

***Submissions/Publications*:**
1. Shearer WT, Reuben JM, Mullington JM, Price NJ, Lee B, Smith EO, Szuba MP, Van Dongen HPA, Dinges DF. Soluble tumor necrosis factor-alpha receptor 1 and interleukin-6 plasma levels in humans subjected to the sleep deprivation model of space flight. *J. Allergy and Clinical Immunology*, 2001;107:165-170.
2. Doran SM, Van Dongen HPA, Dinges DF. Sustained attention performance during sleep deprivation: Evidence of state instability. *Archives Italiennes de Biologie: Neuroscience*, 2001;139:253-267.
3. Meier Ewert HK, Ridker PM, Rifai N, Price N, Dinges DF, Mullington JM. Absence of diurnal variation of C-reactive protein. *Clinical Chemistry*, 2001;47:426-430.
4. Rogers NL, Dinges DF. Shiftwork, circadian disruption, and consequences. *The Economics of Neuroscience*, 2001;3 9:1-7.
5. Dinges DF. Sleep in Space Flight: Breath Easy – Sleep Less? (Editorial). *American J. Respiratory and Critical Care Medicine*, 2001;164:337-340.
6. Gazendam JAC, Freedman NS, Van Dongen HPA, Dinges DF, Pack AI, Schwab RJ. Light–dark patterns in the intensive care unit. *Aviation, Space, and Environmental Medicine*, (in press).
7. Meier Ewert HK, Ridker PM, Rifai N, Price N, Dinges DF, Mullington JM. Association of sleep loss and C-reactive protein: Evidence for a counter-inflammatory activity of sleep. (submitted).
8. Kloss JD, Szuba MP, Dinges DF. Sleep Loss and Sleepiness: Physiological and Neurobehavioral Effects (Chapter) American College of Neuropsychopharmacology: Fifth Generation of Progress (in press).
9. Dinges DF, Maislin G, Van Dongen H. Chronic sleep restriction: relation of sleep structure to daytime sleepiness and performance. *Sleep*, 2001;24:A28.

10. Maislin G, Rogers NL, Price NJ, Mullington JM, Szuba MP, Van Dongen HP, Dinges DF. Response surface modeling of the effects of chronic sleep restriction with and without diurnal naps. *Sleep*, 2001;24:A242.
11. Mallis MM, Neri DF, Oyung R, Colletti L, Nguyen T, Dinges DF. Factors associated with behavioral alertness in pilots flying simulated night flights. *Sleep*, 2001;24:A123.
12. McConnell KJ, Maislin G, Rogers NL, Price NJ, Mullington JM, Szuba MP, Brodnyan CG, Cerceo L, Van Dongen H, Dinges DF. Sleep efficiency during chronic nocturnal sleep restriction with and without diurnal naps. *Sleep*, 2001;24:A431.
13. Orthmann JL, Rogers NL, Price NJ, Mullington JM, Szuba MP, Van Dongen H, Dinges DF. Changes in plasma growth hormone levels following chronic sleep restriction. *Sleep*, 2001;24:A248.
14. Shah AD, Van Dongen J, Maislin G, Brodnyan CG, Dinges DF. Dynamics of slow-wave activity during chronically restricted sleep. *Sleep*, 2001;24:A247.
15. Dinges DF, Van Dongen HPA, et al. Cumulative sleep loss in space flight: Consequences and countermeasures. *Proceedings of the 52nd International Astronautical Congress, Toulouse, France* (in press).

Grant Proposals:

1. Title: Neurobehavioral Effects of Partial Sleep Deprivation
   Agency: National Institutes of Health (NINR)
   Duration: Four years
   Total Direct Costs: $1,074,184.00
   Funding Status: Submitted

2. Title: Individual Differences in Response to Sleep Deprivation
   Agency: National Institutes of Health (NHLBI)
   Duration: Five years
   Total Direct Costs: $1,270,015.00
   Funding Status: *Funded*

Presentations:

1. *Inflammation, Cytokines & Neurobehavioral Functions Workshop* sponsored by Schering, October 2000, Washington, D.C. "Assessing neurobehavioral functions in relation to cytokines".
2. *Colloquium at the University of Arizona*, November 2000, Tucson, A.Z. "Can Sleep need be eliminated (or at least reduced) in the new millennium".
3. Keynote Presenter, *National Science Teachers' Association*, March 2001, St. Louis, M.O. "Challenges to human behavior and performance during prolonged space flight".
4. *Science of Mind-Body Interactions: An Exploration of Integrative Mechanisms* sponsored by the John D. and Catherine T. MacArthur Foundation Network on Mind-Body Interactions, NIMH, NINDS, and OD OIR NIH, March 2001, Washington D.C. "Chronically reduced sleep: Do we cope, adapt or deteriorate?".
5. Invited Lecture, *Mount Sinai School of Medicine*, May 2001, New York City, N.Y. "Preventing neurobehavioral deficits from cumulative sleep loss during space flight: Evidence for behavior, pharmacology and technology countermeasures".

6. Invited Lecture, *NIH Behavioral and Social Science Seminar Series*, May 2001, Washington D.C. "Sleep debt: Neurobehavioral consequences of chronic partial sleep loss".
7. Invited Lecture, *National Advisory Council for Nursing Research*, May 2001, Washington D.C. "Chronically reduced sleep: Do we cope, adapt or deteriorate?".
8. *Air Transport Association Symposium on "Enhancing Aviation Safety,"* May 2001, Washington, D.C. "Fatigue: Latest scientific findings/technology approaches".
9. *Association of Professional Sleep Societies*, June 2001, Chicago, I.L. "Sleep Debt: Initial discoveries -- many questions".
10. *Association of Professional Sleep Societies*, June 2001, Chicago, I.L. "Chronic Sleep Restriction: Relation of Sleep Structure to Daytime Sleepiness and Performance".
11. Invited Lecture, *Groningen Graduate School for Behavioral and Cognitive Neurosciences Summer School*, July 2001, Groningen, Netherlands. "Human performance and fatigue in modern society".
12. Invited Lecture, *Groningen Graduate School for Behavioral and Cognitive Neurosciences Summer School*, July 2001, Groningen, Netherlands. "Performance, fatigue and motivation".
13. Invited Lecture, *Groningen Graduate School for Behavioral and Cognitive Neurosciences Summer School*, July 2001, Groningen, Netherlands. "Consequences of acute total sleep deprivation and cumulative partial sleep loss".

Current Progress:

1. Recruitment and screening of subjects underway for experiment 2, with the first data acquisition run in progress. Delay in initiation of data acquisition was associated with two University administrative processes: (i) requirement that funding be received to initiate project; and (ii) FDA-mandated revisions to IRB and GCRC review procedures, as well as monitoring and compliance for investigator-initiated research.
2. During the administrative delays, experimental preparations were completed for the protocol, including the acquisition of non-thrombogenic catheters for the collection of blood using the Carmeda ConFlo blood pump system for assessment of plasma melatonin, cortisol and growth hormone; and the acquisition of new ambulatory physiologic recording equipment that provides enhanced ability to conduct EEG and PSG recordings 24/7.
3. Continued development of response surface models (RSMs) of neurobehavioral and physiological (e.g., sleep efficiency) data obtained in experiment 1 (chronic sleep restriction via nocturnal anchor sleep + diurnal nap sleep). Results presented at the national and international scientific meetings.

Major Findings:

Identification of elevated levels of C-reactive protein in the plasma of subjects deprived of sleep both acutely (88 hr without sleep) and chronically (4.2hr anchor sleep for 10 nights), relative to a control condition (8.2 hr anchor sleep for 10 nights). Using high sensitivity C-reactive protein (HSCRP) assays, a dose-response relationship was found between increasing sleep deprivation and increasing plasma concentrations of HSCRP. The finding is consistent with earlier work we have performed showing that sleep deprivation increases white blood cell counts (Dinges et al., 1994) and the presence of pro-inflammatory cytokines (Shearer et al., 2001). C-reactive protein is a stable marker of inflammation and has been shown to be a predictor of cardiovascular morbidity. As a result of this finding we have added the evaluation of HSCRP as a new objective in our current research in an effort to determine if

chronic sleep restriction combined with naps can prevent the development of the inflammatory response. Funding for the cost of the addition of HSCRP to the protocol is currently being sought. A manuscript reporting to new findings has been submitted for publication (Meier Ewert et al.: Association of sleep loss and C-reactive protein: Evidence for a counter-inflammatory activity of sleep).

Project 4: Primate Circadian Rhythms in the Martian Environment

PI: *Charles A. Fuller, Ph.D.*
University of California, Davis

***Research Focus*:** Bright light pulses, Period vs. Gravitation

***Specific aims*:**
Test the hypotheses that:

1. Rhesus macaques will not entrain to the Martian solar day when exposed to ambient light available on Mars, resulting in performance decrements and sleep and circadian dysfunction;
2. Some, but not all rhesus macaques will be able to entrain to a Martian solar day under a lighting environment proposed for the Martian Habitat;
3. The rhesus circadian period will change as a direct function of G level in hypergravity;
4. Daily evening pulses of bright light will synchronize all rhesus monkeys to the proposed Habitat environment

Current Progress:
Animals are being trained and experiment set up is in progress.

Project 5: Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure

PI: *Megan E. Jewett, Ph.D.*
Brigham and Women's Hospital/Harvard Medical School

***Research Focus*:** Use of light and performance mathematical models in schedule design

***Specific aims*:**

1. Further develop and refine the dynamic stimulus processing model by using data from existing four studies;
2. Perform validation analyses on the revised models;
3. Incorporate these refinements into models of neurobehavioral performance;
4. Develop a user-friendly predictive performance software program that can be used in-flight as a self-directed countermeasure.

***Submissions/Publications*:**

1. Brown EL, Barger LK, May CD, Jewett ME. A transformation function can equate readings of wrist-worn light measuring devices to those of hand-held light monitors. Sleep, 2001;24:A102.

1. Dean II DA, Jewett ME. Circadian Performance Simulation Software (CPSS) provides a tool for validation of circadian and neurobehavioral mathematical models. *Sleep*, 2001;24:A103.
2. Ritz-De Cecco A, Jewett ME, Duffy JF, Shanahan TL, Czeisler CA. Assessment of phase shift of melatonin rhythm to a single bright light stimulus is confounded by masking effects of scheduled sleep:wake and/or dim light:dark cycles. *Sleep*, 2001;24: A85.
3. Jewett ME, Wright JR KP, Duffy JF, Rodriguez DM, Czeisler CA. Practice effects observed over a month-long 28-hour forced desynchrony protocol in a cognitive throughput task are well described by a saturating exponential function. *Sleep*, 2001;24: A4.
4. Kahlsa SBS, Jewett ME, Duffy JF, Czeisler CA. The timing of the Human Circadian Clock is Accurately Represented by the Core Body Temperature Rhythm following Phase Shifts to a Three Cycle Light Stimulus Near the Critical Zone. *J Biol Rhythms*, 2000;15 6:524-530.

Grant Proposals:

1. Title: Spaceflight Fatigue Assessment Tool
   Agency: NASA Wyle Laboratories
   Duration: One month
   Total Direct Costs: $39,049
   Funding Status: *Funded*

2. Title: Development and Validation of a Computational Model for Intra-Cellular Circadian Oscillators
   Agency: DARPA
   Duration: Three years
   Total Direct Costs: $1,499,873
   Funding Status: *Funded*

Presentations:

1. Poster Presentation, *NSBRI Third Year Review*, November 2000, Houston, TX "Prerequisite studies for utilizing wrist-worn Actiwatch-L light recordings as input to mathematical model of the effect of light on the human circadian pacemaker".
2. Invited Workshop Speaker, *Associated Professional Sleep Societies*, June 2001, Chicago, IL "Models of sleep/wake and circadian processes and their contribution to neurobehavioral performance".
3. Invited Panel Member, *Associated Professional Sleep Societies*, June 2001, Chicago, IL "Markers of 'sleep debt' accumulation and recovery: evidence for SWA, REM, TST?".
4. Poster Presentation & Panel Member, *Associated Professional Sleep Societies*, June 2001, Chicago, I.L. "Circadian Performance Simulation Software (CPSS) provides a tool for validation of circadian and neurobehavioral mathematical models".

Current Progress:

1. Hired Premananda Pai Indic as a Post Doctoral Fellow.
2. Introduced exponential scaling constants to allow for amplitude recovery in the statistical model that is currently used by Dr. Czeisler and other circadian researchers to assess circadian phase and amplitude from core body temperature data collected under Constant Routine (CR) conditions.

3. Compared the fits of one- and two-constant amplitude recovery models with data from a previously-published circadian amplitude suppressing experiment (n = 15 subjects, 30 CRs).
4. Studied the solution space for both of the amplitude recovery models by determining each model's goodness-of-fit to the CR temperature data for a range of scaling factors.
5. Compared the fitted amplitude growth rates to the fitted initial circadian amplitudes of the CR's in order to determine whether our lower- or our higher-order pacemaker model better describes circadian amplitude recovery dynamics in humans.

Major Findings:

1. When compared to a two-harmonic model that uses a single scaling factor for both harmonics, a model that uses independent scaling factors for each harmonic provides a better fit to CR core body temperature data in amplitude suppression studies.
2. Three-dimensional plots of the fit error for a range of scaling factors for each harmonic shows that in most cases a single amplitude growth rate provides the best fit for a given CR. However, in some CRs the fit error does not indicate any one best fitted rate of amplitude growth (see Fig. 1).
3. A preliminary comparison of the model-predicted amplitude recovery rates with the scaling factors computed from the CR temperature data shows that the experimental results are quite noisy in comparison to the model predictions (see Fig. 3). We therefore plan to expand our sample size using CR temperature data from other amplitude suppressing studies in order to more clearly determine whether our lower- or our higher-order model better describes the amplitude recovery dynamics of the human circadian pacemaker.

Project 6: A Model of Circadian Disruption in the Space Environment

PI: *Michael Menaker, Ph.D.*
University of Virginia

***Research Focus*:** The effect of meals, melatonin, exercise, and dark pulses dysphasia

***Specific aims*:**

1. Evaluate the effects of constant conditions and of shift work schedules on both the maintenance of circadian rhythmicity in central and peripheral structures, and on temporal synchrony among them in a transgenic rat model system;
2. Ameliorate or prevent dysphasia by manipulating meal timing, melatonin administration, forced exercise and short pulses of darkness.

Current Progress:

Research is continuing.

Project 7: Circadian and Vestibular System Relationships

PI: *Lawrence P. Morin, Ph.D.*
State University of New York, Stony Brook

Research Focus: Three dimensional motions to stimulate the vestibular and circadian system

Specific aims:

1. Identify efferent and afferent anatomical connections between the vestibular nuclei and the intergeniculate leaflet;
2. Test the hypothesis that patterned moving light (an optokinetic stimulus) will functionally activate the vestibular and circadian systems;
3. Test the hypothesis that a non-locomotor, non-photic three-dimensional motion stimulus will functionally activate the vestibular and circadian systems, as measured by FOS induction in the IGL and circadian phase shifts.

Grant Proposals:

1. Title: Intrinsic anatomy of the circadian rhythm system
   Agency: NIH
   Duration: Four years
   Total Direct Costs: $600,000.00
   Funding Status: Submitted

Current Progress:

1. Seth Horowitz hired as a research assistant professor with the prime responsibility of managing the project.
2. Constructed an oscillatory acceleration-deceleration stimulator to cause vestibular stimulation during the proposed experiments.
3. Initiated a study evaluating the effects of heavy water or ethanol on novel wheel-induced phase shifts.
4. Began a tract tracing study to determine the connections between the vestibular nuclei and the circadian rhythm system.

Major Findings:

All animals studied thus far (N=4) have a group of 6-8 neurons in the medial vestibular nucleus that project to the intergeniculate leaflet of the circadian rhythm system. There are a few other, similarly projecting neurons, scattered loosely throughout the medial vestibular nucleus, as well as in the nucleus prepositus.

Project 8: Long-Term Exposure to Dim Light Desynchronizes the Circadian System of Rats

PI: *Gianluca Tosini, Ph.D.*
Morehouse School of Medicine

***Research Focus*:** Use of melatonin to synchronize rhythms

***Specific aims*:**
1. Determine the effect of long-term exposure to constant conditions on circadian rhythms.

***Submissions/Publications*:**
1. Fukuhara C, Dirden JC, Tosini G. Regulation of period1 expression in the rat pineal. *J. Appl. Physiol.* 2001; submitted.

Grant Proposals:
1. Title: Photic and circadian regulation of retinal melatonin synthesis
   Agency: NIH RO1
   Duration: Five years
   Total Direct Costs: $1,372,087.00
   Funding Status: Submitted

2. Title: Regulation of gene expression in the pineal gland
   Agency: NIH RO3
   Duration: Two years
   Total Direct Costs: $143,000.00
   Funding Status: Submitted

3. Title: Temporal biology training program
   Agency: NIH T32
   Duration: Five years
   Total Direct Costs: $ 2,286,401.00
   Funding Status: Submitted

Current Progress:
Research is continuing.

Project 9: Animal Model for Sleep Loss and Circadian Disruption

PI: *Fred W. Turek, Ph.D.*
Northwestern University

***Research Focus*:** The effect of melatonin on circadian phase

***Specific Aims*:**
1. Determine the effects of 12 hours of imposed wakefulness during the normal active and inactive periods on circadian rhythms, the sleep-wake cycle and neurobehavioral and motor performance measures in the mouse;

2. Treatment with either a physiological or pharmacological dose of melatonin at the beginning of the imposed period of wakefulness will alter the effects of this temporal desynchrony on the circadian clock, the sleep-wake cycle and/or neurobehavioral and motor performance measures;
3. Access to a wheel (exercise) when in the home cage, will alter the effects of the imposed periods of wakefulness on the circadian clock, the sleep-wake cycle and neurobehavioral and motor performance measurements.

Grant Proposals:

1. Title: Importance of sleep and genotype in drug abuse studies
   Agency: NIH
   Duration: Three years
   Total Direct Costs: $300,000
   Funding Status: Submitted

2. Title: Mice lacking H3 receptor: model for early PD detection
   Agency: NIH
   Duration: Two years
   Total Direct Costs: $250,000
   Funding Status: Submitted

3. Title: Regulation of circadian rhythms and sleep in familial DSPS and ASPS
   Agency: NIH
   Duration: Five years
   Total Direct Costs: $1,000,000
   Funding Status: Submitted

4. Title: Sleepiness, performance and autonomic function in EDS
   Agency: NIH
   Duration: Four years
   Total Direct Costs: $800,000
   Funding Status: Submitted

Presentations:

1. Information Booth, *National Sleep Awareness Week*, March-April 2001, Northwestern University and Northwestern Memorial Hospital, Evanston, I.L. The importance of sleep and sleep disorders.
2. Invited Panel Member, *Chicago Tonight - Chicago WTTW – PBS*, April 2001, Chicago, I.L. "Sleep Deprivation in America".
3. *5th International Meeting on Sleep Disorders*, April 2001, Bordeaux, France. "Genetics and circadian rhythms".
4. *Center for Sleep and Circadian Biology - outreach program:* Take your daughters to work day, April 2001, Evanston, I.L. "Animal care in the sleep research environment".
5. *Bristol Myers Squibb*, April 2001, Hopewell, NJ. "Animal models of sleep disruption".
6. *Illinois High School & College Driver Education Association*, May 2001, Effingham, I.L. "Sleep Deprivation & Drowsy Driving".

7. Co-Chair - Discussion Group, *Associated Professional Sleep Societies 15th annual Meeting*, June 2001, Chicago, I.L. "Drowsy Driving a Public Policy Perspective".
8. Invited Lecture, *Associated Professional Sleep Societies 15th annual Meeting*, June 2001, Chicago, I.L. "Analysis of sleep/wake states in mice".
9. Discussion Member, *Associated Professional Sleep Societies 15th annual Meeting*, June 2001, Chicago, I.L. "Analysis of sleep/wake states in mice".
10. *National Sleep Foundation Meeting*, June 2001, Washington, D.C. "Animal models for sleep disruption".
11. *Universities Space Research Association*, June 2001, Hampton, V.A.
12. *MBL Physiology course*, July 2001, Woods Hole, M.A. "Animal models of sleep and circadian disruption".
13. *DARPA CAP Teaming Workshop*, August 2001, Las Vegas, N.V. "Novel and feasible ENU mutagenesis to screen and discover novel genes that affect the need to sleep".

Current Progress:

Setting up system to begin collection of data from mice in the next 1-2 months.

Major Findings:

Data from experiments in which rats were subject to chronic partial sleep restriction over 1-9 days indicate that there is a gradual alteration in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. In addition, when these animals are subject to a stressor there are changes in neuroendocrine reactivity and subsequent responses to stress.

Related Project working in Cooperation with the HPFSC Team: Microgravity and Circadian Cardiovascular Rhythms

PI: *Vincent M. Cassone, Ph.D.*
Texas A&M University

***Research Focus*:** Integrating circadian physiology with cardiovascular medicine.

***Specific aims*:**

1. Determination of SCN Efferents Controlling Circadian Variations of Cardiovascular Function in Long-Term, Conscious Rats;
2. Role of Circadian System on Daily and Circadian Variation in Regional Blood Flow;
3. Sex Differences in Circadian Variation of Cardiovascular Function.

Grant Proposals:

Title: NASA SPACE GRANT
Agency: NASA
Duration: Three years
Total Direct Costs: $30,000.00
Funding Status: *Funded*

Current Progress:

1. Equipment has been purchased and installed.
2. First Experiment in Specific Aim #1 has been partially completed. The effects of deafferentation of the suprachiasmatic to sub-paraventricular pathways on the

circadian variation in body temperature, activity and heart rate were determined in 12 Long Evans rats. Sham surgeries resulted in only temporary disruption of all parameters.

3. These rats will be place in a hindlimb suspension apparatus next to determine the effects of simukated weightlessness on the circadian variation of heart rate. Then, they will be sacrificed for histological verification of lesions.

Major Findings:

Sub-Paraventricular Knife-cuts reduce the amplitude or abolish the circadian pattern of heart rate but have no effect on body temperature or activity rhythms in rats.

V. FUTURE PROGRAM DIRECTIONS

The Human Performance Factors, Sleep and Chronobiology Team has nine ground-based projects currently funded until 2003. In addition to the progress toward countermeasure development anticipated from the currently funded research projects, it is anticipated that the following four research questions which are not being addressed by the current research program will be addressed in the coming years:

Physical effects. Research is needed to determine how space flight or exposure to chronic sleep restriction and/or circadian disruption affect sleep- and/or circadian-mediated neuroendocrine, metabolic, neurologic or autonomic functions, particularly those relevant to risk mitigation (e.g., growth factors, nutrition, glucocorticoids, monoamines) during extended duration missions.

Monitoring the status of sleep, sleep homeostasis, circadian rhythmicity and/or neurobehavioral performance during space flight. Research is needed to to validate methodologies that are portable and non-intrusive in the space flight environment to assess sleep and/or circadian rhythms.

Novel countermeasure development. Research is needed to determine how recent advances in the neurobiology of sleep and/or circadian rhythms (orexin/hypocretin system, circadian photoreception, output pathways that regulate sleep or circadian rhythms) can be used to develop countermeasures to adapt to and thereby maintain optimal neurobehavioral performance during exploration class space missions.

Age, gender and inter-individual differences. Research is needed to determine how age, gender and individual biological and behavioral characteristics alter sleep- and/or circadian-mediated physiologic responses to, and risk mitigation for, prolonged space flight.

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE
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2. Viral Infections and Mucosal Immunity – Janet S. Butel, Ph.D.

3. Suspension, the HPA Axis, and Resistance to Infections – Gerald Sonnenfeld, Ph.D.

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SIGNATURE: William T. Shearer 11-04-01

William T. Shearer, M.D., Ph.D. — Date

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I. EXECUTIVE SUMMARY

1. Research Problem and Basic Approach to Achieve Goals

The reconstituted Immunology, Infection, and Hematology Team (October 1, 2000 to September 30, 2003), in its six approved projects, has achieved new common themes and unity of purpose. Because of congressional delay in passing appropriations, the three new projects (Sonnenfeld, Gewirtz, Shi) required funding later than the October 1, 2000 start-up date. Nevertheless, the three new team project leaders have met by monthly conference calls and an in-person retreat with the three previously funded project leaders (Shearer, Butel, Fox) and formed a solid and productive research team with enthusiastic participation in team goals. These common goals include the investigation of altered immunity, infection, altered microbes, radiation-induced malignancy, and neuroendocrine abnormalities in earth-bound, space-equivalent models. The team is using both human subjects in isolation studies in the Antarctic winter-over and a Russian space capsule, human bone marrow pleuripotent stem cells *in vitro*, and animal subjects in hind-limb suspension (zero gravity effect) and radiation/viral challenge studies. There is an extraordinary synergy among the separate projects, in that the investigators are sharing information within and without the team structure. For example, the Shearer and Butel projects are looking at the immunological and virological consequences, respectively, of irradiating mice and challenging them with a latent virus in studies that include non-NSBRI investigators at Loma Linda University (LLU), where a synchrotron linear proton accelerator is available. The LLU investigators are funded through other federal research grants that enable them to offer the NSBRI teams a collaborative use of their radiation facilities. The LLU investigators also participate with the NSBRI Radiation Effects Team (Dr. John Dicello, Leader) in collaborative studies and intend to submit an application for NSBRI funding at the next request for proposals. The Immunology, Infection, and Hematology Team investigators are also collaborating with non-NSBRI researchers in pathology at Baylor College of Medicine and immunologists at M.D. Anderson Cancer Center to evaluate tumorgenesis in animals given radiation and viral challenges. Another example of the synergy approach is that of the Sonnenfeld project, that is collaborating with the NSBRI Radiation Effects Team at the Brookhaven National Laboratory (Dr. Marcello Vazquez) in planning for animal experiments involving both zero gravity and radiation effects. Similarly, Dr. Alan Gewirtz is collaborating with radiation biologists (Dr. Elizabeth Sutherland) at the Brookhaven National Laboratory in planning for his radiation experiments involving heavy ion radiation of human stem cells *in vitro*. Finally, Dr. Yufang Shi has consulted with Dr. Gerald Sonnenfeld in preparing to begin experiments with the hind-limb suspended mouse model.

2. Strengths, Key Findings, and Discoveries of the Team's Research Program, Including Synergisms Among Projects and Between Teams

As a team, the three continuing projects renewed in the present grant cycle and the three new projects coming on line have a very sharp focus of detecting host resistance mechanisms that are likely to be weakened by space conditions and the consequences of this immunodeficiency that result in the inability to contain infection and the appearance and spread of malignant cells. These investigations have been given new impetus by the awareness of additional information on the serious nature of infections (e.g., sepsis) suffered by some astronauts in space travel. Also, a new appreciation of the universal threat of deep-space radiation has prompted a careful examination of the potential for chronic infection and cancer in space travelers. The team's full

complement of funded projects will be better implemented next year, but significant progress has been made by the team in discovering alterations in immune function and latent virus reactivation in human models of space flight and in the improved methods of detecting microbial contamination of spacecraft. The detection of microbial contamination has included the collaboration of the Fox project with the Butel and Sonnenfeld projects within the Immunology, Infection, and Hematology Team, the Klempner project in the Smart Medical Systems Team, and the Pierson research project at the NASA-funded Johnson Space Center. The continuing collaboration of the Australian National Antarctic Research Expedition (ANARE) has provided a rich source of specimens for NSBRI investigators. Significant progress has been made by Dr. Sonnenfeld in further understanding the role of the neuroendocrine system in regulating resistance to infection using Klebsiella-challenged hind-limb-suspended mice, where an approximate two-fold difference lethality was observed in comparison to control mice.

3. Gaps in the Team's Program That Need to be Filled

There is an important need for another project (Radiation Immunobiology) to be added to the Immunology, Infection, and Hematology Team. The expertise and capabilities of the investigators (Gridley, Nelson) at LLU, for example, would greatly benefit the other projects in the team and speed up the synergy projects that are beginning. Because of other federal funding, the LLU investigators are able to provide use of the synchrotron proton linear accelerator, but there is no current funding mechanism for personnel and animal purchases, other than what can be supplied through the NSBRI projects. Funding of investigators at LLU would provide a much more active participation in the team's overall research plan.

4. Implications of Key Findings for Future Program Plans

The team's findings have validated the ground-based models of space flight, in that they confirm the hypothesis originally proposed. That is, space flight conditions, as they exist in these ground-based models, pose significant risks for the health of long-term space travelers. The team's overall goals of obtaining evidence of altered immunity, activation of latent viral infection, and potential serious health consequences have been achieved using both human and animal subjects. Improvement of a detection system for spacecraft microbial contamination is another achievement that indicates the team's success in preparing for long-term human space travel. The beginning of the team's research on the human bone marrow pleuripotent stem cell promises to yield a new understanding of the pathogenesis of space flight conditions on the innate and acquired forms of immunity. Implicit in all of the team's research is the development of a countermeasures program that will prevent or significantly ameliorate the effects of space flight on immune resistance mechanisms and prevent or lessen the incidence of viral infection and possibility of cancer formation.

II. INTRODUCTION

The Immunology, Infection, and Hematology Team is dedicated to understanding the risks posed to the human immune system by conditions of long-term space flight and to devising a countermeasures program that will ameliorate or eliminate the harmful consequences of infection and cancer that result from a weakened immune system. From numerous clinical examples, such as congenital immune deficiency, AIDS, and radiation sickness, it is abundantly clear that there

is a reciprocal relationship between the strength of the immune system and the occurrence of infection and cancer: a child born without T-cells succumbs to opportunistic infections by one year of life; an AIDS patient with low $CD4^+$ T-cell counts develops fungal sepsis and Kaposi sarcoma; an irradiated human dies from lymphoma. The known conditions of long-term space travel that impact the immune system are radiation, microgravity, microbial contamination, isolation, containment, sleep deprivation, and stress. There are already unpublished clinical data on astronauts which suggest that astronauts have an increased risk of death from lymphoid malignancies and skin cancers, conditions that the healthy immune system controls. There have been several incidences of bacterial sepsis associated with local infections, two of which aborted space flights. The skepticism of only a few years ago has yielded to the realization that space flight conditions will most certainly depress the human immune system. A most responsible reply to this threat is to make quantitative and controlled studies that will better delineate these risks to health and to adapt the measures of immunoreconstitution that we have on Earth to interplanetary space travel.

The six-pronged attack of the Immunology, Infection, and Research Team is to carefully assess different aspects of certain conditions of space flight using ground-based, space equivalent models. The team investigators include immunologists, hematologists/oncologists, molecular biologists, and microbiologists. These individuals have formed projects involved with several common risks of long-term space travel: immunodeficiency, cancer, reactivation of latent virus infections, mucosal immunity breakdown, interruption of the hypothalamic-pituitary-adrenal (HPA) axis, premature death of T-cells, and genetic alteration (superstrain) of microbes. The projects of the team address all of the Critical Questions contained in the Space and Life Sciences Directorate of the Critical Path. An example is Critical Question 7.33: "Are there currently accepted measures to treat immunodeficiencies (antibodies, immunizations, cytokines, T-cells, stem cells) that can be adapted to space flight?" The first step in answering this question is to determine to what extent innate and acquired immune responses are compromised by space flight conditions. With this information in hand, a careful approach can be made toward applying known methods of immunoprevention or immunoreconstitution to astronauts at risk.

III. RESEARCH PROGRAM STRUCTURE AND DESIGN

The co-investigators of the six projects of the Immunology, Infection, and Hematology Team have met by teleconference and in at a face-to-face retreat session and have planned the following team approach to coordinate and amplify the strengths of the individual projects and investigators. This team approach also includes non-NSBRI investigators, such as Dr. Daila Gridley at LLU (linear proton accelerator) and Dr. Elizabeth Sutherland at Brookhaven National Laboratories (facility for heavy ion radiation).

1. Radiation Studies

a. Murine Latent Virus Model. Co-investigators will include Drs. Shearer, Butel, Ling, Conner, Reuben, and Rosenblatt from the Immunodeficiency and Viral Infection team projects and Dr. Daila Gridley from LLU. Selected strains of mice (e.g., BALB/c, C57 black) will be exposed to proton and gamma ray radiation and subsequently to murine viruses (e.g., gamma 68, polyomavirus), in an attempt to determine the combined effects of space radiation and latent virus infection on the immune function of study animals. This first approach will examine the

simultaneous effects of radiation and infection and will then be followed by a sequential approach of infection first and radiation second, the likely scenario for human space travelers to Mars. The dose of radiation that will be utilized initially (3Gy, the estimate of radiation received by astronauts on a Mars Mission) will be that used by Dr. Gridley and her colleagues who have demonstrated rapid and profound alterations in immune cells and immune responses in murine subjects. Replicate and controlled experiments will be performed by both the LLU site and the Baylor site to insure that the same methods are followed at both sites and that the results of the experiments at Baylor confirm those of LLU. If gamma radiation proves to be equivalent to proton radiation, in terms of effects upon the immune system (e.g., spleen cell T-cell response to non-specific stimuli and specific antigen stimulation; plasma antibody formation to neoantigen; spleen lymphocyte subset distribution), it may be possible to avoid transfer of mice between institutions, as Baylor has a source of gamma radiation.

In addition to examination of the effects of radiation and latent virus infection on immune cells and immune responses, study animals will be evaluated for the development of tumors and blood malignancies. This will be carried out with the assistance of Dr. Cory Brayton, a veterinary pathologist at Baylor, who has agreed to collaborate on this project.

b. Human Stem Cell Model. Dr. Alan Gewirtz at the University of Pennsylvania has begun collaborative NSBRI studies with Dr. Elizabeth Sutherland at the Brookhaven National Laboratory with bone marrow-derived human stem cell lines. These cell lines will be exposed to heavy metal ion (Fe^{56}) radiation and subsequently tested by standard hematologic assays for ability to form colonies of cells in the myeloid series: granulocytes, erythroid cells, and platelets. In the future, similar experiments will be performed at LLU, where the effects of proton and gamma radiation will be evaluated in these same assays. Because the preparation of human stem cells from donor bone marrow also yields precursor cells in the lymphoid system, it will be possible to simultaneously evaluate the effects of the various types of radiation on the development of T- and B-cells. Similarly, macrophages and monocytes and stromal cells could be evaluated. The methods of analysis of these various types of immune cells could include measurement of cell growth factors (e.g., IL-3, IL-6, IL-7, TGF-β), apoptosis gene regulation (e.g., gene array assay), and cell repair pathways. These studies would include the collaboration of Drs. Gewirtz, Reuben, Rosenblatt, and Gridley.

Peripheral Blood Stem Cells. Stem cells will also be harvested by phoresis in subjects given GMCSF to increase the number of circulating stem cells at the M.D. Anderson Cancer Center. Dr. James Reuben will utilize these cell harvests in similar radiation studies and evaluate dendritic cell (#1 and #2 types) function in the presentation of antigens to lymphocytes.

In both bone marrow and peripheral blood stem cell preparations, evidence for genetic damage will be investigated by examination of progenitor cells for chromosomal breaks. These measurements will yield important information on the possibility that radiation of human stem cells might result in leukemogenesis and tumorigenesis.

2. Surrogate Marker Studies

Future collaborative studies were proposed for the Radiation Effects Team, in which the use of surrogate markers could be used to assess the risks of tumor development in irradiated animals. Surrogate markers would greatly reduce the time needed to evaluate tumorigenesis and to

observe exposed animals for cancer development. For the current Fe^{56} irradiated rat breast tumor model, one such surrogate marker might be the appearance of epithelial cells in the peripheral blood that herald the development of breast cancer. In addition to the detection of epithelial cells, it might be possible to examine the gene imprints of these cells by gene array assays. Such studies might yield a characteristic dysregulation of normal gene activation that would be predictive of breast cancer in this animal model. Collaborators include Drs. Shearer, Rosenblatt, Reuben, Butel, Gewirtz, Conner, Shi, and Gridley.

3. Antiorthostatic (Hind-Limb Suspension) Model

It is important that standardized procedures be used by the team investigators to allow for comparison of results across projects. The exact caging and suspension techniques do not have to be identical, but the parameters used for setting up the suspension should be uniform. The experiments using this antiorthostatic mouse model will include bacterial challenge in animals given catecholamines to alter the HPA axis and opioids to alter lymphocyte apoptosis pathways. These experiments will examine the importance of central nervous system control of infection and stress-induced immunosuppression. These same models will be studied in concert with radiation experiments to look at the combined effects of radiation and hind-limb suspension (microgravity) upon infection and lymphocyte depletion. Also, the virulence of microbes will be evaluated after radiation to determine whether increased resistance to immune control has emerged. Collaborating investigators will be Drs. Sonnenfeld, Shi, Vazquez (Radiation Effects Team), Fox, and Conner.

4. Commitments Made for Synergy Interactions among Investigators

These commitments are as follows (see also **Table 1**):

a. Drs. Shearer, Butel, Ling, Conner, Reuben, Rosenblatt, and Gridley for studies on radiation, viral infection and immune responses.

b. Drs. Sonnenfeld and Vazquez (also involving Drs. Shearer, Butel, Conner, and Gewirtz as plans develop) for studies on radiation and immune responses.

c. Drs. Sonnenfeld, Butel, Ling, Conner, and Shi for studies of the effects of neuroendocrine hormones and opioid mediators on viral growth and lymphocyte apoptosis.

d. Drs. Gewirtz, Sutherland, and Reuben for radiation and hematopoietic stem cell research and development of surrogate markers for development of malignancy.

e. Drs. Sonnenfeld, Fox, and Willson for studies to determine effects of neuroendocrine hormones of gene expression of bacteria by array analysis.

f. Drs. Fox, Willson, Butel, and Ling for studies of rapid detection of viruses.

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

The Immunology, Infection, and Hematology Team has made substantial progress in the overall goal of defining the risks due to exposure to conditions of space flight. The team has used human and animal experimental subjects to obtain research information in the Antarctic winter-over (Shearer, Butel), the closed chambers (Butel), and antiorthostatic models of space flight (Sonnenfeld, Butel). In addition, the team has made progress with *in vitro* radiation studies (Reuben, Gewirtz) and *in vitro* lymphocyte apoptosis studies (Shi). Finally, substantial progress has been made in perfecting microbial detection systems involving nucleic acid fingerprints (Fox). These accomplishments are described in detail in the six individual project reports by principal investigators Shearer, Butel, Sonnenfeld, Gewirtz, Shi, and Fox. However, these findings can be summarized in terms of the common themes of the Immunology, Infection, and Hematology Team (**Table 2**):

1. Abnormal Immunity, Infection

By using the same human specimens of plasma, saliva, and urine of the Antarctic winter-over and closed chamber models of space flight, the Shearer and Butel projects have obtained evidence of latent virus (polyomavirus) activation and T-cell activation in study subjects as compared to control subjects (**Tables 3 and 4**). This research was begun during the first cycle of NSBRI funding (1997-2000) and continued during Year 1 of the present funding cycle (2000-2003), in accord with the previous recommendations of the NSBRI External Advisory Council and the NSBRI Board of Scientific Counselors. Because of the large number of specimens (>1,500), these studies are ongoing, particularly in light of the concordance of the virologist and virologic assessments indicating latent virus reactivation and immune system perturbation in the Antarctic winter-over model. In studies performed in specimens from the closed chamber model at the Russian Institute for Biomedical Problems in Moscow, there was again evidence of latent virus activation, but the plasma cytokine and cytokine receptor levels need to be measured. These exciting preliminary findings demonstrate the synergistic power of the team approach to space immunology research. In accord with the previous recommendations of the NSBRI Board of Scientific Counselors, the studies of preserved lymphocytes from the Antarctic winter-over were not pursued after it was discovered that the viability of the cells was less than 50 percent. Preserved lymphocytes were not collected in the Russian closed chamber study because of local problems. Should the originally planned, but later postponed, NASA closed chamber study at the Johnson Space Center in Houston be reactivated, functional lymphocyte studies of proliferation to virus-specific antigen and T-cell cytotoxicity studies to virus-specific cell targets will be planned.

2. Antiorthostasis, Neuroendocrine Abnormalities, Altered Microbes

Even without full funding for the new projects, the Immunology, Infection, and Hematology Team has moved forward with experiments involving the antiorthostatic mouse model (**Table 5**, Sonnenfeld; **Table 6**, Shi). In addition, significant progress has been made in devising molecular and genetic probes for new and emerging strains of microbial organisms (**Table 7**, Fox). Catecholamines have been shown to increase their virulence. These findings suggest that the HPA axis may play a role in the stress-induced antiorthostatic mouse model. Challenge experiments with Klebsiella organisms produced death in suspended animals at twice the rate of incidence of control animals. Analysis of the TH1 vs. TH2 cytokine balance in these suspended

mice will yield useful information, particularly in light of the shift to a TH1 cytotoxic plasma cytokine profile observed in Antarctic subjects **(Table 3)**. It will be important to use the new bacterial detection probes developed by the team to use in the altered organisms seen in the suspended mouse model.

3. Radiation, Cancer

With partial and late funding of the new radiation project, only preliminary work has been accomplished (**Table 8**, Gewirtz; **Table 3**, Shearer). Radiation experts at Brookhaven National Laboratory and M.D. Anderson Cancer Center have been consulted to begin to plan for the *in vitro* heavy metal ion and gamma radiation of human stem cells.

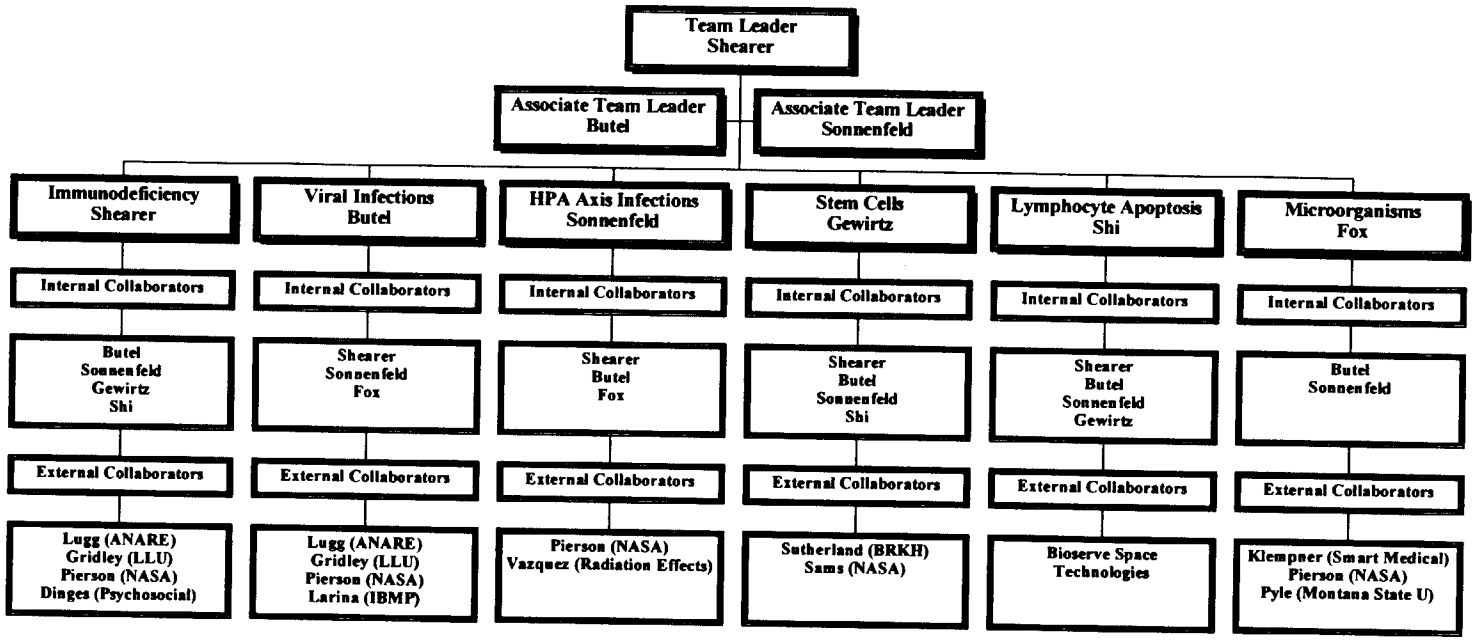
V. FUTURE PROGRAM (TEAM) DIRECTIONS

The NSBRI Immunology, Infection, and Hematology Team has recommended that the Critical Path risk of carcinogenesis caused by immunodeficiency due to space radiation be assigned a Risk Type of I (known and demonstrated serious problem without a proven countermeasure). The NASA IPT Immunology Team has concurred (October 30, 2001). Because of the preeminence of the risk factor of radiation, the team's future is primarily concerned with the immunodeficiency that radiation produces with the consequences of reactivated viral infection and development of tumors **(Table 9)**. Addition of the risk factors of microgravity, containment, isolation, and stress would only compound the effects of space radiation on the immune system. The countermeasures program that the team is developing is expected to be significantly advanced from the present condition by the year 2003 and fully operational in the year 2006 **(Table 10)**.

TABLE 1

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

PROGRAM DIAGRAM



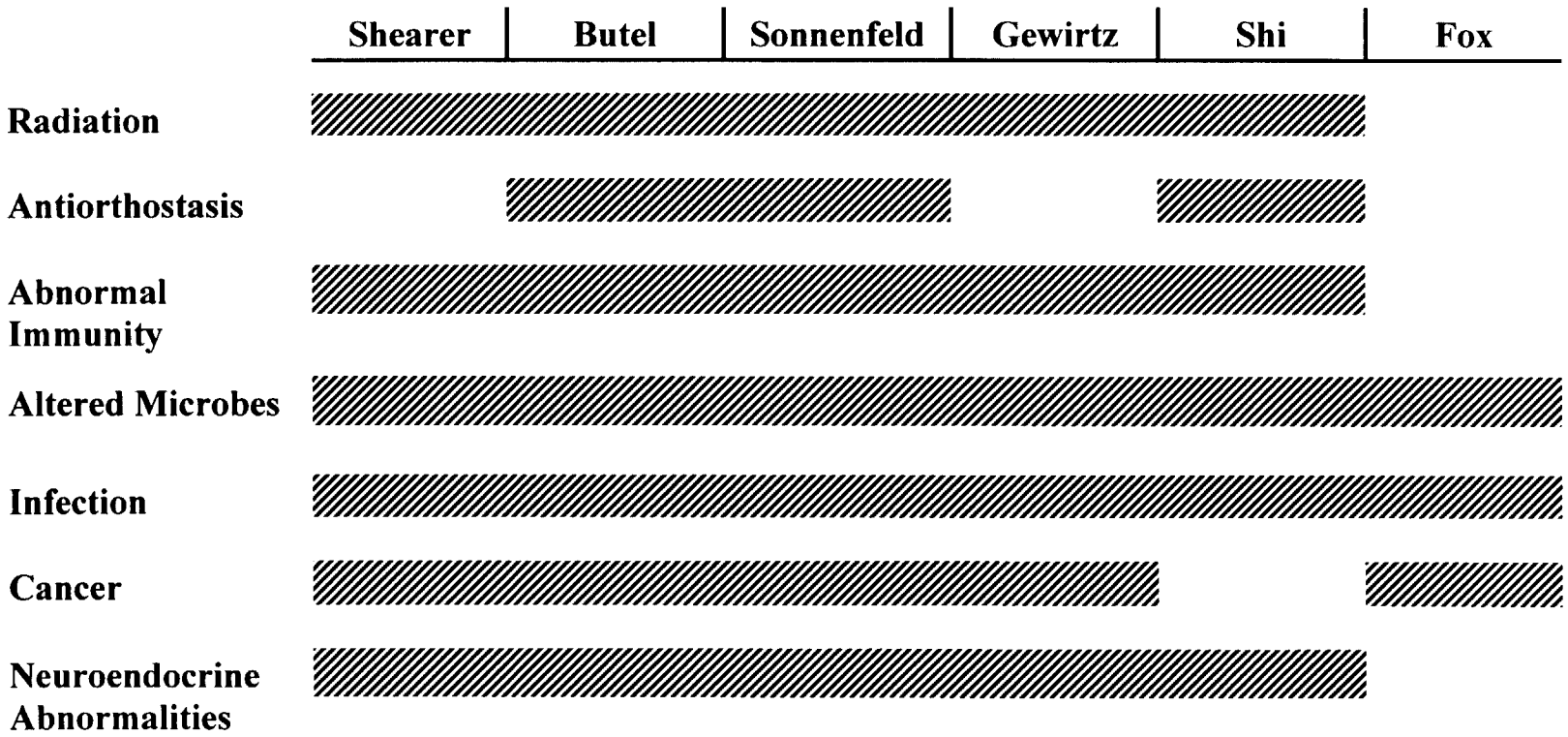
Key:

ANARE - Australian National Antarctic Research Expedition
NASA - National Aeronautics and Space Administration
IBMP - Russian Institute for Biomedical Problems
LLU - Loma Linda University
BRKH - Brookhaven National Laboratory

TABLE 2

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

PROGRAM STRENGTHS AND SYNERGIES AMONG PROJECTS



William T. Shearer, M.D., Ph.D.
Team Leader

TABLE 3

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

TEAM PROJECT: IMMUNODEFICIENCY (SHEARER)

NEW DISCOVERIES/ACTIVITIES SINCE FEBRUARY 2001

- **Antarctica Cytokine Study**
  - Δ **The TH1 cytotoxic cytokine (interferon-gamma)) appears to be elevated in Australian volunteers in the Antarctic winter-over space model, suggesting that the stressful conditions produce an alteration in the balance of key cytokines important in host defense against virus infection.**
  - Δ **Also, interleukin-12 (T-cell activating cytokine) appears to be elevated in these Antarctic volunteers, suggesting a possible response to reactivation of latent virus infection. Current publications and presentations indicate that Epstein-Barr virus, and polyomavirus become reactivated, and interleukin-12 may be a response to activated virus-specific T-cells in response to this infection.**
- **Using a radiation murine model of space flight, collaborative studies will be performed with Dr. Daila Gridley at Loma Linda University that will attempt to show that radiation-induced immunosuppression leads to reactivation of the murine-equivalent of Epstein Barr virus infection.**
- **Mobilization of human peripheral blood progenitor (stem) cells with GCSF and intracytoplasmic cytokines has been made as a prelude to performing radiation studies.**
- **Preliminary discussions with the United States Submarine Service to use this space model in experiments have been put on hold because of the war on terrorism.**

William T. Shearer, M.D., Ph.D.
Team Leader

TABLE 4

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

TEAM PROJECT: VIRUS INFECTIONS (BUTEL)

NEW DISCOVERIES/ACTIVITIES SINCE FEBRUARY 2001

- **1-Year Study of Normal Subjects**
  - Δ **All shed herpes EBV in saliva.**
  - Δ **JCV shedding age-dependent (≥ 40 yrs).**
  - Δ **Variable patterns among subjects.**
  - Δ **EBV, JCV, CMV reactivate independently.**
  - Δ **EBV shedding - seasonal periodicity.**
- **Antarctica Winter-Over**
  - Δ **JCV shed by younger persons.**
  - Δ **Will correlate with cytokines.**
- **Russian Chamber Study**
  - Δ **Analysis on-going.**
  - Δ **Apparent differences among crews.**
- **HIV Infection**
  - Δ **JCV shedding no longer age-dependent.**
  - Δ **EBV and JCV shedding ↑ as immune function ↓ (CD4 counts).**
  - Δ **Slight ↓ in immune function in normals correlates with virus reactivation.**
- **Murine Radiation/Virus Study**
  - Δ **Houston flood delayed start.**
  - Δ **Plans made with D. Gridley (LLU).**
  - Δ **Immune responses, cancer, infection to be studied.**

TABLE 5

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

TEAM PROJECT: HPA AXIS INFECTIONS (SONNENFELD)
NEW DISCOVERIES/ACTIVITIES SINCE FEBRUARY 2001

- **Murine Anti-Orthostatic (AOH) Suspension Model**
  - Δ **AOH-suspended mice have faster mean day of death and almost 2X greater mortality than controls when they are infected with *Klebsiella pneumoniae*.**
  - Δ **Potential pathogens (*Klebsiella pneumoniae*, *Escherichia coli*, *pseudomonas aeruginosa*) grow faster and produce virulence factors when grown in the presence of catecholamines. This points to possible involvement of the neuroendocrine system in enhanced mortality of AOH-suspended and infected mice.**
  - Δ **Studies beginning with anaerobic bacteria.**
  - Δ **Dr. Shi came to laboratory to learn how to develop AOH-suspension in his lab and standardize the procedure.**
- **Murine Radiation Study**
  - Δ **Dr. Sonnenfeld visited Brookhaven National Laboratory and met with Dr. Marcello Vazquez to discuss joint suspension, radiation, and immune response study.**

William T. Shearer, M.D., Ph.D.
Team Leader

TABLE 6

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

TEAM PROJECT: AOS - IMMUNE SYSTEM (SHI)
NEW DISCOVERIES/ACTIVITIES SINCE FEBRUARY 2001

- **Dr. Shi's competitive NIH grant funded.**
- **Specific aims for Dr. Shi's new NSBRI grant application:**
  - Δ **Study the cytotoxic cytokine (TH1) pattern vs. the inflammatory cytokine (TH2) pattern in the anti-orthostatic mouse model of space flight.**
  - Δ **Study the early programmed cell death (apoptosis) in this model to determine effects upon the education and selection (self vs. non-self) of lymphocytes as they pass through the thymus.**
  - Δ **Study lymphocyte mediators upon the osteoblast/osteoclast regulation of bone metabolism.**
- **Dr. Shi consulted with Dr. Gerald Sonnenfeld at Morehouse School of Medicine to gain expertise with the anti-orthostatically suspended mouse model.**

TABLE 7

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

TEAM PROJECT: MICROPROBES (FOX)
NEW DISCOVERIES/ACTIVITIES SINCE FEBRUARY 2001

- **Longer capture nucleic acid probes and molecular beacon probes have been devised to increase the specificity of identification tests of bacterial contamination of space potable water.**
- **An immunohybridization assay has been devised to identify the 16S rRNA sequences of pathogenic bacteria.**
- **Protocols for probe identification of bacteria under zero gravity conditions are being developed.**
- **Anion exchange and compaction precipitation are being adapted to rapid RNA determination.**

TABLE 8

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

TEAM PROJECT: STEM CELLS (GEWIRTZ)
NEW DISCOVERIES/ACTIVITIES SINCE FEBRUARY 2001

- **Dr. Alan Gewirtz has recently received NSBRI funding to begin his research project on the survival of bone marrow pleuripotent stem cells and their decreased differentiated cell types (neutrophils, monocytes, lymphocytes).**
- **Dr. Gewirtz has hired a postdoctoral fellow and organized his laboratory to prepare for this project.**
- **Dr. Gewirtz has collaborated with Dr. Elizabeth Sutherland at the Brookhaven National Laboratory to secure the access to radiation sources.**
- **The University of Pennsylvania will be able to provide Dr. Gewirtz with ground radiation sources to complement those at Brookhaven.**

William T. Shearer, M.D., Ph.D.
Team Leader

TABLE 9

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

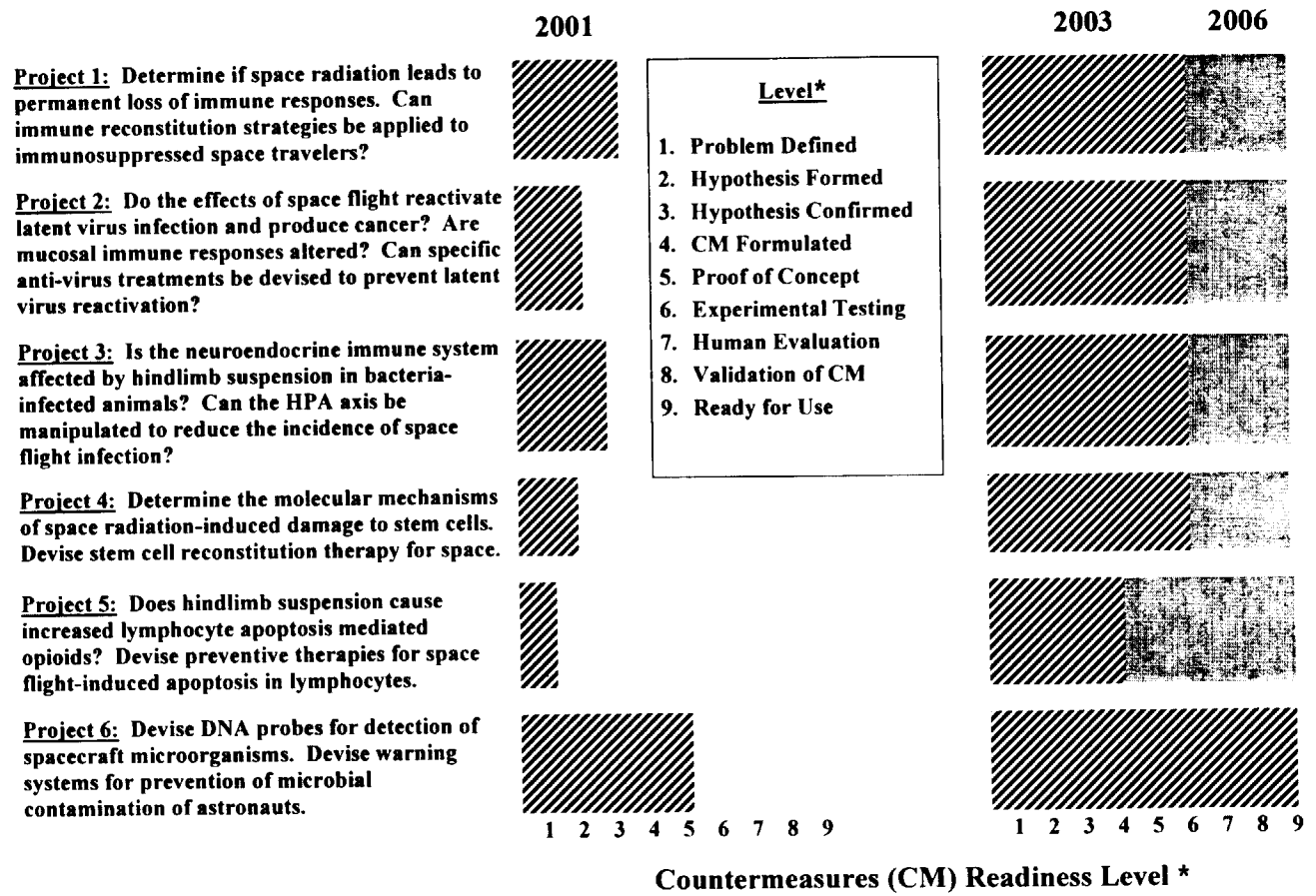
TEAM STRATEGY FOR COUNTERMEASUREMENT DEVELOPMENT

- **Evaluate if Radiation Damages Stem Cells or Differentiated Cells (Projects 1-5).**
  - Δ **Differentiated T-cell damage would indicate need for stem cell differentiation factors, e.g., IL-7, IL-12.**
  - Δ **Damage to thymus would indicate need for thymic factor treatment.**
  - Δ **Damage to primordial stem cell would indicate need for autologous stem cell replacement.**
- **Adapt DNA Probes to Viral Organisms (Project 6).**

TABLE 10

NSBRI IMMUNOLOGY, INFECTION, AND HEMATOLOGY TEAM

PROGRESSION OF COUNTERMEASURES READINESS LEVEL



NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE
INTEGRATED HUMAN FUNCTION TEAM
FY 2001 ANNUAL REPORT

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Prepared by MJ Kushmerick in consultation with JE Coolahan on 29 October, 2001 describing the work of the team in its initial operations.

Martin J. Kushmerick

TEAM PROJECTS		
Integrating Human Muscle Energetics and Mechanics Started Feb. 2001		
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Distributed Simulation of Integrated Human Function Started Dec. 2000		
James E. Coolahan, Jr., Ph.D.	Johns Hopkins University Applied Physics Laboratory 11100 Johns Hopkins Road Laurel, MD 20723-6099	Voice: (240) 228-5155 Fax: (240) 228-5910 James.Coolahan@jhuapl.edu
Integrative Cardiac Myocyte Model: Ion Channels, Ca and Contraction Started August 2001		
Donald M. Bers, Ph.D.	Loyola University Stritch School Of Medicine 2160 South First Ave. Maywood, IL 60153	Voice: (708) 216-1018 Fax: (708) 216-6308 dbers@lumc.edu
Metabolic Adaptations of Skeletal Muscle to Training/Detraining: A Systems Model Started June 2001		
Marco E. Cabrera, Ph.D.	Case Western Reserve University 11100 Euclid Ave.,RBC-380N Cleveland, OH 44106-6011	Voice: (216) 844-5085 Fax: (216) 844-5478 mec6@po.cwru.edu
Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle Started April 2001		
P. Bryant Chase, Ph.D.	Florida State University Department of Biological Science Biology Unit One Tallahassee, FL 32306-4370	Voice: 850-644-0056 Fax: 850-644-0481 chase@bio.fsu.edu
Integrated Modeling of Cardiac Mechanical and Electrical Function Started August 2001		
Andrew D. McCulloch, Ph.D.	University of California, San Diego 9500 Gilman Drive La Jolla, CA 92093-0412	Voice: (858) 534-2547 Fax: (858) 534-6896 amcculloch@ucsd.edu

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I. Executive Summary

The goals of the Integrated Human Function Team are to advance our description and understanding of human systems and their parts, to improve the ability to predict human responses to functional stresses - especially those encountered in long-duration space-flight - and to assess the potential efficacy of countermeasures as they interact in multiple cells and organ systems. The loss of homeostasis and/or appropriate dynamic responses in humans under stress involves the interaction of varied functions and mechanisms at many levels (e.g., molecules to organ systems). Thus, the goals of this team require the development of mathematical models and simulations in order to account for the interaction of this large number components. These models, based on known mechanisms and established data, will encapsulate our current understanding of the functions simulated. So when the models are compared to experimental data, missing information will stand out and will help to prioritize the tasks of gathering needed data.

The deleterious effects experienced by astronauts occur in all body systems, and integration is needed to cope with this complexity. Modeling ensures quantitative evaluation of astronaut performance in terms of underlying mechanisms and provides a way to judge possible widespread effects of proposed countermeasures. Molecules, intracellular organelles, cells, tissues, organs and systems are tightly inter-related and in the most basic meaning of the words, always operate as a unit. That is, human function for any particular system is an integrated unit. Therefore, it is essential that all modeling of human function be based on this hierarchical structure and integrate vertically as well as horizontally. Because of genetic and experiential differences among individuals, this integration needs to be able to address generic human responses as well as individual functional characteristics.

The term *"Digital Human"* captures this very ambitious vision of a whole-body, quantitative and physiological model. To make significant progress toward this goal, it will be necessary to be very detailed and specific with respect to the purposes of the models and to the major questions that the integration would answer. It will also be necessary to develop specific components and projects that are feasible and for which progress in a timely manner can be monitored. The work of this team will specify the key aspects of current knowledge in its areas of research and the congruent research in the other teams. It will also serve *predictive goals* for defined physiology, *analytical goals* as a check on the validity of the growing model, and *normative goals* for identifying missing data and novel questions. Each stage in the program is designed to make tangible and measurable progress toward the development of the *Digital Human.*

There are six projects in the current program. These projects were selected because they received very high peer review scores and because they formed a well integrated and complementary group of projects, for which opportunities for synergistic evolution were apparent. All projects focus on metabolism and on cardiac and skeletal muscle at the level of molecular, cellular and organ properties, and demonstrate a desirable balance of experimental work and synergistic modeling. It was anticipated that modeling projects would emerge as a natural outcome of the work of the other teams and that future calls for proposals would attract highly meritorious projects in other systems. Cell electrical properties, control of intracellular calcium dynamics, cross-bridge properties in different cell types, cellular energy metabolism, whole-body substrate distribution and metabolism that may be altered during space travel, and convergence of cellular to organ mechanics are being experimentally studied and modeled by this team.

The projects selected for inclusion in the team focus their research at what we call the "mesoscopic" level of organization, at the cell and tissue level, allowing meaningful, logical and mechanistic extensions down to the subcellular and molecular level and up to the organ and system level. In this way mechanisms are summed and integrated appropriately and naturally lead to emergent phenomena, which are the whole organ and system responses. These responses are not easy - and may even be impossible - to predict from the properties of lower levels of organization, unless the essential features of the components at that lower level are included, quantified and integrated. Without this logical and mechanistic integration, data regarding the higher level phenomena would not be interpretable in terms of the underlying cellular and molecular mechanisms and thus would remain purely descriptive.

This team has been operating for less than a year, some projects for only several months. Nonetheless, we envision successful accomplishment of our initial milestones within one year of beginning work.

First-year milestones for the cardiac group (Bers, Coolahan and McCulloch projects)

- Bers' and Puglisi's model will be made portable for operation in PC environments for the entire team and for any others upon request, with extensive text help files.
- Coolahan and McCulloch will demonstrate the ability to model electromechanics between their two sites and assess the potential for and extensibility of one piece of high-level software architecture for this purpose. The initial goal is to pass the results from electrical simulation at JHU for mechanical simulation at UCSD. Besides joining the research of these two groups, success will constitute a proof of the concept for this kind of distributed computation (see systems engineering below).
- A cellular and multicellular model of electromechanical coupling will demonstrate the principles for interconnectivity within the heart, which is the basis for conduction of electrical signals throughout the human ventricle.

First-year milestones for the limb muscle and metabolism group (Cabrera, Chase and Kushmerick)

- Cabrera will add a skeletal muscle compartment as the dominant volume of actively metabolizing tissue in his whole body metabolic model and join this with Kushmerick's cellular energy balance model, connecting mechanical output with cellular metabolic fluxes.
- Chase will develop his generic cross bridge models to include fast- and slow-twitch types of myosin heavy chains and add Ca^{++} control applicable to known troponin isoforms.
- Chase, Kushmerick and members of the Bers project will investigate muscle economy and efficiency.

Progress to date has implications for further research. Concepts are clear to bridge the differences between cardiac and skeletal muscle function at all the levels studied in the individual projects. The work in skeletal muscle at the myofibrillar level, uniting mechanics and energetics, and at the cellular level uniting energetics with whole body metabolism, will be readily transferable to the myocardium. Similarly, work in the myocardium on electrophysiology and biomechanics is transferable to skeletal muscle. The reasons for this synergy include common cellular principles and functions (some components differ but the basic structure of these processes is similar), common computer algorithms (some already developed and used) and common investigative tools for the experimental parts of the projects.

II. Introduction

The central hypothesis that permeates the Integrated Human Function team's research is that many of the physiological problems astronauts encounter in space can be attributed to the virtually complete inactivity of the lower body. Astronauts are highly active, athletic and fit, so this major reduction in activity has profound effects on all systems and loss of homeostasis and/or appropriate dynamic responses even under normal stress conditions. The reduction of metabolic activities results in altered nutrition and fluid needs and in reduced demands on the cardiovascular and respiratory systems in particular; but the effects occur in all systems. Acute exposure to 0G starts adaptive processes which lead (with various time courses from hours to weeks) to fluid shifts and reduction in body fluids and red cell mass, cardiovascular changes including the sympathetic and parasympathetic nervous systems, altered proprioceptive, vestibular, visual and other sensory inputs to the central nervous system, sleep and immunologic disturbances, muscle and bone atrophy, altered nutritional needs and intake, and overall metabolism.

These alterations of human physiologic systems and performance in the environment of space travel are appropriate adaptive responses to weightlessness and reveal that the human phenotype is extraordinarily robust and capable of adapting suitably even to stresses it has never previously faced. This adaptive capacity is remarkable given the fact that the totality of human evolutionary development has occurred under conditions of normal gravity. The crucial problems arise because of the need for astronauts to adapt rapidly to shifts from zero-gravity or micro-gravity conditions to normal conditions upon return to Earth. All of these normal adaptive mechanisms lead to sub-optimal performance in the environment of space and to poor or even life-threatening performance upon rapid re-entry to 1G conditions. But the key conclusion to be drawn from these observations is that countermeasures ought not to be developed that attempt to block these normal adaptive signals and responses. Instead the goal ought to be to utilize and exploit them to our advantage. The deleterious effects experienced by astronauts occur in all body systems and integration is needed to cope with this complexity.

The research of the Integrated Human Function team is organized around a strategy to advance the development of a whole-body, quantitative and physiological model, the *"Digital Human."* The research is designed to facilitate the development of needed tools for validating models and simulations, based on authoritative data, to represent integrated human function. Based on known mechanisms and established data, the models developed will capture current understanding of the function simulated, and thus when compared to experimental data will point out missing information on mechanisms and prioritize the tasks of gathering needed data. Ultimately, as the research progresses and the required data are attained, the models developed by this team will prove invaluable in predicting human responses to stresses encountered during space-flight and in assessing potential countermeasures.

III. Research Program Structure & Design

The goals of the Integrated Human Function Team have not changed since its formation just about a year ago and during its operation of its projects, variously for 3 to 10 months of funding: to advance our description and understanding of human systems and their vertically organized parts (molecules to systems) as an integrated unit, the intact human individual; to improve the ability to collect data, analyze that data, and use models to predict human responses to functional stresses, especially those encountered in long-duration spaceflight; and to use these integrated models and the resultant understanding to assess the potential effects on the whole body of countermeasures developed for one particular system. The projects in this program are designed to lead to paradigms for combining experimentation and simulation and software approaches needed for other systems and teams. The first-year goals of these projects and synergies within the team are given in the following section.

The projects selected for inclusion in the team focus their research at the cell and tissue level, which allows meaningful, logical and mechanistic extensions down to the subcellular and molecular level and up to the organ and system level. In this way mechanisms are summed and integrated appropriately and naturally lead to emergent phenomena: the whole organ and system responses. Without this logical and mechanistic integration, the higher level phenomena cannot be interpreted properly in terms of the underlying cellular and molecular mechanisms. This is why specific countermeasures are usually developed on the basis of understanding of these fundamental mechanisms. The problem with approaching integration of human function from the bottom up - the fundamental tenet of much of contemporary reductionist science - is that the molecular components themselves do not contain information regarding the functional purpose for their operation even one level higher in the hierarchy. This is the principle of teleonomy: processes in living organisms can only be understood in terms of the goal to be accomplished by that function. A purely top down approach uses high level abstractions of human function (such as cardiac output, renal clearance, motorunit recruitment, sympathetic nervous system output) and connects them logically. These high level functional abstractions are themselves emergent phenomena arising from lower hierarchical levels; they can be quantitatively organized and simulated only using ad hoc descriptive equations, not ones derived from the underlying biochemical, physical chemical, kinetic and thermodynamic functions and mechanisms. Such models exist for cardiorespiratory function, thermal balance and other physiological systems as currently useful teaching tools. Thus, modeling at both extremes of the hierarchical arrangement of human function is unlikely to succeed. For these reasons our team decided to focus on what can be termed the "mesoscopic" scale of function. This is typically the cellular level in which sufficient knowledge exists to allow meaningful, mechanistic and quantifiable connections with both the next lower level of organization and the next higher level. In this way a mesoscopic based plan of experimental research, simulation and model validation has the intrinsic ability to connect molecular and supramolecular mechanisms via cellular function to synthesize logically, in a scientifically testable manner, with tissue and organ properties. Our progress since the beginning of operation of our team has already demonstrated the fruitfulness of this strategy.

Scope of current activity and milestones

The team's focus and interactions are based on scientific research that is likely to have useful integrative results. Productive interactions have already developed among the project personnel. The current projects arose because muscle tissues and organs (cardiac and skeletal) provide outstanding examples of molecular to higher integration. The work of the presently constituted

six projects will produce two results of general utility to NSBRI and NASA. First, the current topics, when integrated, will be highly relevant to the overall research within NSBRI. Second, the process of working out the specific research of the team is likely to lead to paradigms for working out other systems, to outline needed research for other teams/areas, and to define the software approaches needed.

The six projects in the current program focus on metabolism and on cardiac and skeletal muscle at the level of molecular, cellular, and organ properties and demonstrate a desirable balance of experimental work and synergistic modeling. Ion channels, electrical properties, control of intracellular calcium dynamics, cross-bridge properties in different cell types, cellular energy metabolism, whole-body substrate distribution and metabolism that may be altered during space travel, and convergence of cellular to organ mechanics are experimentally studied and modeled. The current projects fall into two groups: studies of cardiac function and studies of limb muscle function with a project on systemic and intermediary metabolism further linking the two. First-year milestones within each of the two groups are listed below. All projects incorporate experimental studies and model simulations and analytical development.

First-year milestones for the cardiac group (Bers, Coolahan and McCulloch projects)
- Bers' and Puglisi's model will be made portable for operation on PC environment for the entire team and any others upon request with extensive text help files.
- Coolahan and McCulloch will demonstrate ability to model electromechanics between their two sites and assess the potential for and extensibility of one high-level software architecture for this purpose. The goal is to pass the results from electrical simulation at JHU for mechanical simulation at UCSD, returning new mechanical/anatomical model to JHU, and so forth. Besides joining the research of two groups, success will constitute proof of concept for this kind of distributed computation (see systems engineering below).
- A cellular and multicellular model of electromechanical coupling will demonstrate the principles for interconnectivity within the heart, which is the basis for conduction of electrical signals throughout the human ventricle.

First-year milestones for the limb muscle and metabolism group (Cabrera, Chase and Kushmerick)
- Cabrera will add a skeletal muscle compartment as the dominant volume of actively metabolizing tissue in his whole body metabolic model and join this with Kushmerick's cellular energy balance model, connecting mechanical output with cellular metabolic fluxes.
- Chase will develop his generic cross bridge models to include fast- and slow-twitch types of myosin heavy chains and add Ca^{++} control applicable to known troponin isoforms.
- Chase, Kushmerick and members of the Bers project will investigate muscle economy and efficiency.

Systems engineering in the IHF Team

Developing a solution to the complex problem faced by the IHF team requires an appropriate *systems engineering* approach. Lessons and tools for such solutions have been developed effectively and successfully over the past five decades to design, develop, and manufacture complex systems in government, business and academia. The systems engineering process begins with a statement of need, proceeds to a definition of system requirements, and then undertakes a successive multi-level decomposition of component functions which, when designed and implemented, will meet the overall need. Examination of the component functions

identifies components that are readily in hand and gaps needing development. This process is iterative, especially as new knowledge becomes available. Necessary interfaces between the components are also identified; this step is crucial. As individual components are developed, *systems integration* is performed to ensure that the components operate in unison as a system.

The long-term systems engineering and software engineering goal for the IHF Team is to develop a persistent, readily available set of models and simulations that can be composed as required to address long-duration space flight biomedical issues associated with the NASA Critical Path Road Map, providing predictive capabilities and the ability to assess proposed countermeasures from an Integrated Human Function perspective. Although there are many useful software engineering techniques, one important software engineering practice, object-oriented software development, is critical in developing a composable approach to software systems, such as those models and simulations needed to answer particular questions in the IHF area. Simply stated, an object-oriented approach involves developing software modules for specific functions with well-defined external interfaces, but which hide the internal methods by which the functions are performed. This methodology greatly enables integration (or, perhaps better stated, *interoperability*) of multiple software modules and applications, whether executing in a tightly coupled fashion on a single computer or more loosely coupled across a local- or wide-area-network. It also facilitates *reuse* of individual software modules across multiple applications composed to answer different questions.

First-year milestones for IHF Team for inter-project integration:

- As a starting point for electronic collaboration, utilize the existing NSBRI secure site to post and exchange relevant research information, plans, and interim results, and to make models and simulations available for sharing. This and the milestone below form the beginning of our bioinformatics efforts within current projects.
- Participate in the development of a limited data element dictionary to ensure common understanding of the semantics of data elements that could be exchanged by the models and simulations being developed. This is important in ensuring meaningful integration and interoperability of models and simulations as the work of the Team expands.

IV. Research Program Accomplishments

To initiate the team and start its work, six projects focus on models of the heart and limb muscle function and metabolism because these tissues and organs are centrally involved in, and form a causal mechanistic link among the adaptive responses occurring in microgravity cited above. Information on these cells and organs is extraordinarily rich so that these topics are ripe for quantitative simulation and analytical modeling. The projects are designed to lead to paradigms and software approaches needed for other systems and teams. The first-year goals of these projects and synergies within the team are given below. Although we have just begun and have done so in a time-staggered manner, it is clear that important progress has been made. This progress is due in large measure to three features of the projects in this team:

- soon after the team was formed but before all projects actually began funding, our retreat made reality out of merely anticipated interactions. Unanticipated synergies were uncovered. Common approaches were selected.
- each NSBRI project is operating in a large and generally well funded laboratory so that work commenced earlier in many cases that did the actual funding by redirecting efforts from the time the team was announced.
- the scientists in each project were scientifically well known to each other prior to forming the team, and in some cases already had productive interactions and collaborations.

The six projects in the current program focus on metabolism and on cardiac and skeletal muscle at the level of molecular, cellular and organ properties and demonstrate a desirable balance of experimental work and synergistic modeling. Ion channels, electrical properties, control of intracellular calcium dynamics, cross-bridge properties in different cell types, cellular energy metabolism, whole-body substrate distribution and metabolism that may be altered during space travel, and convergence of cellular to organ mechanics are experimentally studied and modeled. The current projects can be grouped several ways:

- type of cell and organ: studies of cardiac function and studies of limb muscle function with a project on systemic and intermediary metabolism further linking the two.
- experimental and theoretical simulations; all projects combine both for reasons given above.
- development of modeling tools versus use of modeling tools: while each project will develop its own simulation and model, the projects led by Coolahan and McCulloch also will develop generally adaptable software tools, which they will need for their own work in the first instance, but ipso facto are applicable to the whole team and for the other teams.

ACCOMPLISHMENTS BY THE TEAM AS A WHOLE

We consider the development of the following broad notions as important contributions of collective work of the team:

- Mesoscopic level is the most appropriate to begin functional and mechanistic integration:

The development of each of our projects independently indicated the general tendency to work at similar levels of organization within each project's particular subject material. This level was generally at the level of individual cells and organized collections of cells in tissues and organs. Out of these individual judgements, we formed a clear vision at our April retreat that the most appropriate scientific and productive approach to our work was at the "mesoscopic" level. This is the level that each project naturally gravitated to and can be defined as the middle level of

organization. It is scientifically appropriate because current investigations occur at that level. It is productive because it is relatively easy to extend, experimentally and by modeling, information both down towards and to the molecular level and up towards and to the organ and system level. Examples are given under the progress list for each project.

• Approach to systems engineering should start at an intermediate level of complexity:

Developing a solution to the complex problem faced by the IHF team requires an appropriate *systems engineering* approach. Lessons and tools for such solutions have been developed effectively and successfully over the past five decades to design, develop, and manufacture complex systems in government, business and academia. The systems engineering process begins with a statement of need, proceeds to a definition of system requirements, and then undertakes a successive multi-level decomposition of component functions which, when designed and implemented, will meet the overall need. Examination of the component functions identifies components that are readily in hand and gaps needing development. This process is iterative, especially as new knowledge becomes available. Necessary interfaces between the components are also identified. As individual components are developed, *systems integration* is performed to ensure that the components operate in unison as a system. Current practice shows that the iteration works well when the starting point is intermediate, i.e. a mesoscopic level of biological organization, working towards the top in a systems-level integration and towards the cell and molecule components. Thus the six current projects in the IHF Team are working at this mesoscopic level of organization in their experimental work as well in the modeling and simulation work.

• Modeling and integration needs to have a specific goal and to be targeted. Integration and models need to account for specific functions in specific circumstances and over specific time and spatial domains (and possibly other landscape features). The Critical Path Analyses should provide these when developed more fully. This is the practical application of the principle of teleonomy to NASA's goals.

• Modern biomedical science requires that all models ultimately be based on known mechanisms, which are largely at the molecular and cellular level (due to the enormous success of reductionist biology over the past several decades). Simply put, this is where the science is now and where it will remain.

Research accomplishments for each project in the team.

INTEGRATING HUMAN MUSCLE ENERGETICS AND MECHANICS
M. J. Kushmerick, principal investigator

• Cellular biomechanics in human muscle.

We have analyzed the tibialis anterior muscle with a 3D method which is superior to all current 2D analyses which give only projections of solid angles. The tibialis anterior muscle has a bipennate anatomy forming a central tendon that twists distally. Correct anatomical details are needed because the force that the cells develop equals the force in the tendon times $1/\cos\theta$, where θ is the angle between the fascicle bundle of cells and the tendon. Obviously larger forces are borne by the muscles at large angles, and the cellular force may change significantly with shape changes during contraction and with atrophy. All angles are measured normal to the plane

of the tendon at the fascicle. The angle of insertion of the fascicles appears to be invariant along the muscle. The fascicle angles (measured in approximately 1/3rd of the muscle) at rest were not significantly different anywhere in the measured volumes, and averaged 11° ± 5° (stdev).

Work is underway to evaluate the fascicle angle during active muscle contraction. This information is needed to assess the change in muscle cell forces due to anatomical factors and to enable better interpretation of the functional energetics and metabolism at the level of the cell from measurements in whole muscle. Also these results provide important data for the biomechanical modeling to be started in year two of this project.

• Modeling intracellular energy metabolism

We recently published a relatively simple yet powerful algorithm which accounts for the time course of energy changes during and following muscle activity. We made progress in three directions with the modeling component of this project this year:
- include a term to account for glycolysis in the current published model
- test generality of the model for transient as well as steady state exercise
- include a function relating force and ATPase.

The advanced model is given by the following differential equation:

$$\frac{dATP(t)}{dt} = -k \bullet force \bullet ATP(t) + OxPhos(t) - \frac{dPCr(t)}{dt} + \text{glycATP(t)}$$

We tested the application of this more complete energy balance model as expressed in the equation above for the analysis of a variety of types of contractions. Three types of exercise were analyzed successfully, using the model with the same biochemical parameters: brief (90 sec) maximal twitches both in normal and with ischemic conditions and steady state normal exercise for 7 minutes. These initial results demonstrate that the energy balance model is robust and applicable to a range of physiological states in human exercise. In other applications we have demonstrated this model also applies to rat and mouse muscle, with appropriate changes in parameter values but not in the basic algorithm. We are in position now to apply this analysis to a number of normal individuals to quantitatively and mechanistically assess the differences in the biochemical parameters and system performance that exist. The model also serves as a basis for analysis of working contractions; these will be carried out and analyzed during the rest of this year and the next.

Another goal is to implement a detailed model for glycolysis and oxidative phosphorylation. This is planned to be the intracellular counterpart to the whole body metabolic work in the Cabrera project in the Integrated Human Function team. We realize that much of the intracellular energetic model will be directly applicable to the myocardium and interfaced with the work of the Bers, McCulloch and Coolahan projects. As soon as the Chase project develops a working crossbridge model that will replace the simple term for ATPase in our current model.

• Functional anatomy of microvasculature and visualization of intramuscular vascular dynamics gives kinetics of recovery

Three dimensional ultrasound scans of intramuscular arteries and veins (~ 1 mm and smaller) near the middle of the tibialis anterior muscle were obtained. These vessels were demonstrated to change their size and blood velocity in response to exercise. These scans produced images of vessels analyzable by a number of criteria, including 1) the number of pixels

showing vessels with a velocity above a threshold flow, 2) the velocity within the vessels, and 3) the Doppler power within the vessels. Thus, functional imaging of the dynamic response intramuscular vasculature is feasible. 3D US images were obtained after several minutes at rest and during the 15 minute period of recovery following 1 minute of exercise (foot dorsiflexion/plantarflexion). At each time point, 120 images were captured over an approximately 5-cm length of the muscle. Dense cross-sectional views of the anterior tibial muscle were captured in power Doppler mode with the gray scale background included. The position and orientation of the scanhead were recorded during image acquisition by a magnetic tracking system so that the scanned volume could be reconstructed in 3D. Within the volume the 3D images showed a large vasodilation after exercise which returned to baseline. The time course of these changes could be fitted to an exponential decay. In 9 subjects, the mean time constant was 2.3 ± 1.1 minutes. These transient responses are twice the duration as the directly measured blood flow in the nutrient artery by color doppler and by the dynamics of intracellular energetics as determined by observation of the time course of chemical changes and flux calculations from energetics modeling. We are working on further experiments and analyses to determine the reason for this apparent difference. These results establish a firm basis for proceeding with a detailed structural-functional analysis of exercise hyperemia at 1G that is readily extensible to 0G environment with technology planned for the ISS.

DISTRIBUTED SIMULATION OF INTEGRATED HUMAN FUNCTION
James E. Coolahan, Jr. principal investigator

- Work progressing on human heart imaging and human myocyte model (Winslow).

We have developed a relational database (built on DB2) and user interface (Java) for visual interaction with, and querying, of the anatomic data sets. We have also developed a suite of Matlab tools that work using this interface, and which can be used to fit finite element models of both cardiac geometry and fiber fields. The advantage of this tool is that it allows interactive visual guidance of the fitting process by the user. MRI imaging methods are adapted to image cadaver human hearts.

- Developed simplified high-speed cardiac electrical/mechanical cardiac simulation and integrated using High Level Architecture (HLA) as proof-of-concept

As an early demonstration of the ability to construct an interoperable simulation of cardiac electrical and mechanical function, a simple electrical activation model and a simple mechanical deformation model were constructed. The electrical model utilizes a 2D monodomain reaction-diffusion partial differential equation (PDE) representation of the excitable tissue with a piecewise-continuous, nonlinear voltage-current source term to represent the local excitation and recovery properties of the cells. The grid points for the PDE solutions are arranged on a 10 cm x 10 cm grid with periodic boundary conditions in one direction (parallel to the fast axis) and no-flux boundary conditions in the other direction (slow axis). Topologically the elements form a cylinder with the cardiac "fiber" directions oriented circumferentially. The mechanical model of the tissue consists of an array of square elements arranged in a rectangular grid with the same cylindrical topology as in the electrical model. A simple Windkessel electrical circuit analog with ideal diodes is used to represent the aortic and mitral valve behaviors. Solutions for the set of differential equations for the equivalent electric circuit were obtained and yielded ventricular

and systemic pressures at successive time points. The instantaneous ventricular pressures were then used to compute the radii of each concentric tissue fiber ring comprising the whole ventricle model.

To demonstrate the applicability and viability of the High Level Architecture (HLA) for purposes of IHF simulation, the simplified electromechanical heart model discussed above was converted into an HLA federation. The electromechanical model was broken down into two different components that would form the basis of two HLA federates. The first federate was composed of the simple electrical activation model and also performed ECG computations, while the second federate contained the simple mechanical deformation model and the Windkessel electrical circuit analog. Next, a distributed federation execution was successfully completed with each federate executing on a separate machine and all data being passed by the HLA Run-Time Infrastructure (RTI) over the JHU/APL network.

- Work progressing on integration of JHU electrical model and UCSD mechanical model of cardiac function.

In support of the effort to achieve the distributed high-fidelity simulation of electromechanical cardiac function via interoperation of the UCSD and JHU models, most initialization and configuration steps have been completed. The simulation software used by the JHU (Winslow) electrical model has been installed. The mechanical model is simulated using the Continuity 5.0 continuum modeling software package developed by UCSD. Simulations have been run remotely on UCSD's Origin 2100 server and configured so that a JHU/APL-based computer performed all visualization functions. Using this configuration, several remotely executed mechanical simulations have been performed, including passive and active inflation of a symmetric ellipsoidal 3-element model of a canine left ventricle; passive and active inflation of a symmetric ellipsoidal 3-element model of a canine left ventricle using a JHU/APL-generated input data sequence; and passive inflation and systolic deformation of a non-symmetrical high-order 24-element finite element (FE) model of the 3D canine left ventricle.

- Cardiac and cardiovascular system models being integrated.

A collaboration has begun with members of the NSBRI Cardiovascular Alterations team at the Massachusetts Institute of Technology (MIT). A more advanced (compared to the simple model discussed previously) electrical-mechanical simulation of the left and right ventricles is being integrated with a new cardiovascular system model currently under development at MIT that is based on the "CVSIM" cardiovascular simulator that is used as a teaching tool in the Harvard-MIT Division of Health Sciences and Technology. The original MIT model was derived from experimental data and is a generalization of the simple Windkessel lumped circuit model of the heart and circulation. The model parameters have been adjusted so that the model yields a heart rate variability spectrum comparable to the average spectrum obtained from 15 NASA astronauts.

This electro-mechanical model of the heart is being integrated with the MIT cardiovascular system model using the HLA, replacing the simple sinusoidally-varying capacitance model of the ventricles currently used. The integrated cardiovascular system model will initially be used to study hemodynamics and reflex responses to abnormal cardiac rhythms, such as electrical alternans, runs of ectopic beats, and also tachycardia. These behaviors can most directly demonstrate the capabilities of an integrated model.

INTEGRATIVE CARDIAC MYOCYTE MODEL: ION CHANNELS, CA AND CONTRACTION

Don Bers, Principal Investigator

Our modeling effort is currently being implemented in a user-friendly fashion through development of an interactive computer program, LabHEART© . It was developed to simulate the action potential, ionic currents and Ca handling mechanisms in a rabbit ventricular myocyte. User-oriented, its design allows switching between voltage and current clamp, easy on-line manipulation of key parameters to change the original formulation. The model reproduces normal rabbit ventricular myocyte currents Ca transients and action potentials (AP).

We can also change parameters to simulate data obtained from heart failure (HF) myocytes, including reduced transient outward and inward rectifying K currents (I_{to} and I_{K1}), enhanced Na/Ca exchange expression and reduced SR Ca-ATPase function, but unaltered Ca current density. These changes caused reduced Ca transient amplitude and increased AP duration (especially at lower frequency) as observed experimentally. The computer model showed that the increased I_{NCX} in heart failure lowers the intracellular [Ca] ($[Ca]_i$) threshold for a triggered AP from 800 to 540 nM. Similarly the decrease in I_{K1} reduced the threshold to 600 nM. Changes in I_{to} have no effect. Combining enhanced Na/Ca exchange with reduced I_{K1} (as in heart failure) lowered the threshold to trigger an AP to 380 nM. These reproduce experimental results in heart failure where the contributions of different factors is not readily distinguishable. We conclude that the triggered APs that contribute to nonreentrant ventricular tachycardia in heart failure are due approximately equally (and nearly additively) to alterations in I_{NCX} and I_{K1}. A free copy of this software can be obtained at http://www.meddean.luc.edu/lumen/DeptWebs/physio/bers.html and this work is currently in press in the American Journal of Physiology.

On the development end of the project, we have developed a new detailed mathematical model for Ca^{2+} handling and ionic currents in the ventricular myocyte. The model includes the following novel features: 1) Three functional cytosolic compartments (junctional, subsarcolemmal and bulk) since ion channels in the junction and elsewhere sense ion concentrations which differ from bulk (figure below). 2) A reversible SR Ca pump as suggested by the results of Shannon, *et al. (Biophys. J.* 78:322-333), 3) A scheme for Na-Ca exchange transport which is [Na]i-dependent and allosterically regulated by $[Ca]_i$ as proposed by Weber, *et al. (J. Gen. Physiol.* 117(2):119-31) and 4) A phenomenological model of SR Ca release (Fabiato, *J. Gen. Physiol.* 85:247-289) including both inactivation/adaptation (Cheng, *et al.*, *Science* 267:2009-2011, Stern, *et al.*, *(J. Gen. Physiol.* 113:469-489) and SR Ca load-dependence (Shannon *et al.*, *Biophys. J.* 78: 334-343). The data describes normal electrical activity and Ca handling characteristics of the cardiac myocyte and the SR Ca load-dependence of these processes. A particular emphasis is placed upon reproducing the non-linear dependence of fractional SR Ca release upon load and we conclude that this model is more robust than previously existing models that do not adequately account for SR Ca load-dependence. A manuscript from this work is currently in preparation.

Also on the development end, we are currently working upon a model that emulates the Ca dependence, cooperativity and force-generating capacity of the myofilaments of the cardiac myocytes. Extensive work has been done upon these aspects of the project and the outlook is promising. This aspect of the project interfaces directly and synergistically with the Chase project.

METABOLIC ADAPTATIONS OF SKELETAL MUSCLE TO TRAINING/DETRAINING: A SYSTEMS MODEL

Marco Cabrera, Principal Investigator

This project is developing a computational model of skeletal muscle metabolism that incorporates the necessary components (enzymes, substrates, reactions, and pathways) and subsystems (slow-twitch and fast-twitch fibers) participating in the acute and chronic metabolic adaptation to training and detraining. The resulting computational model may ultimately assist in the design and evaluation of optimal exercise training protocols to counteract the deleterious effects of weightlessness on skeletal muscle structure, metabolism and function. In parallel, we started the development of a computational model of cardiac metabolism that could be incorporated into our proposed whole-body model of energy metabolism. Thus, over the last five months we have focused on developing a detailed model of metabolism for skeletal muscle and an initial model of metabolism for cardiac muscle.

Skeletal Muscle Metabolism. Training Responses.

We have worked on identifying morphological/structural, biochemical/metabolic, and functional characteristics of skeletal muscle fibers that are affected by endurance training. Accordingly, we have redesigned the model subsystems and their elements. The new model of skeletal muscle is subdivided into slow- and fast-twitch fiber type compartments in order to show the effects endurance training has on the morphology and metabolism of each specific type of muscle fiber. To obtain a more accurate description of the metabolic changes that occur during endurance training, various substrates, enzymes, and metabolites have been added (See Table). In the previous model, there was no differentiation between fiber types and many intermediate substrates and enzymes were lumped into a single summarizing reaction.

At this point of the project, a map of the pathways of energy metabolism in skeletal muscle has been created, and essential parameters in skeletal muscle adaptation to training have been identified. Yet to be determined is the link between gene expression and activity of the various enzymes affected by training. The activity of many enzymes with endurance training increases before any increase in mitochondrial density is seen in muscle cells. This means there must be some factor other than an increase in the number of mitochondria that plays a role in increased enzyme activity. Once we link together the factors affecting enzyme activity, the next step is to mathematically integrate the different metabolic pathways in muscle using this redesigned model.

Cardiac Metabolism.

As part of developing a whole-body model of metabolism we are developing a computational model of cardiac metabolism. Currently, we are estimating model parameters from experimental studies and validating some of the model predictions.

In addition, Marco Cabrera visited Dr. Babs Soller at the Medical Center of the University of Massachusetts, Worcester to explore the possibility of building a collaboration. Dr. Soller is a PI member of the Smart Medical Systems team. The collaboration will investigate the links between interstitial pO2, pCO2, pH, lactate metabolism and work capacity in order to develop and monitor high intensity training programs for the International Space Station. The aim of this proposal is to develop optimal training programs to minimize time invested in counteracting the effects of microgravity on skeletal muscle function.

CELL AND MOLECULAR BIOMECHANICS: CARDIAC AND SKELETAL MUSCLE
P. Bryant Chase, Principal Investigator

Work progresses on two fronts:

- isoform dependence of product inhibition of cellular biomechanics at the cross bridge level

Human and animal muscles are composed of a mixture of cell types. To correctly model the interaction between cellular biomechanics and energetics in heterogeneous systems, it is necessary to know the properties of the individual cell types. We have made comparison in single fibers from five skeletal muscles of the rat. The sensitivity to inorganic phosphate (a metabolite that accumulates during strenuous exercise and fatigue) and to pH (which also decreases with contraction and fatigue). While all fibers were inhibited by Pi and lower pH, there were striking fiber-specific differences. Soleus fibers which contain the highest proportion of slow myosin heavy chain were inhibited by Pi at lower concentrations that in other fibers; half maximal inhibition occurred at 6 – 7 mM Pi in soleus. Fibers with high proportion of fast myosin heavy chain showed the greatest pH inhibition to force development. We also confirmed the differences in Ca^{++} sensitivity to development of contractile force but the new information showed a fiber-specific alteration in this sensitivity.

- molecular modeling of cellular biomechanics

Our initial work incorporates into a Monte-Carlo simulation of cross bridges protein compliances derived from measured values and Ca^{++} regulation. An important new addition is that the model contains a 3-dimensionally accurate representation of the myofilament lattice structure. This model produced activation curves similar to measured force-pCa curves. The shape of these curves depends on the compliance of the proteins in the filament; this is a property which is not usually considered in thinking about the effects of protein isoforms (both in the thin and thick filaments). This result suggests that part of the well known cooperativity in the Ca^{++} activation is due to mechanical properties in addition to protein-protein interactions. Experimental work is characterizing the properties of various isoforms. The combination of experiment and modeling will develop a unique and useful model of muscle chemomechanics at the cross bridge and single cell level; these can be summed and integrated over the fiber types comprising anatomical muscles to yield accurate predictions of function.

INTEGRATED MODELING OF CARDIAC MECHANICAL AND ELECTRICAL FUNCTION
Andrew D. McCulloch, Principal Investigator

Funds for this project were only received from the agency beginning August 1st, 2001, but substantial progress has already been made in the following areas:

Software engineering:
We have almost completed the alpha release of *Continuity 6.0,* which has been specifically designed to facilitate model integration. The computational server and user interface client have been completely separated and communicate across any network through standardized XML-based data encodings. The user interface client is scriptable, programmable and includes a

graphical user interface with powerful embedded visualization. The model computational server has been modularized so that different model subsystems can be readily loaded and integrated. See http://cmrg.ucsd.edu/modelling/modelling.html. We expect the new release to be ready by the Fall.

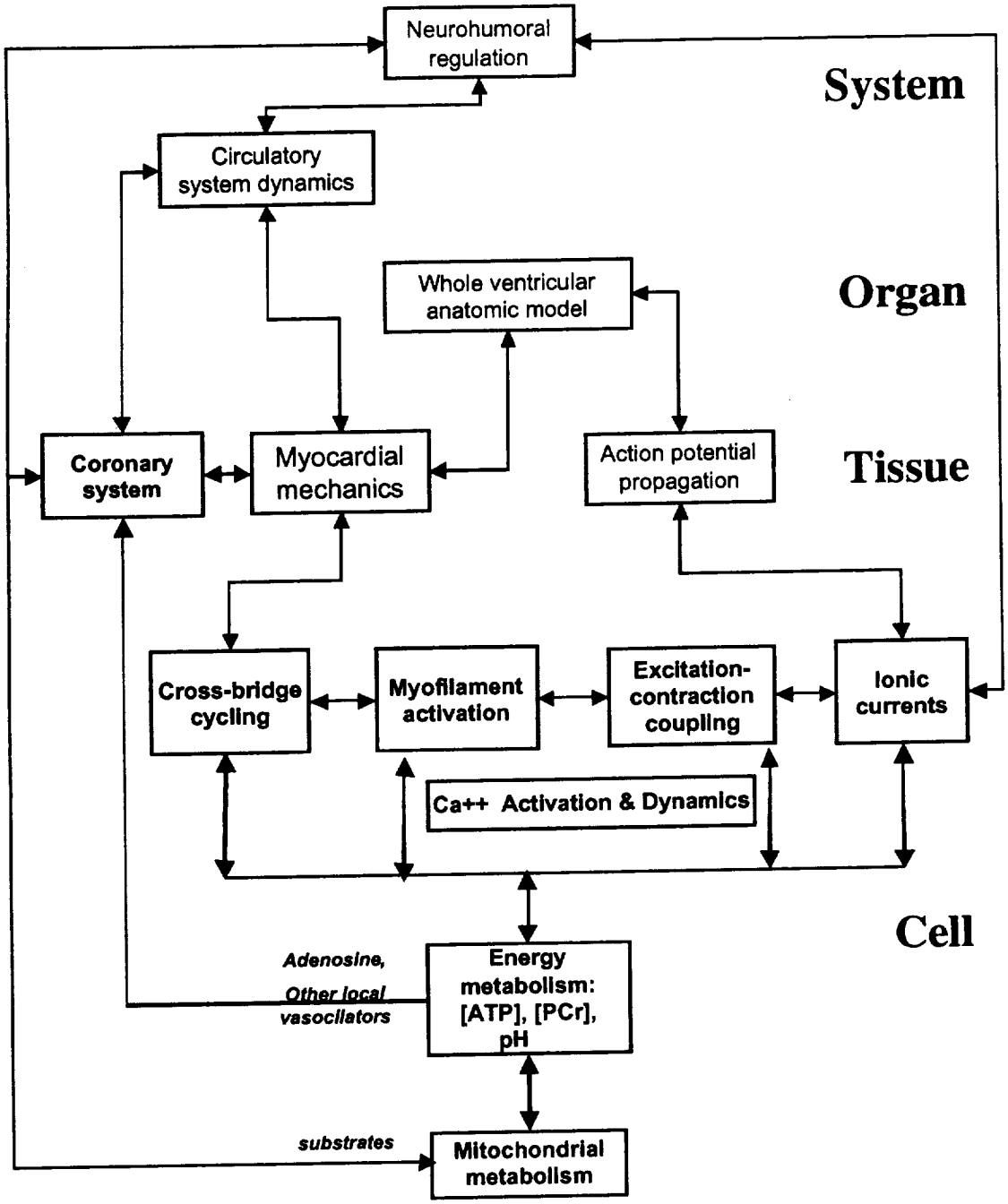
Parallel computing:
Excellent progress on developing parallel algorithms for large-scale modeling of three-dimensional tissue mechanics and electrophysiology.

Excitation-contraction-metabolic coupling:
We have recently published in *Biophysical Journal* a new model that is the first to couple cardiac action potential propagation with intracellular calcium cycling and intracellular concentrations and fluxes of ATP and ADP. A web site allows users to run the model interactively via their browser (http://bionome.sdsc.edu).

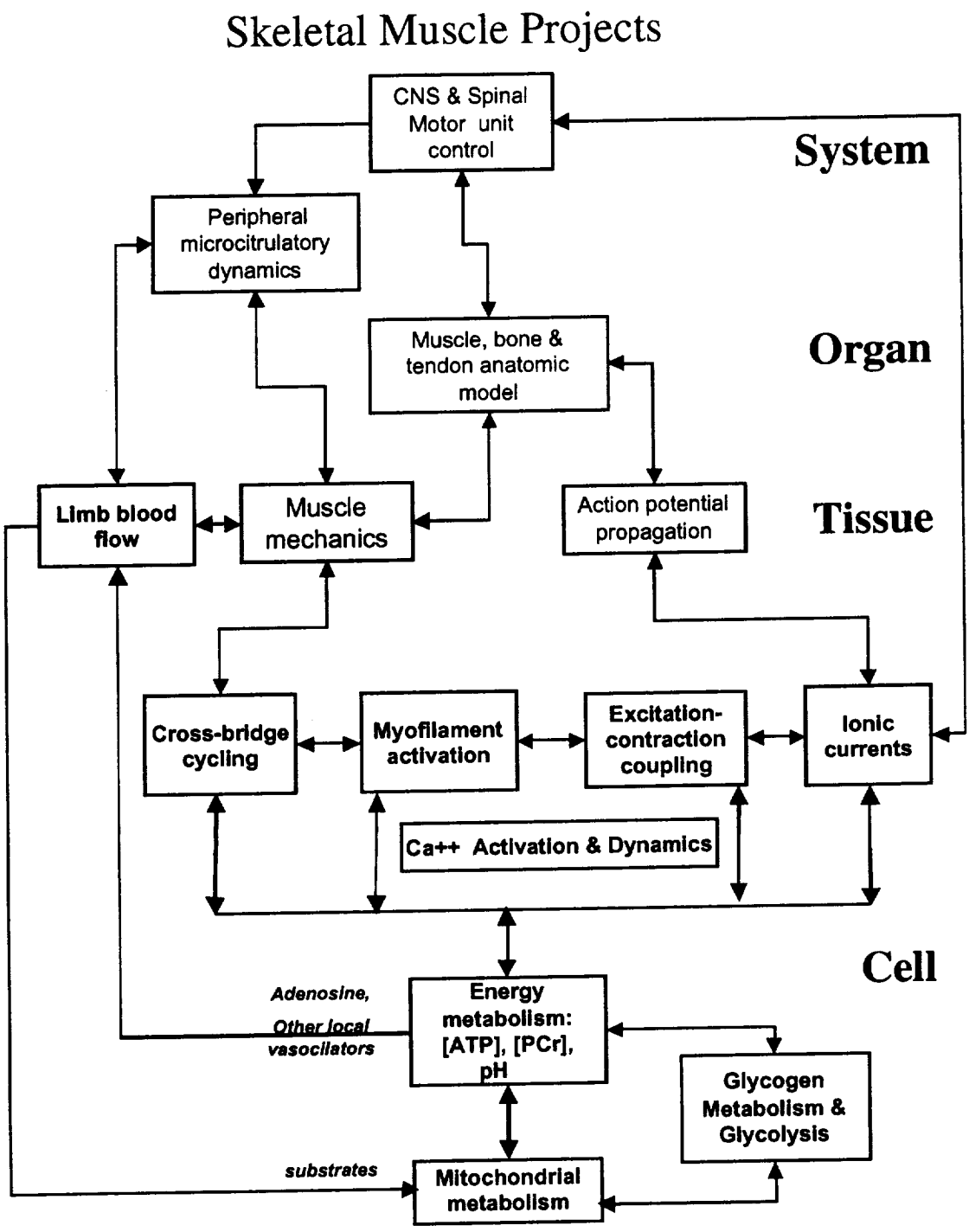
Electromechanical coupling: We have developed a new three-dimensional model of ventricular mechanoelectric feedback showing that regional alterations in action potential morphology are not consistent with a simple uniaxial strain determinant of stretch-activated current that may underlie arrhythmogenesis in microgravity. A combination of strains in the muscle fiber and transverse directions were required to reproduce experimental observations in rabbits. We have also developed a new model of electromechanical coupling that predicts how the three-dimensional sequence of action potential propagation influences global and regional right and left ventricular mechanics. This model also include the anatomy of the Purkinje fiber network and a paper has been recently submitted.

Interactions: With the other members of the team we have formulated a systems model of the principal cardiac physiological interactions that we wish to focus on in the team project

Cardiac Muscle Projects



Using the same hierarchical organization and basic set of interactions, a very similar chart can be made for integrating skeletal muscle components of the projects in this team.



V. Future Program Directions

The work of this team is only beginning; no project has been operational for a full year. The primary tasks are to complete the specific aims of each project at least within the expected three year duration of each project. A listing of the milestones for the first year is given in the section above on the design of the research program for the team. Here we outline the long term milestones which capture our current vision.

Five-Year Research Strategy

In the 2002-2006 time frame, the IHF Team will complete its six initial projects. Additionally, new research projects are being sought within the integrated function team and from the other teams to expand the physiologic basis of the team, to foster development of models within other teams as synergistic activities and to develop systems-level models that will be joined to provide a view of the overall physiology of the astronaut.

Five-year milestones for the current IHF Team:

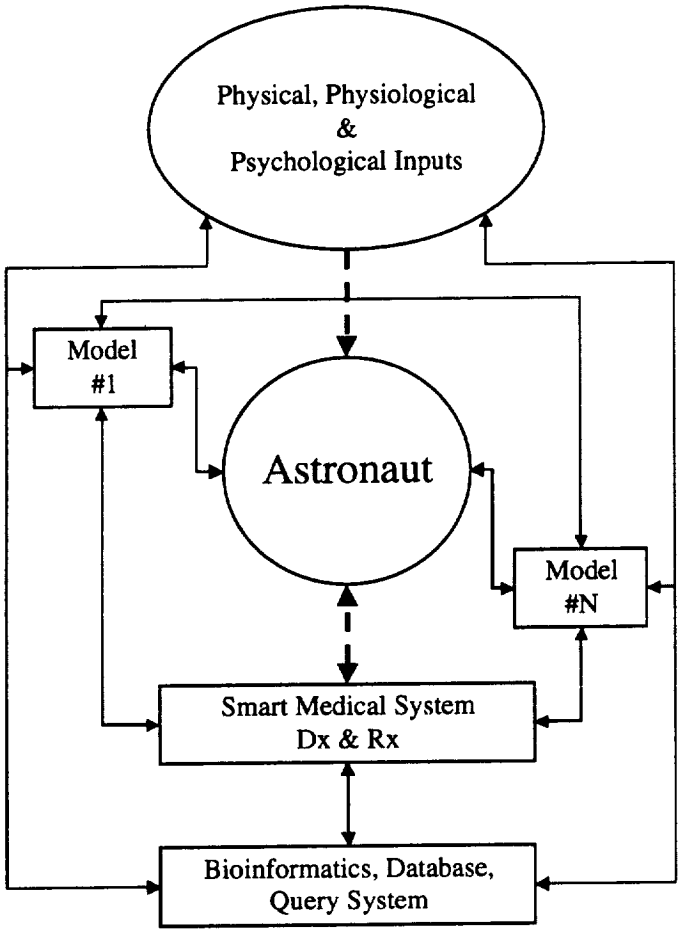
- Concepts and modeling of Ca^{++} control and electromechanical coupling will be made common for cardiac and skeletal muscle, using common cellular and molecular components where possible. This will demonstrate common and distinguishing features between the two types of muscle, predict the consequences of adaptive isoform switching and predict Ca^{++} transients in both, which will be essential to evaluate the Ca^{++} and other signals involved in normal and mal-adaptive effects.
- Concepts and modeling of cellular and whole-organism energetics and metabolism will be extended to include normal 1G exercise requirements and altered requirements in 0G. Evaluation of the model predictions with actual exercise experience at 1G, in bed rest and with astronaut data will be possible and used to validate the model.
- Several software tools and approaches will be developed. We will explore and demonstrate methods of standardizing data interchange among models and simulations, making use of commercial standards wherever possible. We will explore and demonstrate methods of integrating models and simulations, whether being executed in a tightly coupled fashion on a single computer, or loosely coupled across a network. From this information decisions will be made on the approaches to be used throughout the IHF program and on strategic interfacing with other teams within NSBRI.

This anticipated progress represents a unique contribution to the NSBRI program and to physiological modeling in general. The reason is its tight thematic focus and, within that focus, a solid hierarchical integration from molecules to organs. Integration of intracellular signaling and metabolism, intercellular mechanics, metabolism and signaling, and resultant organ functional output will have begun. This thematic and hierarchical working knowledge is expected to direct the development of similar integration along other cell types and organ systems by the concepts and software developed.

All of the above will enable a quantitative test of the basic premise: that insufficient lower limb and hip muscle activity is a major factor in reduced muscle activity and hence muscle atrophy, reduced cardiovascular output and function, and altered metabolic and nutritional needs.

Five-to-Ten-Year Research Strategy

A long term effort is required to unite the necessary physiological systems into an integrated whole. Iteration will be constantly required. This work will develop the range of physiological systems and models, integrated both vertically and horizontally, and develop the software systems engineering to perform simulations of the components and of the ensemble. The plan is depicted in the figure below. We will have a number of models of system components, vertically integrated to include as much of the organ to molecule information as available, appropriate for the countermeasures developed for each system and for the specific challenges and stresses envisioned by NASA's risk analysis system. These models will be developed primarily by the Integrated Human Function Team, but models developed by other teams will be fostered, facilitated and interfaced with. Models are interconnected to provide realistic simulation of astronaut behavior. Physiological signals and stresses provide input to the homeostatic and dynamic systems of the astronaut and prior knowledge for the models. We will form intimate connections with the work of the Smart Medical Systems and envision shared simulation modules with medical evaluation and therapy planning. All are interconnected with a continuously evolving bioinformatics program. In the systems and software engineering area, the mid-term (5-10 years) objective is to develop an electronically-enabled collaborative environment, linking subject matter experts and composable sets of models and simulations that can assist in answering specifically-posed subsets of biomedical questions associated with long-duration space flight. Elements of such a collaborative environment would include the appropriate modeling and simulation tools and data, and associated computer hardware platforms; standards for data element definitions, data interchange and modeling/simulation interoperability within an overall framework; network resources for communication, electronic collaboration, and remote/distributed model/simulation execution; and processes for configuration management and assessment of model validity for specific uses.



Muscle Atrophy and Alterations Team Annual Report 2001

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I. EXECUTIVE SUMMARY

In the Fall of 2000, the National Space Biomedical Research Institute's (NSBRI) Muscle Alterations and Atrophy Team (MAAT) began its second three-year funding cycle on research dealing with the structural and functional deficits of the skeletal muscle system in response to prolonged exposure to space flight or the environment of microgravity. Of the eight original projects that were selected in the first period of funding (1997-2000), only one project was selected for continuation. This project was headed by Dr. Alfred Goldberg, Harvard Medical School; and Dr Ken Baldwin, University of California, Irvine served as a co-investigator on that original project.

In the Fiscal-Year 2000-2003 funding cycle, seven new projects were selected for funding in addition to Dr. Goldberg's project. Two projects were selected for a funding cycle that started on October 1, 2000. These selections involved projects headed by Dr. Baldwin and Dr. Goldberg, who now serve as the Team Leader and Co-Team Leader, respectively, for the MAAT. The remaining six projects were recently initiated in funding cycles at different starting dates in the 2001 calendar year, as detailed in the section on Research Program Accomplishments.

The research mission of the MAAT is to ascertain the underlying mechanisms associated with the loss of muscle mass, strength, and endurance that are the cornerstones of the structural and functional deficits that occur when individuals (human and animal) are subjected to prolonged states of inactivity or skeletal muscle unloading. A key element of this research mission is to elucidate countermeasures that can effectively ameliorate these deficits using a variety of strategies such as exercise, nutritional and pharmacological interventions, as well as the unique strategy of human powered artificial gravity.

Muscle Deficits to Be Addressed by the Muscle Team.

The following deficits have been identified in the critical pathway of understanding astronaut health and safety during prolonged spaceflight. These include:

- Reduced muscle mass (atrophy), which is thought to be due to an imbalance in protein synthetic to protein degradation activity within targeted fibers. The mechanism for such a response is largely unknown.

- Reduced muscle strength leading to a decrease in physical activity performance and high power output capacity. Deficits in strength often exceed the loss in muscle mass suggesting that more complex mechanisms are responsible for the reduced performance.

- A slow-to-fast shift in the contractile protein phenotype, e.g., shifts to faster myosin heavy chain (MHC) and calcium cycling proteins. These alterations induce the muscle fibers to become less economical in sustaining force output.

- A decreased resistance to fatigue,(which could have functional implications in the performance of extra vehicular activity in space and in performing emergency egress activity upon space craft landing.) This problem is relevant to the other deficits outlined above.

- A proneness to muscle injury, which is due to the atrophy and loss of strength. An additional outcome of the muscle weakness could cause increased susceptibility to accidents that could cause damage to other systems, e.g., bone fractures.

- Changes in muscle properties are closely linked to changes in the ability of nervous system to accurately control movements and thus such changes affect safety when performing any type of work.

Current Research Themes (PI's):

Listed below are the research topics and the associated Principal Investigators that form the backbone of the MAAT. These include:

- Role of Muscle Loading Conditions on Mechanisms of Protein Translation and Their Impact on Unloading-Induced Atrophy -(PI: K. M. Baldwin; University of California, Irvine)

- The Activation of Protein Breakdown Upon Unloading and Possible Countermeasures—(PI: A.L. Goldberg; Harvard Medical School)

- Calpains in Simulated Microgravity-induced Muscle Atrophy -(PI: P. B. Antin: University of Arizona)

- Genomics of Human Skeletal Muscle During Bedrest and Exercise --(PI: M. Hamilton; University of Missouri, Columbia)

- Gene Expression Profiling of Unloaded Skeletal Muscle -(PI: S. Kandarian: Boston University)

- In Vivo Stress Strain Dynamics in Human Muscle -(PI:S. Sinha; University of California, Los Angeles)

- .Redux Modulation of Muscle Fatigue and Atrophy Processes in (Simulated) Microgravity -(PI: M. Reid: Baylor College of Medicine)

- Calcium Homeostasis and Muscle Phenotype -(PI: R. Wiseman: Michigan State University)

Based on the above descriptions, it is evident that the current research team is addressing relevant issues linked to the identified risks associated with NASA's critical pathway for

extending human space exploration for prolonged duration as defined above in the Executive Summary.

- All projects in some way impact either directly or indirectly on the critical problem of muscle atrophy and the corresponding loss in muscle strength and human performance.

- The Baldwin, Goldberg, and Antin projects seek a better understanding of the mechanisms associated with the imbalance in protein synthesis and protein degradation.

- The Goldberg and Antin Projects, while addressing mechanisms of degradation, focus on different, but complementary processes that impact protein loss.

- The Baldwin project directly addresses mechanisms of protein balance in the context of defining the underlying factors that support resistance exercise as a fundamental countermeasure to ameliorate muscle atrophy in response to space flight.

- The Kandarian (rodent) and Hamilton (human) projects deal with parameters of functional genomics that can potentially affect mechanisms related to key genes involved in both atrophy and hypertrophy processes.

- The Wiseman project will provide insight into cell signaling and regulatory factors that control the protein phenotype and the metabolic capacity of muscle cells.

- The Reid project will dissect the role of both ionizing radiation and reactive oxygen species on mechanisms of fatigue, as well as muscle atrophy processes.

- The Sinha/Edgerton project will use humans to dissect the effects of unloading on stress/strain function in skeletal muscle and attempt to dissect the mechanisms of how muscles may become prone to injury in the face of atrophy and loss of strength.

In addition, investigators within the MAAT are initiating interactions with PIs in other NSBRI teams. For example Dr. Baldwin is part of a research project headed by Dr. Shapiro of the Bone Team in investigations on bone and muscle wasting in spinal injured patients. Also, Drs. Hamilton and Baldwin are beginning collaborations with Dr. Per Tesch of the Karolinska Institute in Stockholm, Sweden on mechanisms of muscle wasting in human subjects exposed to prolonged inactivity. Finally, interactions are underway between members of the muscle team and individuals in the Nutrition and Fitness team to explore common problems facing these research entities.

Research Accomplishments:

Although it is rather early in the current funding cycle, there have been significant research accomplishments thus far among members of the muscle team. These include the following highlights summarized by work by the teams headed by Dr. Baldwin and Dr. Goldberg.

In collaboration with Dr. V. R. Edgerton's group at UCLA, Dr Baldwin's group has been studying a unique model to induce marked atrophy of the skeletal muscle system called spinal isolation (SI). The SI model induces marked atrophy by 40 –50% in both slow and fast rodent muscle. The atrophy response is brought about by marked decreases in transcriptional activity of sarcomeric genes (myosin heavy chain and actin), which lowers the amount of mRNA available for translation. Total RNA concentration and content, of which the majority is ribosomal, is markedly reduced. Although the activity of enzyme systems involved in protein translation remain activated in the inactive muscles, it appears that the decrease in transcription and the increased expression of enzyme systems involved in protein degradation (enzymes of the ubiquitin proteasome and calcium activated calpains) are up-regulated to create net protein loss. These findings clearly point to the importance of maintaining transcriptional activity in order to generate sufficient substrate for protein translation in models of marked atrophy.

Research in Dr. Goldbergs group has been using a comprehensive picture of the transcriptional adaptations that occur during various types of muscle atrophy, and that may be responsible for the activation of protein breakdown. They have used Incyte cDNA microarrays to compare mRNA levels in normal mouse muscles with those from several types of atrophying muscles (manuscript in preparation). Because much is known about the enhancement of proteolysis in muscle and this tissue's other metabolic adaptations to fasting, they initially performed microarray experiments comparing polyA$^+$RNA from muscles of normal and food-deprived mice, and have identified a group of genes whose transcripts change markedly in the atrophying muscles.

One EST was of particular interest because its level increased most dramatically (8-12 fold) upon fasting. Therefore, they have cloned this protein and defined its properties (Gomes, et al, Proc Natl Acad Sci, In Press). The team demonstrates here that this protein has the properties of an E3 (ubiquitin protein ligase) of the SCF class, and it is unusual in being expressed selectively in striated muscle. they have also studied further the expression of this gene upon food deprivation, with hind limb suspension, and in several other models of human diseases in which there is a marked acceleration of muscle proteolysis. These studies demonstrate the existence of a novel ubiquitination enzyme that appears to increase whenever muscles undergo atrophy.

This gene is expressed specifically in skeletal muscles and to a much lower degree in heart. Because this mRNA also markedly increases in leg muscles atrophying due to diabetes, cancer and renal failure, as well as disuse (hind-limb suspension) and denervation, the Goldberg team has named it atrogin.

Future Program Directions

During the next five-year time frame the Muscle Team will evolve a countermeasure strategic plan that is predicated on ground based models using both animal and human subjects. The following projects are envisioned:

- Interact the ground-based models of limb suspension and resistance training paradigms to prevent muscle atrophy using human subjects. This project will likely be expanded to involve the bed rest model and expand the project to include nutritional interventions in collaboration with the Nutrition and Fitness Team (see below).

- Develop in-depth studies on the effects of artificial gravity on skeletal muscle structure and function. This project will also make use of the unilateral limb suspension model.

- Test pharmacological approaches identified in the present funding period separately and in combination with resistance training using animal models for inducing muscle atrophy.

- Amplify how resistance loading and unloading affects stress-strain reactions in human subjects.

- Explore interactions with the Nutrition and Fitness Team concerning how the separate and combined effects of nutritional modification and physical exercise impacts muscle homeostasis and protein balance in bed rest subjects.

II. INTRODUCTION

Previous research involving both humans and animals clearly indicate that the skeletal muscle system is negatively impacted by prolonged exposure to states of unloading (bed rest; space flight). The following deficits have been identified in the critical pathway of understanding astronaut health and safety during prolonged spaceflight. These include:

- Reduced muscle mass (atrophy), which is thought to be due to an imbalance in protein synthetic to protein degradation activity within targeted fibers. The mechanism for such a response is largely unknown.

- Reduced muscle strength leading to a decrease in physical activity performance and high power output capacity. Deficits in strength often exceed the loss in muscle mass suggesting that more complex mechanisms are responsible for the reduced performance.

- A slow-to-fast shift in the contractile protein phenotype, e.g., shifts to faster myosin heavy chain (MHC) and calcium cycling proteins. These alterations induce the muscle fibers to become less economical in sustaining force output.

- A decreased resistance to fatigue,(which could have functional implications in the performance of extra vehicular activity in space and in performing emergency egress activity upon space craft landing.) This problem is relevant to the other deficits outlined above.

- A proneness to muscle injury, which is due to the atrophy and loss of strength. An additional outcome of the muscle weakness could cause increased susceptibility to accidents that could cause damage to other systems, e.g., bone fractures.

- Changes in muscle properties are closely linked to changes in the ability of nervous system to accurately control movements and thus such changes affect safety when performing any type of work.

The Muscle Atrophy and Alterations Team (MAAT) was formed as an integral component of the National Space Biomedical Research Institute (NSBRI) in order to evolve both a strategic plan and comprehensive research program in order to 1) gain insight into the causes of muscle dysfunction in microgravity environments and 2) to generate effective countermeasures to ameliorate the structural and functional deficits in this important organ system under conditions of muscle unloading.

III. RPROGRAM STRUCTURE AND DESIGN

In Fiscal Year 2000-2001, the Muscle Team research program was totally restructured, due in part to the turnover of team members via the peer review process. The current team is comprised of eight principal investigators, with five of them being new NSBRI investigators. This caused a significant refocus and a shift in the team's research objectives, which now are more closely aligned with addressing issues more relevant to the critical pathway as defined above in the Introduction section.

Current Research Themes (PI's):

- Role of Muscle Loading Conditions on Mechanisms of Protein Translation and Their Impact on Unloading-Induced Atrophy -(PI: K. M. Baldwin; University of California, Irvine)

- The Activation of Protein Breakdown Upon Unloading and Possible Countermeasures—(PI: A.L. Goldberg; Harvard Medical School)

- Calpains in Simulated Microgravity-induced Muscle Atrophy -(PI: P. B. Antin: University of Arizona)

- Genomics of Human Skeletal Muscle During Bedrest and Exercise --(PI: M. Hamilton; University of Missouri, Columbia)

- Gene Expression Profiling of Unloaded Skeletal Muscle -(PI: S. Kandarian: Boston University)

- In Vivo Stress Strain Dynamics in Human Muscle -(PI:S. Sinha; University of California, Los Angeles)

- .Redux Modulation of Muscle Fatigue and Atrophy Processes in (Simulated) Microgravity -(PI: M. Reid: Baylor College of Medicine)

- Calcium Homeostasis and Muscle Phenotype -(PI: R. Wiseman: Michigan State University)

The Current Research Strategy:

Risk #→ Projects	**# 1 Muscle Atrophy**	**# 2 ↓Strength**	**# 3 Phenotype Change**	**# 4 Fatigue Properties**	**# 5 Injury/ Repair**	**# 6 Movement Accuracy**
K. Baldwin UCI	X	X	X	X	X	
A. Goldberg Harvard	X	X				
S. Kandarian Boston U.	X	X	X	X	X	
M. Reid Baylor	X		X	X		
M. Hamilton Missouri	X	X	X	X	X	
P. Antin Arizona	X					
R. Wiseman Washington			X	X		
S.Sinha UCLA	X	X		X	X	X

Based on the above matrix, it is evident that the current research team is addressing relevant issues linked to the identified risks associated with NASA's critical pathway for extending human space exploration for prolonged duration.

- All projects in some way impact either directly or indirectly on the critical problem of muscle atrophy and the corresponding loss in muscle strength and human performance.

- The Baldwin, Goldberg, and Antin projects seek a better understanding of the mechanisms associated with the imbalance in protein synthesis and protein degradation.

- The Goldberg and Antin Projects, while addressing mechanisms of degradation, focus on different, but complementary processes that impact protein loss.

- The Baldwin project directly addresses mechanisms of protein balance in the context of defining the underlying factors that support resistance exercise as a fundamental countermeasure to ameliorate muscle atrophy in response to space flight.

- The Kandarian (rodent) and Hamilton (human) projects deal with parameters of functional genomics that can potentially affect mechanisms related to key genes involved in both atrophy and hypertrophy processes.

- The Wiseman project will provide insight into cell signaling and regulatory factors that control the protein phenotype and the metabolic capacity of muscle cells.

- The Reid project will dissect the role of both ionizing radiation and reactive oxygen species on mechanisms of fatigue, as well as muscle atrophy processes.

- The Sinha/Edgerton project will use humans to dissect the effects of unloading on stress/strain function in skeletal muscle and attempt to dissect the mechanisms of how muscles may become prone to injury in the face of atrophy and loss of strength.

The collective projects thus provide insight into the current problems in muscle structure and function that impact the critical pathway.
However, our current research team efforts and resources are deficient in addressing issues related to neuromuscular function.

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

Role of Muscle Loading on Mechanisms of Protein Translation and the Impact on Unloading-Induced Atrophy—K. M. Baldwin; PI.

Grant Activation Date: October 1, 2000-2001

Specific Aims of the Grant:

- To determine how changes in mechanical loading impact fundamental signaling pathways and regulatory processes that control protein translation capacity/efficiency in the context of skeletal muscle hypertrophy and atrophy.

- To systematically develop a rodent resistance training program designed to attenuate the atrophy process and blunt slow to fast transitions in contractile protein phenotype.

- To determine the potential interaction of amino acid (leucine and cysteine) therapy and resistance training as a countermeasure.

Progress To Date:

In FY 2000-2001 four projects were initiated in order to establish cellular/molecular profiles in skeletal muscle reflecting anabolic/catabolic states based on net protein balance in skeletal muscle using models that cause either marked atrophy or hypertrophy. Project #1 examined changes in rodent slow and fast skeletal muscle in response to complete muscle inactivity, as induced by the novel technique of spinal isolation (SI) in which the spinal cord is severed and all afferent input into the motor pathways to the muscles are eliminated. Project #2 examined adaptive hypertrophy responses to the intervention of chronic functional overload (FO)in which the target muscles are induced to increase their weight bearing activities by the surgical elimination of synergists. Project #3 examined the effects of an intermittent isometric resistance training (IRT)on hypertrophying processes in slow and fast skeletal muscles. Project #4 examined cellular/molecular markers of anabolic/catabolic processes in human skeletal muscles that were subjected to unilateral lower limb suspension (ULLS), resistance training (RT), and ULLS plus RT. This latter project was performed in collaboration with Dr. Per Tesch at the Karolinska Research Institute in Stockholm, Sweden.

Key Findings:

The SI model induces marked atrophy by 40 –50% in both slow and fast rodent muscle. The atrophy response is brought about by marked decrease in transcriptional activity of sarcomeric genes (myosin heavy chain and actin), which lowers the amount of mRNA available for translation. Total RNA concentration and content, of which the majority is ribosomal, is markedly reduced. Although the activity of enzyme systems involved in protein translation remain activated in the inactive muscles, it appears that the decrease in transcription and the increased expression of enzyme systems involved in protein degradation (enzymes of the ubiquitin proteasome and calcium activated calpains) are up-regulated to create net protein loss. These findings clearly point to the importance of maintaining transcriptional activity in order to generate sufficient substrate for protein translation in models of marked atrophy.

With functional overload, the opposite occurs in that transcriptional activity of sarcomeric proteins is increased. Also, enzyme systems and activation of prteins involved in protein translation are increased relative to control states. Further, there is increased expression of muscle growth factors such as IGF-1and myogenic growth factor, which are thought to increase signaling for protein translation. Preliminary studies suggest that there is decreased expression of the proteins involved in protein degradation. Thus in this robust model of hypertrophy there is net positive protein balance by increasing the transcriptional/translational events while blunting degradation processes.

The initial findings of the IRT study suggests that slow skeletal muscle is more responsive than fast muscle to the anabolic effects of resistance training as performed under isometric conditions. Additional experiments are being performed to further evaluate this finding.

Finally, in humans undergoing muscle atrophy, the patterns of change of mRNA for both myosin heavy chain and actin are reduced, which is consistent with the type of changes observed in rodent models of muscle atrophy. This observation suggests that human and animal muscle may respond to altered loading via similar mechanisms.

The activation of Protein Breakdown in Muscle Upon Unloading and Possible Countermeasures : Alfred Goldberg, PI

Grant Start Date: October 1, 2000-2001
Specific Aims of Grant:

- To clarify the mechanisms that activate the Ubiquitin (Ub)-proteasome pathway during muscle atrophy induced by hindlimb suspension and by glucocorticoids, which may contribute to muscle wasting.

- To determine whether pharmacological inhibitors of the Ub-proteasome pathway could be useful as countermeasures to reduce muscle proteolysis and atrophy and to synthesize novel types of inhibitors of this pathway.

- By using a gene micro-array analysis, identify the spectrum of genes whose transcriptions rises or falls during muscle atrophy induced by hind-limb suspension or glucocorticoid treatments.

- To identify possible non-pharmacological approaches to reduce protein breakdown by investigating biochemical adaptations that occur in certain animals to suppress muscle proteolysis and preserve muscle mass.

Key Findings:

Muscle wasting is a debilitating consequence of inactivity, fasting, cancer, and other systemic diseases that result primarily from accelerated protein degradation by the ubiquitin-proteasome pathway. The present studies were undertaken to identify key factors that may be important in the acceleration of muscle proteolysis in catabolic states. To establish a comprehensive picture of the transcriptional adaptations that occur during various types of muscle atrophy, and that may be responsible for the activation of protein breakdown, we have used Incyte cDNA microarrays to compare mRNA levels in normal mouse muscles with those from several types of atrophying muscles (manuscript in preparation). Because much is known about the enhancement of proteolysis in muscle and this tissue's other metabolic adaptations to fasting, we initially performed microarray experiments comparing polyA$^+$RNA from muscles of normal and food-deprived mice,

and have identified a group of genes whose transcripts change markedly in the atrophying muscles. As expected, there was a general reduction in the mRNAs for many contractile proteins and for glycolytic enzymes and a 2-3 fold increase in mRNAs for ubiquitin and multiple proteasome subunits, as we had previously found by Northern blot analysis.

One EST was of particular interest because its level increased most dramatically (8-12 fold) upon fasting. Therefore, we have cloned this protein and defined its properties (Gomes, et al, Proc Natl Acad Sci, In Press). We demonstrate here that this protein has the properties of an E3 (ubiquitin protein ligase) of the SCF class, and it is unusual in being expressed selectively in striated muscle. We have also studied further the expression of this gene upon food deprivation, with hind limb suspension, and in several other models of human diseases in which there is a marked acceleration of muscle proteolysis. These studies demonstrate the existence of a novel ubiquitination enzyme that appears to increase whenever muscles undergo atrophy.

This gene is expressed specifically in skeletal muscles and to a much lower degree in heart. Because this mRNA also markedly increases in leg muscles atrophying due to diabetes, cancer and renal failure, as well as disuse (hind-limb suspension) and denervation, we named it atrogin. It contains a functional F-box domain that binds to Skp1 and to the other components of SCF-type Ub-protein ligases, Roc1 and Cul1 (E3s). Atrogin also contains a nuclear localization sequence and PDZ-binding domain. Upon fasting, atrogin mRNA levels increase specifically in skeletal muscle and rise before atrophy occurs. Atrogin is one of the few examples of a ubiquitin-protein ligase (E3) expressed in a tissue specific manner, and it appears to be a critical component in the enhanced proteolysis leading to muscle atrophy in diverse diseases. Presumably, atrogin plays a key role in the breakdown of essential growth-related proteins (e.g. regulators of protein synthesis and proteolysis). The major goal of future work will be to clarify its precise role in the atrophy process and to identify its substrates in muscle cells.

Calpains in Simulated Mircogravity-Induced Muscle Atrophy--P. B. Antin, PI

Specific Aims of the Grant:

- Understand mechanisms regulating protein turnover in normal muscles and to develop methodologies for modulating muscle protein homeostasis in normal and abnormal conditions.

- Investigate whether target over expression of calpastatin will reduce muscle atrophy in a transgenic animal model.

- Investigate the efficacy of using mutated forms of calpains to inhibit muscle atrophy.

Key Findings:

Project Goals for Year 1:

As part of the long term goals of this project to investigate the role that calpain proteolysis plays in muscle atrophy, the objectives for year 1 are to develop and characterize a line of transgenic mice in which expression of calpastatin in skeletal muscles can be induced by administration of the antibiotic doxycycline.

Experiments are utilizing two gene constructs. The first contains a cDNA coding for the "tet-on" transcriptional regulatory protein (rtTA) under control of the muscle creatine kinase (MCK) gene promoter, which has been mutated to eliminate expression in the heart and is therefore active only in skeletal muscle cells. This construct has been designated "MCK-rtTA". A second gene construct consisting of a bi-directional "tet inducible" promoter driving the calpastatin cDNA in one direction and the luciferase reporter gene in the other direction is also being used. This construct has been designated "TRE-LucCalp".

During this first year, we have successfully generated several transgenic mouse lines containing each gene construct. The first line, designated the "transactivator line", contains MCK-rtTA construct. These mice should express the rtTA protein only in skeletal muscles. Three independent lines of mice containing the MCK-rtTA construct have been generated. A second transgenic line, designated the "transresponder line", contains the TRE-LucCalp construct. Four independent transresponder lines of transgenic mice have been generated.

MCK-rtTa mouse lines should constitutively express the rtTA transcription factor only in skeletal muscles. RNA was isolated from MCK-rtTA transgenic mice and assayed by RT-PCR for the presence of rtTA mRNA in skeletal muscles versus other tissues. Results showed that the rtTA protein was present in skeletal muscles but not in any other tissues, except the testes. The reason for testes expression is not known but should not affect our experiments.

Because the TRE promoter is only active in the presence of both the rtTA transcription factor and tetracycline (or its analogue doxycycline [DOX]), the TRE-LucCalp lines can only be tested by crossing to the MCK-rtTA lines. Mice containing both constructs were therefore bred. Some double transgenic mice were fed DOX (in the drinking water) for five days, while others received only sugar water. After five days, mice were sacrificed and a panel of tissues assayed for both luciferase and calpastatin (the TRE promoter is bi-directional and drives the luciferase reporter gene in one direction and calpastatin in the other direction). Mice were then sacrificed and various tissues assayed for both luciferase and calpastatin protein levels. As shown in Fig. 1, luciferase was dramatically induced only in skeletal muscle cells. Induction varied between muscles and between mice, but in general luciferase activities were at least 8000 fold higher in the skeletal muscles of mice fed DOX compared with those that received only sugar water.

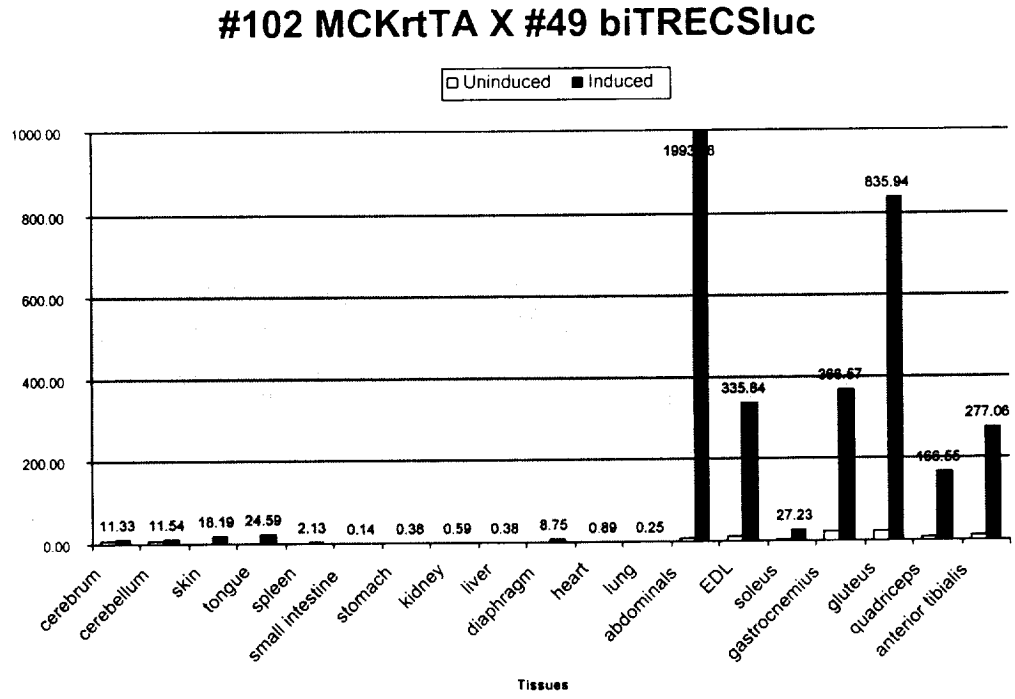


Figure 1: Luciferase Assays for tissues isolated from mice containing both the MCK-rtTA and TRELucCalp constructs. Luciferase values for from control (uninduced, white bars) versus doxycycline treated (Induced, black bars) mice are shown for each tissue. Doxycycline inducible expression was detected almost exclusively in skeletal muscles. Preliminary western analysis to detect calpastatin expressed from the transgene show that transgenic calpastatin is undetectable in uninduced mice but is expressed at extremely high levels in mice treated with DOX for five days. A manuscript is in preparation describing this tetracycline inducible transgenic mouse model.

Goals for the Coming Year:
With the muscle specific, inducible transgenic mouse lines fully characterized, experiments will begin during the coming year to investigate the effects of calpastatin over expression on muscle atrophy. The hindlimb unweighting model will be used for these experiments. Hindlimb weighted and unweighted control mice or mice over expressing calpastatin will be subjected to detailed analyses for changes in overall muscle size, fiber diameter, nucleus/cytoplasmic ratio and protein turnover rates.

Genomics of Human Skeletal Muscle During Bedrest and Exercise-- M. Hamilton, PI

Start Date of Grant: July 1, 2001

Specific Aims of Grant:

- Integrate the cellular and molecular responses in skeletal muscle during physical inactivity that shape the phenotype of skeletal muscle as it pertains to human health.

- Identify the responsive genes associated with a "microgravity phenotype" using micro-array and bioinformatics methodologies.

- Investigate the effects of bed rest and a one-leg exercise model as a countermeasure to attenuate the effects of inactivity on the soleus muscle.

- Provide a human data base on microgravity induced muscle gene profile using bioinformatics tools.

Progress To Date:

Summary of goals of project.
This goal of this project is to identify candidate genes and clusters of related genes that are differentially expressed during unloading of skeletal muscle, and the impact of exercise as a countermeasure on the global gene expression pattern. This potentially has impact in providing basic insights for other microgravity researchers to base novel hypotheses and interpret their work in the context of global genomic influences of simulated microgravity and exercise. This may also have high impact in understanding the genomic influences responsible for the common and unhealthy responses to reduced muscle use during physical inactivity on earth.

In the first 3 months of year 1 we have been laying the foundational bases for the studies to be completed in subsequent years. The goals for year the year 1 that we began within the last 3 months include:
1- Purchase and set-up Affymetrix equipment and other equipment
2- Use our existing microarray data of skeletal muscle to establish better set of rules regarding reproducibility and work with statistical experts in better approaches for analysis of unloading studies
3- Establish better methods of cRNA preparation
4- Have firmly established collaborative human project with other(s) performing unloading/countermeasure projects
5- Validation of novel unloading responsive mRNAs obtained from microarray results obtained in the ongoing studies with our new quantitative RT-PCR assays.
6- By the end of year 1: have prepared a first manuscript on unloading and loading countermeasure; begin recruiting and establishing the early phases year 2 data collection

1. Purchase and set-up Affymetrix equipment "in house" and other equipment. **This is a major advancement of this project for allowing collaboration and better quality control of the postdoctoral fellow performing the analysis, and less time traveling.** This was a substantial element of our time and progress because this purchase was for $206,000, and required a proposal to the MU Vice Provost for Research, a second proposal to the Veterinary Science Research Equipment Committee for matching funds,

and negotiations with the Molecular Biology Core for costs of upkeep and service. This is now in the procurement office for the bid phase. Dr. Hamilton will contribute to this purchase within the limits of his NSBRI equipment budget and also using his university start-up funds, and the University will supplement the rest. This includes the fluid station, oven, and the scanner. Also, software for running the equipment, the basic analysis, and the informatics. A new computer dedicated to the NSBI work was set up in Dr. Hamilton's laboratory to test the newest software before purchase.

The costs for cDNA preparation are being negotiated to substantially reduce those costs and allow for us to use more replicates per sample. We also are negotiating for the cycle ergometer for one leg countermeasure during unloading and thermocycler.

2. Use our existing microarray data of skeletal muscle to establish better set of rules regarding reproducibility and work with statistical experts in better approaches for analysis of unloading studies.

Variability in measuring array-based skeletal muscle gene expression has been studied. This is not a trivial issue and requires both empirical and statistical approaches we have been developing. To do this, we have been analyzing tissue obtained from multiple muscle replicates. One major question is to understand the technology well enough to know how many replicates are necessary for a given treatment. At the most fundamental level, for one to generate good quantitative data comparing treatment to control muscle, the data needs to be "reproducible". We thus took muscle tissue, pooled the RNA to obviate issues about biological variation, and then made up to 9 comparisons on samples. The same type of array from the same production lot was used to minimize variations from manufacturing. The starting cRNA was of very high quality as confirmed by test arrays and standard molecular biology methods. Several layers of analysis have been performed. The primary goal of our research is to identify the skeletal muscle mRNAs that are increased and decreased in expression during unloading. Using the algorithms published for Affymetrix arrays containing up to 20 probe pairs for each gene, we identified the mRNAs called out as being differentially expressed between treatment and control. We found an exponential reduction in the error rate as the number of replicates was added. The statistics are currently being analyzed to describe quantitatively this relationship.

3. Establish better methods of cRNA preparation from small tissue samples.

The members of this new grant in Dr. Hamilton's laboratory have made progress in analysis of microarray data. This was facilitated by several factors. We have been comparing methods to improve upon what we have established in our prior work. With the cRNA prepared in our laboratory, we have been generating preliminary data and learning new procedures for hybridization and analysis.

One of the technical improvements has been redefining the starting amount of tissue to use. This is especially important in our human biopsy work, because it sets a limitation to collaborative projects we share tissue for other's physiological measures. This is also

important because when we prepare cRNA for microarrays, it is important to have enough to use on several arrays developed now, and even more expansive ones in the future. There is typically a wide range in the amount of amplification in the step from total RNA-to-cRNA synthesis. This creates a significant problem because we need to be able to tell collaborators exactly how much tissue is needed for our assays alone. Thus, our laboratory has recently optimized procedures to produce cRNA from both total RNA and polyA RNA.

4. Established synergistic collaborative human project with other(s) performing unloading/countermeasure projects

Dr. Per Tesch who is leading a group performing a prolonged bedrest study in France. Dr. Tesch has the desire to send muscle biopsy material to us for analysis. Dr. Hamilton has shared his NRSBI proposal with Dr. Tesch, and Dr. Tesch reciprocated. This study appears to be a promising collaborative effort because a good set of phenotypic measures will be obtained to put the microarray work in context of our global gene expression data. The first group of subjects should be completing their bedrest phase in September 2001, and the second group will begin immediately afterwards.

5. Validation of microarray with independent methods. We are developing these assays to be performed using quantitative RT-PCR or both human and rat muscle. We have now established assays for 3 new mRNAs that are highly responsive to unloading and countermeasure responsive, as revealed in preliminary data. A goal in the next several months will be to expand upon this list so that some of the positive microarray results can be confirmed in both rats and humans with unloading.

Redox Modulation of Muscle Function In (Simulated) Microgravity-- M. B. Reid, PI

Start Date of Grant: May 1, 2001

Specific Aims of Grant:

- To determine if oxidative stress contributes to muscle fatigue during handgrip exercise (human subjects).
- To determine whether ionizing radiation accelerate reactive oxygen species production and fatigue in skeletal muscle (rodent subjects).
- To evaluate oxidative stress as a mediator of muscle atrophy caused by gravitational unloading (rodent subjects).
- To determine if ionizing radiation stimulates atrophic signaling in muscle cells (rodent subjects).

Progress To Date:

Immediate Past Goals:

1. Recruit project personnel.
2. Establish conditions for a proton radiation field to mimic the conditions encountered in low earth orbit.
3. Evaluate proton radiation as a stimulus for reactive oxygen species (ROS) formation in biological systems.
4. Develop the equipment and procedures for conditioning of mice by hindlimb suspension.

Results of Past Period:

Progress in this period has been seriously hindered by the effects of Tropical Storm Allison. On June 4, the Texas Medical Center was badly hit by the storm with massive flooding at Baylor and many of our sister institutions. The flood destroyed much of the College infrastructure, with loss of electricity, heating/cooling, potable water, and sanitary facilities. Baylor was closed to all but essential personnel for three weeks, then underwent a phased reopening in July and August. Areas of some buildings remain closed for renovation but Baylor research and educational activities are now fully operational.

Our laboratory did not experience direct water damage but we lost virtually all of our frozen and refrigerated materials: solutions, reagents, cell lines, antibodies, conditioned tissues, etc. All experimental animals were drowned. And experiments in progress at the time of the flood were ruined. We lost several experienced lab personnel who either chose not to return to Baylor or finished their research commitments in July. Since the lab reopened, we have been aggressively rebuilding our program. We have recuited new lab members, including a research assistant and postdoctoral fellow dedicated to this project. Commercially available reagents have been replaced, cell lines are being re-derived, and experiments are in progress. Thus, despite Allison, the project is back on track and moving forward at the fastest possible pace. Project-specific progress is outlined below according to past goals:

1. Personnel: We have recruited an experienced senior research assistant and a postdoctoral fellow to work on the project. The staff member is in place and actively working on the Project. The research fellow is an experienced clinician scientist from France who will join our group as soon as visa issues can be resolved.

2. Proton radiation conditions: In May, we began a productive collaboration with Dr. Carlos Gonzalez, Asssociate Professor of Radiology and Executive Director of the Cyclotron Facility at the University of Texas Medical School. Located across the street from Baylor, the facility directed by Dr. Gonzalez was well equipped for the experiments we proposed. We made rapid progress identifying the cyclotron settings needed to obtain a reasonable proton radiation field (30 MeV, LET 1.88 x 10^{-2}, 100 electrons/cm^2/s). Custom-built muscle chambers were fabricated for use in the radiation field and our

instrumentation was adapted for use in the cyclotron facility. We conducted our first experiment on May 30. Later that week, Allison destroyed the cyclotron, which was located in the University basement. It is not known when the facility might be rebuilt.

3. Biological effects of proton irradition. In May, we conducted pilot studies using cytochrome c assay to measure superoxide anion levels in buffer solution exposed to proton radiation. The initial results were equivocal. Continuation of this experiement and planned studies of other ROS molecules and of oxidants in muscle tissue were precluded by the flood.

4. Hindlimb suspension technique. We are currently developing this method, fabricating cages and acquiring the needed supplies in consultation with several experienced groups at Baylor. We anticipate formal experiments will begin within the next month.

Representative Data: None suitable for presentation.

Goals for the Coming Period: (Nov '01 - Apr. '02)

1. We expect to complete a series of experiments using hindlimb suspension to evaluate the adaptive response to unloading. We will assess markers of oxidative stress and catabolic signaling in unloaded antigravity muscles. We also will begin testing antioxidant supplements for their capacity to inhibit these changes and slow protein loss in the affected muscles.

2. We plan to schedule proton radiation studies at the cyclotron facility operated by Texas A&M University. This facility is 90 mi north of Houston and will be more costly than conducting studies in the Texas Medical Center. Advantages of this new arrangement is the expertise at A&M, where a staff of research physicists are available for consultation.

3. We will begin preparations to test N-acetylcysteine effects on handgrip fatigue in human subjects. These experiments will be conducted at Baylor in close association with Dr. Jeff Jones of NASA Johnson Space Center.

Gene Expression Profiling of Unloaded Skeletal Muscle-- S. Kandarian, PI

Start Date of Grant: February 1, 2001

Specific Aims:

- To Conduct a temporal analysis of global gene expression in mechanically unloaded mammalian skeletal muscle.

- To Identify candidate factors and pathways involved in the regulation of unloding-induced muscle atrophy.

- To perform quantitative analysis f candidate factors and pathways involved in regulating unloading-induced atrophy

- To identify conserved regulatory elements in co-regulated genes.

Results & work performed to date:

Year 1 Project Goals

Aim 1: *To conduct a temporal analysis of global gene expression in mechanically unloaded mammalian skeletal muscle.* Affymetrix RatU34A GeneChips will be used to probe mRNA expression in rat soleus muscle after 1, 4, 7 and 14 days of hindlimb unloading. GeneChips allow for the parallel analysis of ~7,000 full-length annotated genes and several thousand expressed sequence tag (EST) clusters.

Total RNA isolation and GeneChip hybridization

Total RNA was isolated from rat soleus muscles from all time points using a Trizol based protocol recommended by Affymetrix (n= 32 samples, right and left muscles were pooled). A second "clean-up" step was used improve the quality of total RNA using columns from Qiagen, also recommended by Affymetrix. RNA quality was judged based on the ratio of absorbance at 260nm and 280nm. Further, all RNA samples were size fractionated using agarose gel electrophoresis. The gels were stained with EtBr and viewed on a transilluminator to check for integrity of the 18 and 28S RNA. Stained gels were photodocumented. RNA was transferred onto **nylon membrane, UV cross-linked** to the membrane, and blots were prepared for northern analysis. Our laboratory has characterized the upregulation of SERCA1 as a robust indicator of the adaptations associated with unloading induced atrophy. Thus, with each sample we performed SERCA1 northern blots to assure normal progression of this change. We then sent all 32 samples to a GeneChip core facility in the Boston area that is affiliated with Affymetrix. The core facility completes the remaining steps in the creation ds cDNA and of biotin labeled cRNA, and hybridizes the labeled cRNA to the GeneChips. This facility is used by many investigators in the Boston area and has a reputation for quality sample processing of a wide array of tissue types and organisms.

Initial Experiment – Gene expression analysis of rat soleus muscles following 6 days of hindlimb unloading

In an initial experiment we examined highly parallel gene expression in soleus muscle RNA samples from 3 control rats and 3 rats hindlimb unloaded for 6 days. This experiment was done to test the quality of the data obtained from the core facility we have chosen for the labeling and hybridization of GeneChips.

We carried out the RNA isolation and northern blotting as described above for the large timecourse experiment. The results of our analysis were returned to us by the core facility on a CD containing the Affymetrix output files. With 3 control and 3 unloaded samples we were able to do a total of 9 iterative (pairwise) comparisons. Using the Affymetrix data analysis software, a query was performed to extract genes that were up- or downregulated having >2 fold changes associated with them. The genes that made this call in 6 out of the 9 iterative comparisons were saved to a list. For each of the genes on this list, a student's t-test was performed on the average difference between the control and unloaded samples. The genes that had p-values less than 0.05 where kept and exported to the lists of up- and downregulated genes. The technical support person from Affymetrix carried out these analyses as we have ordered, but do not yet have, the Affymetrix data mining software.

In table I, we have organized some of the results into groups based on Gene Ontology (GO) functional classifications. Most genes are classified under the "Biological Process" GO category while the "chaperones" designation is taken from the "Molecular Function" GO category. This list is by no means exhaustive. It merely shows some significant results from our stringent initial analysis. Moreover, in the changes reported, 1 of the 3 control GeneChips and 1 of the 3 HU GeneChips had high background. Therefore the changes reported are likely fewer than what will emerge when these 2 samples are re-analyzed with 2 new GeneChips. The number of probe cells that were significantly increased ($p<0.05$) with unloading was 18, and 16 of these are unique gene products. The number of probe sets showing significantly downregulated genes was 50, and 44 of these represented unique gene products. Also, we identified all the ESTs for genes that were significantly up- or downregulated by searching the rat Unigene database.

Most of the genes that were upregulated with 6 days of unloading are involved in proteolysis. These observations are consistent with the literature showing that components of both the ubiquitin-proteosome pathway and the lysosomal proteolytic pathway are upregulated. We have not yet compared these outcomes, gene by gene, with the literature, but, when we do it is possible that there may be increases in some "proteolysis mRNAs" that have not been previously reported. With respect to components of the protein synthetic pathway, the eukaryotic translation initiation factor 4E was upregulated by 14-fold with unloading. This is consistent with an inhibition of translation seen with muscle atrophy since unphosphorylated 4E-BP1 inhibits translation. Taken together, these data are consistent with the increased degradation and decreased synthesis which is characteristic of muscle disuse atrophy.

The upregulated genes also showed a 3-fold increase in GST, which plays a role in glutathione metabolism, and is known to be involved in responses related to metabolic stress. Glutamine synthetase is also upregulated by 2.4-3.4 fold. The 3 repeats of this probe indicated that different sequences were used to design the different oligonucleotides, but the fact that all 3 sequences showed similar regulation adds confidence to the expression data. This enzyme is involved in the production of the amino acid glutamine. Its upregulation may be related to its role as an intermediate in metabolic pathways and its common use as a carbon source. We will know more after

additional analysis of GeneChips from the time course study. There was one transcription factor upregulated (MRG1) and one immediate early response gene called PRG1 (or IEX). IEX has been shown to inhibit apoptosis during NF-kB mediated cell survival. This is of high interest to us as we are currently submitting a paper for publication on the activation of NF-kB transcription during unloading atrophy but we do not know the immediate gene targets. Genes that we have examined so far that we know are upregulated with unloading using traditional analysis but that were not revealed on the GeneChip were due to the fact that the genes were not represented on the GeneChip. This further emphasizes the need to employ the RatU34B and RatU34C GeneChips which contain many additional gene products. Our expression timecourse analysis will also help us with this type of evaluation.

Table 2 shows the genes that were significantly downregulated with 6 days of unloading. Fifty probe sets were downregulated by 2-fold or greater with $p<0.05$. Of these 50 probe sets, 44 represented unique gene products. Five different gene products which act as chaperones were downregulated from 2.1 to 7.2-fold. Several of these are heat shock proteins that have been previously shown to be downregulated with unloading. Some of these gene products are also involved in maintenance of the cytoskeleton (B-crystallin) and alpha1 tubulin, a component of the cytoskeleton was markedly downregulated (25-fold). Moreover, mRNAs whose protein products are involved with growth control such as those in the GO categories "Cell shape & cell size control," "Positive control of cell proliferation," and "Oncogenesis" were also downregulated. In this way, the atrophy phenotype looks opposite as that seen in tumor tissue, which would be expected. Moreover, ornithine decarboxylase was decreased by 63-fold, and it is the rate limiting enzyme in the synthesis of polyamines which regulate DNA synthesis. This is consistent with the reduction in non-muscle cells in atrophied muscle and possibly to the loss of myonuclei. Four different mRNAs whose protein products have roles in signal transduction appeared on the downregulated gene list but none appeared on the upregulated gene list.

Many gene products involved in energy metabolism or energy pathways were downregulated but no genes involved in these processes were upregulated. This is consistent with the metabolic profile with muscle atrophy; less energy production overall and less reliance on oxidative metabolism. GPX1 is reduced with HU and this may be consistent with a lower capacity to oxidize glutathione (GSH) and increase susceptibility to oxidative stress. Also of note, is the 3.7-fold decrease in aquaporin 7, a water channel forming integral protein. This is in contrast to a report of an unloading-induced increase in the expression of aquaporin 4, a different isoform, which is highly expressed in skeletal muscle (Frigeri et al., FASEB J, 2001).

Contractile proteins genes were also downregulated. MLC2slow was decreased and this is consistent with the slow to fast transition in twitch properties. Cardiac calsequestrin was downregulated, consistent with this idea, but fast (skeletal muscle) calsequestrin is not represented on this GeneChip. We previously showed upregulation of fast calsequestrin, SERCA1, and alpha subunit of the L-type calcium channel, not of which are represented on this GeneChip. The Na/K ATPase beta 1 subunit was decreased with

unloading and this has been previously reported. Lastly, 3 different probe sets with slightly different sequence all showed that alpha1 collagen was downregulated by 2.5 fold. This is consistent with the loss of fibroblasts and connective tissue during disuse atrophy.

Gene expression analysis of rat soleus muscles following 1, 4, 7 and 14 days of hindlimb unloading

We have just received our data from the core facility for the large timecourse experiment of unloading (1, 4, 7, 14 days). After we have evaluated the quality of these GeneChip data we will precede with Aims 2 and 3. The quality control is assessed by several parameters from the chip analysis given to us in a summary report. In addition to organizing up- and downregulated genes by functional grouping we will also perform the complex analysis methods described in our original application (see below). We have purchased the Affymetrix software suite and are waiting for it to arrive. We have purchased the new computer for GeneChip analysis and already have GeneSpring software running on it. We have done some preliminary analysis of the 1 and 4 day data. We are planning to attend the next Boston-based GeneSpring class (3 days in length). We have also been to several classes hosted by Affymetrix in the Boston area, and these have been very helpful in teaching us how to extract the most information from our datasets. Finally, we have been downloading the latest information on characterized ESTs (Affymetrix website & Unigene databases) that are contained on our GeneChips as well as downloading relevant GO categories for the genes on our Chips. The latter will be used for standardized functional group clustering.

Other bioinformatics efforts

In our continuing effort to develop tools for mining large datasets we have established collaborative relationships with researchers in the Boston University Bioinformatics department in analyzing our data as well as developing our skeletal muscle gene expression database Sarcogene (http://www.sarcogene.org).

Collaborations with other NSBRI investigators

Our laboratory has met with Dr. Fred Goldberg's group at Harvard Medical School and we currently have a second meeting planned. We are in the process of developing collaborative projects with Dr. Goldberg related to both of our NSBRI projects. We are also particularly interested in collaborations with Dr. Reid's group and Dr. Wiseman's group.

Goals for the next year of work

Aim 2: *To identify candidate factors and pathways involved in the regulation of unloading-induced muscle atrophy.* First, data analysis software will be used to identify genes that are sensitive to unloading by plotting expression of genes in known functional categories and pathways over time. Clustering algorithms will then be used to elucidate

sets of genes, with known or unknown functions, that are co-regulated based on temporal expression patterns. These approaches will provide insight into possible gene associations and candidate players in the pathways that regulate the atrophy process, thereby proposing them for further study.

Aim 3: *To conduct quantitative analysis of candidate factors and pathways involved in regulating unloading induced atrophy.* Interesting candidate factors or pathways identified in Aim 2 will be reconfirmed using more quantitative and focused methods. These will include northern analysis or RNase protection assays to reconfirm observations from the chips, western assays to quantify protein levels, activity assays for enzymes, immunohistochemistry to localize protein expression, and in vivo overexpression experiments of candidate regulatory proteins.

Aim 4: *To Identify conserved regulatory elements in co-regulated genes.* Local sequence alignment algorithms (e.g. AlignACE) will be used to identify regulatory sequences conserved among genes that are co-regulated. The identification of such *cis*-elements will expedite attempts to determine unloading-sensitive regulatory sequences, the transcription factors that bind them, and whether there are synexpression groups or transcriptional programs that regulate transcriptional machinery in response to change in activity pattern.

Table 1 - Upregulated Genes

Proteolysis and Peptidolysis

Gene Name	Genbank	Fold
CTSL; Cathepsin L	Y00697	2.6
PSMB3; proteasome subunit RC10-II	D21800	2.7
PSMB4; Proteasome RN3 subunit; Proteasome (prosome, macropain) subunit, beta type, 4	L17127	2.7
PSMB4; Proteasome RN3 subunit; Proteasome (prosome, macropain) subunit, beta type, 4	L17127	2.3
PSMD1; 26S proteasome, subunit p112	AJ006340	2.2
UBB; polyubiquitin (four repetitive ubiquitins in tandem)	D16554	2.4

Translation Regulation

GeneName	Genbank	Fold
PHAS-I; EIF4EBP1; Eukaryotic translation initiation factor 4E binding protein 1	U05014	14.5

Stress Response

GeneName	Genbank	Fold
GSTA3; glutathione S-transferase A3 subunit	K01932	3
GSTYC1; glutathione S-transferase Yc1 subunit	S72505	2.7
GSTA3; glutathione S-transferase A3 subunit	X78848	3.1

Glutamine Synthesis (Amino Acid Metabolism)

GeneName	Genbank	Fold
GLUL; Glutamine synthetase (glutamate-ammonia ligase)	M29579	2.4
GLUL; Glutamine synthetase (glutamate-ammonia ligase)	M91652	2.7
GLUL; Glutamine synthetase (glutamate-ammonia ligase)	M91652	3.4

Transcription Regulation

GeneName	Genbank	Fold
ESTs, MRG1; Weakly similar to melanocyte-specific gene 1 protein [R.norvegicus]; CITED2; Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain	AF361476	2.6

10-formyltetrahydrofolate catabolism

GeneName	Genbank	Fold
FTHFD; 10-formyltetrahydrofolate dehydrogenase	M59861	3.8

Other

GeneName	Genbank	Fold

hypertension-related	U57050	2.1
PRG1; PACAP-responsive gene 1; IEX	X96437	4.2

Table 2 - Downregulated Genes

Chaperones

GeneName	Genbank	Fold
CRYAB; alpha B-crystallin (ocular lens tissue)	M55534	-2.6
CRYAB; alpha B-crystallin (ocular lens tissue)	X60351	-3.1
HSBX; alphaB crystallin-related protein; Heat-shock 20 kDa like-protein P20	D29960	-2.1
HSP70; heat shock protein 70	Z27118	-4.7
ESTs, Weakly similar to HS9B RAT HEAT SHOCK PROTEIN HSP 90-BETA [R.norvegicus]	AI176546	-3.2
ESTs, Moderately similar to HS9B RAT HEAT SHOCK PROTEIN HSP 90-BETA [R.norvegicus]	AA944397	-7.2

Cell Shape and Cell Size Control

GeneName	Genbank	Fold
CLP36; PDZ and LIM domain protein 1	U23769	-2.5
ESTs, Highly similar to TBA1 MOUSE TUBULIN ALPHA-1 CHAIN [R.norvegicus]	AA800948	-25.7

Positive Control of Cell Proliferation

GeneName	Genbank	Fold
VEGFB; vascular endothelial growth factor B	AF022952	-2.8

Oncogenesis

GeneName	Genbank	Fold
DRAL; FHL2; Four and a half LIM domains 2	AA891527	-5.3
PPP2CA; Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	M33114	-2.4

Signal Transduction

GeneName	Genbank	Fold
ESTs, MGST3; Moderately similar to microsomal glutathione S-transferase 3 [H.sapiens]	AA892234	-2.3
PPP2CA; Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	M33114	-2.4
VEGFB; vascular endothelial growth factor B	AF022952	-2.8
VESL2; Vesl-2 (delta 11); HOMER	AB007690	-3.8

Energy Generation

GeneName	Genbank	Fold
ATP5J; ATP synthase coupling factor 6, mitochondrial	X54510	-2.3
CKMT2; Creatine kinase, sarcomeric mitochondrial	X59736	-2
CYCS; cytochrome c nuclear-encoded mitochondrial gene	K00750	-2.3
ESTs, Weakly similar to HS9B RAT HEAT SHOCK PROTEIN HSP 90-BETA [R.norvegicus]	AI176546	-3.2
HADHB; Trifunctonal enzyme beta subunit, mitochondrial	D16479	-2
MDH; cytosolic malate dehydrogenase	AF093773	-2.2

Energy Pathways

GeneName	Genbank	Fold
AQP7; aquaporin 7	AB000507	-3.7
ATP5J; ATP synthase coupling factor 6, mitochondrial	X54510	-2.3
CKMT2; Creatine kinase, sarcomeric mitochondrial	X59736	-2
ESTs, Moderately similar to NADH-ubiquinone oxidoreductase subunit CI-SGDH [H.sapiens]	AA893185	-1.9
HADHB; Trifunctonal enzyme beta subunit, mitochondrial	D16479	-2
LPL; lipoprotein lipase	L03294	-2.1
LPL; Lipoprotein Lipase	L03294	-2.7
MDH; cytosolic malate dehydrogenase	AF093773	-2.2

Lipid, Fatty-acid and Sterol Metabolism

GeneName	Genbank	Fold
DBI; Diazepam binding inhibitor; Acyl-CoA-binding protein	M20268	-3.1
ESTs, MGST3; Moderately similar to microsomal glutathione S-transferase 3 [H.sapiens]	AA892234	-2.3
ESTs, Moderately similar to HS9B RAT HEAT SHOCK PROTEIN HSP 90-BETA [R.norvegicus]	AA944397	-7.2
ESTs, Moderately similar to NADH-ubiquinone oxidoreductase subunit CI-SGDH [H.sapiens]	AA893185	-1.9
FABP3; low molecular weight fatty acid binding protein	J02773	-6
HADHB; Trifunctonal enzyme beta subunit, mitochondrial	D16479	-2
HBACH; Cytosolic acyl coenzyme A thioester hydrolase	Y09332	-29.8

Polyamine Biosynthesis

GeneName	Genbank	Fold
ODC; ornithine decarboxylase	J04792	-63.6

Leukotriene Metabolism

GeneName	Genbank	Fold
CLP36; PDZ and LIM domain protein 1	U23769	-2.5

Peroxidase Reaction (glutathione oxidation)

GeneName	Genbank	Fold
GPX1; glutathione peroxidase	X12367	-2.3
GPX1; glutathione peroxidase	X07365	-2.4

Muscle Contraction

GeneName	Genbank	Fold
ACTA2; actin, alpha, cardiac	X00306	-5.5
ACTC; alpha-actin cardiac protein	X80130	-2.8
ACTC; alpha-actin cardiac protein	X80130	-3.8
CASQ2; calsequestrin - cardiac	U33287	-3.7
CKMT2; Creatine kinase, sarcomeric mitochondrial	X59736	-2
CRYAB; alpha B-crystallin (ocular lens tissue)	M55534	-2.6
CRYAB; alpha B-crystallin (ocular lens tissue)	X60351	-3.1
ESTs, ACTA2; actin, alpha, cardiac	X00306	-11.4
FXYD1; PLM; Phospholemman; FXYD domain-containing ion transport regulator 1	U72246	-1.9
HSBX; alphaB crystallin-related protein; Heat-shock 20 kDa like-protein P20	D29960	-2.1
MYH; skeletal muscle myosin heavy chain	L13606	-4.8
MYL2; MLC2; Rat heart myosin light chain 2	X07314	-4
MYL2; MLC2; Rat heart myosin light chain 2	X07314	-4
MYL2; MLC2; Rat heart myosin light chain 2	X07314	-4

Synaptic Transmission

GeneName	Genbank	Fold
DBI; Diazepam binding inhibitor; Acyl-CoA-binding protein	M20268	-3.1
SC2; synaptic glycoprotein	S45663	-2.3

Collagen Type I

GeneName	Genbank	Fold
COL1A1; collagen alpha1 type I	Z78279	-2.7
COL1A1; collagen alpha1 type I	Z78279	-2.5
COL1A1; collagen alpha1 type I	Z78279	-2.5

pH Buffering

GeneName	Genbank	Fold
CA3; Carbonic anhydrase 3	AF037072	-4.2

Small Molecule Transport

Gene Name	Genbank	Fold
ATP1B1; ATPase Na+/K+ transporting beta 1 polypeptide	J02701	-2.2
ATP5J; ATP synthase coupling factor 6, mitochondrial	X54510	-2.3

In Vivo Stress Strain Dynamics in Human Muscle-- S. Sinha, PI

Start date: Aug-01-2001

Specific Aims:

To understand the role of strain within the muscle tendon units of humans by determining:

- The level of strain and spatial distribution of strain within the triceps surae muscle complex during isometric plantar flexion contractions under normal weight bearing conditions.

- The extent that during periods of low level weight bearing activity (unilateral lower limb unloading model) in vivo strain is reduced during maximal isometric contractions due to atrophy even though the spatial distribution of strain may be unchanged.

- To determine stress-strain relationships in triceps surae complex during recovery from atrophy.

Project goals for Year 1:
To design, develop and test the feasibility of methods of imaging non-invasively and in-vivo, the magnitude and distribution of strain in human calf muscle (Triceps surae complex), during isometric contraction, using Magnetic Resonance Phase Contrast, Velocity encoded imaging techniques. In the first year, the focus is on development of technique and testing it in normal human subjects.

Technical progress:
A considerable degree of progress has already been achieved as of now towards the above goal. Even though the funding was officially instituted only from August of this (2001) year, our efforts at developing this technique has been going on for quite sometime, and we are pleased to report the status of the project to be at a stage when we are ready to start our official human subject trial series. Reports of this progress have already been made at several international conferences. All IRB approvals necessary for human subjects trials are in place, and we are scheduled to start our first subject in the

first week of November, 2001. In the following we give details of various aspects of this technique and the progress achieved.

MR Imaging

Towards the objective defined above, we quantified the changes in stress-strain dynamics, during an in vivo isometric contraction of the TSMC in 6 normals and 2 patients, 3 months post surgery following complete Achilles tendon rupture. They were scanned on a 1.5T LX scanner (GE, Milwaukee), using a gated, phase contrast, velocity encoded, fast segmented sequence, with 4 phase-encoding levels per segment. The subject's leg was placed in a fiberglass cast immobilizing the knee at full extension (180°), with both legs in the head coil. Calibrated strain gauges placed at appropriate points in the cast generated a signal proportional to force exerted. The subject was trained to exert 50% of maximal force, timed to an audio cue generated from a computer and fed through headphones. The force signal was used to trigger the gated acquisition as well as displayed on an LED display mounted on the scanner and visible by the subject. This helped the subject exert the same amount of force repeatedly through the phase encoding cycles of the MR acquisition. This force signal was also digitized and recorded for subsequent analysis in stress strain curves. Velocity was generally encoded only in S/I direction with VENC values of 10 cm/sec in order to increase accuracy. Dynamic images were acquired in both the axial and saggittal planes at each of 17 to 25 time points (phases) between each trigger during the muscular contraction. Acquisition matrix was 256x128, FOV 22 cm, TE: 5.3ms, TR: 11.3ms, flip angle 30°, slice thickness 10mm, Avg. 2, bandwidth 32 kHz and total acquisition time of about 1.5 mints.

A set of high resolution axial images were initially acquired, volume rendered (using Vitrea, MN), and utilized to identify the correct anatomical locations for strain analysis such as the lateral gastroc, medial gastroc, soleus, and the triceps surae aponeurosis and Achilles tendon, using an indigenous software. Briefly, the strain $\Box s = t^*(v_1-v_2)$, where v_1 and v_2 are the velocities of the two endpoints of a small segment selected by the user on the saggittal image, t is the time interval between phases. Baseline correction for gross motion was also incorporated. The axial velocity encoded images were also compiled to produce a dynamic video reconstruction of the entire isometric contraction.

Velocity Calibration

Considerable amount of effort was spent in calibrating the velocities as determined by the MR technique against other standards. Calibrations were performed using a 'flow phantom' with known flow rates through glass tubes in place of a limb in the coil. A linear flow pump with a Cole-Palmer flow regulator provided a constant flow of different but known flow rates of NiCl2-doped water, to render it MR-visible. In order to provide a "gold-standard", the output of the flow phantom system was directed to a measuring cylinder and the volume collected over a known time was measured. This value was then used to calculate the average flow-rate through the tube of known diameter. The velocity readings from a cross section of the tube were measured from a phase contrast image and the average velocity across the tube was calculated by averaging the velocity of each pixel in the tube. The actual velocities displayed a 'parabolic' profile, consistent with laminar flow inside the tube at the low velocities used for the calibration.

Phase shading
The phase contrast images acquired in our system demonstrated a gradation of apparent velocity across the image of a stationary object due to eddy currents in the coil. This has been reported previously and correction methods have been discussed. We chose to correct our phase contrast images by subtracting one image, known to have acquired in a period of no movement from all other images in a cine sequence.

Data processing
Software was developed to quickly review all magnitude and phase contrast images in a cine sequence, to digitally process images, to calculate actual velocities from phase contrast images and to measure and graph the velocities. This has provided a clear understanding of the images and allowed us to better understand and correct sources of error in the image data.

Automated software was developed to track the movement of tissue throughout the sequence of cine images at near-pixel resolution. The software reads a tab-delimited text (spreadsheet) file of x, y coordinates of pixels identifying the locations of tissue in the first image of the cine sequence. The software reads the pixel value, coding velocity and predicts the pixel location of the same tissue in the following image from the velocity and the time interval between images. The image sequence may be pre-processed by image subtraction as described in the preceding section on phase shading and by pixel averaging over a user-defined block of pixels to provide velocity averages over a region of interest (ROI) defined by the block size. No major differences have been noted between results of analysis using different ROI dimensions, suggesting that our techniques are relatively robust and provide meaningful data down to pixel resolution.

Force measurement
Measurements of plantarflexor force were used to correlate muscular effort with tissue movements within the calf and to provide feedback to the subject in order to maintain constant plantarflexor forces during the scans. Initial analysis of the force data indicated considerable (+/- 100ms) variability between the start of the MRI scan and the start of the contractions. It was felt that better synchronization may be possible by triggering the scans from the initial rise of plantarflexor force and this became a third use for our force measurements.

Force triggering
The casts were originally instrumented with resistive silicon strain gages which required electrical signals within the MRI bore. The high magnetic fluxes during the MRI scan induced noise into the force signals and frustrated our efforts to use the force as trigger. We have now tested and are in the process of purchasing a system using optical strain sensors (Luna Innovations, Fiberscan 2000 EFPI system) which provide a high level of immunity to electromagnetic interference and allows us to reliably trigger MRI scans from the force transient.

Results

The superior synchronization of the force signals and MRI gating yielded a much better reproduction of the contraction cycle and much sharper images compared to our previous method of triggering using an external functional generator. The two strain curves of Fig. 1 generated from the strain analysis of the aponeurosis and muscle of the lower extremity, during isometric contraction (%-scale on the left hand side Y-axis) show very effectively the comparative magnitude and "sign" of strains for each unit and their variations with respect to force exerted.

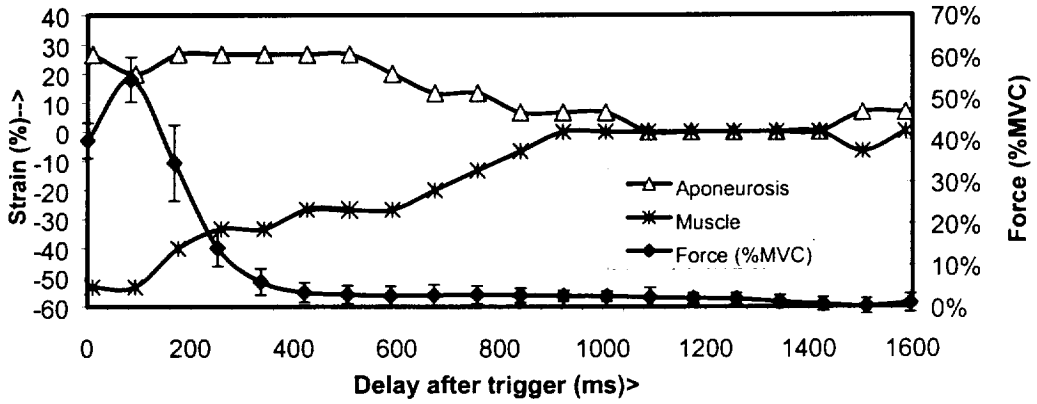


Fig. 1. Percent Strain in Soleus compared to Aponeurosis during Isometric Contraction.

The same figure shows the recording of the force (with error bars) exerted by the subject (scale given on the right hand side Y-axis). The cycle starts with a contraction, with peak strains, which relaxes to zero for both units in the latter part of the cycle. This demonstrates that (i) in-vivo strains can be measured using MRI, (ii) in-vivo recording of the actual force exerted by the subject is possible, in spite of the sensitivity of an MR scanner to noise, and the production of noise in the leads by the fluctuating magnetic field gradients, and (iii) that the MR acquisition can be triggered based on a threshold value of the force exerted.

Shown below in Figure 2 are still frame images of a cine reconstruction of a typical isometric contraction. The images have a grid superimposed upon the sagittal section of the lower leg so that the strain data and the corresponding morphology data can be viewed in the same image throughout the different phases of the contraction. Image A is at the start of the contraction, note no deformation of the grid since the muscle tendon unit is at rest. Image B is in the middle of the contraction, note the deformation pattern with the strain being highest at the aponeurosis interface between the gastrocnemius and soleus muscle groups. Image C is at the end of the contraction, no the return of the grid essentially back to baseline.

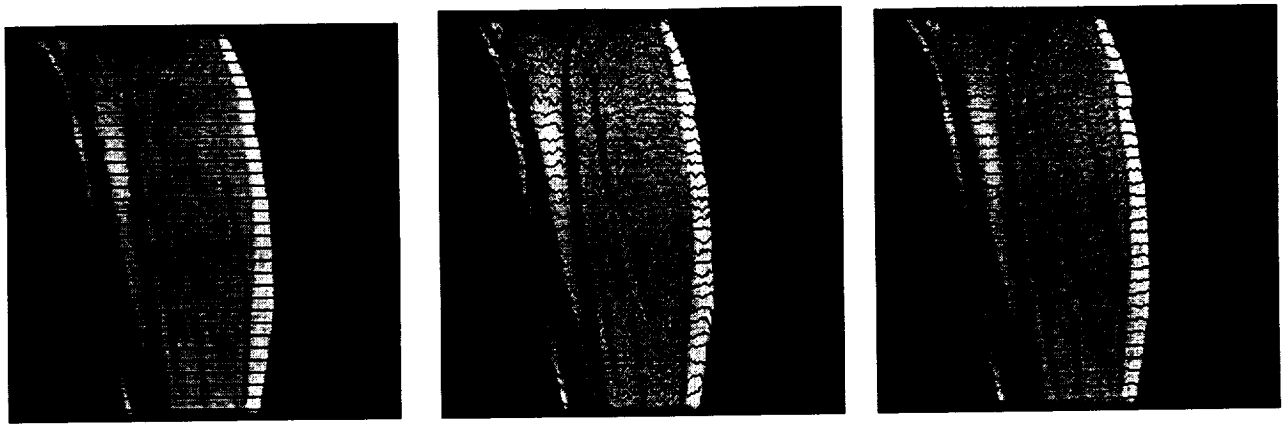


Fig. 3 Velocity Graphs of Normal vs. Achilles Tendon Rupture

Normal

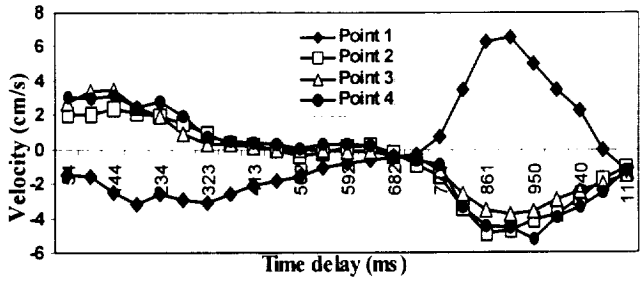


Fig.4: Velocities of Different Muscle-Tendon Structures in a Normal Uninjured Subject.

Rupture

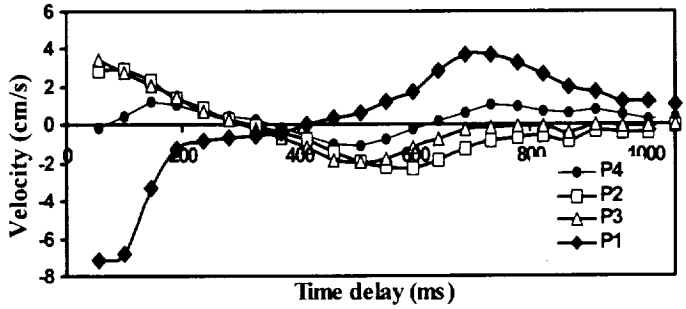


Fig.8: Velocities of Muscle-Tendon, 2 months post Achilles Tendon rupture and Repair.

A B C

Figure 2. Examples of a sagittal section of the lower leg during an isometric contraction with strain data shown by the grid lines

In the axial velocity encoded images for the normals and patients, the clarity with which the different parts of the muscle-tendon units can be distinguished by their velocity values allowed for qualitative identification of temporal patterns of movement between muscles and connective tissue (aponeuroses and tendon) within a muscle. The comparison (Fig.3) shows that even after 2 months the strain rates of the plantarflexors of the injured subject are still not moving at the same velocity as the normal (peak~-5 cm/sec vs. ~-1.5 cm/sec). This is one possible measure by which recuperation of patient can be monitored. The dynamic video reconstruction of the entire isometric contraction revealed that there are definite temporal and spatial differences in the overall movement patterns of the ankle plantar and dorsiflexors of the normal vs. injured lower extremity.

In conclusion, using this technique, we could quantify strain during isometric contraction with sufficiently good accuracy to distinguish between different muscle groups. The technique was sensitive enough to distinguish between spatially specific stress-strain dynamics in ankle plantarflexors in Achilles ruptured patients compared to normals. The cine reconstructions show tremendous clinical potential because they have the level of sensitivity which can distinguish between normal and injured muscle-tendon units

Project goals for next year/phase
Given our progress this year, we anticipate we can complete our projected goal of examining 6 normal subjects in the coming year. We hope to complete analysis of the distribution of strain within the triceps surae muscle complex of the normal subjects pre-suspension so that alterations in the distribution of strain can be identified following the four weeks of lower limb suspension.

Calcium Homeostasis and Muscle Phenotype: Role of Cellular Energetics--R. Wiseman, PI

Requested start date: 07/01/01
NSBRI release date: 09/11/01
MSU budget release: no funds dispersed as of 10/09/01 expected 11/01/01

Specific Aims of Grant:

- To mechanistically link the physiology of muscle use/disuse to the stability or transition in slow-to-fast phenotype conversions.

- Determine the sensitivity of sarcoplasmic reticulum ATPase function and calcium handling to metabolic stress (ATP free energy maintenance).

- Determine to what extent that calcium-sensitive transcription factors are altered in response to increased calcium loads and their involvement in regulating muscle phenotype.

Project Status:

In June of 2001, the PI accepted a full time faculty appointment in the departments of physiology and radiology at Michigan state university. Thus, formal connections with the University of Washington have been reduced to an affiliate appointment in June of 2001 and the laboratory moved on June 11th. The laboratory at MSU opened at the end of June. During this period, no funds have been dispersed from the NSBRI to MSU and all progress towards the goals for this project have been funded by Michigan State University. As a result of the PI moving institutions there are key personnel changes. Jereoen Jeneson, Ph.D. has moved back to the Netherlands and is no longer associated with the current work. Ronald Meyer, Ph.D. is now added as a co-investigator and brings expertise in cellular energetics, whole muscle physiology and modeling to the project.

Project Goals yr 0.5:

The project goals for the first 6-month period were three-fold: hire personnel; construct a calcium imaging system at MSU; and begin to map the calcium transients for

representative fast and slow muscle phenotypes. To date we have generated the progress in the following areas.

Hire personnel: Two new postdoctoral fellows have joined the laboratory.

Roop Jayaraman, PhD is an exercise physiologist. He brings expertise in whole muscle physiology and magnetic resonance imaging and spectroscopy techniques to the laboratory.

Stefanie Carroll is a PhD cell biologist studying calcium homeostasis and fiber type transformation in wild type and transgenic animals. She brings expertise in optics, muscle physiology and molecular biology to the laboratory.

FUTURE PROGRAM DIRECTIONS

The Five Year Strategy (2002-2006)

During this time-frame the Muscle Team will evolve a countermeasure strategic plan that is predicated on ground based models using both animal and human subjects. The following projects will be targeted:

- Interact the ground-based models of limb suspension and resistance training paradigms to prevent muscle atrophy using human subjects. This project will likely be expanded to involve the bed rest model and expand the project to include nutritional interventions in collaboration with the Nutrition and Fitness Team (see below).

- Develop in-depth studies on the effects of artificial gravity on skeletal muscle structure and function. This project will also make use of the unilateral limb suspension model.

- Test pharmacological approaches identified in the present funding period separately and in combination with resistance training using animal models for inducing muscle atrophy.

- Amplify how resistance loading and unloading affects stress-strain reactions in human subjects.

- Explore interactions with the Nutrition and Fitness Team concerning how the separate and combined effects of nutritional modification and physical exercise impacts muscle homeostasis and protein balance in bed rest subjects.

. The 5-10 Year Research Plan (2007-2011)

The Muscle Team views this period most critical to translating the ground- based derived countermeasures into space flight readiness on the International Space Station (ISS). It is

anticipated that the ground-based research will mature three fundamental countermeasure strategies to ameliorate skeletal muscle dysfunctions. These include:

a) different forms of physical exercise, with the resistance type being the primary focus;
b) human powered artificial gravity (gravity equivalent exercise), which could serve as an overarching countermeasure because of its potential to maintain/improve the homeostasis of multiple health systems;
c) novel pharmacological and hormonal approaches that are currently being explored in the current funding period.

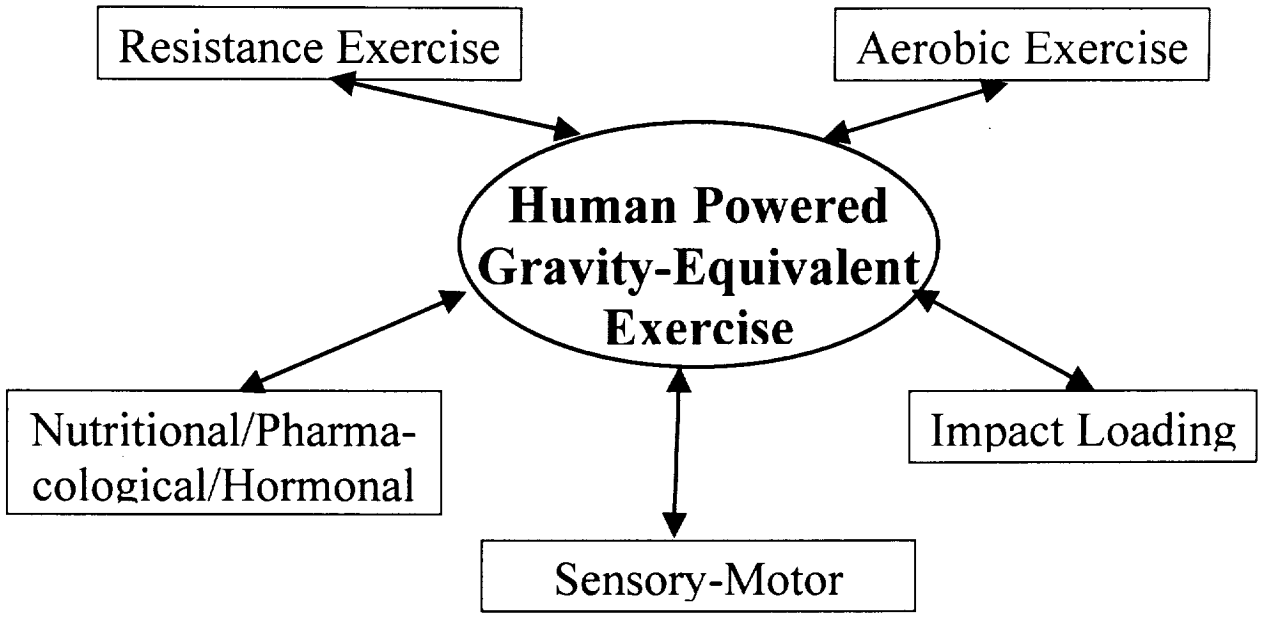
We visualize that exercise related countermeasure to be tested on the ISS platform will include a number of activity-unique prescriptions:

- A resistance training prescription to maintain muscle mass and strength.
- An aerobic exercise paradigm that would improve both cardiovascular fitness and skeletal muscle endurance.
- An activity paradigm that would specifically target the sensory-motor pathways to maintain posture, balance and locomotor skills.
- An impact loading paradigm that could conceivably affect both the skeletal muscle system and the skeletal systems to stimulate/maintain bone homeostasis.

Human powered gravity equivalent activities need to be a high priority for flight testing, because this activity paradigm has the potential to encapsulate all four of the exercise-type prescriptions as well as have a positive impact on the bone, cardiovascular, vestibular, and nutritional/fitness countermeasure strategies.

In addition to these primary activity-related strategies, additional an additional major research objective will be to incorporate other countermeasure activities that fall into the categories of nutritional, pharmacological, and possibly growth factor/hormonal interventions.

Presented below is a schematic diagram that outlines the interaction of these various countermeasure activities with the artificial gravity serving as the potential epicenter of this interaction.


Resistance Exercise
Aerobic Exercise
Human Powered Gravity-Equivalent Exercise
Nutritional/Pharma-cological/Hormonal
Impact Loading
Sensory-Motor

ANNUAL PROGRAM REPORT

NSBRI Team: NEUROBEHAVIORAL AND PSYCHOSOCIAL FACTORS

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Projects and Principal Investigators:

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Project 2. **Psychosocial Performance Factors in Space Dwelling Groups**
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Project 4. **Designing a Smart Medical System for Psychosocial Support**
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Project 5. **Optical Computer Recognition of Performance Under Stress**
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Project 7. **Quick Assessment of Basic Cognitive Functions**
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Project 8. **Stress, Performance and Locus Coeruleus**
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Projects 1-8 are ground-based experiments. Projects 9 and 10 are flight experiments that are not described in detail, since they are not yet funded, but are in feasibility evaluation at JSC.

10-30-01

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I. EXECUTIVE SUMMARY

Prolonged habitation in space will involve exposure to behavioral challenges for a much longer period of time than have been experienced thus far. Stressors and risk factors include confinement for a year or more with the same small group of people; isolation from family and friends; limited communication with Earth including potentially long delays in bi-directional communications; loss of privacy due to habitability constraints; and interacting without the normal gravitational orientation that facilitates cognitive and emotional interpretation of the world. There are also risks to neurobehavioral capability and emotional stability posed by prolonged exposure to microgravity, radiation, physical illness, interpersonal strife, and equipment failure in space. Differences in language, culture, gender, and work roles will pose challenges to crew communication and effectiveness. Therefore the success of prolonged human habitation in space will depend on prevention, identification and mitigation of individual neurobehavioral and interpersonal psychosocial risks to crew health, safety and productivity. Such risks have been identified in NASA's Critical Path Roadmap involving human behavior and performance in space flight. The NSBRI Neurobehavioral and Psychosocial Factors Team conducts research on countermeasures to ensure that astronaut behavioral health is maintained during prolonged space missions, and that both individual astronaut and crew functioning are optimized. Currently 10 projects—eight funded ground-based experiments and two proposed flight experiments—divided equally between psychosocial and neurobehavioral scientific emphases make up the Team. Investigators bring considerable breadth of scientific acumen and techniques required to address scientific questions that range across biological mechanisms; cognitive and affective processes; individual differences in vulnerability to impairment; selection and leadership criteria; and team problem solving and optimization of communications. Approaches used in the projects include studies in analog environments (Antarctica, Mt. Everest), computer simulations of space-based and Earth-based teamwork, and basic laboratory experiments in humans and animals. The breadth of scientific approaches notwithstanding, the overarching focus of the eight projects underway is on the impact of stress—individual and interpersonal—on behavioral functions. Collectively, the projects seek to identify (1) the causes of stress, and its consequences for astronaut cognitive, affective and social functioning; (2) techniques and technologies to objectively detect stress reactions and performance deficits in individuals and groups in the remoteness of space; and (3) countermeasures to prevent and otherwise mitigate the occurrence of stress reactions and their adverse effects on individual and crew performance. The eight ground-based projects have been underway for less than a year, and therefore no change is planned in the future strategy of these projects until their experimental results are clear. On the other hand, it is recognized that there are important areas and technologies that have not yet been covered by the current projects. Future plans should focus on adding research projects that seek to identify the effects of long-term exposure to the major factors in the space environment on emotions, cognition, performance, and behavioral health. In addition, novel neuroscience technologies (e.g., neuroimaging; transcranial magnetic stimulation) or novel behavioral methodologies (e.g., virtual reality, experimental manipulation of small group microsocieties in isolation and in tandem) should be included to search for new countermeasures for the psychosocial and neurobehavioral effects of prolonged space flight. Finally, work is needed on the behavioral strategies, scheduling strategies and habitat design elements that can maintain or enhance crew performance and prevent the development of hostility between crews and ground-support personnel during long-duration space flight. In addition to preventing behavioral problems in space flight, the countermeasure research being performed on the Team may also aid in helping to solve neurobehavioral and psychosocial problems on Earth, ranging from early objective detection of cognitive and emotional impairment, to development of new behavioral and pharmacological treatments for stress, to

better communication systems that prevent errors and misunderstandings, to techniques for improving group performance and cohesion.

II. INTRODUCTION

The success of prolonged human habitation in space will depend on prevention, identification and mitigation of neurobehavioral and psychosocial risks to crew health, safety and productivity. Risks to interpersonal psychosocial functioning and individual neurobehavioral functioning during prolonged space flight have been identified in NASA's Critical Path Roadmap involving human behavior and performance in space flight. In fact, due to their demonstrated occurrence in USA and Russian space missions, problems involving poor psychological adaptation to the space environment currently share the highest risk rank (level I) with bone loss, radiation exposure and emergency medicine in space flight. The NSBRI Neurobehavioral and Psychosocial Factors Team—a new team in its first year—has been charged with conducting research on countermeasures that will ensure that astronaut behavioral health is maintained during prolonged space missions, and that both individual astronaut and crew functioning are effectively optimized.

Prolonged habitation in space will involve exposure to behavioral challenges for a much longer period of time than have been experienced thus far in microgravity. Stressors and risk factors include confinement for a year or more with the same small group of people; isolation from family and friends; limited communication with Earth including potentially long delays in bi-directional communications; loss of privacy due to habitability constraints; and interacting without the normal gravitational orientation that facilitates cognitive and emotional interpretation of the world. There are also risks to neurobehavioral capability and emotional stability posed by prolonged exposure to microgravity, radiation, physical illness, interpersonal strife, and equipment failure in space. Differences in language, culture, gender, and work roles will also pose challenges to crew communication and effectiveness. Without mitigation these stressors individually and collectively have the potential to erode cognitive performance; change neuroendocrine, cardiovascular, and immune responses; disrupt appetite, sleep, and other basic regulatory physiology; lead to neuropsychiatric impairment through anxiety and depression; and potentiate serious interpersonal problems among crewmembers and with Earth-based mission support personnel.

The overarching scientific goal in this area is to mitigate these risks through research on the development of novel ways to monitor individual astronaut brain functions, as well as group behaviors, and to provide preventive and operational countermeasures to enhance crew cognitive performance, motivation, emotional well being and quality of life. Therefore, the scope of this research area includes: (1) Identification of the neurobehavioral and psychosocial risks to crew health, safety, well being, performance and productivity during long-duration space missions; (2) Evaluation of the effects of space-related stressors (i.e., habitability constraints, microgravity, radiation, work requirements, sleep deprivation, perceived risks, restricted communication with Earth and boredom) on physiological and psychological functions of individuals and crews; (3) Development of accurate, practical techniques and approaches to monitor behavior and performance capability during missions; (4) Development and validation of countermeasures to manage or mitigate space-related risks to neurobehavioral functions and to enhance health, motivation, safety and performance during such missions; (5) Identification of strategies to maintain motivation and ensure an effective quality of life in space; and (6) Development of procedures to determine optimal leadership style, crew composition, organization and communication with

Earth. There is therefore considerable breadth of scientific acumen and techniques required to address scientific questions that can include biological mechanisms, cognitive and affective processes, individual differences in vulnerability to impairment; selection and leadership criteria, team building and optimization, and management systems that affect crew capability.

III. RESEARCH PROGRAM STRUCTURE & DESIGN

The research program and structure of the Neurobehavioral and Psychosocial Factors Team was defined by the peer-review selection conducted in 2000, of eight ground-based studies and two possible flight experiments (not yet funded but in feasibility evaluation at JSC). These eight funded ground-based projects (and two as yet unfunded flight studies) collectively address four interrelated team goals. Despite the breadth of projects, there is considerable synergy among them relative to the goals they seek to address, such that some projects address more than one of the following goals. Below are listed only the primary goal of each project.

1. Identify salient features, needs, and predictors of behavioral functioning in isolated groups.
   - **Project 1.** **Individuals and Cultures in Social Isolation (Antarctica)**
   - P.I. JoAnna Wood, Ph.D.
   - **Project 4.** **Designing a Smart Medical System for Psychosocial Support**
   - P.I. James A. Carter, Ph.D.
   - Project 10. (Proposed flight project: Psychosocial Education (PSE) Training for ISS Missions)
   - P.I. (Nick Kanas, M.D.)

2. Identify factors influencing communications and problem solving in small groups.
   - **Project 2.** **Psychosocial Performance Factors in Space Dwelling Groups**
   - P.I. Joseph V. Brady, Ph.D.
   - **Project 3.** **Distributed Team Decision Making for Long Duration Space Missions**
   - P.I. Judith M. Orasanu, Ph.D.

3. Identify ways to objectively detect affective and neurocognitive functions in remote locations.
   - **Project 5.** **Optical Computer Recognition of Performance Under Stress**
   - P.I. David F. Dinges, Ph.D.
   - **Project 6.** **Speech Monitoring Cognitive and Personality Alterations**
   - P.I. Philip Lieberman, Ph.D.
   - **Project 7.** **Quick Assessment of Basic Cognitive Functions**
   - P.I. Stephen M. Kosslyn, Ph.D.

4. Identify pharmacological interventions for neurobiological responses to distress in space flight.
   - **Project 8.** **Stress, Performance and Locus Coeruleus**
   - P.I. Gary Aston-Jones, Ph.D.
   - Project 9. (Proposed flight project: Effect of Spaceflight on Pharmacokinetics of Psychotherapeutic Agents)
   - P.I. (Lane J. Brunner, Ph.D.)

The eight ground-based projects making up the Neurobehavioral and Psychosocial Factors Team have been underway an average of 7 months during year 01. The projects are described below relative to their respective themes, countermeasure goals, and their synergies and collaborations within and between projects. Projects 1 through 4 deal primarily with research questions on

"Human performance failure because of poor psychosocial adaptation," which is area 18 from the Critical Path Roadmap. Projects 5 through 8 are concerned primarily with "Human performance failure because of neurobehavioral dysfunction," which is area 21 from the Critical Path Roadmap.

Project 1. Wood, J., Helmreich, R., Lugg, D.: Individuals and Cultures in Social Isolation. This project focuses on psychosocial critical path questions. The goal is to increase understanding of the effects of personality, culture, and group characteristics on both individual and group performance in an extreme environment (Antarctica) that parallels many of the conditions likely to occur in long-duration space missions. ***Countermeasure goals*** include identifying those elements of leadership that maximize crew functioning in extreme environments, and increase understanding of how individual and group factors affect physical health under prolonged group isolation in Antarctica. The resulting information can be used in identification of optimal flight crew configurations. ***Synergy and collaboration within the project and with other projects:*** Like many of the other projects on the team, this project on the psychological and physical health of people wintering over in the Antarctic is possible through a strong multidisciplinary collaboration. Dr. JoAnna Wood is an experienced applied experimental psychologist located at Johnson Space Center and on the faculty of Baylor College of Medicine. She conceived the project to answer questions about the roles of personality, culture, leadership and individual and group adaptation in an extreme environment (i.e., isolation, confinement, severe environmental conditions). The project is possible through her collaboration with Dr. Desmond Lugg, Head of Polar Medicine for the Australian Antarctic Division, who coordinates psychological and serum data acquisition (for neuropeptides) in the Antarctic. Dr. Robert Helmreich, the project Co-PI and a renowned social psychologist, developed and manages the psychological assessment instruments, and he will be involved in the hierarchical linear modeling of the data. Analyses of serum samples for neuropeptide levels is being shifted from the laboratory of Dr. Terry Philips at NIH where there is a backlog of work, to the laboratory of Dr. William Shearer, Team Lead for the NSBRI Immunology, Infection and Hematology Team, at Baylor College of Medicine. Dr. Shearer has had prior synergistic collaborations with the Team lead, Dr., Dinges, and his laboratory's involvement with Dr. Wood's project offers a new synergy between the neurobehavioral and psychosocial factors team and the NSBRI Immunology Team.

Project 2. Brady, J., Hursh, S., et al.: Psychosocial Performance Factors in Space Dwelling Groups. This project focuses on psychosocial critical path questions. The goal is to determine the effects of variations in the structure and function of communication channels within and between simulated space-dwelling and Earth-based groups. It addresses the effects on psychosocial performance effectiveness of (1) stressful environmental and behavioral interactions; (2) variations in the appetitive and aversive characteristics of incentive control systems; and (3) selection, training and experience. The research methodology will involve development of a distributed interactive multi-person simulation in computer-generated environments as an experimental test bed for modeling psychosocial performances within and between space-dwelling and Earth-based groups. The simulation approach provides an automated means of setting the context for the analysis of performance in space-dwelling groups and monitoring electronically the effects of varying experimental conditions that alter psychosocial interactions. ***Countermeasure goals*** include identifying ways to use distributed interactive multi-person simulation to optimize crew communication and performance, and to identify the roles of selection, training and experience in effective communications, as well as crew psychosocial and ecological stability. ***Synergy and collaboration within the project and***

with other projects: Like Project 1, this project is lead by investigators experienced in space behavioral science, and it is possible through a strong multidisciplinary collaboration. Dr. Joe Brady has 50 years experience in space behavioral biology, including providing the first two primates to fly in orbit in the 1960's. He will head the project and manage the simulated environment. His collaborator, Dr. Steven Hursh, the Program Area Manager for Biomedical Modeling at Science Applications International Corporation (SAIC) leads SAIC's development of the communication software that will provide the simulated space-dwelling and Earth-based scenarios in which communications contingencies and related countermeasure goals will be evaluated. The investigators intend to show the SAIC distributed interactive multi-person simulation to other project P.I.s on the Team to determine if the simulation would have utility in other projects. The simulation is expected to be complete and ready for testing by November, 2001.

Project 3. Orasanu, J., Fischer, U., Montgomery, L.et al: Distributed Team Decision Making in Exploration Missions. This project focuses on psychosocial critical path questions. The goal is to examine how team structure and communication medium affect the nature and quality of small team interaction, distributed decision making strategies, and problem solving under a variety of stressful conditions (i.e., time pressure, risk level, information accuracy/completeness). It assesses autonomic nervous system markers and the Specific Affect Coding System technology for detecting when crew interactions and decision-making are degrading. ***Countermeasure goals*** include identifying ways to optimize crew problem solving performance during demanding and non-demanding periods. ***Synergy and collaboration within the project and with other projects:*** Dr. Judith Orasanu is a senior research psychologist at NASA Ames Research Center. She has extensive experience studying decision making and under stressful and non-stressful conditions in both laboratory and field settings in aviation. Dr. Orasanu and the project Co-P.I., Dr. Ute Fischer of Georgia Institute of Technology, have developed coding schemes for measuring team communication and decision making. These will be utilized in the proposed work to examine factors that affect the nature and quality of team interaction and decision making strategies under a variety of stressful conditions, and to assess a technology for detecting when crew interactions are degrading, so that appropriate interventions (countermeasures) can be introduced to prevent further deterioration of crew performance. Project 3 research complements that of Project 2 (Brady et al.) and Project 4 (Carter et al.). Project 2 will also investigate media effects on team collaboration and decision making, focusing on team outcome measures such as task success, time to completion, etc., while Project 3 will emphasize process characteristics such as how team members build a shared problem understanding, structure their problem solving and decision making efforts, respond to each other's contributions, and deal with disagreement. Project 3 will complement Project 4 by identifying factors that predict or contribute to dysfunction and conflict in teamwork. Thus, Project 3 research could help identify individuals or teams who could benefit from the interactive multimedia system for diagnosis and management of psychosocial problems being developed in Project 4.

Project 4. Carter, J., Buckey, J., Holland, A., et al.: Designing a Smart Medical System for Psychosocial Support. This project focuses on psychosocial and neurobehavioral critical path questions. The goal is to to develop a prototypical smart medical system for psychosocial and neurobehavioral support in space flight. The computer-based system will include the systems infrastructure and basic functions of three modules—self-diagnosis of psychological problems, treatment of depression, and conflict management. The prototype will apply IML's Virtual Practicum model, creating an immersive, welcoming environment in which to seek assistance for

psychosocial problems. The computer-based system will address neurobehavioral issues, including the assessment of affect and suggested interventions, and provide a training module on interpersonal conflict resolution. The system will be developed and evaluated with experienced users and content experts. If it proves effective, the prototype could be expanded to include additional modules for diagnosis, treatment, patient management, and prevention of any possible psychosocial problems that might arise on space missions. ***Countermeasure goals*** include using the system to help detect (via standardized questions) behavioral dysfunction in individual astronauts and among flight crews, and having the computer-based system offer suggestions to crews for remediating problems and conflicts. ***Synergy and collaboration within the project and with other projects:*** Like Projects 1, 2, and 3, this project involves a multidisciplinary collaboration. Dr. James Carter, a clinical psychologist who helps direct an interactive media lab at Dartmouth has teamed up with physician and former astronaut, Dr. Jay Buckey at Dartmouth, and Dr. Al Holland, Chief Psychologist at NASA Johnson Space Center. A key feature to developing a system that astronauts will accept involves getting their input early in the development phase. This project team has recently secured NASA-Johnson Space Center's Committee for the Protection of Human Subjects approval to interview astronauts, flight surgeons, flight directors, and Capsule Communicators who have experience with long-duration space flight, on Shuttle-Mir and ISS missions to establish. As the prototype takes shape, the P.I. intends to show it to P.I.s on the Neurobehavioral and Psychosocial Factors Team for additional input.

Project 5. Dinges, D., Metaxas, D., et al.: Optical Computer Recognition of Behavioral Stress. This project focuses on neurobehavioral critical path questions. The goal is to determine whether a state-of-the-art optical computer recognition algorithm based on facial expression can be developed that will objectively discriminate when subjects are undergoing behavioral stressors and negative affect. It also evaluates the effects of behavioral stressors on physiological responses, on psychological responses, and on performance responses, and explores the magnitude of stress responses relative to the accuracy of the optically based computer recognition algorithm of the face. ***Countermeasure goals*** include using the system to objectively detect distress and negative affect in astronauts when verbal and self-report communications are not possible or not reliable, in order to identify when appropriate behavioral and pharmacological countermeasures are needed for stress responses and affective dysfunction. ***Synergy and collaboration within the project and with other projects:*** Like Projects 1-4, Project 5 is made possible by a novel collaboration. In order to provide countermeasures for stressor-induced impairments in astronauts, objective, unobtrusive measures of the presence of stress reactions are needed. This project seeks to achieve such a measure, through a collaboration between two established laboratories at the University of Pennsylvania—one with expertise in the evaluation of behavioral and physiological responses under stressful and non-stressful conditions (Dr. David Dinges, Department of Psychiatry), and the other with expertise in optical computer recognition of human subjects' facial expressions and gestures (Dr. Dimitry Metaxas, Department of Computer Science). Dr. Metaxas' Vision Analysis and Simulation Technologies Laboratory has extensive experience creating novel optical computer recognition approaches (e.g., deformable masks) to human speech (lips) and gestures (sign language). Dr. Dinges' Unit for Experimental Psychiatry Laboratory has experience in stress-induction behavioral biology paradigms in healthy subjects. As part of an additional synergistic collaboration within the Team, Dr. Dinges' laboratory will also simultaneously obtain digital recordings of speech under stressful and non-stressful performance demands for Dr. Lieberman (Project 6), to provide an additional test of the sensitivity of the speech algorithms relative to the optical computer recognition algorithms for detection of stress responses during performance.

Project 6. Lieberman, P., Mertus, J., et al.: Speech Monitoring, Cognitive and Personality Alterations. This project focuses on neurobehavioral critical path questions. The goal is to develop a system that will detect cognitive deficits, changes in personality and emotional disturbances by means of acoustic measures of speech. The project utilizes data from studies of speech and behavior of individuals in a space analog environment (Mt. Everest climbers) as well as patients suffering neurodegenerative diseases (Parkinson patients), to develop and verify techniques for analysis of conversational speech for detection of cognitive changes. ***Countermeasure goals*** include using the system to objectively detect significant personality changes and emotional dysfunction in astronauts when optical communication is not possible or not reliable, in order to identify when appropriate behavioral and pharmacological countermeasures are needed for personality disturbances. ***Synergy and collaboration within the project and with other projects:*** Like Projects 1-5, Project 6 relies on a key collaboration. The P.I., Dr. Philip Lieberman is an internationally renowned linguistic scientist at Brown University, working with a mathematician, Dr. John Mertus, to refine and automate acoustic measures of speech for use in monitoring astronaut neurobehavioral capability. Dr. Mertus developed the BLISS speech analysis system and algorithms to be used in Project 6. Projects 5 and 6 clearly share a similar broad goal—to develop objective, on-line, unobtrusive, reliable measures of the status of astronaut emotional and cognitive distress levels. The research will determine whether either or both techniques yield useful information on affective and cognitive stress levels, and whether these lines of inquiry are worthy of continued pursuit. As described above in Project 5, Dr. Lieberman will also obtain speech samples from Project 5 (Dr. Dinges) when subjects are undergoing stressful and non-stressful performance demands, to provide a comparable test platform for comparing the discriminatory capability of the speech algorithms to that of the optical computer recognition algorithms. Like the latter, the speech information will be gathered and analyzed blind to the stress / non-stress performance condition in Project 5 (i.e., both Project 5 and 6 will prospectively test their algorithms in Project 5 in a double-blind manner).

Project 7. Kosslyn, S., et al.: Quick Assessment of Basic Cognitive Function. This project focuses on neurobehavioral critical path questions. The goal is to develop a set of brief performance tasks on a hand-held device that will be computerized versions of 11 standard tasks from cognitive psychology, which tap the range of basic cognitive abilities. The performance tasks being developed will be very short versions or variants of tasks that will capture the processing differences indicated by scores on the standard tasks and be designed to be self-administered. ***Countermeasure goals*** include using the brief tests to objectively detect cognitive performance deficits in individual astronauts, to alert crewmembers to diminished behavioral capacity and the need for rest or other interventions. ***Synergy and collaboration within the project and with other projects:*** Project 7 is directed by Dr. Stephen Kosslyn, Professor of Psychology at Harvard. Dr. With hundreds of publications, including 12 books, on cognitive performance and mental imagery, Dr. Kosslyn is ideally suited for leading this project, which is designed to develop a "tool box" for assessing the efficacy of specific aspects of cognitive processing. The goal is to have each task delivered by a hand-held Palm Pilot computer very quickly, allowing a fast and accurate "read out" of a person's processing abilities at that moment. To achieve this, Dr. Kosslyn is collaborating with the Palm corporation (Sam Kho, who was the chief programmer for the M100 series) to develop the software "shell" that will be designed to read in a stimulus file, present stimuli for specific amounts of time, and record responses and response times. The responses will be stored, along with a time-and-date stamp. The task can be set to display the results immediately after each test; the results will be presented in a bar graph, comparing errors and response times to norms. The shell will be written to be flexible, allowing

the programming of new tasks quickly and easily as the need arises. It is anticipated that once the basic system (hardware, software and task parameters) is complete, Dr. Kosslyn will collaborate with other projects on the Team (Projects 1 – 6), to evaluate the utility of the hand-held test device in subjects undergoing various behavioral challenges.

Project 8. Aston-Jones, G., Druhan, J., et al.: Stress, Performance and Locus Coeruleus. This project is currently the sole basic neuroscience research (i.e., using non-human species) on the Team. It focuses on neurobehavioral critical path questions. The goal is to analyze locus coeruleus (LC) activity during a continuous performance task, to determine the effects of acute and repeated stress on changes in LC function and performance, and identify pharmacological countermeasures to mitigate stress effects on LC activity and attentional function. ***Countermeasure goals*** identifying promising pharmacological countermeasures to mitigate stress effects on brain activity and attention in flight crews. ***Synergy and collaboration within the project and with other projects:*** Project 8 is directed by Dr. Gary Aston-Jones, an internationally recognized authority on the neurobiology of the LC and its functional significance in stress reactions and performance (vigilance and working memory). Dr. Aston-Jones is Professor of Psychiatry and Director of the Laboratory of Neuromodulation and Behavior at the University of Pennsylvania School of Medicine. He was originally Co-P.I. on the project when it was submitted and peer-reviewed, but he assumed the P.I. position at project initiation, when the previous P.I. (Dr. Druhan) left the University to take a new position. Targeted basic neuroscience research is needed on the Neurobehavioral and Psychosocial Factors Team, because it is the only way to address a number of critical path questions that cannot be pursued in human volunteers. Dr. Aston-Jones' focus on the noradrenergically-mediated locus coeruleus is synergistic with many of the human research projects on the Team that deal with stress as an outcome relative to performance (e.g., Projects 1, 2, 3, 5, 6, 7). He abiliy to record the effects of stress induction on both waking performance and sleep of rodents also provides a bridge between the stress focus of the NSBRI Neurobehavioral and Psychosocial Factors Team and Sleep loss focus of the NSBRI Human Performance Factors, Sleep and Chronobiology.

(Project 9. Proposed flight project (in feasibility evaluation at JSC, but not yet funded). Brunner, L., Feldman, S., et al.: Effect of Spaceflight on Pharmacokinetics of Psychotherapeutic Agents.) This project focuses on a neurobehavioral critical path question. The goal is to determine the effects of space flight on the pharmacokinetics, pharmacodynamics and the underlying physiologic processes (gastric motility and drug absorption), of the anti-anxiety drug, lorazepam (Ativan®), and the anti-depressant drug, venlafaxine (Effexor®). ***Countermeasure goals*** include studying both oral and intravenous use of both drugs, to determine ways to maximize their effectiveness for affective disorders and stress reactions that may develop in prolonged space flight, while minimizing their toxicity. ***Synergy and collaboration within the project and with other projects:*** The P.I., Dr. Lane Brunner is a pharmacologist in Pharmaceutics at The University of Teaxas at Austin. The project addresses a fundamental issue regarding the application of psychoactive substances in space flight. If the project is judged to be feasible by JSC and supported on a future space flight, the work will be relevant to the Team projects that seek to develop ways to objectively detect affective dysfunction in space flight (Projects 5, 6) and offer ways to treat it (Project 4, 8).

(Project 10. Proposed flight project (in feasibility evaluation at JSC, but not yet funded). Kanas, N., Marmer, C., et al.: Psychosocial Education (PSE) Training for ISS Missions.) This project focuses on a psychosocial critical path question. The goal is to evaluate the effectiveness in five International Space Station (ISS) crews and their support personnel of a 5-

hour, pre-launch Psychosocial Education (PSE) training program designed to reduce tension and displacement of dysphoria to outside personnel, and to increase cohesion, leader support, expressiveness and personal growth. ***Countermeasure goals*** include determining whether the PSE training program can reduce hostility among astronauts and between astronauts and mission ground support personnel while increasing group satisfaction and behavioral effectiveness. ***Synergy and collaboration within the project and with other projects:*** The P.I., Dr. Nick Kanas, Professor of Psychiatry at UCSF, has worked on space flight psychiatry issues for 30 years, including collaborations in recent years with Russian behavioral scientists studying the psychosocial effects of prolonged flights on Mir. Dr. Kanas' project addresses a fundamental question of whether crew tension and displaced feelings to ground controllers can be prevented or mitigated by psychosocial training. If the project is judged to be feasible by JSC and supported on a future space flight, the work will be relevant to the Team projects that seek to develop ways to prevent psychosocial dysfunction during space flight (Projects 1, 2, 3, 4).

Maintaining and expanding the Teams' synergy and collaborations. Since its inception as a new NSBRI Team in early 2001, the Neurobehavioral and Psychosocial Team continues to work diligently to cross-educate projects and integrate its scientific goals, countermeasure objectives, and methodologies. Team P.I.s had a face-to-face meeting at the University of Pennsylvania in March, 2001, and since then they have instituted and carried out a monthly 1-2 hour telecons to review progress and developments. There is also extensive e-mail communication among projects and team leaders. The Team will meet again in January 2002 at the NSBRI Retreat. The regular meetings, telecons and e-mails have resulted in a number of collaborations among projects on the Team (described above). In addition, the Team continues to successfully develop more extensive communications with the behavioral scientists and Flight Surgeon Psychiatrists at NASA Johnson Space Center (JSC). Associate Team Lead, Dr. JoAnna Wood serves on JSC's Integrated Product Team (IPT) for Human Behavior and Performance, and Dr. David Dinges, Team Lead, has recently been appointed as a non-voting member to the IPT. Dr. Dinges has advised JSC Flight Surgeon Psychiatrist, Dr. Chris Flynn, on development of a fatigue tool for space flight. Dr. Al Holland, JSC Chief Psychologist is on Team Project 4, and participates in the Team's monthly telecon. There are also a number of synergistic and collaborative efforts between Team P.I.s and other NSBRI Teams. Dr. Wood is collaborating with Dr. William Shearer, Team Lead for the NSBRI Immunology, Infection and Hematology Team, on serum measures of neuropeptides from subjects wintering over in the Antarctic for her Project 1. Dr. Dinges also performs research on countermeasures for the adverse neurobehavioral effects of chronic sleep restriction in space flight, as part of the NSBRI's Human Performance Factors, Sleep and Chronobiology Team. Some of the neurobehavioral performance tests used in his Project 5 on optical computer recognition of stress are also used in the sleep restriction research, allowing for a comparison of the effects of chronic sleep loss and acute stressors on these sensitive performance measures. A number of these performance measures were used in behavioral studies aboard Space Shuttle missions STS-90 and STS-95, and showed changes (decrements) in-flight relative to pre- and post-flight assessments (Dijk, et al., 2001), making it possible to compare experimentally induced changes in these performance parameters in stress-induction studies on Earth (e.g., Project 5) to those seen in space flight.

Synergies with other NSBRI Teams. It is the intention and goal of the Neurobehavioral and Psychosocial Factors (NBPF) Team to continue to build active synergies and collaborations with other NSBRI research teams, in relation to the effects of prolonged space flight, and countermeasures for it, on neurobehavioral and psychosocial functions. Some examples discussion underway on inter-Team collaborations and synergies include the following.

Human Performance Factors, Sleep and Chronobiology Team—NBPF Team seeks to determine the interaction of acute and chronic stress effects with sleep loss and circadian physiology.
Immunology, Infection and Hematology Team—NBPF Team seeks to help identify the effects of behavioral and psychosocial stressors on immune function in space flight.
Radiation Effects Team—NBPF Team seeks to assist in identifying the neurobehavioral effects of CNS damage from space radiation.
Bone Loss Team and the Muscle Alterations and Atrophy Team—NBPF Team seeks to help understand the psychosocial and behavioral implications of countermeasures that require lengthy daily periods of physical exercise in space (e.g., motivation, time management).
Neurovestibular Adaptation Team—NBPF Team seeks to help identify the neurobehavioral and psychosocial consequences of "sopite syndrome" and the effects of pharmacological countermeasures for space motion sickness on behavioral capability.
Nutrition, Physical Fitness and Rehabilitation Team—NBPF Team seeks to help understand how nutrition can be used to enhance neurobehavioral and psychosocial functioning.

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

The Neurobehavioral and Psychosocial Factors Team is one of the new NSBRI areas. The current eight ground-based projects were peer-reviewed by NASA, and selected for support as an initial core program 1 year ago. However, funding for most of the projects was not available or initiated for 3-7 months into year 1. Therefore the program progress reported below reflects an average of approximately 7 months work on these eight new research projects. The eight ground-based studies cover a considerable breadth of scientific techniques, but the overarching focus of all the research on the team is on the impact of stress—individual and interpersonal—on the functional capability of astronauts on long-duration missions. Collectively, the projects seek to (1) identify the causes of stress during space flight, and its consequences for astronaut cognitive, affective and social functioning; (2) identify techniques to objectively detect performance-impairing stress reactions in individuals and groups in the remoteness of space flight; and (3) identify countermeasures to prevent and otherwise mitigate the occurrence of stress reactions and their adverse effects on individual and flight crew performance. Because of the breadth of scientific techniques and approaches, each project is described briefly below.

Project 1. **Individuals and Cultures in Social Isolation**
Principal Investigator: **JoAnna Wood, Ph.D.**

Project 1. Research Objectives and Plan
This study evaluates a multidisciplinary, multi-level model of individual and group factors that influence health and performance in extreme environments, such as spaceflight, and Antarctic winter stations. This model explicitly incorporates the nested social structure of individuals living in small groups to examine both individual and group level outcomes. The primary hypothesis is that a useful model can be derived from hierarchical linear modeling analyses, but this can be described in more detail by the following hypotheses:

1. Individual factors and group factors combine to predict psychological stress, use of health care services, and changes in serologic markers of physiologic stress.
2. Individual factors and group level factors, as influenced by local events, combine to predict behavior and performance at both the individual and mission levels.

The proposed research is specifically designed to study the roles of personality, culture, and group influences on behavior, performance, and health outcomes in winter-over Antarctic research stations. These remote and isolated habitats provide an environment analogous to long-

duration space missions (e.g., confinement, isolation, risk), such as those planned for International Space Station and eventually a piloted expedition to the plant Mars. The ultimate objectives of this project are to increase understanding of the effects of personality, culture, and group characteristics on both individual and group performance in extreme environments; identify those elements of leadership that maximize crew functioning in extreme environments; and understand how individual and group factors affect physical health under prolonged stress.

Project 1. Year 01 Activity

The first year of data collection is winding down. The pre-isolation data collection at Bronte Park, Tasmania, was successfully completed in November 2000. Blood samples have been collected throughout the year on each station and are being returned to Australia, for delivery to the US, for processing. Data from the electronic questionnaires have been collected throughout the year via a secure web connection to the P.I.'s office, and have been stored there for analysis.

Project 2. **Psychosocial Performance Factors in Space Dwelling Groups**
Principal Investigator: **Joseph V. Brady, Ph.D.**

Project 2. Research Objectives and Plan

The objectives of this research focus upon the development of a distributed interactive multi-person simulation in computer-generated and/or computer-controlled environments as an experimental test bed for modeling psychosocial performances within and between space-dwelling and Earth-based groups. Computer generated simulation scenarios will be designed to permit changes in the parametric features (e.g. objectives, routes, etc.) of the required tasks each time the same scenario is encountered by a given group. Simulated space-dwelling group decisions will be based upon actions and pooled information from individual members as well as Earth-based centers, as required. Psychosocial performance effectiveness evaluation criteria will be developed based upon both process and outcome measures (e.g. error and task completion rates, timing, communication modes, frequencies, durations, and content, etc.). The project has five specific aims.

1. Determine the effects of environmental and behavioral interactions within and between simulated space-dwelling and Earth-based groups upon psychosocial performance effectiveness, Time pressure will be used as a stressful condition and will be evaluated with operational groups in distributed interactive simulation scenarios.
2. Determine the effects of variations in the structure and function of communication channels within and between simulated space-dwelling and Earth-based groups upon psychosocial performance effectiveness. Textual, voice/audio, and personal/video communication channels, singly and in combination, will be evaluated with operational groups interacting via computer terminals in distributed interactive simulation scenarios.
3. Determine the effects of variations in the appetitive/aversive characteristics of incentive systems within and between space-dwelling and Earth-based groups upon psychosocial performance effectiveness. Positive and punitive methods for maintaining on-task time will be evaluated with operational groups in distributed interactive simulation scenarios.
4. Determine the effects of prior training and experience within and between space-dwelling and Earth-based groups upon psychosocial performance effectiveness. Longitudinal group training and problem solving experience involving individual characteristics and psychosocial interactions will be evaluated with operational groups in distributed interactive simulation scenarios.

5. Determine the effects of personality selection factors upon the psychosocial performance effectiveness of simulated space-dwelling groups. Criteria for group composition will be evaluated with operational groups in distributed interactive simulation scenarios.

Five experiments will be conducted to address the specific aims. Groups of three individuals (located at workstations that are isolated both physically and acoustically from each other and from a remotely located monitoring station) will be trained to interact within the simulation environment. During each session, groups will be engaged in tasks that are cognitive in nature, do not require physical exertion beyond computer interactions, and may require repeated exchanges of information among group members and between the group and the Earth-center mission control. The principal objective of the simulation approach is to provide automated means for setting the context of space-dwelling group performances under study. In addition to setting the context, the simulation will provide an electronic means for monitoring psychosocial interactions and a method for varying important contextual variables that may alter the quality of group interaction, decision making, and performance effectiveness.

Project 2. Year 01 Activity

The goal for activity in year 01 was to develop the distributed interactive simulation environment and the simulation scenarios. The project was formally initiated April 1, 2001. Over the 6 months since then, a subcontract was established with Science Applications International Corporation and work was on programming a three-person crew simulation of a Mars surface expedition involving an Orbiter/Lander/Rover model. The program represents a range of simulated tasks and decisions relevant to exploration of the Martian surface. The activities involved include finding, collecting, and analyzing geological samples; avoiding hazards; communicating with other crew members and Mission Control; coping with equipment failures; and managing logistical requirements. The simulation incorporates a multi-modal communication system with voice, video, and text messaging. All contact among crew members and between crew members and simulated Mission Control is made via electronic channels of communication which are Internet compatible. The simulation is designed to provide a challenging cooperative work environment with multiple decision requirements, the potential for programming time-pressure stress effects, and a rich context for the emergence of leadership styles and alternative task organizations. Recording and measurement of all activities and communications will provide for an analysis of crew performance effectiveness. Experiments will focus upon the effects of crewmember personality, training, stress, and communication characteristics to include analysis of content, frequency, and patterns of communication.

Communication software has been completed and installed on the networked simulated crew stations. Interactive communication system pre-testing has begun between two of the four functional networked crew stations. The SAIC expeditionary simulation software is 95% complete and is expected to be ready for initial testing by November, 2001, when the entire simulation system, both hardware and software, will be evaluated in the operational pre-testing phase of the research project. A special laboratory facility for the research has been constructed at the Institute for Behavior Resources Headquarters Building in Baltimore City in the vicinity of the Johns Hopkins University Homewood Campus. The laboratory provides for three acoustically controlled workstations plus an additional separate 'Mission Control' station for operational implementation of the simulated Orbiter, Lander, Rover expedition for simulated exploration of the Martian surface. Essential professional and technical support personnel have been recruited for computer system maintenance and troubleshooting as well as the personality testing and evaluation phases of the project.

Project 3. **Distributed Team Decision Making for Long Duration Space Missions**
Principal Investigator: **Judith M. Orasanu, Ph.D.**

Project 3. Research Objectives and Plan

This project has two specific aims.

1. Conduct laboratory experiments to study the effects of task-related stressors on team performance in challenging distributed decision-making situations. Effects of national culture and gender on team interactional processes will be examined. Teams will work together for several days in 4-hour sessions to give them time to get to know each other and to build working relationships.
2. Monitor affective responses by individual team members as they work on team tasks that present both task and interpersonal stressors. Techniques will be developed to monitor team performance in order to detect when a team's cohesion and effectiveness are deteriorating so that countermeasures can be applied (specific countermeasures will not be developed at this time). Interpersonal friction can cause anxiety, which may be discernible through physiological indicators, as well as facial expression and verbal content of communication.

The project addresses the following four research questions. (i) How do gender and cultural background influence decision-making strategies and interactional behaviors in team problem solving situations? (ii) How do communication media and collaborative tools influence team decision-making strategies and interactional processes? (iii) Do communication media and technologies have similar effects over gender and cultural groups? (iv) Does physiological arousal in team situations show similar relations to interactional and decision strategies across gender and national culture groups?

Project 3. Year 01 Activity

The project was scheduled to begin on April 1, 2001, but funding was not available until August 15, 2001. Initial planning and development was undertaken prior to funding, but the project was limited in what could be done without the funding in place. Since project initiation, Distributed Dynamic Decision Making (DDD) software was acquired from Aptima, Inc., who previously developed software for NASA that supports distributed team decision making tasks in dynamic environments. The software presents graphical displays of evolving problem scenarios and supports manipulation of task features to vary the level of challenge or pressure inherent in a task. It also supports communication among team members via e-mail messages or voice (either open microphone or restricted communication, such as when subjects can only talk with the base station, which will relay messages). The DDD Search and Rescue scenario that will be used in this project involves searching for a lost research team in the Antarctic. Other members of the team must search for the team under a variety of task constraints. The DDD system will enable examination of collaborative behaviors among team members in two phases of the task: (a) during planning how to go about locating the lost party, and (b) executing the search task under a variety of task constraints such as time limitations, unexpected obstacles, etc.

Scenario development has also been underway. The software permits manipulation of a variety of potential stressors in two categories: task difficulty and team conflict. Ambulatory physiological monitoring devices (Biologs) will be used for recording ECG, PPG, EMG, respiration, skin conductance, skin temperature, and activity level. Video recordings of participants' faces while engaged in team tasks will be synchronized in time with physiological monitors and audio recordings using techniques developed by Drs. Gottman and Carrere at the University of Washington. Dr. Carrere is a consultant on the project. Affect assessment will

involve coding and analysis of facial expressions and communication from video recordings of participants' faces. Both on-line and post-hoc versions of the Specific Personal Affect Coding system (SPAFF) developed by Drs. Gottman and Carrere will be used.

A collaborative relationship has been established with Norbert Kraft, M.D., who works for the Japanese Space Agency, NASDA. Discussions are underway on the possibility of collaborating on a planned ground-based ISS simulation study in Japan that would involve multicultural crews. Dr. Kraft was the Commander of the multicultural crew in the recent SFINCSS isolation study conducted in Russia. His personal experience of 117 days in this environment has given him considerable insight into a number of factors relating to team interaction and performance in space missions. Most significant was the occurrence of interpersonal difficulties, especially those involving gender or cultural interactions. Specifically, these turned on specific cultural views of women's roles in society; norms for working together; priorities and goals; and sense of time. One major problem area was the relation between the space crew and ground support—development of an "us/them" relationship, lack of trust, and reduction of communication over time were evident. Much of this relates to problems with developing shared mental models, which is difficult when team members are physically separated, have different goals and priorities, perhaps different perceptions of risk, and different pressures and constraints. A final area of concern for Dr. Kraft was family support for space crews and maintenance of relationships during long periods of separation. Dr. Kraft designed a set of team problem solving tasks that were used in the SFINCSS study. These dealt with ethical issues in emergencies, medical problems, resource allocation problems etc. The suitability of these tasks for use in Project 3 is being evaluated, as additional vehicles for examining team interaction, primarily in face-to-face but also in mediated communication modes.

Project 4. **Designing a Smart Medical System for Psychosocial Support**
Principal Investigator: **James A. Carter, Ph.D.**

Project 4. Research Objectives and Plan

This project addresses the need to develop a set of on-board and pre-flight tools to train astronauts how to prevent and manage psychosocial problems. The goal is to develop a prototype of a smart medical system for psychosocial support. A fully developed system would include resources to assist space crews in preventing, assessing and managing an array of problems. The prototype will include programs on conflict, depression, and assessment of psychological problems. There are 3 phases: Background research, multimedia production, and evaluation.

In phase I background research, interviews will be conducted with astronauts, flight surgeons, flight directors, and Capsule Communicators (Capcoms) who have experience with long-duration space flight, on Shuttle-Mir and ISS missions. These interviews will be consultative, with the goal of obtaining input from persons who have experienced the stresses of long-duration flight from both space and the ground. Design and content ideas will be presented, including potential training scenarios, and feedback will be solicited as to their importance and appropriateness, as well as their own suggestions on content and design. Input from "end-users" of the system (i.e., astronauts) is critical in developing tools that are both useful and acceptable to them. Phase I also includes input from subject-matter experts in the areas of space psychology (Al Holland, Ph.D., NASA-JSC), depression and psychological evaluation (Mark Hegel, Ph.D., Dartmouth-Hitchcock Psychiatric Associates), and conflict resolution (Leonard Greenhalgh, Ph.D., The Amos Tuck School of Business, Dartmouth College).

In phase II media production will occur. The approach to prototyping is to fully develop small sections of each part of the system. This approach will enable end users and other reviewers to understand how key components of the fully developed system would function and look, and what content it would contain. Multimedia production includes designing the program from start to finish "on paper" and soliciting feedback from relevant parties along the way. Dr. Carter is designing the functionality, look and feel of the system and overseeing the production of the media components. Through formative evaluation, input from subject-matter experts and end users will be obtained at key points in the process to ensure that the final product meets the needs and requirements of all parties involved. The conflict and depression programs will include interactive modules on prevention, assessment, and management/treatment. Simulated encounters using actors to play the roles of crewmembers will be developed and videotaped to help users of the system try out skills that are taught in the conflict management program. The depression program will walk users through the process of "problem-solving treatment," a behavioral treatment for depression that has been empirically validated in primary care settings. Both programs will include a mentor, on video, who guides users through the program, offering encouragement and feedback. Media components will include original video, audio, graphics and text. Video and audio will be recorded in various locations. Post-production, graphics and programming will be done at The Troupe, Inc., a multimedia production company.

Evaluation phase III of the prototype will be conducted at NASA-Johnson Space Center's Usability Testing and Analysis Facility. Dr. Mhiriban Whitmore, the facility's Director, will oversee this evaluation. Testing will include usability, acceptability, and perceived value. Because the prototype is inherently incomplete, no efficacy evaluation will be possible in this study. Subjects will include the same population interviewed in Phase I: current and former long-duration space and ground crewmembers. The program will also be sent to external (non project-related) experts in the fields of Problem-Solving Treatment and conflict management for review. Data from this study will guide further development of the system in future studies.

Project 4. Year 01 Activity

Due to delays in initial funding, work on this project began on May 1, 2001. Since that time, the majority of effort has been dedicated to securing approval from NASA-Johnson Space Center's Committee for the Protection of Human Subjects (CPHS; formerly the IRB). The Committee has provided helpful feedback and recommendations and granted permission to interview NASA-JSC personnel on September 13, 2001. In order for the proposal to be accepted by the CPHS, several changes were made to the phase I and phase III work plans, although the aims of the study are unchanged. Project investigators are now awaiting permission from NASA-JSC's Integrated Projects Team (IPT) to conduct phase I interviews with crewmembers. In the meantime, they are contacting retired crewmembers to solicit their input in interview to begin in December, 2001. In addition to securing CPHS approval to conduct Phase I interviews, the investigators have developed the basic teaching points to be included in the depression and conflict programs, as well as multiple design concepts for the overall system.

Project 5. **Optical Computer Recognition of Performance Under Stress**
Principal Investigator: **David F. Dinges, Ph.D.**

Project 5. Research Objectives and Plan

The goal of this project is to develop and test optically-based computer recognition algorithms of the human face to reliably detect the presence of stress during performance demands. The project specifically addresses the following question from the Critical Path Roadmap: What techniques

and methods can be developed for use in flight to effectively monitor and detect emotional distress and other forms of neuropsychiatric function during a prolonged mission? The project has two specific aims.

1. Experimentally establish whether optical computer recognition algorithms based on facial expression can be developed that can objectively, independently and reliably discriminate when subjects are undergoing behavioral stressors, and whether a high degree of accurate categorization can be achieved for both male and female subjects; for both younger (22-32 years) and older (33-45 years) subjects; and for subjects of different ethnic backgrounds.
2. Evaluate the extent to which the magnitude of the stress response as assessed by cortisol secretion, heart rate, and subjective stress and mood ratings, relates to the accuracy of the optically based computer recognition algorithm of the face.

The computer-based optical recognition system builds on the research of Dr. Dimitris Metaxas (Vision Analysis and Simulation Technologies Laboratory, Department of Computer Science, University of Pennsylvania) utilizing automatic optical tracking of human subjects' subtle anatomical and motoric changes in facial expressions during non-verbal performance tests. Video input to the system is provided from experiments performed in the laboratory of the Dr. Dinges (Unit for Experimental Psychiatry, Department of Psychiatry, University of Pennsylvania), in which healthy adults (males and females of different ages and ethnic backgrounds) are exposed to laboratory simulations of behavioral performance stressors.

A single-blind, repeated-measures cross-over, controlled trial will be used to achieve the specific aims and to provide the data required to test the hypothesis that an objective, unobtrusive, optically based computer recognition algorithm of the face can be developed for each subject to reliably discriminate between the presence of high stress versus low stress performance conditions. A total of 60 healthy adults will be evaluated in the laboratory during three phases: I—screening session; II—training session for development of the optical computer recognition algorithm for each subject; and III—prospective test session of the predictive utility of the optical recognition algorithm to discriminate high-stress versus low-stress states associated with behavioral performance demands. Stress reactions will be tracked during both control (low stress) and high stress conditions in sessions II and III, by measurement of salivary cortisol, heart rate, subjective mood/stress responses, and neurobehavioral performance. Videos of subjects' faces in the low and high stress conditions of session II will be used in Dr. Metaxas' laboratory to develop a predictive optical algorithm for each subject that will be tested blind to stressor level (i.e., high vs. low) in the behavioral stressor conditions of session III.

Project 5. Year 01 Activity

During the first 6 months of year 01, various stress-induction procedures were developed; IRB and GCRC approvals were acquired for the stress-induction protocols; special camera equipment and ECG holter monitors were acquired; salivary kits for cortisol assessments were acquired. The second 6 months were devoted to completing software programming of performance tests and mood scales in the computerized batteries; implementing the facial cameras with the neurobehavioral test batteries; and preliminary tests on the behavioral stressor paradigms. This latter activity was devoted to assessing the applicability and strength of the stressors to be used in the protocol. The specific type of stressor is less important at this early phase of feasibility testing than is the extent to which the stressor induces subjective (self report), facial (affective expressions), hormonal (salivary cortisol), and cardiovascular (ECG) responses indicative of mild to moderate acute stress reactions. These pilot efforts have revealed that the three behavioral stressors that were to be used separately in the optical computer recognition test phase

did not consistently induce subjective distress or physiological distress in all subjects. As a result, the procedures were modified to combine stressors to create high stress conditions at both the optical computer algorithm-training phase and the prospective test phase, relative to low/no stress conditions at those times. The result was that behavioral stressor 1 (sleep inertia) and 3 (anticipation of venipuncture) were abandoned, but behavioral stressor 2 (work demands) was markedly expanded. The resulting high stressor conditions use a combination of performance demands (via high workload, task difficulty, time pressure, uncertain future demands), and frustration (via competing demands, false feedback, and remote social ratings) to induce distress. Dr Metaxas and colleagues have been refining the deformable facial masks and the optical computer algorithm, to increase the likelihood the algorithm will detect facial expressions of distress in subjects.

Project 6. **Speech Monitoring Cognitive and Personality Alterations**
Principal Investigator: **Philip Lieberman, Ph.D.**

Project 6. Research Objectives and Plan

The goal of this project is the development of a system that will detect cognitive deficits, and changes in personality and emotional state during space flight by means of acoustic measures of a person's speech. The system will monitor the flow of normal conversation, deriving relevant acoustic parameters using automated procedures that will be developed and verified in this project. Speech and behavior of individuals will be studied in a space-analog (i.e., Mt. Everest climbers, as well as in patients suffering neurodegenerative diseases (i.e., Parkinson's Disease). To different degrees these populations exhibit degraded neural processes regulating behaviors.

Mt. Everest climbers have been used as a space analog in previous NASA-sponsored research conducted by the P.I. Expedition members selected will be comparable to space-flight crews—extremely fit, intelligent and highly motivated. The climbers experience hypoxic insult and periodic high levels of stress resulting from the life-threatening environment on Everest. The project seeks to develop and verify techniques that will allow analysis of conversational speech, collect additional acoustic parameters that have been associated with cognitive-linguistic deficits in other studies, assess changes in mood and personality relative to speech measures. Each Mount Everest expedition takes approximately 3 months, which allows for repeated formal test sessions at successively higher altitudes and the opportunity to monitor many radio-transmitted conversations. A total of 30 subjects will be studied, 10 in each year of the project (Everest expeditions generally are limited to 10 members).

In addition, comparisons will be made to patient's with Parkinson's Disease, which is a population having more severely compromised dopaminergic circuit function affecting cortico-striatal circuits involved in motor control, but also in cognitive and emotional problems such as depression, apathy, irritability, disinhibition, mood changes and obsessive-compulsive behavior. Techniques are being developed that reveal these deficits through study of patients exhibiting different degrees of cognitive and emotional changes. Dr. Joseph Friedman (Chief of the Neurology, Memorial Hospital of Rhode Island) will identify and evaluate each of the 90 volunteers with Parkinson's Disease.

Speech signals for the Everest climbers are recorded on Sony NT-1 micro digital audio tape recorders. Barus Laboratories Interactive Speech Analysis System (BLISS) is being used to monitor and modify parameters at virtually all stages of analysis, thereby minimizing artifacts that can be introduced by many other speech analysis software systems. The spectral analyses

and F0 analyses features of the BLISS system allow derivation of acoustic parameters associated with stress and affect. Another variable of interest is voice-onset-time (VOT), which is highly correlated with cognitive and sentence comprehension deficits. Automated computer-implemented procedures for on-line VOT monitoring will also be developed and validated in the project. Linguistic and cognitive tests will be administered to determine whether the speech measures track cognitive deficits, particularly those factors involved in decision making and language comprehension. Additional measures include verbal working-memory span; tests that assess a subject's ability to use information conveyed by syntax in the comprehension of sentences; and a test that assesses the ability to learn new problem-solving modes and maintain a cognitive set, then shift sets. Fundamental frequency contours of spontaneous speech produced during episodes in which Everest climbers are subject to high levels of stress will also be studied. Speech analysis techniques for stress will also be analyzed from voice samples obtained in Project 5.

Project 6. Year 01 Activity

This project began February 1, 2001. During the 8 months it has been underway, work has progressed on data acquisition from both Mount Everest climbers and patient's with Parkinson's Disease. Members of the National Federation for the Blind's 2001 Mount Everest expedition were tracked as they ascended the mountain. Samples of isolated words and fluent speech were obtained, and tests of cognition and language comprehension were performed in at Dingboche (elevation 4, 300m) and Base Camp (5,300m) after 3 weeks acclimatization; shortly after subjects returned from Everest's summit; and at Camps 2, 3, and 4 (6,300m, 7,150m, and 8,000m, respectively). Verbal working memory span was determined, cognitive sequencing was tested, and comprehension of sentences was tested with procedures that have been normed for Parkinson's Disease patients. Emotional state was monitored through questionnaires, as were symptoms of acute mountain sickness. Spoken word lists and the responses to the cognitive and linguistic test battery and conversations were transmitted for digital tape-recording at Base Camp. Spontaneous speech transmitted in radio communications throughout the climb, particularly those occurring in stressful situations, was also recorded for analyses.
Data acquisition from 40 patients with Parkinson's Disease has also been completed. Preliminary analyses show that these patients have diminished verbal working memory span, sentence comprehension errors and difficulty in shifting cognitive sets. An eye-tracking procedure used with these subjects in a sentence comprehension experiment is confirming that the off-line response techniques used on Mount Everest subjects are valid. This study is presently in the final stages of preparation for journal submission.

Project 7. **Quick Assessment of Basic Cognitive Functions**
Principal Investigator: **Stephen M. Kosslyn, Ph.D.**

Project 7. Research Objectives and Plan

This project seeks to develop a cognitive testing "tool box" using a Palm Pilot (hand-help) PC platform that administers abbreviated yet valid versions of standardized cognitive tests (with known psychometric properties and identified neurological bases), and records and compares responses to a pre-defined normative database. Each of the tasks will be delivered on the hand-held Palm Pilot computer very quickly, allowing a fast and accurate "read out" of a person's processing abilities at that moment. The specific aim of the project is to design short, easy-to-administer variants of standard information processing / cognitive tasks that will assess very specific types of cognitive functions. Automatic data analysis will be programmed into the

computerized tests, so that final scores can be available immediately (or can simply be stored, for later analysis; all scores will have time stamps).

Based on the literature in cognitive psychology and cognitive neuroscience, initial set of tasks have been selected that tap key features of information processing. The tasks themselves are modeled after corresponding, well-validated tasks in the scientific literature. The specific tasks to be implement initially on the Palm Pilot tool box platform will assess attention (vigilance, divided attention, and filtering), spatial relations encoding (categorical and coordinate), working memory (verbal and spatial), spatial working memory, long-term memory (verbal and visual), and problem solving (verbal and spatial). The new short-duration versions of the tasks will be validated against the traditionally longer versions as administered by a Macintosh computer. The goal is to keep the tasks as brief as possible while maintaining their validity to assess the targeted cognitive functions. Consequently, the tool box tasks much not differ substantively from the validated standard tasks in overall difficulty. The most straightforward way to approach this is to select the "best" items from a longer task (i.e., select the items that correlate most with the overall score and that are equated for difficulty with items in other tasks). Because many items are needed, it will be necessary to not simply select existing items per se, but instead to adopt specific ways to create items (which produce items that correlate with overall task performance and are of specific levels of difficulty). This will be the approach taken.

Project 7. Year 01 Activity

Funding for this project was received on July 1, 2001. During the 3 months the project has been underway, a programmer at Palm corporation (Sam Kho, chief programmer for the M100 series) was commissioned and engaged in a collaborative effort to develop the software "shell" that will run the project cognitive tests. The shell will be designed to read in a stimulus file, present stimuli for specific amounts of time, and record responses and response times. The responses will be stored, along with a time-and-date stamp. The task can be set to display the results immediately after each test; the results will be presented in a bar graph, comparing errors and response times to norms. The tasks differ only in the instructions (which are presented in initial screens), stimuli, and the number of response keys used. The shell will be written to be flexible, allowing one to program new tasks quickly and easily as the need arises (e.g., to test a specific aspect of functioning that is needed for a particular task). However, the current version of the shell has the following limitations: (i) only visual stimuli can be presented; (ii) the stimuli are presented either in a random order of fixed order (adaptive testing is not possible); and (iii) the responses are limited to key presses (with up to 4 keys being used).

The prototypical cognitive tool box being developed will have three general applications. First, it will provide a practical means for assessing the effects of other variables on key cognitive processes. For example, it could be used to assess the effects of motion sickness, sleeplessness, work schedules, exercise, diet, or performance demands on cognitive processing. Second, the tasks can be used to assess the effectiveness of "countermeasures," such as specific pharmacological interventions to protect against the effects of space radiation. The tasks can be administered before and after interventions or training, which will provide a measure of how an intervention affects basic cognitive functions. Finally, the tasks could be used by people "on the job" (astronauts in space) or others to inform themselves about the current state of their cognitive processes. For example, before an EVA to repair an external part, an astronaut might find it useful to know how effectively she or he was reasoning logically and spatially. If one was not doing as well as usual, a countermeasure might be administered, or someone else might go, or one might go later, or one might go but make a concerted effort to be very careful. The essential

point is that the cognitive tool box may be able to provide astronauts with a reliable estimate of their cognitive capabilities prior to undertaking a hazardous activity that demand optimal neurobehavioral performance capability.

Project 8. **Stress, Performance and Locus Coeruleus**
Principal Investigator: **Gary Aston-Jones, Ph.D.**

Project 8. Research Objectives and Plan

This project is focused on understanding the impact of acute and chronic stress on neurobiological processes underlying attention and cognitive performance. The ability to sustain attention is critical to the successful performance of complex tasks over extended periods of time. Studies by the P.I. have determined that optimal attentional performance in non-human primates is associated with moderate basal firing rates of noradrenergic (NA) neurons in the locus coeruleus (LC), and phasic activation of these cells in response to imperative discriminative stimuli. In contrast, poor attentional performance is associated with either high or very low basal activity in LC neurons, and an absence of phasic activation by task stimuli. Studies in this project will determine whether disruptive effects of stress on attentional performance result from stress-induced tonic activation of the LC, and whether these effects become more pronounced after repeated exposures to stress. The ability of pharmacological treatments affecting noradrenergic function to improve attentional performance will also be assessed to identify potential countermeasures that may be used to prevent lapses in attention and cognitive performance during long-duration space flight. There are three specific aims in this project.

1. Analyze LC activity during a continuous performance task. The PI's previous studies linking attentional performance to LC activity were conducted using non-human primates. While primates provide an excellent model for assessing cognitive function, cost factors and ethical considerations prevent the use of these subjects for studies involving large numbers of animals and prolonged exposure to stress. Therefore, a behavioral task for measuring attentional performance in rats was developed that captures the essential cognitive demands of the task used in prior primate studies. Experiments in Aim 1 of this project will measure the activity of LC noradrenergic neurons in rats engaged in this continuous performance task to confirm that the relationship between LC activity and behavioral performance observed in the primate is also present in the rat. Subsequent experiments will examine the effects of directly manipulating LC activity on performance in this task to verify the causal role of the LC system in performance.
2. Determine the effects of acute and repeated stress on changes in LC function and performance. Experiments in this aim will examine the effects of acute and repeated exposures to stress on LC activity and behavioral performance to determine whether the disruptive effects of stress on performance are associated with changes in LC activity. It is hypothesized that acute stress will increase basal firing rates of LC noradrenergic neurons and decrease phasic responses to task-related stimuli, and that these changes will be accompanied by decrements in performance. Furthermore, as central NA systems undergo sensitization following repeated stress, we predict that LC function and behavioral performance will be more susceptible to dysregulation after prolonged exposure to stressful events.
3. Identify pharmacological countermeasures to mitigate stress effects on LC activity and attentional function. The activity of LC noradrenergic neurons is regulated by several transmitter systems, including serotonin, corticotropin releasing factor (CRF), and glutamate. These neurons are also self-regulated through NA actions at alpha$_2$ NA autoreceptors.

Experiments in this aim will specify pharmacological treatments acting at these transmitter systems that can attenuate the effects of stress on LC activity and behavioral performance without affecting baseline LC function and performance.

The results of these studies will provide important information concerning the neural basis for stress effects on performance. This information will lead to the development of effective pharmacological countermeasures for reducing performance decrements produced by stress during long-duration space missions.

Project 8. Year 01 Activity

The project was funded July 1, 2001. The original P.I., Dr. Jon Druhan, left the university for a position elsewhere. Dr. Aston-Jones, the Co-P.I. on the project and the Director of the Laboratory in which the research was to be undertaken, received permission from the NSBRI to assume the P.I. role. In the past 3 months significant progress has been made toward the project goals. A continuous performance task for rats was developed that captures the essential cognitive demands of the task used in earlier primate studies. A central hypothesis in this proposal is that manipulations that increase the basal firing rates of LC neurons should lead to disruptions in performance on tasks that require focused attention. In particular, the P.I.'s studies in monkeys found that tonically increased LC activity were associated with a higher rate of false alarm responses to nontarget stimuli. Here, we tested this prediction by pretreating four rats with the alpha2 NA receptor antagonist idazoxan (1 or 2 mg/kg) or vehicle solution after the task had been acquired. Idazoxan increases LC firing rates and increases NA terminal release by blocking autoreceptors on LC neurons. Thus, idazoxan would be expected to disrupt performance if behavioral responding on this continuous performance task were related to tonic levels of LC activity. Interestingly, we found that the effects of idazoxan depended on the baseline level of performance of each animal. Preliminary results suggest that moderate levels of tonic LC activity are critical for maintaining focused attentiveness to task stimuli and performing optimally, and that behavioral performance declines when tonic LC firing rates are increased.

Another goal of the project is to manipulate noradrenergic LC neurons and examine effects on performance. Selective lesions of these neurons would be one important manipulation to test their role in this and other tasks. Antibodies against dopamine beta hydroxylase (DBH) linked to the ribosomal toxin saporin (anti-DBH-saporin) is a newly described method for lesioning NA-LC cells. We examined the utility of this approach by making focal microinjections of anti-DBH-saporin directly into the LC of rats. Injections were guided by electrophysiological recordings, so that the accuracy of injection placements was very high. Results indicated that although these injections effectively lesioned LC neurons, they also lesioned other DBH-containing cells, such as NA neurons in the A5 and A7 cells groups, and epinephrine cells in the C1 cell group. This apparently occurred as a result of retrograde transport of the toxin by DBH-containing neurons that innervate the LC. These findings rule out the use of this method for selective lesions of the LC. The new plan is to examine the use of focal microinjections of the selective neurotoxin 6-hydroxydopamine into the LC.

It is well established that, in addition to affecting performance, stressors also disrupt sleep. Therefore, a method was implemented for recording rat electroencephalogram, electromyogram, and body temperature by telemetry, and performing computer-based automated scoring of sleep stages in rats. This technical effort may prove important in the ongoing experiments as an additional insight into how stress alters the neurobiology of behavior, and it may provide a

bridge between the stress focus of the NSBRI Neurobehavioral and Psychosocial Factors Team and Sleep loss focus of the NSBRI Human Performance Factors, Sleep and Chronobiology.

V. FUTURE PROGRAM DIRECTIONS

The NSBRI Neurobehavioral and Psychosocial Factors Team encompasses the Critical Path Roadmap scientific questions from two of the four areas subsumed under Behavior and Human Performance: (1) Human performance failure because of poor psychosocial adaptation (area 18), and Human performance failure because of neurobehavioral dysfunction, (area 21). The demonstrated occurrence in USA and Russian space missions of problems involving poor psychological adaptation to the space environment have resulted in area 18 being rated at the highest risk rank (level I) in the Critical Path Roadmap. The Neurobehavioral and Psychosocial Factors Team is charged with conducting research on the development of countermeasures that will ensure that astronaut behavioral health is maintained during prolonged space missions, and that both individual astronaut and crew functioning are effectively optimized. As described above, the research program of the Team was defined by the peer-review selection conducted in 2000. The eight ground-based projects making up the Team have been underway an average of 7 months. The studies are divided equally between psychosocial and neurobehavioral scientific questions. They cover a considerable breadth of scientific techniques—projects range from studies of humans living in an analog environment to neurobiological studies of rodents performing under conditions of stress. This breadth notwithstanding, the overarching focus of all eight projects is on the impact of stress—individual and interpersonal—on behavioral functions. Collectively, the projects seek to identify (1) the causes of stress, and its consequences for astronaut cognitive, affective and social functioning; (2) techniques to objectively detect stress reactions and performance deficits in individuals and groups in the remoteness of space; and (3) countermeasures to prevent and otherwise mitigate the occurrence of stress reactions and their adverse effects on individual and crew performance. Given that the projects are still early in development, no change is planned in the future strategy of these projects until their experimental results are clear. On the other hand, it is recognized that there are important areas and technologies that have not yet been covered by the current projects. To this end, future plans should focus on adding research projects that seek to identify the effects of long-term exposure to the major factors in the space environment on emotions, cognition, performance, and behavioral health. In addition, novel neuroscience technologies (e.g., neuroimaging; transcranial magnetic stimulation) or novel behavioral methodologies (e.g., virtual reality, experimental manipulation of small group microsocieties in isolation and in tandem) should be included to search for new countermeasures for the psychosocial and neurobehavioral effects of prolonged space flight. Finally, work is needed on the behavioral strategies, scheduling strategies and habitat design elements that can maintain or enhance crew performance and prevent the development of hostility between crews and ground-support personnel during long-duration space flight. In addition to preventing behavioral problems in space flight, the countermeasure research being performed on the Team may also aid in helping to solve neurobehavioral and psychosocial problems on Earth, ranging from early objective detection of cognitive and emotional impairment, to development of new behavioral and pharmacological treatments for stress, to better communication systems that prevent miscommunication, to improved techniques for improving group performance and cohesion.

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

Research Team Annual Report
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Projects and Principal Investigators

- Context-Specificity and Other Approaches to Neurovestibular Adaptation
  Mark J. Shelhamer, Sc.D., Johns Hopkins University School of Medicine
- Neuro-Vestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation. Laurence R. Young, Sc.D., Massachusetts Institute of Technology
- Modification of Eccentric Gaze-Holding
  Millard F. Reschke, Ph.D, NASA Johnson Space Center
- Visual Orientation and Spatial Memory: Mechanisms and Countermeasures
  Charles M. Oman, Ph.D., Massachusetts Institute of Technology
- Advanced Techniques to Assess and Counter Gait Ataxia
  Conrad Wall III, Ph.D., Massachusetts Eye and Ear Infirmary
- Understanding Full-Body Gaze Control During Locomotion
  Jacob J. Bloomberg, Ph.D, NASA Johnson Space Center

- Pharmacological Countermeasures for Space Motion Sickness
  John L. Dornhoffer, M.D. University of Arkansas for Medical Sciences

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I. Executive Summary

The most overt change affecting an astronaut in space flight is the immediate response of the neurovestibular system to changes in gravity. NSBRI's neurovestibular adaptation research program supports research aimed at developing scientifically-based countermeasures against the vestibular problems associated with space flight: space motion sickness, disorientation, oculomotor deficits, postflight postural instability and gait ataxia. Problems typically arise first when astronauts transition from 1-G to 0-G, unfortunately at a time when their physical and cognitive performance is often critical for mission success and safety. Postflight problems have generally been more severe after 3-5 month Mir and ISS flights than on 1-2 week Shuttle missions, showing that some components of vestibular adaptation to 0-G take place over time scales of months, rather than weeks. Looking beyond ISS to interplanetary exploration missions, operationally significant vestibular problems are anticipated when astronauts make the transition from 0-G to partial G, or from 0-G to an artificial gravity environment.

During the 1980s and 90s, space neurovestibular research largely focused on understanding the effects of unweighting of the otoliths on the vestibulo-ocular reflex (VOR), and predicting space sickness susceptibility. The NSBRI neurovestibular adaptation research program has a broader scope. It is aimed at developing scientifically based countermeasures against the vestibular problems of space flight, and addresses the five major space neurovestibular risks identified in NASA's Critical Path roadmap. These are:

1. Disorientation and reduced performance on cognitive and physical tasks, including vehicle egress, especially during/after g-level changes.

2. Impaired neuromuscular coordination strength upon return to positive G leading to increased incidence of falls and injury during emergency egress and escape.

3. Impaired cognitive and/or physical performance due to spatial disorientation, motion sickness symptoms or treatments as a result of changes in g-level, or use of artificial gravity.

4. Autonomic dysfunction which may be of vestibular origin.

5. Permanent impairment of orientation or balance function due to microgravity or radiation.

The goals of the program are to develop countermeasures that ultimately will to allow crewmembers to avoid disorientation, meet the physical requirements of emergencies, treat motion sickness without side effects and safely control vehicles and systems.

Eight interrelated research areas define the scope of the program:

- Sensory-motor adaptation;
- Artificial gravity;
- Visual (multisensory) orientation, navigation and spatial memory;
- Vestibular/Autonomic/Emetic physiology and drug countermeasures;
- Postflight locomotion and gaze assessment;
- Neurovestibular rehabilitation;
- Effects of weightlessness, immobilization, stress, isolation, and diet on vestibular function; and
- Potential mechanisms for and diagnosis of irreversible neurovestibular changes.

The current research portfolio of seven projects was selected in the spring of 2000 based on independent scientific peer review. Three of the projects are competitive renewals of projects initiated in 1997. The NSBRI neurovestibular adaptation research team consists of 37 investigators associated with these seven projects. They come from 21 different universities and research laboratories, including NASA's Johnson and Ames research centers. Many have concurrent support from NIH, NSF, and foundations. Twelve investigators have previously flown spaceflight experiments. They are an interdisciplinary group of physiologists, physicians, psychologists, and engineers, with background in human and animal sensory and motor neurology, physiology, psychology, navigation, vestibular rehabilitation and technologies such as head and eye movement tracking, locomotion measurement, and virtual reality.

The seven projects and their principal investigators are:

1. Context-Specificity and Other Approaches to Neurovestibular Adaptation
   Mark J. Shelhamer, Sc.D., Johns Hopkins University School of Medicine

2. Neuro-Vestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation. Laurence R. Young, Sc.D., Massachusetts Institute of Technology

3. Modification of Eccentric Gaze-Holding
   Millard F. Reschke, Ph.D, NASA Johnson Space Center

4. Visual Orientation and Spatial Memory: Mechanisms and Countermeasures
   Charles M. Oman, Ph.D., Massachusetts Institute of Technology

5. Advanced Techniques to Assess and Counter Gait Ataxia
   Conrad Wall III, Ph.D., Massachusetts Eye and Ear Infirmary

6. Understanding Full-Body Gaze Control During Locomotion
   Jacob J. Bloomberg, Ph.D, NASA Johnson Space Center

7. Pharmacological Countermeasures for Space Motion Sickness
   John L. Dornhoffer, M.D. University of Arkansas for Medical Sciences

The first year of the current cycle has been a productive one. Highlights include:

- Adaptation to weightlessness, artifical gravity or Mars gravity could be enchanced if it is possible to induce context specific adaptation of sensory-motor responses. Our Context Specific Adaptation project has shown that context switching of saccadic eye movments is more effective if the context includes a motor component – as opposed to a purely sensory one. This suggests that gravity sensing alone may not be a sufficiently powerful cue for context switching.

- Artificial gravity (AG) remains a potentially important multi-system countermeasure for neuromuscular, bone, cardiovascular and neurovestibular dysfunction in 0-G. However, head movements made in a rotating environment produce nauseogenic vestibular Coriolis illusions and oculomotor reflexes. Limb movements are disturbed by biomechanical Coriolis forces. Our artifical gravity project has found encouraging evidence that adaptation to Coriolis head movements acquired at low RPM generalizes to higher RPM stimuli. Subjects are able to rapidly adapt simple arm movements to compensate for disturbing Coriolis forces, regardless of the rotation rate of the centrifuge.

- Absent gravity, visual landmarks inside spacecraft frequently provide the only cues to static orientation, and establish a "visual vertical". Our Visual Orientation project has shown that visual cues to the vertical can be so compelling, even in 1-g, that most gravitationally supine subjects viewing the interior of a furnished room tilted 90 degrees feel subjectively upright if the visual frame and polarity cues align with their body axis. Motion about the gravitational axis potentiates the illusion. If they are rolled about the gravitational axis, they feel tilted from the vertical, but underestimate the angle. The latter effect may be another manifestation of an "idiotropic" tendency to align the subjective vertical with the body axis, believed responsible for the well known Aubert illusion on Earth, and which causes visual reorientation illusions (VRIs) in astronauts. Such experiments may prove useful in understanding and predicting VRIs in astronauts

- Returning astronauts have difficulty walking around corners and compensating for gait disturbances. Our Gait Ataxia project has shown that compensatory eye, head and body movements stabilize gaze during straight walking, while visual and vestibular orienting mechanisms direct the eyes, head and body to tilts of the resultant of gravitational and centripetal acceleration in space during turning. Our Full Body Gaze Control project has further refined their method for measuring dynamic visual acuity during treadmill walking. Both projects are collaborating on the development of a portable device which moves the foot laterally during the support phase of walking which may prove useful for assessing locomotor deficits in astronauts.

All seven projects are inter-institutional, and several of them involve inter-project collaborations. Several team members are collaborating with JSC clinical colleagues to develop a postflight neurological assessment battery. One of our associate team leads serves on the JSC Critical Path Project Review Panel. We also coordinate our research with

several neurovestibular related projects underway on other NSBRI research teams. Several of the investigators in the separately administered extramural NIH-NIDCD/NSBRI Vestibular Research Program are also actively participating.
The current project portfolio collectively addresses four of the five critical path risks and five of the eight potential research areas. The remaining gap areas which we hope to be able to fill include:

- Vestibular/autonomic/emetic physiology
- Postflight neurovestibular rehabilitation
- Mechanisms of long term spaceflight effects on otolith end organ function and vestibular reflexes.

Between 1997 and 2000, the original 21 investigator Neurovestibular Resarch Team published 14 journal articles, 3 reports, 6 graduate theses, 23 abstracts, with 15 manuscripts accepted or in review. Eleven graduate students and 10 postdoctoral trainees participated. Two students interned at NASA JSC. Working with JSC colleagues, we prepared a summary of Mir program neurovestibular episodes which was distributed to neurovestibular specialists. The team developed a web site to provide project science details, and developed a high school vestibular case study in collaboration with the NSBRI Education and Outreach Team. Lecture materials on vestibular function and disorientation have also been prepared. The team is preparing a special issue of the Journal of Vestibular Research, and is involved in planning the 6th Symposium on the Role of the Vestibular Organs in Space Exploration in October, 2002.

II. Introduction

The NSBRI Neurovestibular Adaptation research program at developing scientifically based countermeasures against the vestibular problems of space flight, and addresses five major space neurovestibular risks which were identified in 1999 by the NASA Critical Path roadmap project (http://criticalpath.jsc.nasa.gov). In priority order, these are:

1. Disorientation and reduced performance on cognitive and physical tasks, including vehicle egress, especially during/after g-level changes (associated with acute spontaneous and head movement contingent vertigo, nystagmus, oscillopsia, saccadic errors, reduced dynamic visual acuity)

2. Impaired neuromuscular coordination strength upon return to positive G leading to increased incidence of falls and injury during emergency egress and escape (gait ataxia, postural instability).

3. Impaired cognitive and/or physical performance due to spatial disorientation, motion sickness symptoms or treatments (including short term memory loss, reaction time changes, drowsiness, fatigue, torpor, irritability, ketosis) as a result of changes in g-level, or use of artificial gravity.

4. Autonomic dysfunction (including cardiovascular, respiratory, gastrointestinal, sleep and mood changes) which may be of vestibular origin.

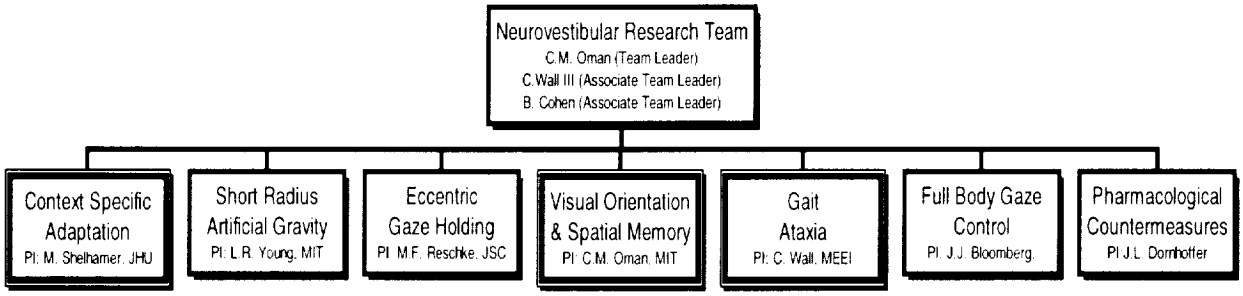
5. Permanent impairment of orientation or balance function due to microgravity or radiation (causing chronic imbalance, gait ataxia, vertigo, eye movement disorders, chronic vestibular insufficiency, poor dynamic visual acuity)

Eight interrelated research areas define the scope of the program:

- Sensory-motor adaptation;
- Artificial gravity;
- Visual (multisensory) orientation, navigation and spatial memory;
- Vestibular/Autonomic/Emetic physiology and drug countermeasures;
- Postflight locomotion and gaze assessment;
- Neurovestibular rehabilitation;
- Effects of weightlessness, immobilization, stress, isolation, and diet on vestibular function; and
- Potential mechanisms for and diagnosis of irreversible neurovestibular changes.

III. Research Program Structure and Design

Since NSBRI's formation in 1997, the neurovestibular research program has been led by Dr. Charles Oman (MIT). Two associate team leaders were subsequently appointed: Drs. Bernard Cohen (Mt. Sinai School of Medicine) and Conrad Wall (Harvard Medical School/Mass Eye and Ear Infirmary). The NSBRI neurovestibular adaptation research team currently consists of 42 investigators from 21 different universities and research laboratories, including NASA's Johnson and Ames research centers. Many have concurrent support from NIH, NSF, and foundations. Twelve investigators have previously flown spaceflight experiments. The current research portfolio of seven projects was selected based on a February, 2000 solicitation (NSBRI 00-01) and independent peer review. Six of the seven are three year projects. Three of the projects (double boxes in the figure below) were initiated in 1997, and competitively renewed in 2000.



The investigators, critical path risks, specific aims, and countermeasure types and countermeasure readiness levels of the seven projects are:

1. Context-Specificity and Other Approaches to Neurovestibular Adaptation. PI: Mark J. Shelhamer, CoIs: Minor, Zee, Angelaki, Zhou, Wu. Institutions: Johns Hopkins U. School of Medicine, Washington U., U. Mississippi Med Ctr. Critical Path Risks: 1 (Disorientation). Research areas: 1 (Adaptation) Countermeasure Types: assessment, prediction, training. Readiness Level: 2

Specific Aims:

- Is torsional eye position a context cue for saccade adaptation ?
- Does a rest interval between stimuli promote adaptive consolidation ?
- Can cyclovergence adaptation provide a countermeasure to ocular torsion changes in parabolic flight ?
- How do pursuit and LVOR deficits correlate in cerebellar lesioned monkeys ?
- How do pursuit and LVOR adaptation transfer across frequencies in humans and monkeys
- Can LVOR adaptation be trained with pursuit stimuli, and how do cerebellar lesions influence adaptation.
- Does head tilt adaptation of saccades and VOR transfer to arm movements in monkeys ?
- What is the best way to induce context specific LVOR adaptation in humans ?
- Does the naso-occipial LVOR also show context specific adaptation ?

2. Neuro-Vestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation
PI: Laurence R. Young. CoIs: Hecht, Oman, Mast, DiZio, Lackner, Paloski, B. Cohen, Dai, M. Cohen, Welch, Stone. Institutions: MIT, Brandeis, NASA-JSC, Mt. Sinai Hospital, NASA-Ames. Critical Path Risks: 1. (Disorientation), 3 (Impaired performance due to AG). Research areas: 2 (Artificial G), 4 (Drug countermeasures). Countermeasure Types: assessment, training, environmental manipulation, drugs. Readiness Level: 4
Specific Aims: Using short and medium radius centrifuges and rotating chairs, to determine:

- How context cues influence VOR, perception and motion sickness adaptation.
- What is the role of sensory-motor (non-vestibular) adaptation to AG ?
- What types of sensory conflict drive adaptation ?
- What are the optimal duty cycles and inter-session intervals ?
- Does body orientation re gravity provide a context cue ?
- In what way does adaptation generalize to different rotating environments?
- How does intermittent training influence the accuracy of head movements ?
- How does promethazine affect adaptation and eye movements in humans and monkeys ?

3. Modification of Eccentric Gaze-Holding, PI: Millard F. Reschke CoIs: Paloski, Kornilova, Wood, Leigh
Institutions: NASA-JSC, IBMP/Moscow, BCM, University Hospitals of Cleveland
Critical Path Risk: 1 (Disorientation and motor performance), 5 (Permanent effects). Research areas: 1 (Sensory-motor adaptation), 8 (Irreversible changes). Countermeasure types: assessment, prediction, training. Countermeasure Readiness Level: 2
Specific Aims:

- Effect of tilt and proprioception on centripetal drift time constant
- How rebound nystagmus provides adaptive compensation.
- How centrifugation influences gaze holding.
- Why adaptation fails in cerebellar patients.
- Whether gaze-holding is impaired immediately following spaceflight.

4. Visual Orientation and Spatial Memory. PI: Charles M. Oman. CoIs: Howard, Shebilske, Taube, Hecht, Harris, Jenkin, Liu, Stuerzlinger. Institutions MIT, York University, Dartmouth Medical School, Wright State University. Critical path risk: 1 (Disorientation). Research area: 3 (Orientation and Spatial Memory). Countermeasure types: assessment, prediction, training, environmental manipulation. . Countermeasure Readiness Level:**5**
Specific aims:

- Human visual orientation. Effects of visual frame, polarity, brightness, motion, and gravireceptor cues on the subjective vertical, eye movements, and limb movements.
- Three dimensional spatial memory and spatial frameworks. Generic and environment specific preflight and onboard virtual reality training methods, interior architectural standards, and escape path countermeasure design and evaluation.

- Neural coding of spatial orientation. How do visual, vestibular, gravireceptive, proprioceptive, and motor pathways drive limbic head direction cells in the rat, as a model for visual reorientation illusions in astronauts.

5. Advanced Techniques to Assess and Counter Gait Ataxia PI: Conrad Wall III, Co-Is:Bloomberg, Oddson, Raphan, Solomon. Institutions: Mass Eye and Ear Infirmary, NASA-JSC, Boston University, Mt. Sinai Hospital, U. Penn. Critical Path Risks: 2 (Impaired neuromuscular coodination), 1 (Disorientation). Research area: 5 (locomotion and gaze). Countermeasure types: assessment, prediction, training, prosthesis. Countermeasure Readiness Level: 5
Specific Aims:

- Quantify body, head, & eye coordination during perturbed straight walking. And also:
- during straight and circular walking on a circular treadmill.
- while ascending/descending stairs.
- while wearing a tactile prosthetic countermeasure.
- assess effect of dynamic balance exercises.

6. Understanding Full-Body Gaze Control During Locomotion
PI: Jacob J. Bloomberg, Jacob, Co-Is:H. Cohen. Institutions: NASA-JSC, Baylor College of Medicine
Critical Path Risks: 1 (Disorientation), 2 (Impaired neuromuscular coodination). Research area: 5 (Locomotion). Countermeasure types: assessment, prediction, training. Countermeasure Readiness Level: 5
Specific Aims: How are eye, head, trunk, and lower limb movements coordinated. Specifically:

1) How is coordination changed following adaptive modification of VOR function?
2) How do these multiple systems reorganize when available degrees of freedom are restricted at the knee and neck?
3) How does adaptive reorganization in locomotor function alter shock-wave transmission to the head and dynamic visual acuity during walking?

7. Pharmacological Countermeasures for Space Motion Sickness. PI: John L. Dornhoffer CoIs: Garcia-Rill, Paule, Van De Heyning. Institutions: U. Arkansas for Medical Sciences, National Center for Toxicological Res., U. Hospital, Antwerp. Critical Path Risks: 3. (Cognitive performance). Research area: 4 (autonomic/drug) Countermeasure types: assessment, prediction, pharmacological. Countermeasure Readiness Level: 5
Specific Aims: (2 year project)

- What are the effects of lorazepam, meclizine, promethazine, and scopolamine on coriolis induced motion sickness symptoms ?
- How do these drugs affect reticular sensory gating (P50 double click auditory evoked potential), time perception, short term memory, and learning ?

Each of the seven projects involves collaborations between investigators at different institutions. In addition, there are several significant inter-project collaborations and coordinations. For example, Drs. Wall and Bloomberg are coordinating their locomotion research, and developing a portable testing platform. Dr. Minor (Shelhamer project) is

assisting Dr. Taube in developing an animal preparation. Drs. Shelhamer, Solomon, and B. Cohen and are working with JSC clinical colleagues on development of a postflight neurological assessment battery. There are also several neurovestibular-related projects underway on other teams: Ray/Cardiovascular; Morin/Chronobiology; Dinges/Psychosocial; Putcha/Smart Medicine.

Five of the seven projects continued or began in October, 2000, and have prepared annual progress reports which are available separately. Funding of Dr. Reschke and Dr. Dornhoffer's projects was delayed for various administrative reasons. They will report on their anniversary dates early in the coming year.

IV. Research Program Accomplishments

Some highlights of our team's current research, and their significance are presented in the Executive Summary. More detailed summaries are available in the individual project annual reports, available separately.

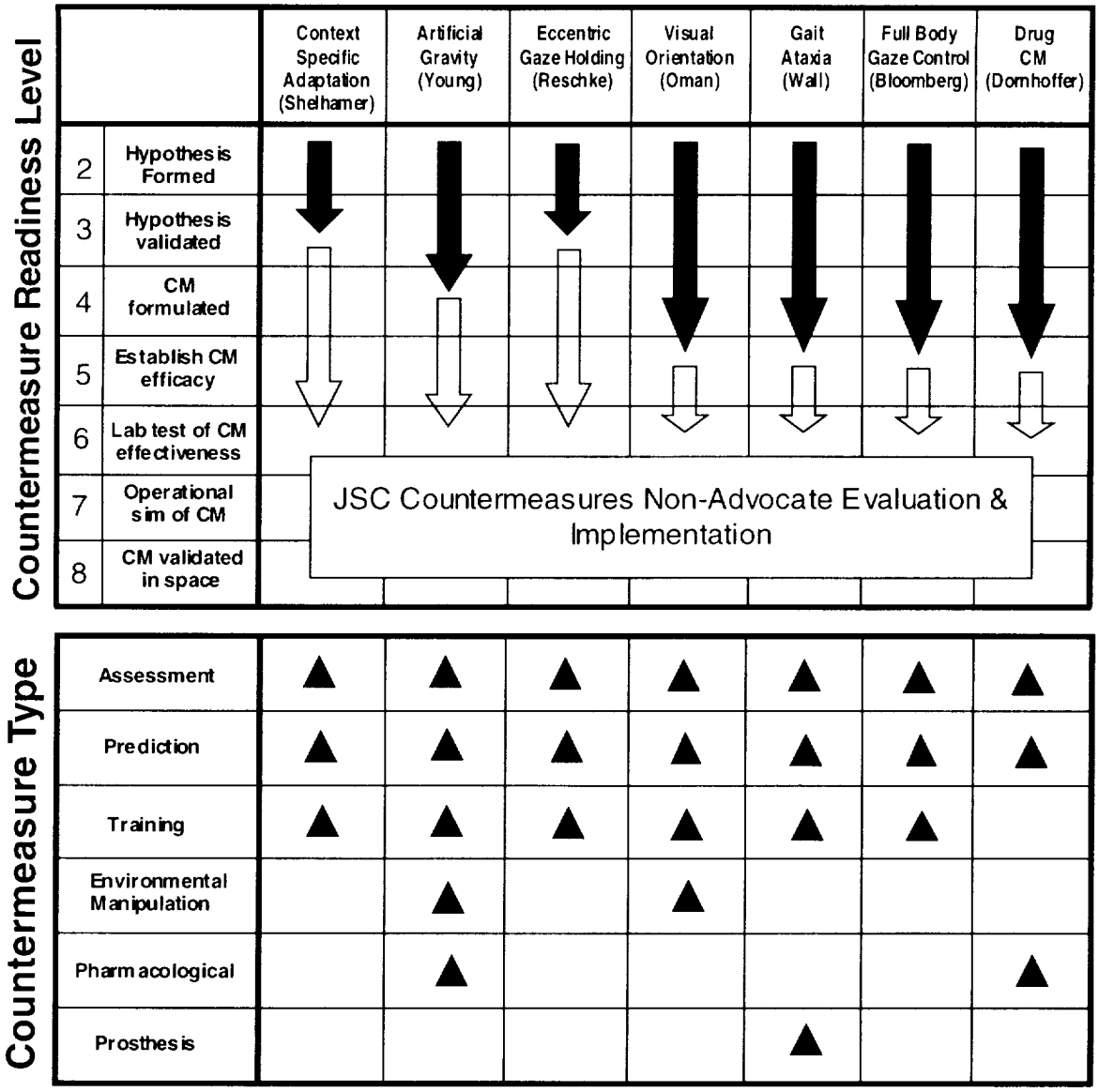
The NSBRI neurovestibular adaptation research program was reviewed in December, 2000 by Dr. Olsen's NASA site visit team. They judged it a well integrated program with strong leadership and an internal review process, evidence of true collaborative research, and clear added value to NASA.

Between 1997 and 2000, the initial 3 projects published 14 journal articles, 3 reports, 6 graduate theses, 23 abstracts, with 15 manuscripts accepted or in review. Eleven graduate students and 10 postdoctoral trainees participated. Two students interned at NASA JSC. Dr. Oman, Mr. J. Richards, Dr. J. Clark (JSC) and Dr. Marshburn (JSC & Smart Med. Team) prepared a detailed retrospective summary of Mir neurovestibular episodes, based on debriefing transcripts, which has been distributed to neurovestibular specialists, along with video clips showing postflight ataxia after shuttle and long durationflights. The team developed a web site to provide project science details (mvl.mit.edu/Neurovestibular/Pages/ home.html), and worked with members of the NSBRI Education and Outreach Team at Harvard Medical School to prepare a vestibular case study for use in high schools ("Cecilia's Story", www.nsbri.org/Education/Cecilia.pdf). Team members haveprepared several lectures on vestibular function on Earth and in weightlessness for an MIT space biomedical engineering course sponsored by NSBRI (www.nsbri.org/Education/2001-2003/NewmanAbstract.html). The team is also preparing a special issue of the Journal of Vestibular Research in 2002.
We are also working with colleagues at NASA and in Portland to plans a 6th Symposium on the Role of the Vestibular Organs in Space Exploration. This symposium will be held in Portland, Oct. 1-3, 2002, as a satellite to the concurrent Barany Society meeting in Seattle, and continues the series initiated at Pensacola in the 1960s and 70s by Dr. A. Graybiel with NASA sponsorship.

V. Future Program Directions

NSBRI research teams normally particpate in steps 2-5 of the countermeasures development process. After the neurovestibular problems are initially discovered and clinically described (step one), our NSBRI research team hypothesizes the physiological mechanisms and/or cognitive processes responsible for neurovestibular risks; performs the neccessary laboratory experiments to validate these hypothesis; formulates potential countermeasures, and then does the ground and/or parabolic flight experiments to establish their efficacy. Most research will be done in ground laboratories or in parabolic flight. We then work with JSC to evaluate whether the countermeasures are practical in an operational environment, sometimes via simulation, and an independent, non-advocate group reviews the case for using the countermeasure. The countermeasure is evaluated on the ground using surrogate subjects, and then ultimately in flight.

The countermeasures development status and type is shown schematically in the figures on the next page for the seven projects in the current portfolio. Filled arrows show the current status of countermeasures development, and the open arrows show the steps required during the 2002-2006 period to complete the preflight portion of the development process.



Short Term Research Strategy

Realistically it is not likely that all of the current countermeasures development efforts will be successful. Those projects which fail to generate countermeasures concepts (Step 3) will be discontinued. When countermeasures concepts prove ineffective (Step 4), the thrust of the project will be redirected. We expect that during the next five years, several candidate countermeasures will enter steps 6 and 7 of the development process. Developing an effective working relationship with our NASA scientific and clinical colleagues is a priority for our team during the next five years. Once countermeasure reliability is established preflight assessment techniques (e.g. locomotion and dynamic acuity tests) is relatively straightforward. Other types of countermeasures (e.g. environmental manipulations such as artificial gravity) can ultimately only be validated via spaceflight. Development of a flight

experiment takes several years, so initial planning of inflight neurovestibular countermeasure validation experiments, and the specification of the required special facilities needs to begin soon.

Artificial gravity (AG) remains a potentially important multi-system countermeasure for neuromuscular, bone, cardiovascular and neurovestibular dysfunction in 0-G. Large radius AG spacecraft systems are likely at least a decade away, but short radius (2-3 m) systems could be developed now which fit inside Shuttle or an ISS module. As a neurovestibular countermeasure, AG is a double-edged sword: it probably can be used to pre-adapt crewmembers for return to Earth or to 3/8 (Martian) gravity, but if crewmembers move their heads out of the plane of rotation, the resulting vestibular Coriolis stimulus potentially will produce disorientation and motion sickness. Establishing the values of AG system radius and RPM, and the duration/repetition rate of AG sessions which are effective for neuromuscular, bone, cardiovascular and neurovestibular therapies should remain both an Institute and Neurovestibular Team priority.

Another goal is to obtain support for at least one new research project initiative in each of our three gap areas: The current project portfolio collectively addresses four of the five critical path risks and five of the eight potential research areas. The significant gap areas include:

- Vestibular/autonomic/emetic physiology
- Postflight neurovestibular rehabilitation
- Mechanisms of long term spaceflight effects on otolith end organ function and reflexes.

The vestibular/autonomic/emetic area is challenging because of its interdisciplinary aspects. The vestibular system thought to play a role in cardiovascular, respiratory, and circadian regulation, but relatively little is yet known about mechanism or functional significance. The physiological basis of the sensory conflict theory and the linkage to emetic centers remains unknown. Existing anti-motion sickness drugs have been empirically discovered. The emetic research community is a small one, but a breakthrough in vestibular/autonomic/emetic physiology could have important implications for development of targeted pharmacologic countermeasures. Recent research on vestibulo-autonomic reflexes should remain a priority. NASA and NIH have not provided major funding for vestibular-emetic research since the early eighties, prior to the development of modern molecular neuroscience and functional imaging techniques. A coordinated NASA/NIH research initiative in this basic research area could yield dramatically important results. Given the almost universal human susceptibility to motion sickness, validated concepts for targeted pharmacologic approaches to motion sickness would likely be of considerable interest to our Industry Forum partners.

Neurovestibular rehabilitation is also a challenging area also because most of the clinical techniques used today are empirical, guided by qualitative theories about the relative advantages of adaptation vs. sensory substitution. Hypothesis-driven research techniques and quantitative assessment techniques are only slowly entering the field. Astronauts differ physiologically from vestibular patients in several important ways. There is no widely

accepted 1-G research analog for the space environment which can be used for neurovestibular rehabilitation studies in a manner analogous to the way bed rest or leg suspension in neuromuscular rehabilitation studies. Further, it is not clear from clinical experience how to optimize the readaptation and functional recovery of returning flight crews, or how neurovestibular, neuromuscular, bone, and cardiovascular rehabilitation techniques may interact.

Although all of the neurovestibular effects of spaceflight encountered to date are apparently reversible, it is now clear that in general the longer a person is in 0-G, the longer lasting the postflight aftereffects, and only a very few people (all of them cosmonauts) have experienced weightlessness for more than six months. We cannot confidently predict the potential long term effects of weightlessness (including systemic changes in body calcium for example) on the vestibular end organs, on the otolith-ocular and otolith-spinal reflexes, and know if they are reversible. We do not yet have a reliable, sensitive method for assessing the function of the human otolith organs, analogous to clinical audiometric testing.

Long Term Research Strategy

If and when the current NASA ISS budget problems are resolved, access to space flight and opportunities for sophisticated experimentation will eventually increase. Several neurovestibular countermeasures concepts will presumably have emerged from JSC evaluation, and be ready for flight testing in astronauts. Also, there are a variety of basic human and animal neurovestibular experiments (associated with the hypothesis testing phase of countermeasures development levels 3) that will require access to ISS and Shuttle crewmembers preflight, inflight and postflight for large n longitudinal studies. Ground facilities needed include a neurovestibular testing laboratory for pre and postflight experiments, equipped with angular, linear, and artificial gravity stimulus devices, 3D eye movmement, whole body kinematic, otolith, posture and locomotion testing and immersive VR display equipment. A need for an immersive VR orientation training facility (perhaps an adjunct to the existing EVA training VR facility) is also forseen. Orbital research facilities include a short or medium radius human centrifuge for both neurovestibular adaptation research and countermeasure use. Also needed will be second generation eye, head, and body movement research equipment with capabilities beyond those currently aboard the ISS-Human Research Facility.

VI. Publications of the Neurovestibular Adaptation Team (1997-2001)

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Journal Articles Accepted

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I. EXECUTIVE SUMMARY

Optimal human performance during space exploration requires the maintenance of all physiological systems such as cardiovascular capacity, bone mineral density, and skeletal muscle function. Adequate nutrition and physical fitness affect all physiological functions and are dependent in part on each other. The critical issues for nutrition are: (1) counteracting the observed anorexia of space flight; (2) determining nutrient needs to meet modified requirements due to space flight stressors including microgravity; and (3) developing new strategies including use of functional foods, supplements, and timing of food intake relative to specific activities that will optimize human performance. Equally important is remaining physically fit. Critical issues for physical fitness include: (1) development of appropriate aerobic and resistive exercises (mode, frequency, duration, intensity) and the appropriate balance for each to maintain aerobic capacity and muscle performance (as measured by strength and endurance); (2) optimizing the appropriate timing of exercise programs with respect to food intake and other activities (e.g. extra vehicular activity; EVA); (3) development of the hardware to most efficiently implement the exercise countermeasures.

Since physical activity will, in part, determine nutrient needs, and the optimization of nutrient delivery will in part depend upon blood flow and muscle mass (which are affected by physical activity) these two disciplines need to be considered together. Examples of relevant risks that may be ameliorated by nutrition and physical activity interventions are:

- Reduced cardiovascular capacity
- Loss of bone mineral density
- Diminution of skeletal muscle function
- Depressed immune response
- Radiation enhanced development of cancer
- Decrease in cognitive function
- Alterations in sleep patterns

Psychosocial factors

The nutrition and physical fitness program is new, becoming operational in 2001. It presently consists of three nutrition countermeasure projects (Lupton, Wolfe, and Tobin) and an "in flight" physical fitness project (Schneider) which has recently been approved for feasibility studies. Briefly, *Nutritional Countermeasures to Radiation Exposure*, JR Lupton, PI, Texas A&M University, is testing the hypothesis that a specific diet intervention in rats should protect against radiation-enhanced colon cancer by targeting DNA damaged cells for apoptotic removal. This project also has a noninvasive component of monitoring changes in gene expression over time as a result of radiation and carcinogen exposure. If validated in rats, the diets and techniques can be modified for future studies in humans. *Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress*, RR Wolfe, PI, University of Texas Medical Branch at Galveston, will test an amino acid supplement designed to ameliorate muscle wasting induced by stress-and-microgravity-induced depression of protein synthesis in a bed rest study. *Nutritional Modulation of Pancreatic Endocrine Function in Microgravity,* BW Tobin, PI, Mercer University School of Medicine, will determine amino acid countermeasure effects on endocrine function of human pancreatic islets of Langerhans with the goal to optimizing insulin synthesis and secretion under microgravity conditions. Finally, *Treadmill Exercise as a Countermeasure for Microgravity Deconditioning*, SM Schneider, PI, NASA, Johnson Space Center will evaluate the effectiveness of two treadmill countermeasures to maintain aerobic capacity, leg strength, and prevent increases in bone resorption and muscle atrophy.

The current strengths of the research program center on its focused effort to ameliorate the muscle wasting and loss of strength observed in space flight. The cornerstone project is the Wolfe bed rest study and the science of this study has been optimized for maximal effect. The Tobin project addresses mechanisms by which insulin secretion and muscle cell response to insulin may be compromised during space flight. One of its interventions will be the same amino acid supplement used in the Wolfe bed rest study. Additionally, this supplement will also be used by the muscle team in a rat project of Ken Baldwin's. A number of important ancillary studies are now underway that will use material (blood, muscle biopsies, urine, saliva) from the Wolfe bed rest study. For example, Helen Lane, NASA JSC, is leading the NMR spectroscopy project for determination of oxidative capacity of calf muscle before, during, and after exercise. Scott M. Smith, (Also a collaborator on the Tobin project) has a supplemental project to the Wolfe project which is designed to measure markers of bone and calcium metabolism in the bed rest subjects. Raymond P. Stowe, University of Texas Medical Branch Galveston, TX is addressing changes in antiviral immunity during bed rest. These measurements are also being performed on the Shuttle and ISS crewmembers through his NASA grant. These include measurement of viral load in blood, urine, and saliva samples using molecular methods. Peter N. Uchakin, Mercer University School of Medicine, Macon, Georgia (Also a lead investigator on the Tobin project) is investigating the effect of bed rest and corticosteroid treatment on the secretion of pro- and anti-inflammatory cytokines. Two other important additions to the bed rest study are pending.

Future plans for the Nutrition and Physical Fitness program are to continue to capitalize on the existing projects by maximizing their effectiveness with ancillary projects and adding synergy projects with other teams. Since this is currently a small program there are, by definition, several important gaps. The most noticeable is an integrated project with both nutrition and physical fitness as dual interventions. Our top priority is to have a physical fitness study which complements the Wolfe bed rest study. Another major area which needs to be addressed is the anorexia of space flight. We plan to actively solicit a study in this area. Finally, data are needed on the timing of food intake and exercise with respect to each other and with the accomplishment of other tasks. This is also a high priority for this team.

In summary, Nutrition and Physical Fitness is a new, currently small program which is working to fully integrate its existing projects, expand by addition of small projects which capitalize on the Wolfe bed rest study, and is poised to solicit new proposals which will fully integrate nutrition with physical fitness. Optimal diet and physical activity protocols for space flight will impact every aspect of astronaut health and performance.

II. INTRODUCTION

Optimal human performance during space exploration requires the maintenance of all physiological systems such as cardiovascular capacity, bone mineral density, and skeletal muscle function. Adequate nutrition and physical fitness affect all physiological functions and are dependent in part on each other. The critical issues for nutrition are: (1) counteracting the observed anorexia of space flight; (2) determining nutrient needs to meet modified requirements due to space flight stressors including microgravity; and (3) developing new strategies including use of functional foods, supplements, and timing of food intake relative to specific activities that will optimize human performance. Equally important is remaining physically fit. Critical issues for physical fitness include: (1) development of appropriate aerobic and resistive exercises (mode, frequency, duration, intensity) and the appropriate balance for each to maintain aerobic capacity and muscle performance (as measured by strength and endurance); (2) optimizing the appropriate timing of exercise programs with respect to food intake and other activities (e.g. extra vehicular activity; EVA); (3) development of the hardware to most efficiently implement the exercise countermeasures.

Since physical activity will, in part, determine nutrient needs, and the optimization of nutrient delivery will in part depend upon blood flow and muscle mass (which are affected by physical activity) these two disciplines need to be considered together. Examples of relevant risks that may be ameliorated by nutrition and physical activity interventions are:

- Reduced cardiovascular capacity
- Loss of bone mineral density
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- Depressed immune response
- Radiation enhanced development of cancer
- Decrease in cognitive function
- Alterations in sleep patterns
- Psychosocial factors

III. RESEARCH PROGRAM STRUCTURE & DESIGN

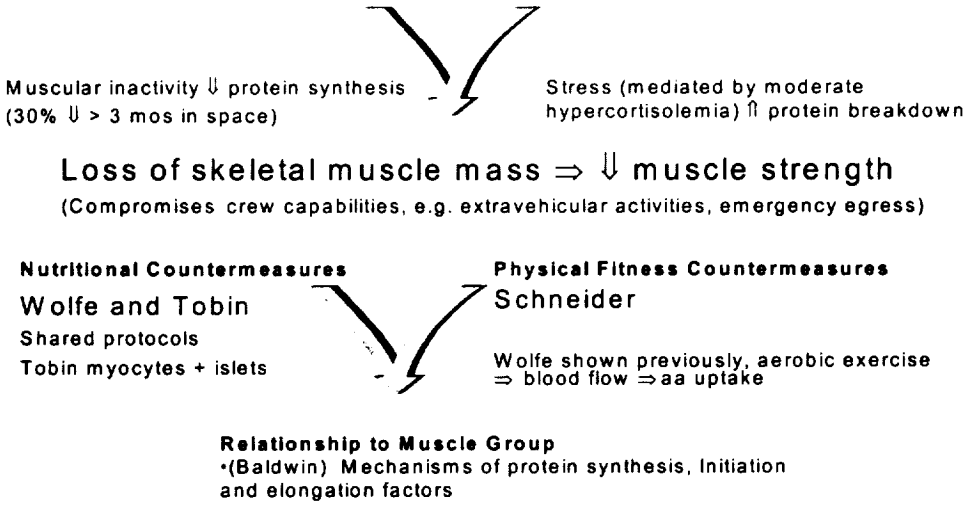
The nutrition and physical fitness program is new, and became operational in 2001. It presently consists of three nutrition countermeasure projects (Lupton, Wolfe, and Tobin) and an "in flight" physical fitness project (Schneider) which has not yet been funded. Figure 1 shows the critical path problem that each project is designed to address (radiation effects; muscle wasting, and muscle strength/aerobic capacity) and the countermeasure readiness level of each project. The last name of the principal investigator is followed by the type of intervention (nutrition or physical fitness).

Briefly, *Nutritional Countermeasures to Radiation Exposure*, JR Lupton, PI, Texas A&M University, is testing the hypothesis that a specific diet intervention in rats should protect against radiation-enhanced colon cancer by targeting DNA damaged cells for apoptotic removal. This project also has a noninvasive component of monitoring changes in gene expression over time as a result of radiation and carcinogen exposure using microarray technology combined with real time PCR. If validated in rats, the diets and techniques can be modified for future studies in humans. *Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress,* R. R. Wolfe, PI, University of Texas Medical Branch at Galveston, will test an amino acid supplement designed to ameliorate muscle wasting induced by stress-and- microgravity-induced depression of protein synthesis in a bed rest study. *Nutritional Modulation of Pancreatic Endocrine Function in Microgravity,* B. W. Tobin, PI, Mercer University School of Medicine, will determine amino acid countermeasure effects on endocrine function of human pancreatic islets of Langerhans with the goal to optimizing insulin synthesis and secretion under microgravity conditions. Finally, *Treadmill Exercise as a Countermeasure for Microgravity Deconditioning*, S. M. Schneider, PI, NASA, Johnson Space Center will evaluate the effectiveness of two treadmill countermeasures to maintain aerobic capacity, leg strength, prevent increases in bone resorption and muscle atrophy markers.

Figure 1. Overview of the Nutrition and Physical Fitness Current Program

Countermeasure Readiness Level			Radiation Effects	Muscle Wasting	Muscle Strength/Aerobic Capacity
	2	Hypothesis formed		Tobin, BW Nutrition	
	3	Validated hypothesis			
	4	Formulate CM			
	5	Establish CM efficacy	Lupton, JR Nutrition		
	6	Lab test of CM efficacy		Wolfe, RR Nutrition	
	7	Operational simulation			Schneider, SM Physical Fitness
	8	CM validated in space			

Figure 2. Integration of three projects



Three of the four projects (Wolfe, Tobin and Schneider) have been modified to use common protocols where applicable. These common approaches are shown in Figure 2. As shown in Figure 2, the combination of muscular inactivity and stress during space flight results in a loss of skeletal muscle mass which leads to decreased muscle strength which may compromise crew capabilities. The Wolfe bed rest study will test if an amino acid supplement can ameliorate these negative effects by increasing protein synthesis. The Tobin project, which addresses both insulin section and myocyte response in an *in vitro* model, will use the same amino acid supplement as Wolfe in one of its test cases. Collaboration will be enhanced with the muscle group (led by Ken Baldwin) due to a similar intervention in a rat study. Although not tested in the present studies, Wolfe has shown previously that exercise, which enhances blood flow to muscle, will enhance the uptake of amino acids in muscle. Thus the Schneider project (if eventually combined with a nutrition intervention) should enhance the nutrition intervention.

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

During the past year excellent progress has been made on each of the 3 funded projects and also on the unfunded "in flight" physical fitness project. Specifically:

Nutritional Countermeasures to Radiation Exposure, JR Lupton, PI, Texas A&M University. Risks to personnel in space from naturally occurring radiations are one of the most serious limitations to human space missions (BEIR V, 1990; BEIR VII, 1998; National Research Council, Washington, DC) and thus considered to be a Tier 1 problem for NASA. One of the most important adverse effects of radiation exposure is increased risk for cancer, and colon cancer is the second leading cause of death from cancer in the US today, striking men and women almost equally. Fortunately, of all the cancers, colon cancer has been shown to be the most responsive to diet. Colon cells are arranged in patterns called crypts (Figure 3) in which cells are born towards the bottom of the crypts and daughter cells may migrate up the side of the crypt eventually being exfoliated into the fecal stream. However, radiation may induced oxidative damage to DNA in these cells, whereas a chemical carcinogen may induce methylation damage to DNA. Once damaged, DNA can be repaired by DNA repair enzymes, or the cell can be removed by programmed cell death (apoptosis), if neither of these events occurs, the cell may go on to become a cancer cell (Figure 4).

Figure 3. Diagram of two colon crypts

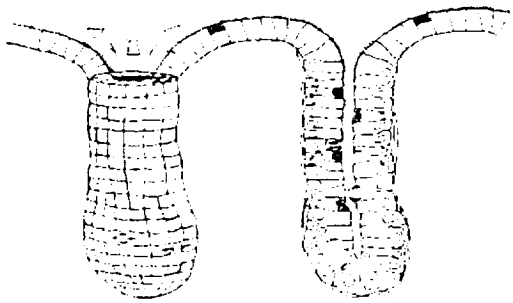
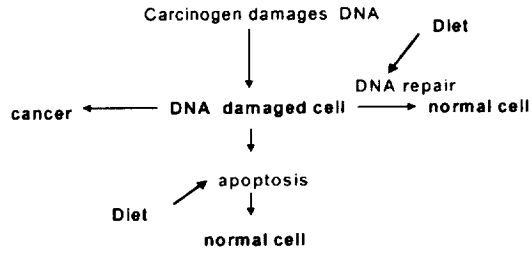


Figure 4. Diet effects on the initiation of cancer



Lupton et al. have shown that a combination of fish oil (high in n-3 fatty acids) and a fermentable fiber (pectin) can upregulate apoptotic removal of DNA damaged cells. The purpose of the NSBRI study is to determine if radiation exposure is promotive of chemically induced colon cancer, thus the experimental design is as shown in Figure 4. The first experiment was initiated in January 2001 in which rats were irradiated with 1 GY heavy iron at Brookhaven National Laboratory and then injected with the colon specific carcinogen azoxymethane (AOM). An equal number of rats were injected with AOM but not exposed to radiation. Results clearly show that there is a promotive effect of the radiation with respect to colon cancer development. Colon cancer proceeds in stages and an intermediate stage is aberrant crypt development. Figure 5 shows a segment of rat colon with several foci of aberrant crypts visible. Figure 6 shows one of the results from this initial study in that the rats that were irradiated and injected with AOM

had significantly greater numbers of aberrant crypts than did those that were injected with AOM but not irradiated (P=0.0009). In addition, there were a greater number of aberrant crypts/focus (considered more predictive of colon tumors) in the irradiated group compared to the non-irradiated group (P=0.0084).

Figure 5. Aberrant crypts in rat colon.

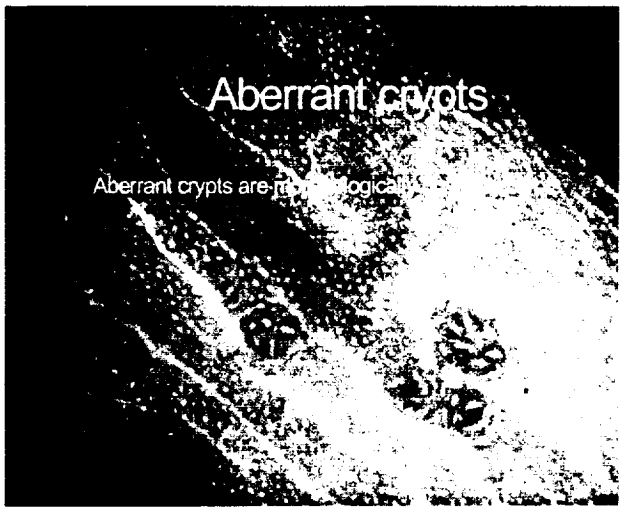
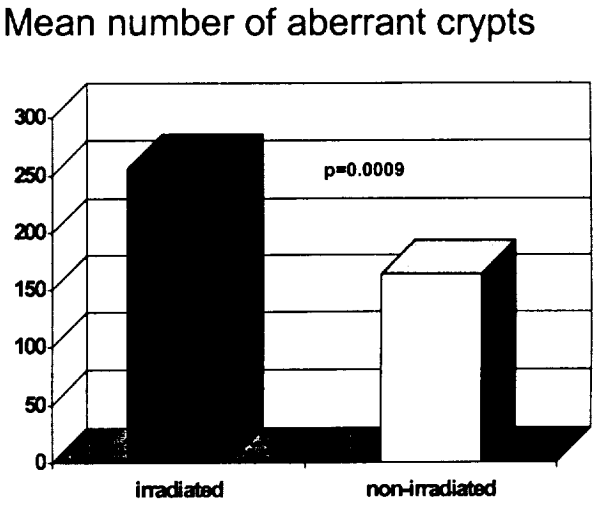


Figure 6. Aberrant crypts in irradiated vs non-irradiated rats



Currently mRNA from the same rats in this experiment is being subjected to both microarray and real time PCR to determine which genes were up or down regulated by the radiation and carcinogen treatments. Also, one half of the control rats for the large NSBRI supported study (Figure 7 shows the overall experimental design) are currently in house, receiving experimental diets.

Figure 7. Experimental Design for NSBRI study

- 560 SpragueDawley rats, 2 x 2 x 2 factorial design
  - Corn oil, fish oil
  - Pectin, cellulose
  - +/- radiation (1 Gy heavy iron)
  - All + AOM (Colon specific carcinogen)
- Specific Aims
  - Effect on initiation, aberrant crypts, tumors
  - mRNA from fecal material predicts the outcomes

In February 2002, half of the rats that will receive radiation treatment will be irradiated at Brookhaven National Laboratory. To summarize, this project is proceeding on schedule, and the initial results confirm the hypothesis that radiation exposure on top of chemical exposure, enhances colon tumor development.

Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress, R. R. Wolfe, PI, University of Texas Medical Branch at Galveston. Figure 8 shows the overall protocol for each subject in the Wolfe bed rest study. The purpose of the study is to determine if an amino acid supplement

can ameliorate the negative effects of bed rest (a proxy for microgravity) on muscle protein synthesis. The composition of the amino acid supplement is shown in Figure 9.

Figure 8. Time Line for each subject in the Wolfe bed rest study

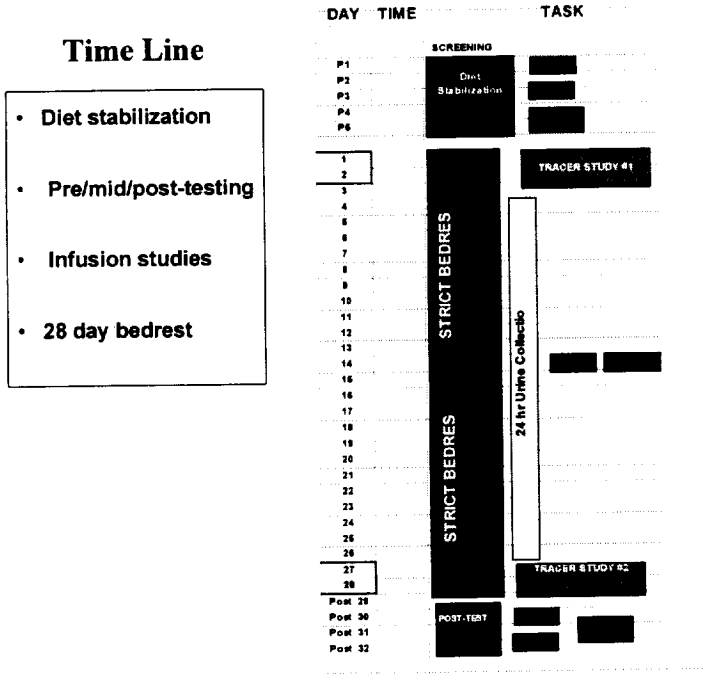
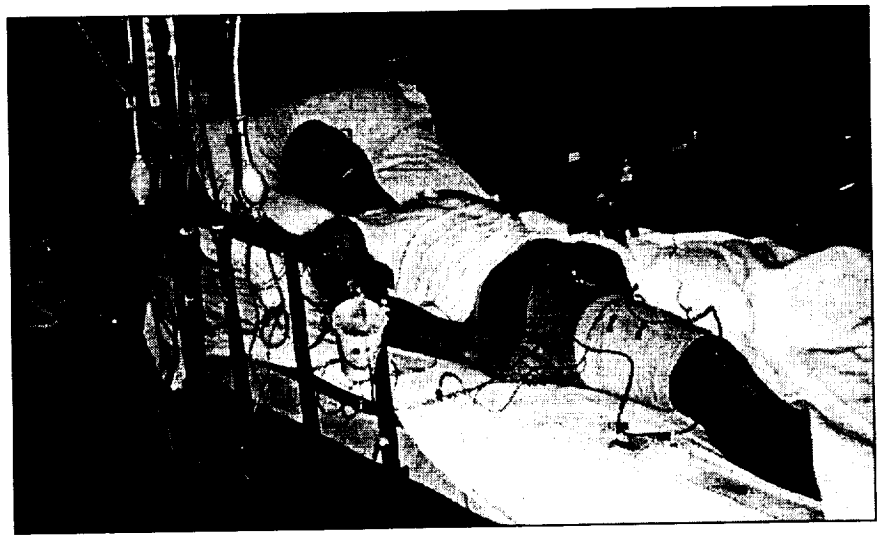


Figure 9. Composition of the supplement

Amino Acid	Amount (g)
L-Histidine	1.70
Isoleucine	1.00
Leucine	3.10
Lysine	2.60
Methionine	0.50
Phenylalanine	2.00
Threonine	2.20
Valine	2.10
Tryptophan	0.60
Glycine	0.70

This research project is proceeding ahead of schedule. To date, two subjects have completed the study, two are currently enrolled and others are scheduled to begin in early 2002. The first subject is shown below in Figure 10.

Figure 10. Subject in Wolfe bed rest study.



Most importantly we have chosen the bed rest study as the cornerstone of our program, and as such have requested and received funding for a number of important additional projects which use samples from this study. The following are descriptions of "add ons" to the Wolfe bed rest study.

1. Helen Lane, Ph.D.
NASA Johnson Space Center
Houston, TX
Title: Response of oxidative capacity in skeletal muscle to prolonged bed rest.
The NSBRI project evaluates a nutritional countermeasure, protein/amino acid supplementation, on leg muscle performance after a month of bed rest. As part of this overall project, Dr. Helen Lane is leading the NMR spectroscopy for determination of oxidative capacity of calf muscle before, during, and after exercise. Phosphorus NMR spectroscopy is a technique to continuously follow high energy metabolism in a localized tissue by determination, using P31, of levels of inorganic phosphate, phosphocreatine, and ATP along with calculation of muscle pH. This technique also allows for determination of creatine kinase reaction at rest and during exercise in skeletal muscles. Creatine kinase catalyses the reversible transfer of high-energy phosphate groups of phosphocreatine to and from adenosine diphosphate. Muscle countermeasures such as training programs changed these parameters both in the level of phosphocreatine as well as the time to recovery of phosphocreatine after fatiguing exercise. The plan is several fold: First, we need to develop the hardware (exercise device, interface for quantification, and other analytical efforts such as precision and reproducibility) and the software interface to determine the oxidative capacity of calf muscle with phosphorus NMR spectroscopy. Next, we will examine the difference in these parameters before and after 28d of bed rest with and without the countermeasure. Our hypothesis is that in subjects without the countermeasure phosphocreatine will be lower at rest and decrease more during exercise than those subjects with a countermeasure. Furthermore, the regeneration of phosphocreatine will be faster in those who have the countermeasure. These measures will be correlated with muscle function (strength measures) and single fiber analyses performed by Bob Fitts (as a separate add-on project). Thus, we will not only determine the effect of bed rest on muscle oxidative capacity, but the relation of oxidative capacity to muscle function. This project is being conducted at no additional cost to NSBRI.

2. Scott M. Smith, Ph.D. **(Also a collaborator on the Tobin project)**
Research Nutritionist
NASA Johnson Space Center
Houston, TX
Title: Markers of bone and calcium metabolism
Dr. Scott M. Smith's supplement to the Wolfe project is designed to measure markers of bone and calcium metabolism in the bed rest subjects. These include serum osteocalcin and bone-specific alkaline phosphatase, and urinary collagen crosslinks. Osteocalcin and Bone-Specific Alkaline Phosphatase are both bone formation markers. The collagen crosslinks are bone resorption markers, and represent a family of compounds which include n-telopeptide, pyridinoline, and deoxypyridinoline, among others. Their analyses will tell us if the Wolfe countermeasure, obviously aimed at muscle tissue, has any impact on the bone side of the musculoskeletal system. At present Dr. Smith has approximately 1/3 of the samples, but is awaiting analysis until after all samples are collected, to analyze these in one batch.

3. Raymond P. Stowe
Assistant Professor (Research), Pathology
University of Texas Medical Branch
Galveston, TX
Title: Changes in antiviral immunity during bed rest
Dr. Raymond P. Stowe's project measures changes in antiviral immunity. These measurements are also being performed on the Shuttle and ISS crewmembers through his NASA grant (Epstein-Barr virus flight grant). These include measurement of viral load in blood, urine, and saliva samples using molecular methods. In addition, he will measure virus-specific immunity by measuring anti-viral antibody titers and antigen (virus)-specific T-cells to correlate with viral load.

4. Brian W. Tobin, Ph.D.
Associate Professor
Mercer University School of Medicine
Macon, GA
Title: Blood amino acid levels from Wolfe project for use in Tobin project
In order to test the same protocols in several model systems, the amino acid blood levels produced by the Wolfe supplement have been quantitated and will be used as one of the interventions in the Tobin protocol. This is at no added expense to NSBRI.

5. Peter N. Uchakin, Ph.D. *(Also a lead investigator on the Tobin project)*
Lead Research Scientist
Division of Basic Medical Sciences
Mercer University School of Medicine
Macon, Georgia
Title: The effect of bed rest and corticosteroid treatment on the secretion of pro- and anti-inflammatory cytokines
Dr. Peter N. Uchakin is investigating the effects of bed-rest and corticosteroid treatment on the secretion of pro- and anti-inflammatory cytokines such as IL-1, IL-6 and IL-10. In addition, Dr. Uchakin will assess immunocyte distribution in whole blood.

6. Robert Fitts, Ph.D. (Pending)
Marquette University
Milwaukee, WI
Title: Single muscle fiber function in response to bed rest and nutritional intervention.
Investigation of the contractile properties and force of single muscle fibers of the soleus (predominantly Type 1) and vastus lateralis (predominantly mixed) in response to prolonged bed rest with and without the nutritional intervention.

7. T.P. Stein, Ph.D. (Pending)
Professor of Surgery
UMDNJ-SOM
Stratford, NJ
Title: Markers of oxidative stress
Oxidative damage from free radicals to DNA and lipids has been implicated in the etiology of a wide variety of chronic diseases and acute pathological states. Dr. Stein and his colleagues had the opportunity to obtain data on the question of whether space flight has any effect on the

oxidative status of astronauts. They measured the urinary excretion of 8-iso-PGF2a and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OH dG) on 6 subjects (2 US astronauts and 4 Russian cosmonauts) before, during and after long duration space flight on the Russian space station MIR. The urinary excretion of the isoprostane 8-iso-PGF2a and 8-OH dG are markers for oxidative damage to lipids and DNA respectively.

There was a trend towards an increase in 8-OH dG excretion in flight. Both 8-iso-PGF2a and 8-OH dG excretion were double post flight indicating that oxidative stress was double. The increase persisted for the two-week observation period. The level was akin to smoking a pack of cigarettes a day. The increased oxidative stress damage post flight most likely reflects impaired endogenous anti-oxidant defenses. (Subjects took vitamin capsules throughout the study period). The down regulation of protein metabolism that occurred on MIR could cause some loss of protein-based antioxidant systems. The likely causes of the compromised protein metabolism are: (i) the combination of reductive remodeling in response to the loss of load and the chronic under-nutrition that occurred on MIR and (ii) competition post flight for amino acids between synthesis of defense related proteins and repleting muscle leading to sub-optimal availability of host defense mechanisms.

The problem being addressed is how to study this problem on the ground. The need is for an acceptable ground based model. At present there is no such model. The objectives of this supplementary project are therefore to determine whether cortisol administration to bed rest subjects reproduces the flight situation. Given a model, counter-measures can be explored. Thus the hypothesis being tested is that bed rest plus cortisol reproduces the flight result. Dr. Stein will use the urinary excretion of the isoprostane 8-iso-PGF2a and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OH dG) to assess oxidative damage to lipids and DNA respectively in the urines from Dr. Wolfe's study.

In summary, the Wolfe/Ferrando bed rest study is proceeding ahead of schedule and all aspects of the study are on target. Important, we have chosen this study as the cornerstone of our program and have acquired additional funding for "add on projects" which will capitalize on this well controlled study. We are very optimistic that definitive data will be obtained from this study which can be used in the very near future to enhance long term travel in space. Examples of answers that will be obtained are: whether or not an amino acid supplement ameliorates the decline in protein synthesis seen in space; if supplements interfere with protein synthesis from meals; if cortisol infusion in addition to bed rest is a better model for what actually occurs in space; if different muscle fiber types are equally affected by bed rest and cortisol; if this nutritional intervention can affect bone loss and muscle strength, among many other questions.

Nutritional Modulation of Pancreatic Endocrine Function in Microgravity, B. W. Tobin, PI, Mercer University School of Medicine, is determining amino acid countermeasure effects on endocrine function of human pancreatic islets of Langerhans with the goal to optimizing insulin synthesis and secretion under microgravity conditions. In addition, he has modified his protocol to include addressing these effects on human myocytes isolated from the same cadavers as the pancreatic islet cells. Figure 11 shows the model used by Tobin et al. Islets or myocytes are cultured in "microgravity" or static plate controls. The HARV rotates at 10 RPM, and islets are eliptical orbit-suspended in media. Measurements are made of medium glucose, lactate, hormones and amino acids. Specific aims of this project are (1) to assess the effect of a microgravity model system on basal amino acid requirements and endocrine secretory function in human islets of Langerhands and (2) to determine human islet endocrine function while testing

amino acid countermeasures. Results from the first set of studies illustrate (1) decreased glucose utilization, (2) enhanced insulin secretion, (3) increased utilization of cysteine, and (4) increased production of ornithine, presumably from arginine. Tobin is now beginning the initial testing of countermeasures, to normalize the hormonal secretory profile. This will consist of two amino acid mixtures: (1) the Wolfe mixture and (2) the Tobin formula which is higher in arginine and cysteine as well as other amino acids utilized at high rates. His experimental design for the studies is shown in Figure 12.

Figure 11. HARV Microgravity Model

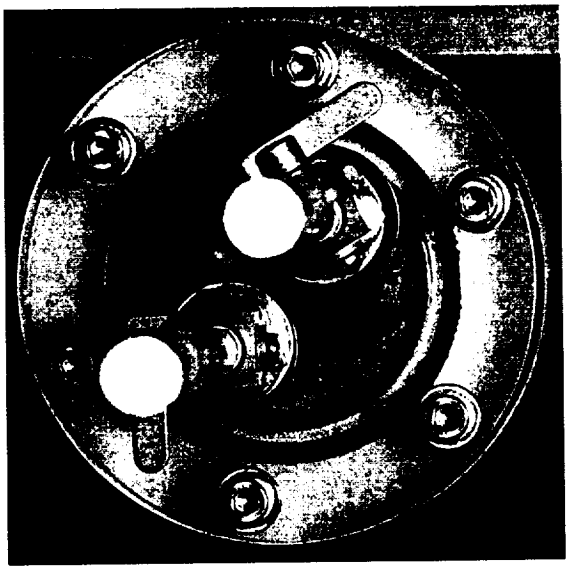
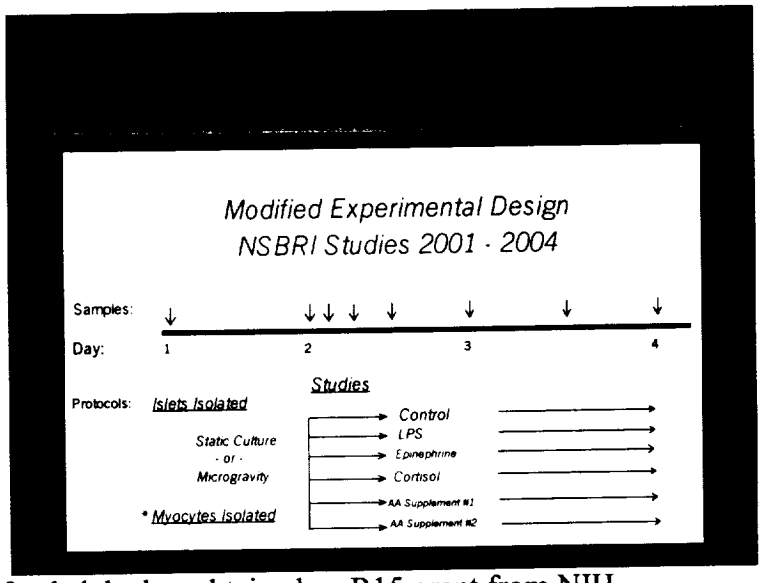


Figure 12. Tobin Experimental Design



In the eight months that Dr. Tobin has been funded, he has obtained an R15 grant from NIH which allowed him to concentrate more on countermeasures with the NSBRI project. He has published two abstracts and has been interviewed on international television on this NSBRI project and on National Public Radio. He is actively collaborating with Wolfe/Ferrando and in fact Peter Uchakin (an investigator on the Tobin project) is now working with Wolfe/Ferrando on site (see the Wolfe/Ferrando summary above).

Treadmill Exercise as a Countermeasure for Microgravity Deconditioning, S. M. Schneider, PI, NASA, Johnson Space Center. This "in flight" study has just been approved for a feasibility phase. Dr. Schneider is an active member of the Nutrition, Physical Fitness and Rehabilitation team and is instrumental in stressing the importance of the combination of physical fitness and nutrition. Her project is very practical and focuses on the amount of time and repetition, etc. that are required to maintain aerobic capacity and to aid in leg muscle strength, prevent increases in bone resorption and ameliorate markers of muscle atrophy. Figure13 shows the set up for the treadmill. In collaboration with Dr. Peter Cavanagh, Penn State University, they have explored harness issues and ground reaction forces in simulated treadmill locomotion at different percentages of body weight. Through appropriate use of the harness, the exercise on the treadmill should not only improve aerobic capacity but also help to maintain muscle strength which is an overall goal of the Nutrition and Physical Fitness Program.

Figure 13. Treadmill used on the International Space Station

V. FUTURE PROGRAM DIRECTIONS

A. Five-year Research Strategy (2002 – 2006)
During this time period research will advance by four mechanisms:

(1) *Completion of existing projects.* The three existing nutrition intervention projects will be completed during this time frame. Our anticipation is that we will have an amino acid supplement ready for testing in space at the end of this time period. It will have been validated in a bed rest study with and without an exercise protocol (See below under Addition of projects). We will also have increased knowledge on the relative contribution of decreased insulin secretion and decreased insulin sensitivity to problems of glucose utilization during space flight. Our nutritional intervention to decrease radiation-enhanced colon cancer will have been validated in rats and ready for testing in humans. If "in flight" projects do occur during this time period, the Schneider protocol for treadmill exercise in space will have been validated.

(2) *Enhancement of existing projects through "add ons" and new solicitations.*
The existing projects will form a research core which will be supplemented by two processes ("add ons" and new solicitations). The plan is to "add on" small projects to two of the existing three nutrition projects as follows:

- Lupton, *nutritional countermeasures to radiation exposure.*

Make tissue from radiation and carcinogen treated rats available to other researchers for complementary studies. Such studies include but are not limited to: effect of the diets on brain; pancreas to Tobin laboratory for analysis; supplemental project on immune response.

- Wolfe, *amino acid supplement as a countermeasure to muscle wasting.*

As noted above, we have already added on a number of important projects to this bed rest study. Two additional projects are pending and our plan is to obtain the necessary funds to do this. These two pending projects are measurements of muscle fiber composition (Robert Fitts) and the studies of Peter Stein on oxidative damage. In addition to the "add ons" a new solicitation, focused on exercise, with the same diet intervention as the Wolfe bed rest study is proposed. The overall goal of the research is to provide a scientific basis for the design and implementation of space based exercise countermeasures. Studies will be designed to determine the optimal as well as minimal prescription for frequency, duration, and intensity of the exercise countermeasure to obtain the most time efficient method to maintain muscle and cardiovascular capacity.

(3) *Addition of projects that are needed but not covered by existing grants.*
Since the nutrition/physical fitness program is incomplete at this time, a workshop was held to write RFPs to solicit proposals to fill important gaps in the research program. In addition to the proposed exercise solicitation noted above, three other areas are considered high priority. They include: (1) a research focus on the anorexia of space flight since despite adequate provision of food and water, inadequate food intake is characteristic of human space flight. This reduction of food intake translates into a significant energy deficit with loss of body mass and diminution of physical fitness. Suboptimal intake of essential macro and micronutrients, and inadequate water intake also occurs. (2) Alterations in nutrient partitioning and metabolism as a function of microgravity and/or other space flight stressors and (3) Meal allocation: nibbling Vs meal eating, supplements Vs whole meals, timing of nutrient intake. The timing and frequency of meals with respect to activity, including sleep cycles and exercise and to the most effective utilization of nutrients may be a key factor in maximizing astronaut health on long duration space flights.

(2) *Activation of synergy grants.*
Although certain projects (as described above) have a primary focus on nutrition and/or physical fitness, these two disciplines underlie overall health and should be a part of every NSBRI program. To that end, we propose to actively solicit and participate in synergy grants that would more fully integrate nutrition and physical fitness with other teams. Already, the muscle team is interested in a collaboration using the same amino acid supplement in rats that we are using in the human bed rest study and in the in vitro Tobin project. In addition, the integrative human function team is planning a collaboration with Helen Lane who is conducting one of our "add ons" to the bed rest study. Plans are to use data from this bed rest study in the modeling conducted by the integrative human function team.

B. Five to ten year Research Strategy (2007-2011)

During this time period, the following strategies will be implemented:
(3) *Full integration of nutrition and physical fitness.*
During the first five years, emphasis will be on development of nutritional and physical fitness countermeasures. However, as these programs develop, emphasis will expand to include an integration of nutrition and physical fitness into each proposal, during years 5 to 10.
(4) *Full integration of nutrition and physical fitness with the other programs.*
We envision a nutrition/physical fitness "clearing house" for every program. In much the same way that projects cannot be implemented without appropriate human or animal use protocols, we will work towards a required section in each proposal in which the diet and physical activity of animals/subjects must be defined. The absence of such protocols has seriously hampered the interpretation of many bed rest and other human studies that otherwise are very carefully controlled.

C. Countermeasure goals to be realized by year ten.
Given appropriate funding, the science exists to be able to solve certain of the important critical issues facing long duration space flight. With adequate support it is realistic to expect the following accomplishments within a 10-year time period:

(1) Optimal physical fitness protocols for space can be developed and tested. Within ten years, capabilities exist to develop the appropriate equipment and protocols (duration, frequency, intensity) of aerobic and resistive exercise to maximize aerobic capacity, muscle mass and strength, and bone mass. Given appropriate financial support, these protocols can be tested in flight during this time period.
(2) An appropriate intervention based on both nutritional and physical fitness criteria will be available as a countermeasure to the documented decrease in protein synthesis, muscle mass, and muscle strength. A diet and physical fitness intervention will be available to ameliorate decreased protein synthesis and loss of muscle mass during space flight. This will have been adequately tested in a bed rest study using both nutrition and physical fitness interventions, and the mechanism behind this effect will be documented in cell culture and animal experiments.
(3) We can have a good understanding of the anorexia of space flight, and preliminary development of countermeasures to combat it. A clear understanding as to whether or not there is a space flight induced reason for the observed decrease in food intake independent of nausea, workload, and psychosocial issues. If there is a physiological basis for this depression in food intake, we should be at the stage to test countermeasures in human ground based studies.
(4) We will have a mechanistically based understanding of whether or not diet can ameliorate radiation enhanced colon cancer. Definitive data in rats, and complementary data in humans,

that a specific diet can reduce the initiation of colon cancer enhanced by radiation will be obtained. Clearly it is not possible to show a decrease in colon cancer in humans during this time period since this disease takes up to 40 years to develop. However, using microarray technology first documented in the rat, and then (years 5 – 10) in the human, preliminary recommendations will be available to support diet interventions in humans.
(5) We will have sufficient knowledge of the timing of food intake, exercise, and other activities to make recommendations for space flight.
(6) The observed insulin resistance and impairment of glucose metabolism will be more clearly understood and proposed countermeasures will be available for testing in human studies.
(7) The potential exists for a phase shift in knowledge on the effects of microgravity on nutrient partitioning. Although not guaranteed, we now have the ability to attract the top nutritional scientists in the world to address basic questions of how microgravity affects cell-cell communication, distribution of nutrients among tissues, signaling events, etc. It is entirely possible that the observed alterations in nutrient partitioning are microgravity related. If so, this would change the way we think about nutritional and physical fitness interventions. If proposals in this area can be solicited during 2001 for activation in 2002 there is a high likelihood that we will have an answer within 10 years as to how microgravity affects nutritional status. Subsequent to that time one would be in the position of suggesting appropriate countermeasures.

National Space Biomedical Research Institute

FINAL PROGRAM REPORT

Team Name. RADIATION EFFECTS

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The information in this report is preliminary, proprietary, and confidential

Signature John F. Dicello 11/19/01

COVER PAGES

National Space Biomedical Research Institute

Radiation-Effects Team

TEAM PROJECTS AND PRINCIPAL INVESTIGATORS

Project Name: **Determination of In-vivo Carcinogenesis with the Sprague-Dawley Rat**

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Project Name: **Chemoprevention and Radiation-Induced Neoplasms**

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Project Name: **Countermeasures for Space Radiation Biological Effects**

PRINCIPAL INVESTIGATOR:

Ann R. Kennedy, D.Sc., Associate Team Lead
Co-Investigators: John E. Biaglow, Ph.D. and X. Stephen Wan, Ph.D.
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Project I, Title: **Risk Assessment and Chemoprevention of HZE Induced CNS Damage**

Project II, Title: **CNS Damage and Countermeasures (In Vivo Studies)**

PRINCIPAL INVESTIGATOR:
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***Project Title*: Charged Particle Radiation-Induced Genetic Damage in Transgenic Mice**

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I. EXECUIVE SUMMARY

Research Problems

Radiation has been categorized in NASA's Strategic Plan as one of four major hazards in space and, frequently the most serious of these four. The major problems are the determination of the level of risk from radiations in space and the development of tolerable but effective countermeasures for the major radiation-induced medical problems, identified in NASA's Critical Path Roadmap to be radiation-induced cancer and CNS damage. Our hypothesis is that the risks can be simulated and countermeasures developed by appropriate ground-based in-vivo studies leading to in-flight verification. It is also expected that, because of the complexity of the interactions resulting from the space environment, non-cancerous heath effects may occur in organ systems other than the CNS, especially in synergy with other risks such as reduced immune responses or increased loss of bone mass..

The main goal of the NSBRI Radiation Program consists of 1) improving the predictions of risks to human health from space radiations and 2) providing effective countermeasures that will significantly reduce these risks. The major approaches being undertaken fall into five categories:

1 Develop countermeasures for mitigating effects of radiation exposure.

2 Develop markers for determining risks and monitoring the efficacy of countermeasures.

3 Determine carcinogenic and CNS effects for space radiation.

4 Determine acute and long-term pathological responses of rapidly renewing organ systems at risk.

5 Characterize differences in cell and molecular mechanisms for pathological effects for high- versus low-LET radiation in defined model systems.

Strengths and Key Findings

Our key findings during the last year include the first completed study of the life-time risk of cancer in an animal model in almost a decade by Dr. John Dicello's group at Johns Hopkins University School of Medicine. We have completed the first two experiments out of a total of six that have been underway underway to measure the incidence of beast cancer in Sprague-Dawley rats. Although these two experiments were originally intended only as baseline studies to establish rough estimates of expected responses for subsequent experiments, the experiments have gone very well and we expect significant data when the analysis is completed.

Although the first experiments of irradiations followed by Tamoxifen as a chemopreventive agent by Dr. David Huso's group at Johns Hopkins University have yet to be completed, the data incidence as a function of time suggest strongly that realistic

chemopreventive drugs can reduce the incidence of cancer even for heavily ionizing particles such as iron ions.

Human neural precursor cells (NT2), neurons (hNT) and rodent glial progenitor cells (CG4) were exposed by Dr. Macelo Vazquez's group at Brookhaven National Laboratory to acute doses of 0.1, to 6 Gy of heavy ions (Fe, C, Si and Ar) and proton irradiation and measurements were obtained for Apoptosis induction,_ cell toxicity, gene expression. Doses as low as 0.1 Gy were able to induce a significant increase of cell damage in comparison to controls. These results for gene expression appear to confirm that p53 gene is involved in the stress pathway induced by low- and high-LET radiation exposures.

The first NSBRI studies for protons and HZEs with the Lac-Z transgenic mouse were intiated during this past year. Dr. Polly Chang's group at SRI studied the dose- and time-dependent radiation-induced responses in *lacZ* transgenic mice after proton radiation. She demonstrated that they can detect cytogenetic damage in circulating reticulocytes at proton doses as low as 0.1 Gy and mutagenic effects in *lacZ* transgene at doses of 0.5 Gy and above.

Dr. Ann Kennedy's group at the University of Pennsylvania is initiating cellular and in-vivo studies of nontoxic dietary supplements as potential means of reducing the incidence of cancers from radiation exposures in space.

Major Gaps

The major gaps in the present program include the lack of:

6 Animal studies of carcinogenesis in relevant tissues other than breast,

7 Interspecies comparisions,

8 Animal studies for protracted exposures,

9 In-vivo data for synergistic effects of mixed fields,

10 Experiments to study synergisms with other environmental factors such as microgravity and bone loss,

11 More comprehensive analysis of human responses to low-dose, protracted exposures,

12 Adequate methods for extrapolating animal data to humans.

13 Ground based clinical trials or in-flight verification.

Summary

In summary, the Radiation Team is on schedule in reference to NASA's Critical Roadmap in terms of obtaining crucial data needed for evaluating risks from radiation,

demonstrating a feasible countermeasure, and in terms of the schedule with which the data are being obtained.

II. INTRODUCTION

Scientific Research Problems

The major problems to be addressed by this Radiation Program are the understanding of radiation risks in space and the development of tolerable but effective countermeasures for the major radiation-induced medical problems, identified to be radiation-induced cancer and CNS damage. Our hypothesis is that the risks can be simulated and countermeasures developed by appropriate ground-based in-vivo studies. It is also possible that because of the complexity of the space environment unanticipated non-cancerous effects may occur in organ systems other than the CNS, especially in synergy with other risks such as reduced immune responses or increased loss of bone mass..

The main goal of the NSBRI Radiation Program consists of 1) improving the predictions of risks to human health from space radiations and 2) providing effective countermeasures that will significantly reduce these risks.

Thus the major aims cover five categories:

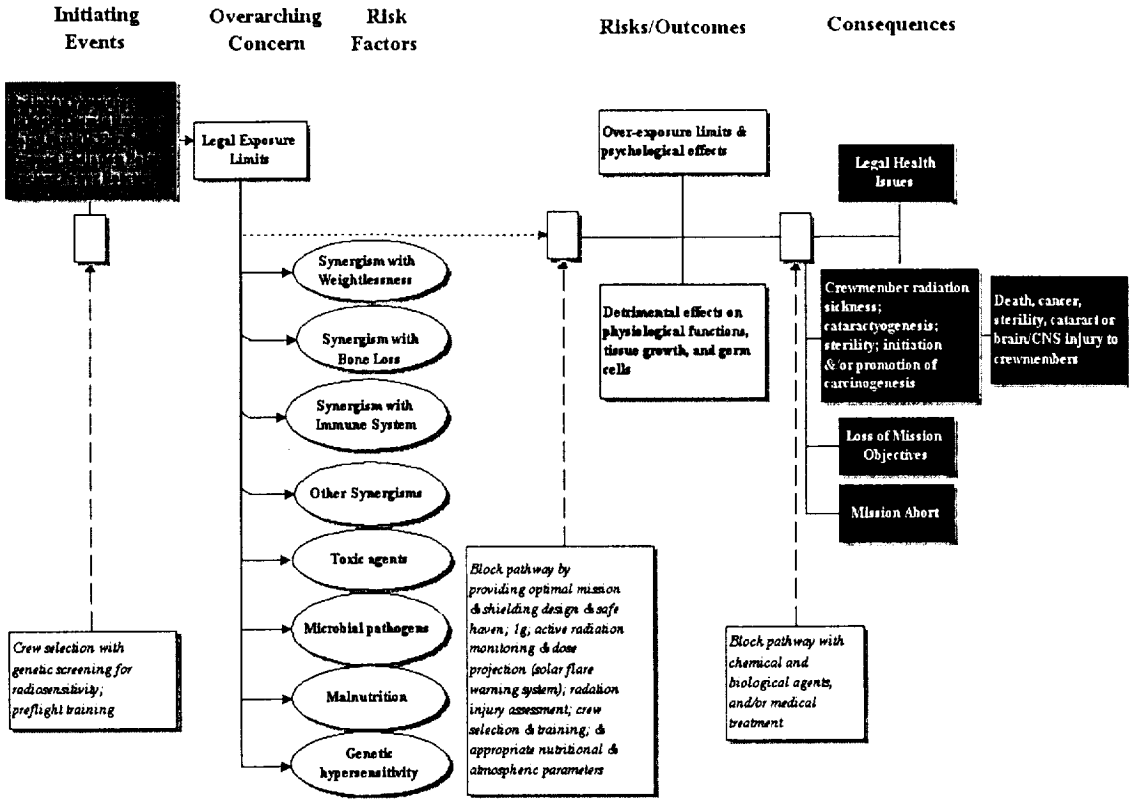
occur in organ systems other than the CNS. Thus the major aims will cover five categories:

1. Develop countermeasures for mitigating effects of radiation exposure.
2. Develop markers for determining risks and monitoring the efficacy of countermeasures.
3. Determine carcinogenic and CNS effects for space radiation.
4. Determine acute and long-term pathological responses of rapidly renewing organ systems at risk.
5. Characterize differences in cell and molecular mechanisms for pathological effects for high- versus low-LET radiation in defined model systems.

II HEALTH CONCERNS AND HAZARDS

The NASA Critical Path Roadmap lists Radiation as one of the four Severe Type I Risks, the most critical type, along with bone loss (acceleration of age-related osteoporosis), human behavior (poor psychosocial adaptation), and clinical capability (trauma and acute medical problems). The radiation risk areas in terms of long-term missions, both low-earth orbit or extraplanetary, and their relation to the overall space program are shown in the following chart adapted from NASA's Critical Path Roadmap:

Radiation Effects Risk Area



The relevant risks and major critical issues according to the NASA Critical Path, in order of priority, are:

Risk	Risk Type	Risk Rank
• **Carcinogenesis caused by radiation**	**I**	**1**
• **Damage to central nervous system from radiation exposure**	**II**	**2**
• **Synergistic effects from exposure to radiation, microgravity, and other spacecraft environmental factors**	**II**	**3**
• **Early or acute effects from radiation exposure**	**II**	**4**
• **Radiation effects on fertility, sterility, and heredity**	**III**	**5**

The main goal of the NSBRI Radiation Program consists of 1) improving the predictions of risks to human health from space radiations and 2) providing effective countermeasures that will significantly reduce these risks.

MAJOR QUESTIONS TO BE ADDRESSED

In summary, the major questions remaining to be addressed include:

6. Animal studies of other relevant tissues
7. Interspecies comparisions
8. Animal studies for protracted exposures.
9. In-vivo data for synergistic effects of mixed fields
10. Synergism with other environmental factors such as microgravity and bone loss
11. More comprehensive analysis of human responses to low-dose, protracted exposures.
12. Improved methods for extrapolating animal data to humans too imprecise.
13. Clinical trials, ground-based then flight-based.

III. RESEARCH PROGRAM STRUCTURE AND DESIGN

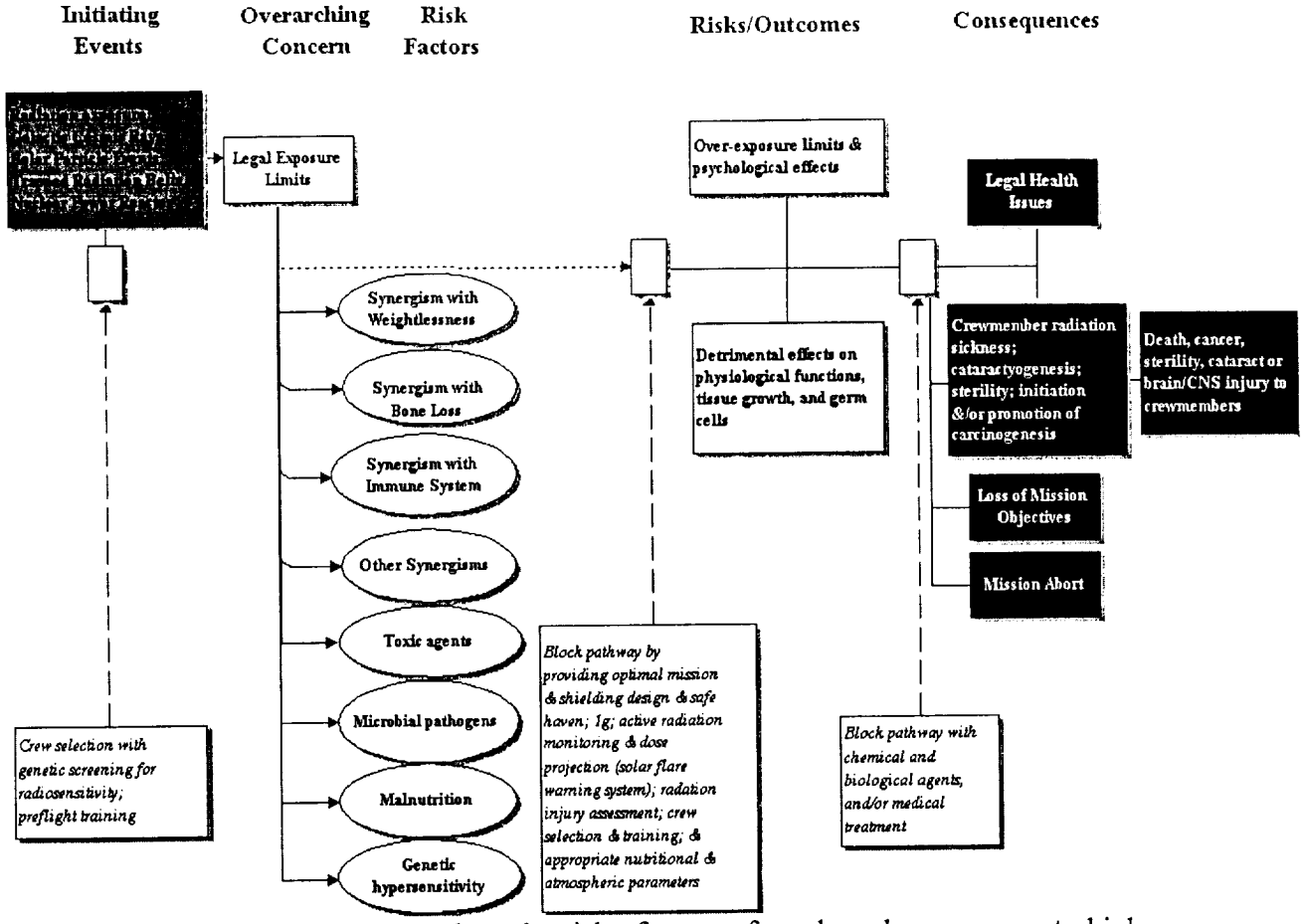
III-1 Structure and Design of Radiation Research Program

Main Goal: **The main goal of the NSBRI Radiation Program consists of 1) improving the predictions of risks to human health from space radiations and 2) providing effective countermeasures that will significantly reduce these risks.**

Overall Strategy: Apply research to risk analysis and assessment for countermeasure development.

The National Space Biomedical Research Institute (NSBRI) was formed to address the medical risks to humans in space as well as the subsequent risk to mission success. One of NASA's goals in supporting the institute is to understand the health effects and performance consequences of space radiation to the astronauts and to mission goals. An essential strategy of the NSBRI has been the Integrated Research Team concept to maximized productivity and cost-efficiency with minimum redundancy to build and balance integrated research programs. A main mission of the NSBRI has been to design and validate countermeasures addressing the major hazards. At the end of the first three years, the Radiation-Effects Team became one of the most productive, cohesive teams focusing on the first four risk factors in NASA's Strategic Plan and one of the few to have shown feasibility of a proposed countermeasure, namely the use of chemopreventive

Radiation Effects Risk Area



agents or dietary supplements to reduce the risk of cancer from low-dose exposes to high atomic-number, energetic charged particles (HZEs) and protons.

RELEVANT RISKS AND CRITICAL ISSUES

The NASA Critical Path Roadmap lists Radiation as one of the four Severe Type I Risks, the most critical type, along with bone loss (acceleration of age-related osteoporosis), human behavior (poor psychosocial adaptation), and clinical capability (trauma and acute medical problems). The radiation risk areas in terms of long-term missions, both low-earth orbit or extraplanetary, and their relation to the overall space program are shown in the following chart adapted from NASA's Critical Path Roadmap:

The relevant risks and major critical issues according to the NASA Critical Path, in order of priority, are:

Risk	Risk Type	Risk Rank
• **Carcinogenesis caused by radiation**	I	1

• **Damage to central nervous system from radiation exposure**	**II**	**2**
• **Synergistic effects from exposure to radiation, microgravity, and other spacecraft environmental factors**	**II**	**3**
• **Early or acute effects from radiation exposure**	**II**	**4**
• **Radiation effects on fertility, sterility, and heredity**	**III**	**5**

The major themes of this program are the understanding of risks and the development of effective countermeasures for the radiation-induced biological effects identified to be of major concern: radiation-induced cancer and CNS damage. It is also possible that because of the complexity of the space environment unanticipated effects may occur in organ systems other than the CNS. Thus the major aims will cover five categories:

1. .Develop countermeasures for mitigating effects of radiation exposure.
2. Develop markers for determining risks and monitoring the efficacy of countermeasures.
3. Determine carcinogenic and CNS effects for space radiation.
4. Determine acute and long-term pathological responses of rapidly renewing organ systems at risk.
5. Characterize differences in cell and molecular mechanisms for pathological effects for high- versus low-LET radiation in defined model systems.

Remaining Issues:

6 Animal studies of carcinogenesis in relevant tissues other than breast,

7 Interspecies comparisions,

8 Animal studies for protracted exposures,

9 In-vivo data for synergistic effects of mixed fields,

10 Experiments to study synergisms with other environmental factors such as microgravity and bone loss,

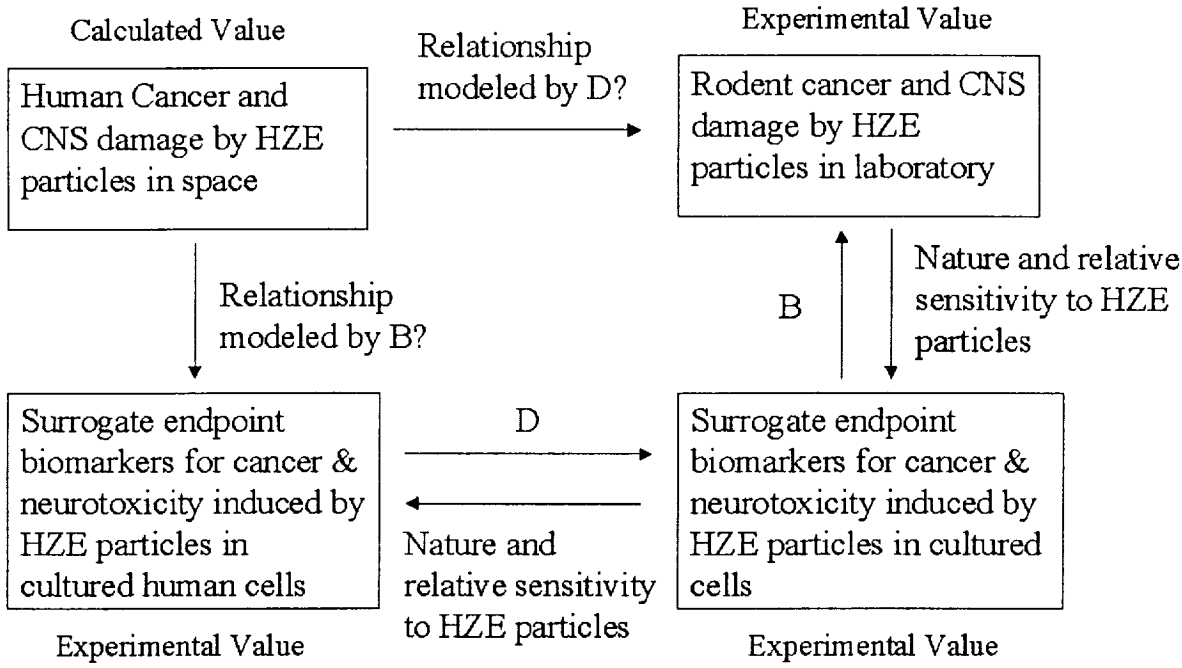
11 More comprehensive analysis of human responses to low-dose, protracted exposures,

12 Adequate methods for extrapolating animal data to humans.

13 Ground based clinical trials or in-flight verification.

III. OVERALL STRATEGY

This program's overall strategy is modeled after that proposed in the NASA report on Modeling Human Risk (1997). The underlying philosophy of the approach is that experimentally determined risks for carcinogenesis and CNS damage in appropriate animal models with corresponding in-vitro measurements can be used to validate theoretical relations between animal results and human response. These theoretical relations, then, can be used to extrapolate known responses of humans to acute exposures of low-LET radiations to expected responses to protracted exposures to protons and HZE particles. When such relations have been established, then this same process and these same animal and cell models can be used to determine the potential of pharmaceutical agents, including both chemopreventive drugs and dietary supplements, for reducing risks. This is illustrated schematically in the following figure which is a revision of that proposed in the NASA report on modeling human risk (1997).



Adapted from Modeling Human Risk: Cell & Molecular Biology in Context, 1997

dietary supplements. The mutation and cytogenetics research gives us cellular and chromosome endpoints for cells irradiated in the animal or in-vitro. In all cases, theoretical models and analysis lead us to

The present Team organization, as outlined in Fig. 1, evolved from the original missions of the NSBRI, the Phase-I and Phase-II NSBRI applications to NASA, recommendations in peer reviews, and administrative directives. The overall philosophy is that *in-vivo* st

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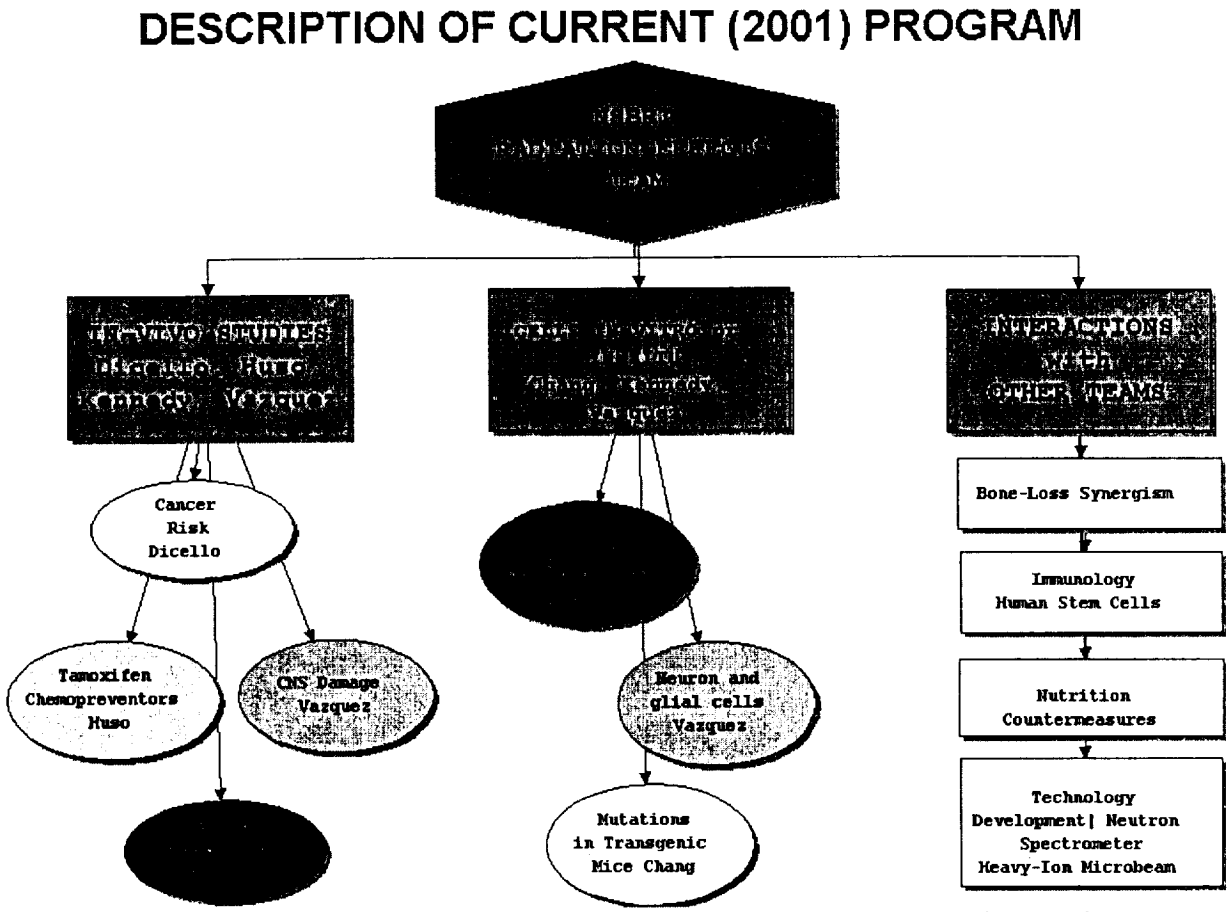
IVIDUAL PROJECTS AND COUNTERMEASURES

Presently, there are six principal projects. A brief description follows of each and the c udies provide



risks of cancer, CNS damage and other diseases and the Chemoprevention project provides the data for risk reduction with pharmaceuticals and/or mechanistic methods for extrapolation to risk assessments for humans for the scenarios in space.

ountermeasures being studied:

PROJECT I: In Vivo Studies of Mammary Carcinomas
John F. Dicello, PhD, *Johns Hopkins University*

Critical Path Risk(s): Carcinogenesis caused by radiation (38:1,3,5,6,7,(8),910,11), Countermeasure Readiness Level: 4

Specific Aim:

Determine risk of carcinogenesis in a relevant animal model and supply exposed animals for chemopreventive studies.

Countermeasure: Chemoprevention of cancers by use of pharmaceuticals administered after high-level exposure to radiations. Improved risk factors can be used to optimize spacecraft design for optimal shielding

PROJECT II: Chemoprevention and Radiation-Induced Neoplasms
David L. Huso, DVM, PhD
Johns Hopkins University

Critical Path Risk(s): Carcinogenesis caused by radiation (38:1,3,5,6,7,(8),910,11) , Countermeasure Readiness Level: 5

Specific Aims: Studies of the pathology of cancer induced by HZE particles and pharmaceutical intervention.

Countermeasure: Tamoxifen as a model for pharmaceutical intervention in the promotion and progression stages of carcinogenesis to reduce risk after exposure

PROJECT III: Countermeasures for Space Radiation Biological Effects
Ann R. Kennedy, PhD
University of Pennsylvania

Critical Path Risk(s): Carcinogenesis caused by radiation (38:1,3,9,10), Early or acute effects from radiation exposure (41:1,3,5,10,11)
Countermeasure Readiness Level: 4

Specific Aims:

(1)Determine the ability of various dietary supplements to reduce radiation induced oxidative stress in cultured cells

(2)For the combinations of agents demonstrating efficacy as antioxidants *in vitro*, determine the ability of these agents to decrease radiation induced oxidative stress in Sprague-Dawley rats.

Countermeasure: Dietary supplements prior to and after exposure to radiation to reduce cancer incidence.

PROJECT IV: Risk Assessment and Chemoprevention of HZE Induced CNS Damage

Marcelo E. Vazquez, PhD, MD
Brookhaven National Laboratory

Critical Path Risk(s): Damage to CNS system from radiation exposure (39:1,3,7,10,11), Early or acute effects from radiation exposure (41:1, 3, 5, 10, 11)
Countermeasure Readiness Level: 3-4

Specific Aims:

(1) Examine cell death in cycling and non-cycling neural cells

(2) To characterize the putative cell signaling cascades induced by high-LET radiation in the apoptotic pathways (ceramide- and p53-dependent).

Countermeasure: Modulate signaling pathways by pharmacological manipulation (trophic factors, free-radical scavengers, p53 modulators)

PROJECT V: CNS Damage and Countermeasures (In Vivo Studies)

Marcelo E. Vazquez, PhD, MD
Brookhaven National Laboratory

Critical Path Risk(s): Damage to CNS system from radiation exposure (39:1,3,7,10,11), Early or acute effects from radiation exposure (41:1,3,5,10,11)
Countermeasure Readiness Level: 3-4

Specific Aims:

(1) Characterize the behavioral, neurochemical and structural changes induced by heavy ions and protons.

Countermeasure: To protect neural cell populations in vivo using pharmaceuticals such neuroprotectants (gangliosides), antioxidants (melatonin) and signal pathways modulators (p53 modulators)

PROJECT VI: Charged Particle Radiation-Induced Genetic Damage in Transgenic Mice

Polly Yee Chang, PhD
SRI International

Critical Path Risk(s): Carcinogenesis caused by radiation, Damage to central nervous system from radiation exposure, Early or acute effects from radiation exposure (41:(3), 5, 10)
Countermeasure Readiness Level: 2

Specific Aims:

1. **Examine both the dose and temporal-dependence of particle radiation-induced mutation in vivo using the LacZ transgenic mice model system. In particular, acute and long-term tissue specific mutagenic responses of CNS and rapidly renewing organ systems will be determined after exposure to protons and HZE particles.**
2. **Examine the impact of genetic backgrounds, eg. p53 on radiation sensitivity using the p53/lac Z double transgenics.**

Potential Countermeasures:
Determine if known radioprotective pharmaceuticals (eg. tamoxifen, anti-oxidants) or cytokines (eg. interleukins) reduce tissue-specific mutation frequencies or genetic damage in vivo. Such alterations in the genome may be precursors of cancer.

In terms of overall strategy, four projects are investigating carcinogenesis (3) and CNS damage (1) in-vivo and three projects address corresponding cellular effects. In addition to these six projects, we also have been collaborating with researchers in other teams where team goals overlap.

Almost every review of the radiation problems in space during the last decade, including the three previous references, has recommended animal studies to quantify the risks to these types of radiation and to pursue likely countermeasures. Until this series of experiments, however, there had been only one comprehensive animal study to investigate the effects of ions of high atomic number and high energy, HZEs. That experiment was conducted by Alpen et al. (1993) with the Berkeley Bevalac, which has been out of commission for almost a decade. It has provided invaluable data on carcinogenesis in the harderian gland of a mouse model as a function of linear energy transfer (LET), and it has been a cornerstone for risk assessments in space during the last decade. No comparable series of experiments until this present series had been conducted to evaluate the use of drugs to reduce the risk of cancer from exposures.

As a result of the scientific reviews, workshops, and discussions with external advisors to the NSBRI during the developmental period of the NSBRI, the Radiation Team focused on the female Sprague-Dawley rat irradiated whole-body with HZEs, protons, or photons. Initially, the biological endpoints included malignant and benign tumors at all sites and and other diseases. The motives for this animal model were multiple and are presented in detail in the Final Report for the Core Project. Perhaps most importantly, this model was that recommended by the members of the External Advisory Council.

Animal experiments of this type were generally not done previously because of the complicated logistics and the large expensive.. Only three facilities in the world, one at Brookhaven National Laboratory in New York State, one in Germany, and one in Japan, produced the necessary accelerator HZE beams. The costs for HZE beam time is millions of dollars a year, far in excess of any funds available from the NSBRI, and only about 150 hours a year are available for all space-biology irradiations in the U.S.A. Finally, no one had ever carrying out experiments with energetic charged particles at multiple facilities , including BNL for HZEs and Loma Linda University with its

energetic proton synchrotron.. The logistics of transporting thousands of animals between multiple facilities in isolated environments and keeping them alive subsequently for three or more years was at best a challenge. To maximize the value of the results, the principal investigators offered support to colleagues in other projects and teams and interested investigators outside of the NSBRI to maximize the production of useful scientific data with the irradiated animals and to provide different information, stressing the importance of data correlated to the same animal species and to humans so that the results could be applied most efficiently to humans in the space environment.

SYNERGISMS AND IMPLICATIONS WITH REGARD TO COUNTERMEASURES

The infrastructure for the NSBRI was designed with the research team as a mission-oriented, focused group. Because of the limited amount and high cost of beam time for radiobiological studies with HZE particles,.collaborating and sharing resources have been essential to achieve the goals of Institute and the team. The immunology studies of Dr. Daila Gridley with protons at LLUMC were funded directly from Dr. Dicello's original Core Project. These studies let to preliminary data, a referreed publication, and funded research on this topic in the Immunology Team (William Shearer, PI). The work of Dr. Dicello in collaboration with Dr. Francis Cucinotta at the NASA Johnson Space Center showed the importance of the neutron secondaries in space leading to a funded project (Richard Maurer, PI) in the Technology Team. The NSBRI Radiation Team (Drs. Dicello, Huso, Williams, and Sinden) developed the procedures and methods for doing large animal experiments concurrently at BNL and LLUMC and the importance of parallel cellular studies, fostering interest in the NSBRI at BNL and its ultimate membership as a consortium member as well as funded research on damage to the centeral nervous system (Marcelo Vazquez, PI). These same methods and procedures as well as the preliminary results formed the basis of a successful application in the Nutrition Team as well (Joanne Luxton). Dr. Dicello, Dr. Huso, and Dr. Jay Shapiro (Team Lead for Bone Loss Team) have done preliminary studies because of the potential synergisms between radiation and bone loss in a microgravity environment. The preliminary results from the previous Core Project (John Dicello, PI) were the basis of a successful proposal to NASA to support the dissertation research of a graduate student, Dustin Simonson, in Dr. Dicello's laboratory. The previous Core Project supported extensive theoretical studies (with Francis Cucinotta's group at JSC) to analyze the experimental results, to evaluate the medical risks, and to optimize spacecraft designs both for Low Earth Orbits and for interplanetary missions.

We continue to work with scientists outside of the NSBRI, such as Dr. Christopher Lange at SUNY Downstate Medical Center, supporting his work to examine the kinetics of double-strand breaks (DSB) using pulsed-field gel electrophoresis and with Dr. Barry Rosenstein at Mt. Sinai Hospital in NYC to examine TP53 mutations. research.

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

IV-1A. Dicello's Project: Determination of In-Vivo Carcinogenesis with Sprague-Dawley Rats

During the last year, we completed the first two experiments out of a total of six to study mammary carcinogenesis in the Sprague-Dawley rat. The remaining four experiments include the animals for the Tamoxifen study by Dr. David Huso's group described below. This is the first such experiment in almost a decade.

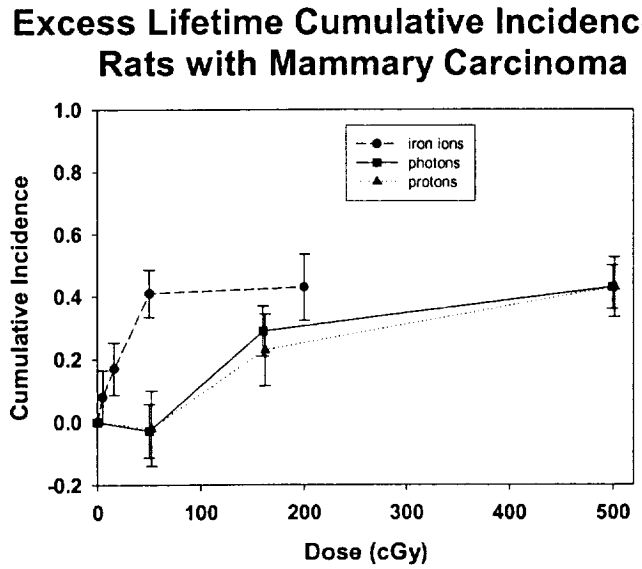


Fig. 1. ***Fraction of mammary carcinomas observed in each group at 514 days after irradiation.***

Approximately three thousand rats have been irradiated with 1-GeV iron ions, 250-MeV protons, and cesium-137 and cobalt-60 gamma rays. Doses for the protons and photons typically ranged between 50 and 500 cGy, and the doses for the iron beams typically ranged between 5 and 50 cGy. Some higher doses were used in the beginning of the experiments because the Relative Biological Effectiveness (RBEs) for the protons and iron ions had not been measured previously, for in the case of the Tamoxifen studies were there is a reduction in the levels of tumor induction with the administration of the drug. Details of the number of animals at each specified dose are delineated in the final report for the Core Project.

Tumors have been observed in primarily the breast and pituitary, but also to a lesser extent in the thyroid and other sites. The Sprague-Dawley rat has a high natural incidence of mammary fibroadenomas and adenocarcinomas; however, with the excision of mammary tumors at a relatively modest size, the major cause of death for our colonies is pituitary-related

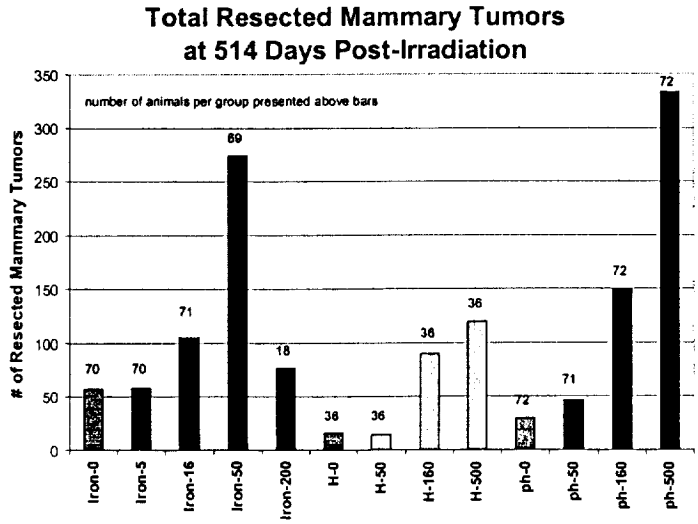


Fig. 2. ***Resected tumors observed as a function of radiation.***

illnesses. The longevity associated with a healthy colony and animals that do not die of the endpoint of interest. lead to more reliable risk factors than studies terminated early because of mortality of diseases. Although an examination of pituitary diseases was not a specific aim of this project originally, we have been examining these tissues and analyzing the results with non-NSBRI support, and these results will be published when they are complete.

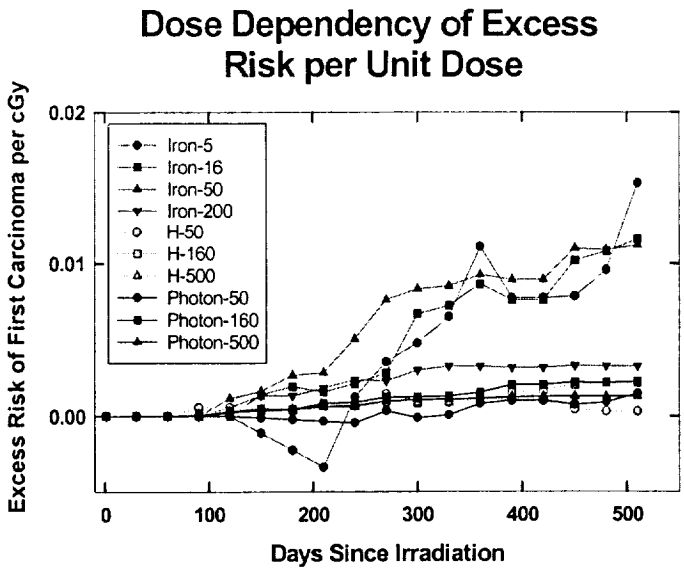


Fig. 3. ***Excess risk per dose unit.***

Figure IV-1 shows the excess cumulative incidence of histologically verified mammary carcinomas as a function of dose and radiation type. Figure IV-2 shows the number of tumors observed as a function of particle type and dose. The number of tumors generally increases with increasing dose of a radiation type. The small count of Iron-200cGy tumors is a reflection of the reduced number of animals in that group. Slightly less than half of all histopathologically classified mammary tumors are carcinomas. There does not appear to be a strong trend in the carcinoma/fibroadenoma ratio with dose or radiation type. It should be noted that each animal has 12 mammary glands, each of which can develop one or more tumors, although recurrences of the same tumor were categorized as a single tumor. However, the same number of tumors in groups of the same size, such as shams and low dose groups, does not mean the same number of animals with tumors.

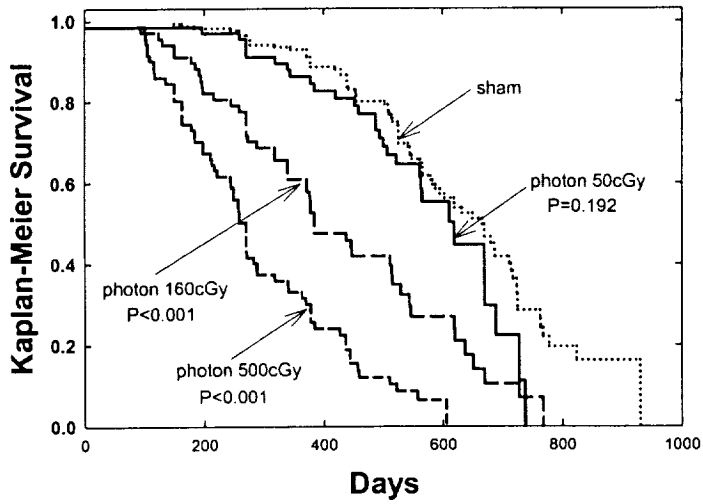


Fig. 4. ***Survival without a mammary carcinoma as a function of time since iron ion irradiation. Survival is calculated using non-parametric Kaplan-Meier method of censor adjustment*** **(E.I. Kaplan, J of the Amer. Stat. Assoc. 53, 457-481).** ***P values are relative to sham response.***

The excess risk of a first mammary carcinoma as a function of time is shown in Fig. IV-3. A statistical analysis of proportions indicates there is no difference between each individual sham group; therefore, at this point, they are being pooled for subsequent analyses. The group of rats

irradiated with Cesium at BNL, those irradiated with Cesium at JHU, and those irradiated with Cobalt at LLUMC were compared within each dose level and also found to be statistically similar and therefore pooled into single photon groups at each dose to increase statistical power.

A paired sample t-test was conducted and all curves for iron irradiation were significantly different from the shams except for iron-5cGy (p=0.54). Similar analyses were performed on the proton and photon curves within each radiation type. It was found that all curves differed significantly from the sham curves, including the curves for the lowest doses of protons and photons. When comparing the proton curve versus the photon curve at the same dose, it was found that only the highest dose of 500cGy resulted in statistically similar curves. This may be significant in that it is frequently assumed in risk assessment that the carcinogenic effects of protons and photons are similar at identical doses.

To evaluate the linearity of effect per unit dose, excess risk of a first carcinoma per cGy was plotted for each dose group in Fig. IV-3. As the animals age, the data for first carcinomas becomes difficult to interpret because of a low population at risk so those data are not being presented at this time. As expected, the effect per unit dose is greater for the iron ions than the protons or photons. The notable exception is the iron-200cGy group in which the excess risk is diminished. This effect is likely caused by the fact that, at this high dose, few animals remain without a carcinoma, so the pool of susceptible animals is diminished. Again, because we did not know responses, we chose doses that would likely bracket the regions of interest. The tight grouping of the 5cGy, 16cGy and 50cGy iron groups is one indication that we are within the linear region of the iron dose scale, at least up to 514 days post-irradiation. At 514 days, RBEs between 1 and 10 appear consistent with the data, depending upon the time and dose-region of interest.

In an effort to correct our analyses for the number of animals at risk at any given time, a Kaplan-Meier survival analysis was performed (Fig. IV-4). A Mantel-Haenszel log rank test of KM survival estimators indicates no difference between shams and iron-5cGy (p=0.07). The lowest-dose proton and photon groups are also not significantly different from the sham response. A comparison between proton and photon groups at the same dose level shows no significant difference.

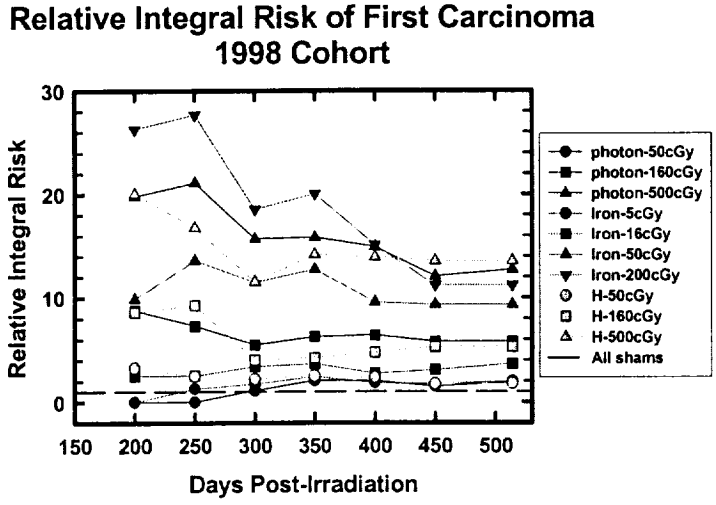


Fig. 5. ***Relative Risk as a function of time since irradiation.***

The relative integral risk (RR) of a first carcinoma (in reference to the sham group) versus time post-irradiation is plotted in Fig. IV-5. The relative risks integrated with time demonstrate the strong dose dependence of time to first carcinoma. The fact that the RR is decreasing with time is an indication that the sham animals are developing mammary carcinomas at a greater relative rate that the highest dose groups. The near linearity of the moderate and low dose groups through time may be

interpreted as support that the relative rate of cancer development is similar to that in the sham group. Such a study of the time-dependence of risk is not possible with most in-vivo animal studies as they typically utilize prevalence at the end of study as the major endpoint.

IV-2A. Huso Project: Chemoprevention of Radiation-Induced Neoplasms

Key findings:
Although our studies are not complete, preliminary trends in our tamoxifen studies have pointed to a proof of principle for a strategy in which chemopreventive agents could play an important role in preventing breast cancer following exposure to radiation during space travel. Confirmation of these trends is still pending the completion of these studies, but considerable progress has been made since Dr. Huso took over as PI of the chemoprevention studies area.

Radiation Effects Study

A) Initial Radiation Effects Study

In order to address the specific aims of the proposal several cohorts of the female Sprague Dawley rat mammary tumor model were irradiated at 60 days of age at Brookhaven National Laboratory (BNL) for heavy ions and Loma Linda University (LLU) for protons. As controls, a portion of each cohort was irradiated with photons at BNL, LLU, and JHU. A summary of the irradiated groups of animals is shown.

All groups are female Sprague Dawley rats exposed at 60 days of age.

IRON ION EXPOSURES (number of rats)	PROTON EXPOSURES (number of rats)
200 cGy (18)	500 cGy (36)
50 cGy (63)	160 cGy (36)
16 cGy (71)	50 cGy (36)
5 cGy (70)	Sham (36)
Sham (70)	

PHOTON EXPOSURES (number of rats) total irradiated at JHU, LLU, BNL

500 cGy (78)
160cGy (72)
50 (72)
Sham (72)

This group consisted of 730 animals. In ongoing studies, mammary biopsies and necropsy tissues have been collected and are being processed and analyzed. Mammary tumor biopsies are classified histopathologically as carcinomas or benign adenomas. Correlations are made between histopathology and radiation dose and quality.

B) Chemoprevention Study Controls

This group is of particular interest since they receive up to 25% dietary restriction to match their weight gains to the corresponding tamoxifen treatment groups. Dietary caloric restriction has been shown to significantly improve the longevity of rats. Therefore in this group a higher percentage of animals should survive to old age and overall they likely will live longer.

Chemoprevention Controls for Radiation Effects Studies (no tamoxifen controls)

IRON ION EXPOSURES(number of rats)	PROTON EXPOSURES(number of rats)
160 cGy (20)	900 cGy (24)
90 cGy (20)	500 cGy (24)
50 cGy (30)	300 cGy (24)
30 cGy (30)	160 cGy (39)

16 cGy (50)
9 cGy (20)
5 cGy (50)
Sham (50)

90 cGy (57)
50 cGy (53)
Sham (34)

PHOTON EXPOSURES (number of rats) total irradiated at JHU, LLU, BNL

500 cGy (50)
300 cGy (50)
160cGy (65)
90 cGy (60)
50 cGy (75)
30 cGy (30)
Sham (60)

The total number of rats that have been enrolled as irradiated chemoprevention controls (receive no tamoxifen) is 915. Lifetime analysis of these will be extremely useful for determining the radiation effects of protons and iron ions, especially at low dose exposures.
All of the above animals were irradiated according to similar protocols. For one cohort, the rats were shipped to each site and rats were irradiated at all three sites (JHU, LLU, BNL) within a 10 day period. Animals were shipped back to JHU following irradiation and animals have been weighed regularly and subjected to 15-25 % dietary restriction to control the rate of weight gain. Additionally the rats are palpated when weighed and mammary tumors detected are sized and recorded. Tumors that grow are removed surgically as needed and all tumors are archived as frozen OCT blocks, formalin or methacarn fixed embedded tissues, or in RNA later for RNA preservation. Animals in good health are returned to the study after surgery. Rats are sacrificed as necessary according to health problems, but otherwise are kept for lifetime follow-up. Complete necropsies are performed and a histopathologic diagnosis is determined for all tumors. Analysis of all potential radiation-induced lesions is underway.

Necropsy and Histopathology

Animals are identified by an ear-tag and by a subcutaneous transponder with a recorded, embedded unalterable random number cross-referenced with an assigned number with relevance to the animal's experimental group. All animals receive identification immediately following successful irradiation. Complete necropsies and microscopic examination have been performed on all at the time of death. At necropsy all organs and tissues have been examined for grossly visible lesions and examples of particular lesions photographed. All mammary tissue was carefully removed with the skin, examined, and then nodules removed for immediate analysis. The remaining mammary tissue was also preserved by fixation. All transponder chips remain with the appropriate archived tissues. Complete histopathologic examinations were performed on all mammary tumors. A database customized to our purposes is being developed (underway) with consultation from an on site expert. Individual animal records and results are accumulated in the database and easily retrieved for analysis of results. Our immediate goals are to fully process all tissue samples and analyze the information we are collecting regarding mammary tumors in the irradiated animals since that is the strength of the model system. We will also obtain information about radiation effects that occur later in life following radiation exposure in other organ systems in additional tissues we are archiving.

Chemoprevention studies

In 1998 the first large scale study demonstrating the effectiveness of tamoxifen chemoprevention for the prevention of breast cancer in humans was reported for the general population. Breast cancer has clear links to radiation exposure as the breast is one of the tissues most sensitive to the carcinogenic effects

of ionizing radiation. Tamoxifen is not an ideal drug. It has been associated with uterine cancer and cataracts and was recently listed as a carcinogen itself. Yet, its benefits apparently outweigh its shortcomings. Tamoxifen is the prototype of a family of estrogen receptor modulators that are the focus of intense developmental efforts. Therefore the chemoprevention studies examining the effectiveness of tamoxifen as a chemopreventative for iron ion or proton-induced mammary tumors may have far reaching implications as a countermeasure for the space program, especially as new, improved estrogen receptor modulator family members reach the market. Perhaps one of the most intriguing questions is, "Can tamoxifen prevent iron ion-induced or proton-induced mamary tumors that may appear much later in life after radiation exposure?"

A) Tamoxifen Chemoprevention Study: Analysis of experimental animals receiving tamoxifen chemoprevention following radiation exposure.

This group of rats receive continuous tamoxifen through an implanted slow-release pellet and they do not receive dietary restriction. Tamoxifen causes varying degrees of decreased weight gains so our goal in dietary restriction of controls is to match the tamoxifen-treated animals for rate of weight gain. The following are the rats currently receiving conitnuous tamoxifen chemoprevention. Within each radiation dosage group, animals were weight-matched as pairs, and then split and assigned as either control or tamoxifen-treated animals.

IRON ION EXPOSURES (number of rats)	PROTON EXPOSURES (number of rats)
160 cGy (20)	900 cGy (24)
90 cGy (20)	500 cGy (24)
50 cGy (30)	300 cGy (24)
30 cGy (30)	160 cGy (39)
16 cGy (50)	90 cGy (57)
9 cGy (20)	50 cGy (53)
5 cGy (50)	Sham (39)
Sham (50)	

PHOTON EXPOSURES (number of rats) total irradiated at JHU, LLU, BNL

500 cGy (50)
300 cGy (50)
160cGy (65)
90 cGy (60)
50 cGy (75)
30 cGy (30/)
Sham (60)

The total number of rats that have been enrolled as irradiated animals receiving tamoxifen chemoprevention is approximately 915 also. This cohort is proving extremely valuable in determining the effectiveness of tamoxifen administration as a chemopreventative against the most relevant mammary tumors likely to be encountered following low-dose proton or iron ion exposure including those that occur later in life. A single cohort of rats was divided and were shipped to each irradiation site directly from a single supplier and rats were irradiated at all three sites (JHU, LLU, BNL) within a 10 day period. For all the three different age cohorts, alls animals were shipped back to JHU following irradiation. One month after radiation exposure, rats were given tamoxifen at 200 ug/day (previously shown effective in preventing photon-induced mammary tumors) as a sustained release subcutaneous pellet. Unlike previous studies the animals then were switched to 20 ug/day for lifetime maintenance chemoprevention using 180 day

sustained release, subcutaneous pellets. The rats have been weighed and palpated regularly to detect mammary tumors. Tumors that grow to a designated size were removed surgically and all tumors archived as frozen OCT blocks, formalin or methacarn fixed embedded tissues, or in RNA later for RNA preservation. Animals in good health were returned to the study after surgery. Rats are sacrificed as necessary according to health problems, but otherwise are kept for lifetime follow-up. Complete necropsies are performed and a histopathologic diagnosis is determined for all tumors. It is important to get a clear idea of both the effects and effectiveness of long term tamoxifen administration. Tamoxifen resistant tumors will be compared with tumors of similar histological type from control animals and in some cases immunohistochemistry was used to assess estrogen receptor expression (see preliminary results). Additionally, immunohistochemistry for vimentin and cytokeratin were used to characterize certain tumors that were difficult to characterize by histological criteria alone. Although results are still not complete, one of the important questions is whether or not tamoxifen's effectiveness against radiation-induced tumors diminishes for tumors that appear later in life and how tamoxifen's effectiveness is influenced by the type of radiation to which the animals were exposed. These are important questions with direct relevance to reducing risks through countermeasures for the space program.

In summary

-Over 2500 total animals have been irradiated with protons, photons, or heavy ions relevant to space studies at the three different locations described and animals shipped back to JHU for enrollment in the mammary cancer chemoprevention studies. This large number increases the chances of finding significant, but subtle effects of low dose radiation and chemoprevention effectiveness at low radiation doses

-Complete necropsies on irraditated rats have revealed a variety of mammary and nonmammary, radiation-induced tumors that are currently being analyzed.

-Over 3000 total tumors have been surgically removed and are in the process of being analyzed of which over 1500 are carcinomas

-All relevant tumors and tissues are being archived for our ongoing studies on the effects and chemoprevention of heavy ion-induced and protoninduced cancer.

Hence the data is truly unique in that:

1) it is directly relevant to space travel (heavy ions and protons),

2)the number of animals that are being studied (750 enrolled in year one, 1200 enrolled in year two, 600 enrolled in year 3) in order to more accurately examine effects of radiation at lower doses

3) the future plans to study multiple body systems (complete necropsies) for radiation effects from sexual maturity through aged animals (lifetime study) and

4) for the plans to test the hypothesis that chemopreventatives can be effective in mitigating risks associated with low dose radiation exposure for cancers that may occur later in life (effects of tamoxifen on breast cancer)

These studies are labor intensive because of the regular palpation of animals required, survival surgery procedures, complete necropsies and histopathology on all study animals, tissue preparation and archiving, and plans for administering tamoxifen continuously for the life of the animals.

Results-Radiation Effects

Histopathology

For our studies we have irradiated young virgin rats at approximately 2 months of age (sexual maturity). The developing mammmary gland in young virgin rats is composed of an interconnecting system of branching tubular structures lined by epithelium and ending as blind sacs terminally as structures called terminal end buds (TEB). The TEB's asynchronously differentiate over time and their development is sensitive to hormones of the female reproductive cycle. They progressively differentiate into alveolar buds and alveolar lobules. In the mammary gland, the number of proliferating cells is greatest and the cell cycle shortest in the least differentiated structure, the TEB, while the alveolar buds have the fewest proliferating cells and the longest cycle. It appears that the TEB plays an important role in giving rise to mammary carcinomas while benign lesions such as adenomas, cysts, and fibroadenomas appear to arise from the more differentiated alveolar buds.

Histologically the mammary gland consists of compound tubuloalveolar glands which form irregular branching tubules with evaginations from their walls and from their blind ends. Secretory portions of the gland are located at the terminal portion of the branches. This terminal secretory portion is referred to as the ductule or alveolus. The ductules form a compact cluster around small intralobular ducts and are collectively referred to as the lobule, the functional unit of the mammary gland. These intralobular ducts join to form interlobular ducts which empty into the main lactiferous duct.

We have encountered non-neoplastic lesions in the mammary glands of the rats under study. These must be distinguished from benign and malignant neoplasms. Lobular hyperplasia consists of enlarged lobules of relatively normal appearing alveoli. The lack of a prominent collagenous stroma has been used in our study to differentiate these lesions from the fibroadenoma, a common radiation-induced benign tumor of the rat mammary gland. In atypical hyperplasia there is cellular atypia of the duct epithelium or alveoli along with papillary infoldings, arches, solid nests or plaques extending inward from the duct wall. Features of cellular atypia have included enlarged cells with vesicular or hyperchromatic nuclei. Cystic changes within the mammary parenchyma have been the most common non-neoplastic change which we have encountered. These thin-walled, epithelial-lined markedly dilated spaces often contain a granular, eosinophilic, secreted material or cholestrol crystals. On palpation, these cysts have been up to several millimeters in diameter and are soft and flucuent.

Fibroadenomas have been the most common benign neoplasm which we have encountered in the rat mammary gland. They are composed of abundant connective tissue, often densely packed, along with clusters of mammary epithelial cells. There has frequently been variation in the proportion of connective tissue to epithelial cells encountered in the rat mammary tumors examined to date. This has varied not only from tumor to tumor, but also on occasion within a single tumor. Fibroadenomas have had one of two patterns. A lobular pattern separated by dense layers of mature collagenous connective tissue. The ductules have been lined by a single layer of epithelium with small nuclei and a single nucleolus. A second pattern in some of the fibroadenomas has consisted mainly of multiple concentric layers of densely packed connective tissue with a small number of widely dispersed ductules with attenuated or atrophic epithelium. On rare occasions we have had fibroadenomas which contained focal areas of atypia or even adenocarcinomas. Depending on the degree of atypia and growth pattern, these have been classified as carcinomas even though the majority of the tumor removed is a fibroadenoma.

Fibroadenomas have been a frequently encountered benign neoplasms in our irradiated animals. These, like carcinomas, are radiation-induced tumors even though they remain benign in their behavior. The benign fibroadenomas, however, continue to proliferate and grow and must be promptly surgically removed in order to follow irraditated female Sprague-Dawley rats for lifespan. The number and size of fibroadenomas, if not removed, eventually becomes a limiting factor for studies of aging, irradiated, female Sprague-Dawley rats.

Additional benign neoplasms which we have encountered are adenomas. Adenomas consisting mainly of glandular epithelial-lined acini with little stroma and with a tubular, secretory, or papillary pattern have also been removed surgically.

Radiation-Induced Breast Cancer

Mammary carcinomas (adenocarcinomas) are a radiation-induced malignant tumor that arises with increasing frequency during aging in our irradiated female Sprague-Dawley rats. These tumors have three main distinguishing features: 1) a loss of the normal tubuloalveolar pattern of the mammary gland, 2) cellular features of malignancy including cellular atypia, increased nuclear to cytoplasmic ratio, altered chromatin content, prominent nucleoli, increased numbers of cells in mitoses and abnormal mitotic figures, and cellular and nuclear pleomorphism. These tumors have frequently been accompanied by a prominent inflammatory infiltrate in the stroma. This usually has consisted of mainly mononuclear cells, but has also on occasion consisted mainly of eosinophils. In addition, a subset of these tumors are locally invasive through the tumor capsule or into surrounding muscle. Additionally, distant metastasis to the lung and other organs also occurs.

Radiation-induced adenocarcinomas have exhibited a broad range of patterns. The most common pattens have included:

1) Papillary pattern consisting of multiple branching papillae covered by one or more layers of cuboidal to columnar epithelial cells orented perpendicular to the fibrovascular core.

2) Tubular pattern characterized by closely packed tubular structures which vary from round to elongated. The tubules have been lined by one or more layers of epithelial cells with the tubular lumina being small and empty.

3) Cribiform pattern has a sieve-like appearance in which sheets of epithelial cells have numerous secondary lumina or small round spaces filled with proteinaceous secretion.

4) Comedo pattern which is characterized by distended ductules filled with sheets of neoplastic epithelial cells and a central cavity filled with necrotic cells, cellular debris, and sometimes calcifying concretions.

5) Solid pattern is characterized by sheets of malignant cells that don't form well-defined acinar structures.

In older rats that have received irradiation, adenocarcinomas also arise within benign fibroadenomas. This is a rare occurrence in spontaneous mammary tumors, but appears to occur more frequently in the irrradiated animals. The tumors have mainly a papillary growth pattern of transformed epithelial cells.

Examples of the histopathology of many of these and additional tumor patterns of interest are included in the appendix.

Results-Chemoprevention

Pilot Tamoxifen Study

Dramatic recent research findings have focused new attention on a class of compounds known as selective estrogen receptor modulators. These agents behave as estrogens in some tissues, but block its action in other tissues. The prototype compound of this class, tamoxifen, has been shown in a large study, reported a little over two years ago, to prevent breast cancer in women who are at high risk for developing the disease. Prior to this no drug had ever been shown clearly to prevent the development of primary breast tumors. A related compound, raloxifene, used for osteoporosis treatment may also protect against breast cancer in a similar way. This is not only relevent to individuals with an increased risk of breast cancer due to increased radiation exposure during space travel, but it also is of importance to the general public. One of eight women will develop breast cancer during their life time. The breast is one of the most radiation-

sensitive organs in the body for the carcinogenic effects of radiation. Radiation exposure is a known risk factor for the development of breast cancer.

A pilot tamoxifen study for the prevention of radiation-induced mammary carcinomas was initiated using a limited number of animals (12) and photon irradiation. This was seen as an important step to take in preparation for large scale tamoxifen chemoprevention studies. Our pilot study results supported use of tamoxifen as a chemopreventative that could mitigate the increased risk of radiation-induced breast cancer that could occur due to increased exposure to radiation in space.

Large scale Tamoxifen studies:

As described previously large scale studies of the effectiveness of tamoxifen against mammary neoplasms induced by photons, protons, and iron ions are underway. The findings of the effectiveness of tamoxifen in chemoprevention of radiation-induced mammary cancer has been demonstrated also in preliminary results from large scale tamoxifen studies that are underway using the Sprague Dawley rat model. The data is summarized below.

Dietary Restriction:

Recently it was shown that decreased weight gains that occur due to tamoxifen administration can have a significant impact on the rate of mammary tumorigenesis beyond the direct effects of tamoxifen. For this reason we placed all control animals on dietary restriction to match the average weight gains seen in the tamoxifen treated animals. The results of weight analysis is seen in a figure in the appendix.

Photon Irradiation

Continuous tamoxifen administration following photon irradiation was remarkably effective in preventing mammary carcinomas in this cohort. Without tamoxifen administration there was a dose-response effect beginning to emerge where the carcinoma incidence correlated with the dose of photon irradiation to which the animals were exposed. These results are in the appendix.

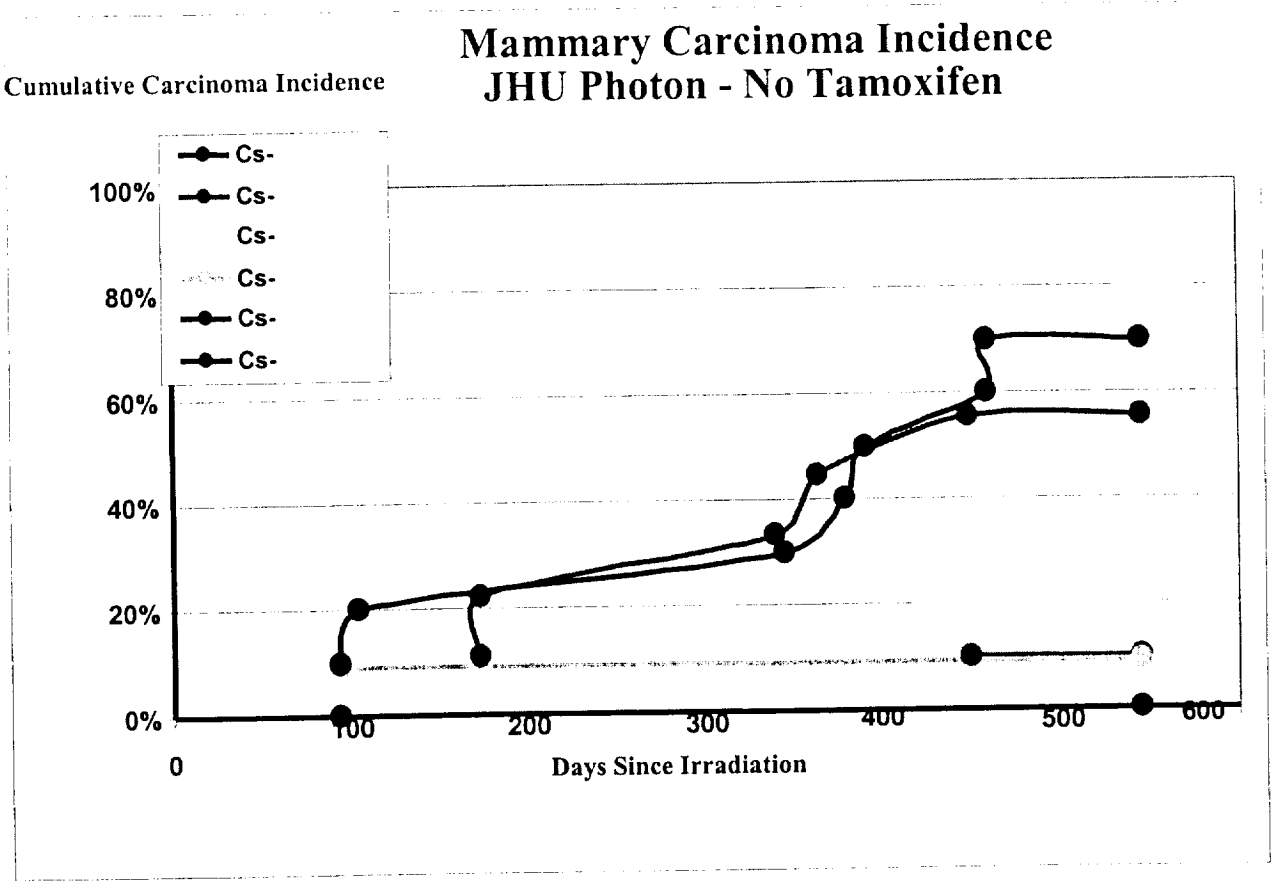
Iron Irradiation

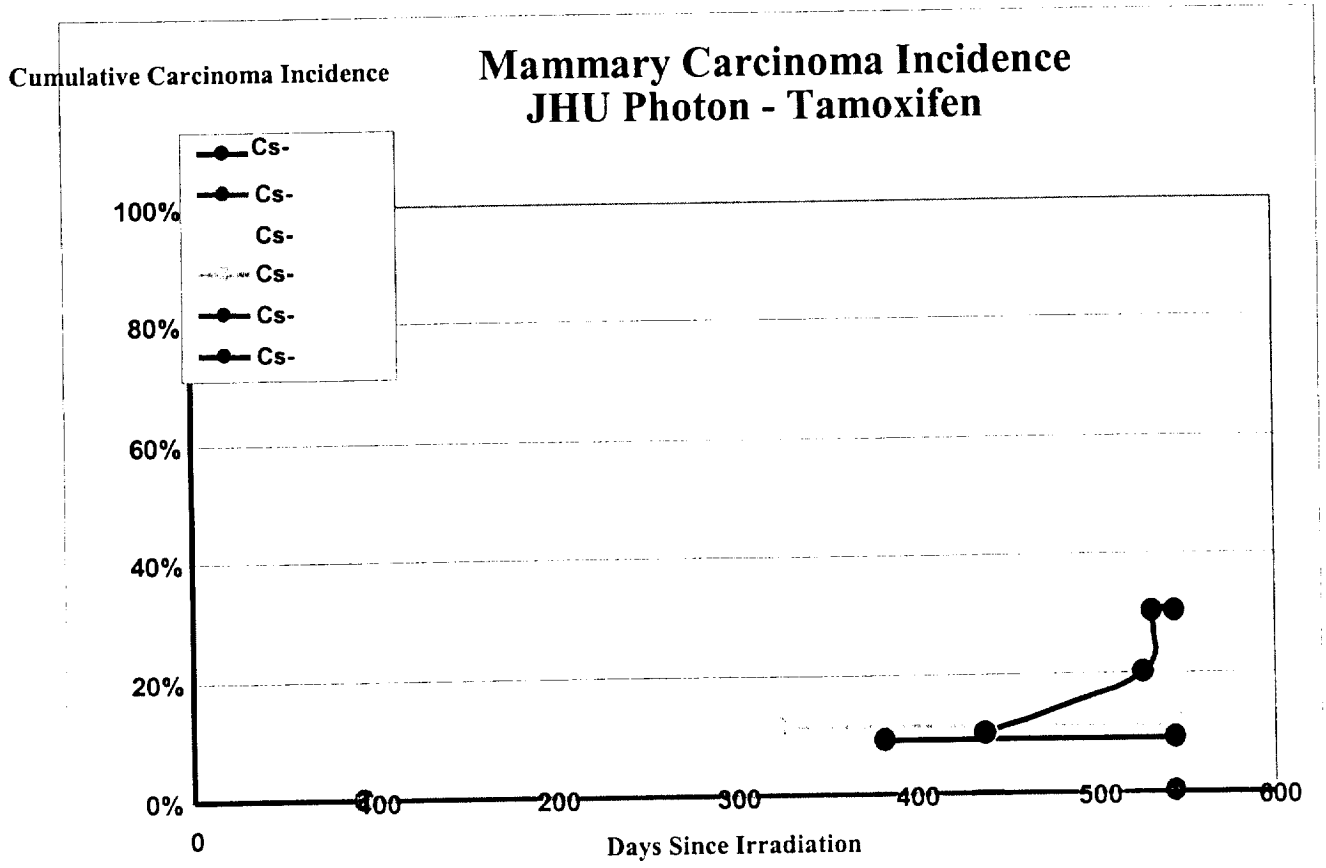
Continuous tamoxifen administration during the first year following heavy ion (iron ion) irradiation was very effective in preventing early carcinomas that arise during this period. A dose-response effect is emerging for irradiation dosage when correlated with the incidence of mammary carcinomas. The figure for these results is shown in the appendix.

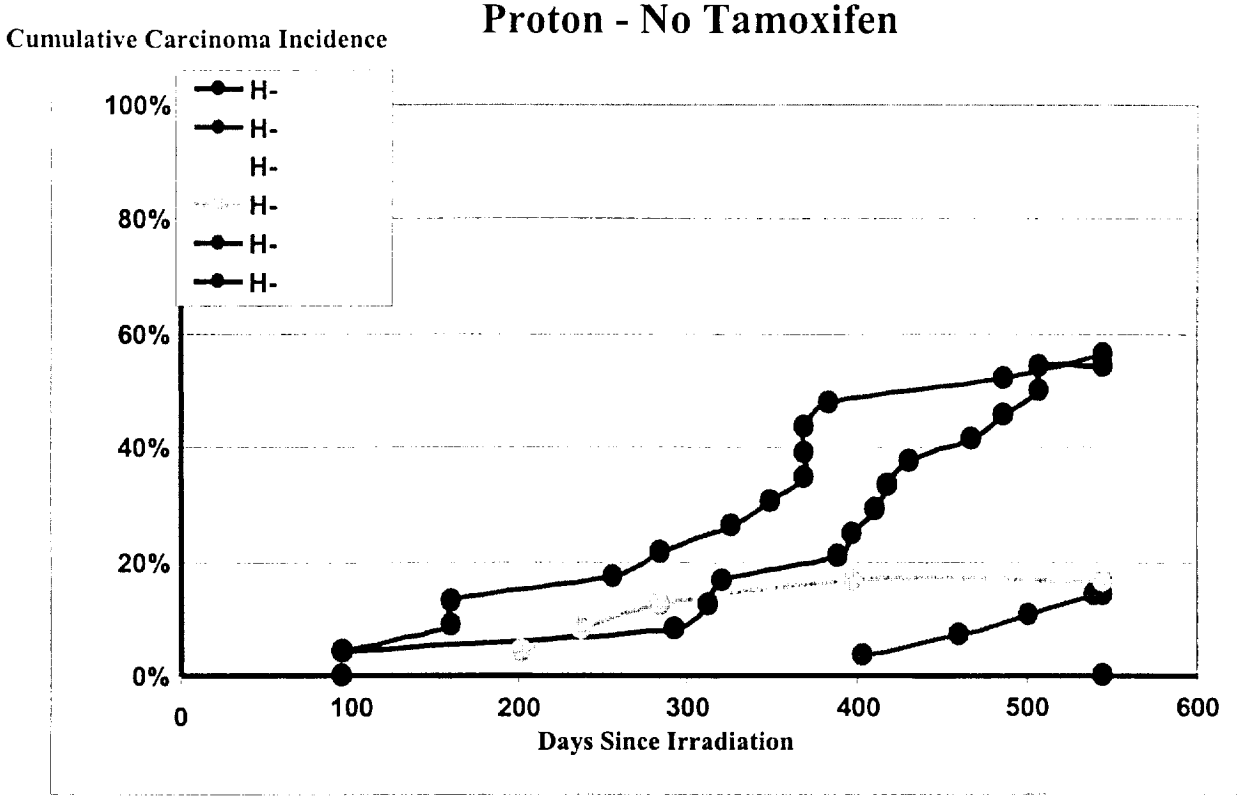
Proton Irradiation

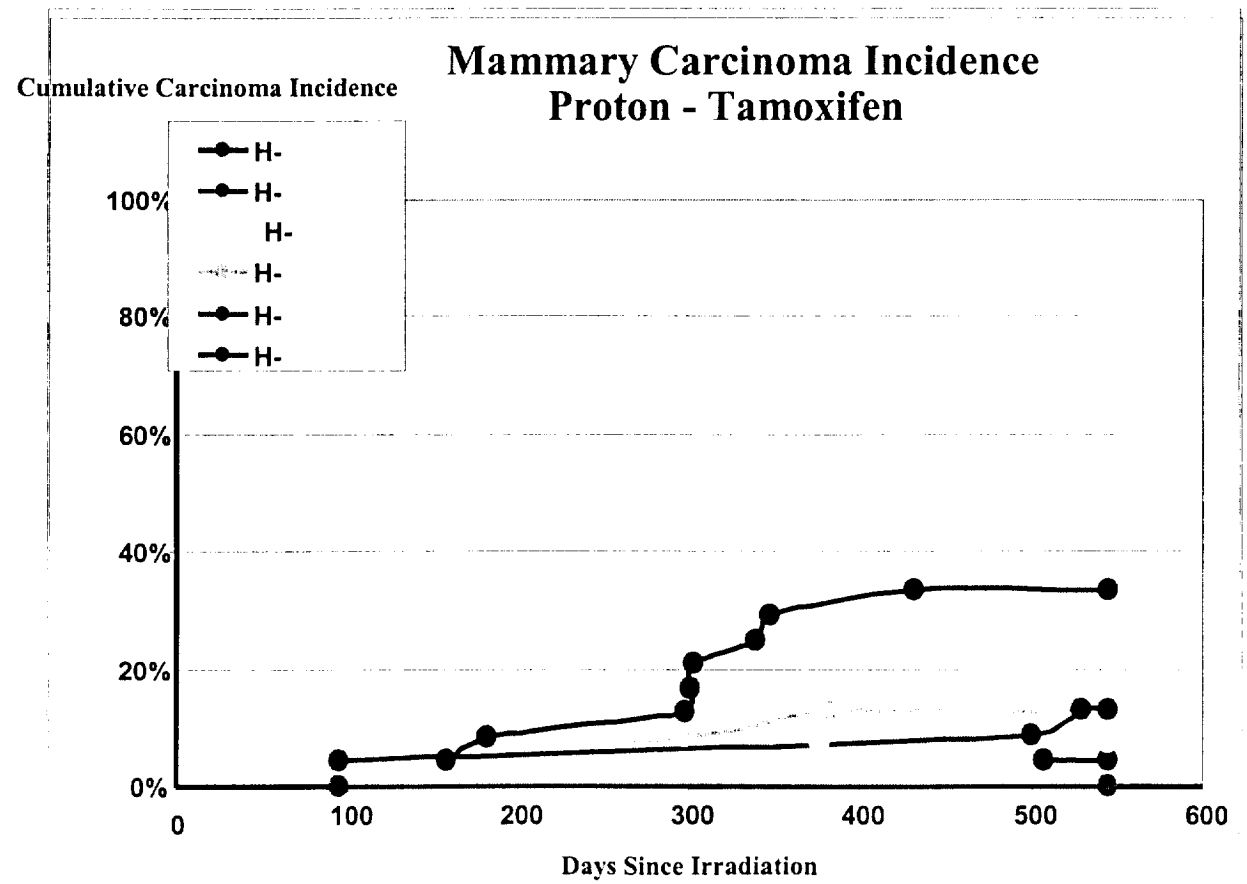
Interestingly, while tamoxifen was mostly effective in preventing proton-induced mammary carcinomas, tamoxifen was completely ineffective in preventing carcinomas induced in one of the high dose groups. It will be of interest to continue to follow these preliminary results over time to see if this trend continues. The reason for these findings are not clear at this point, since tamoxifen appears to be effective in reducing the incidence of early mammary carcinomas induced by low doses of proton irradiation.

A summary of the data for each dosage group follows:

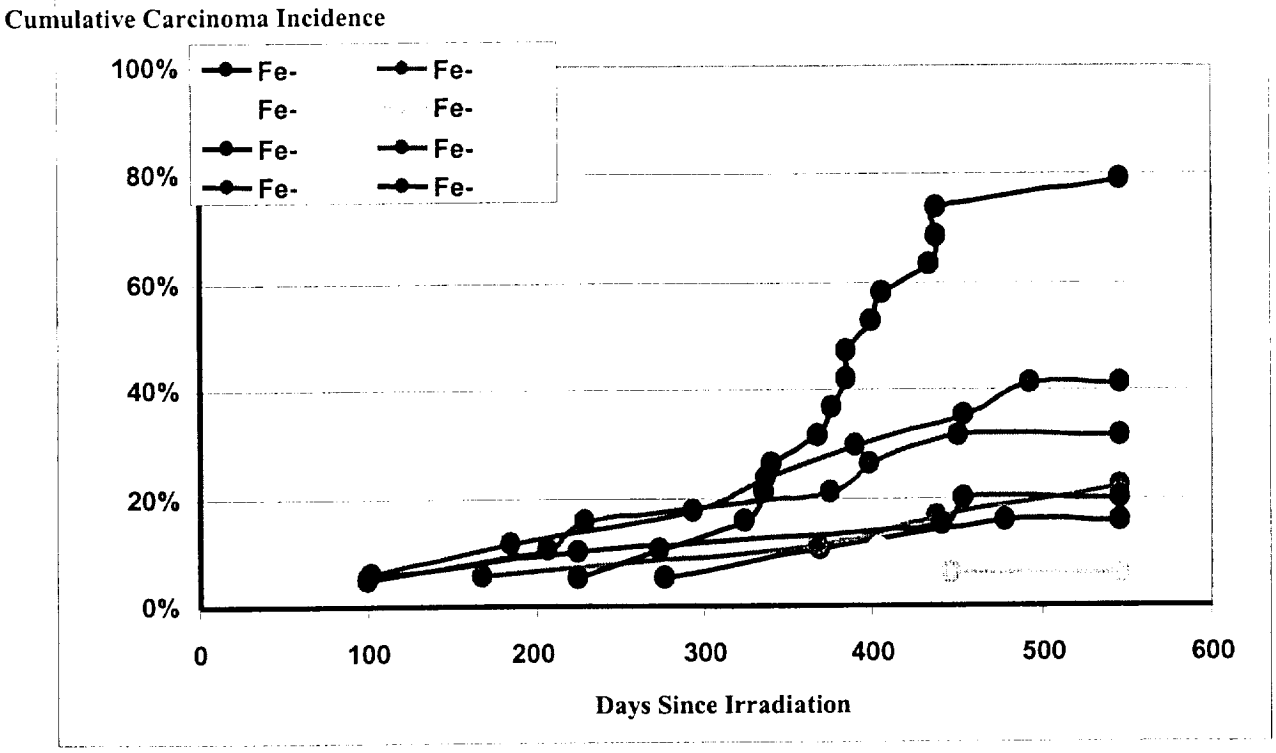




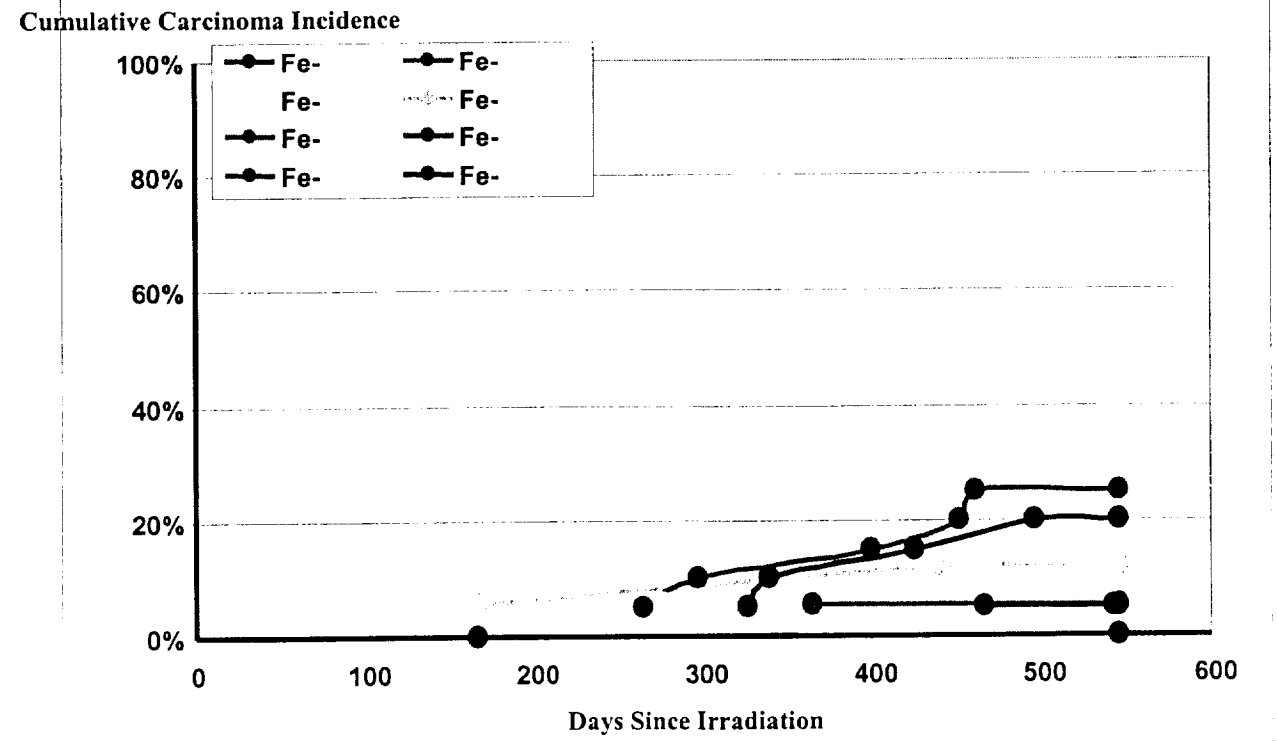

Mammary Carcinoma Incidence
Proton - No Tamoxifen
Cumulative Carcinoma Incidence
H-
H-
H-
H-
H-
H-
100%
80%
60%
40%
20%
0%
0
100
200
300
400
500
600
Days Since Irradiation

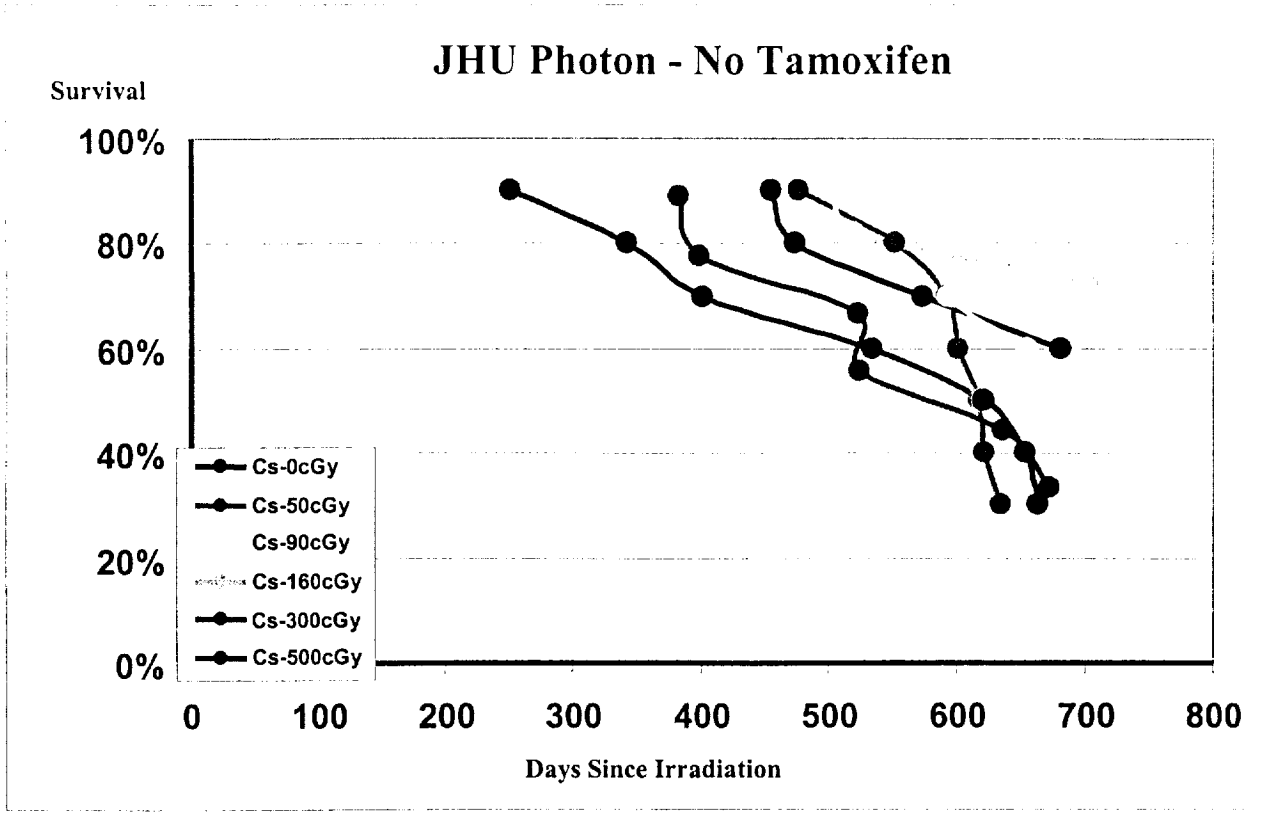

Mammary Carcinoma Incidence
Proton - Tamoxifen
Cumulative Carcinoma Incidence
H-
H-
H-
H-
H-
H-
100%
80%
60%
40%
20%
0%
0
100
200
300
400
500
600
Days Since Irradiation

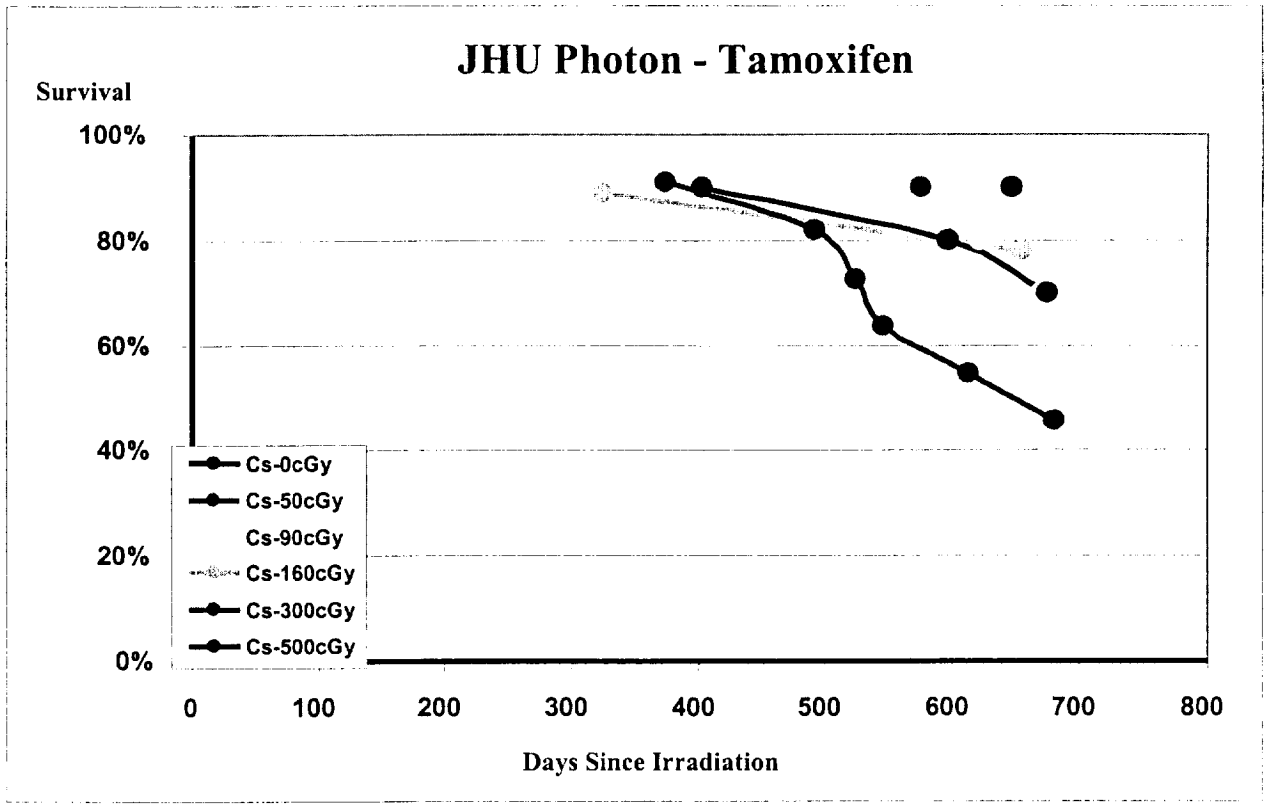
Mammary Carcinoma Incidence
Iron - No Tamoxifen

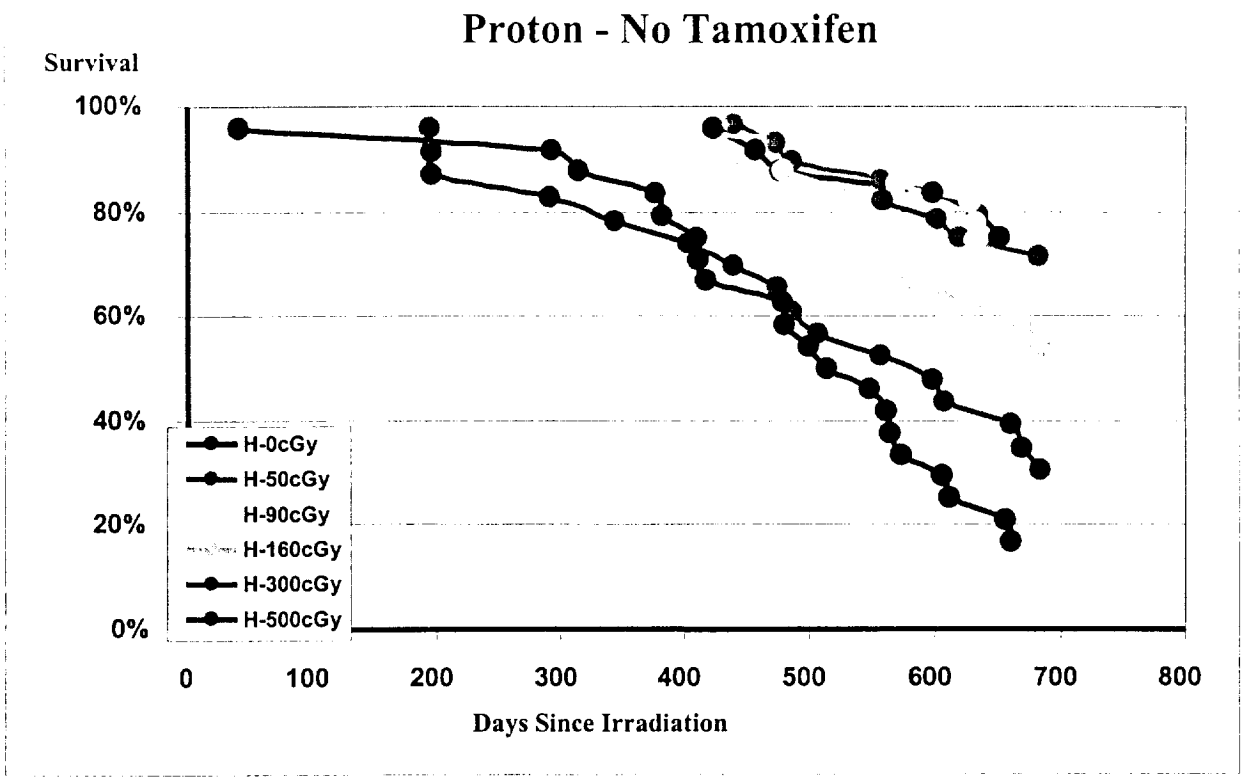


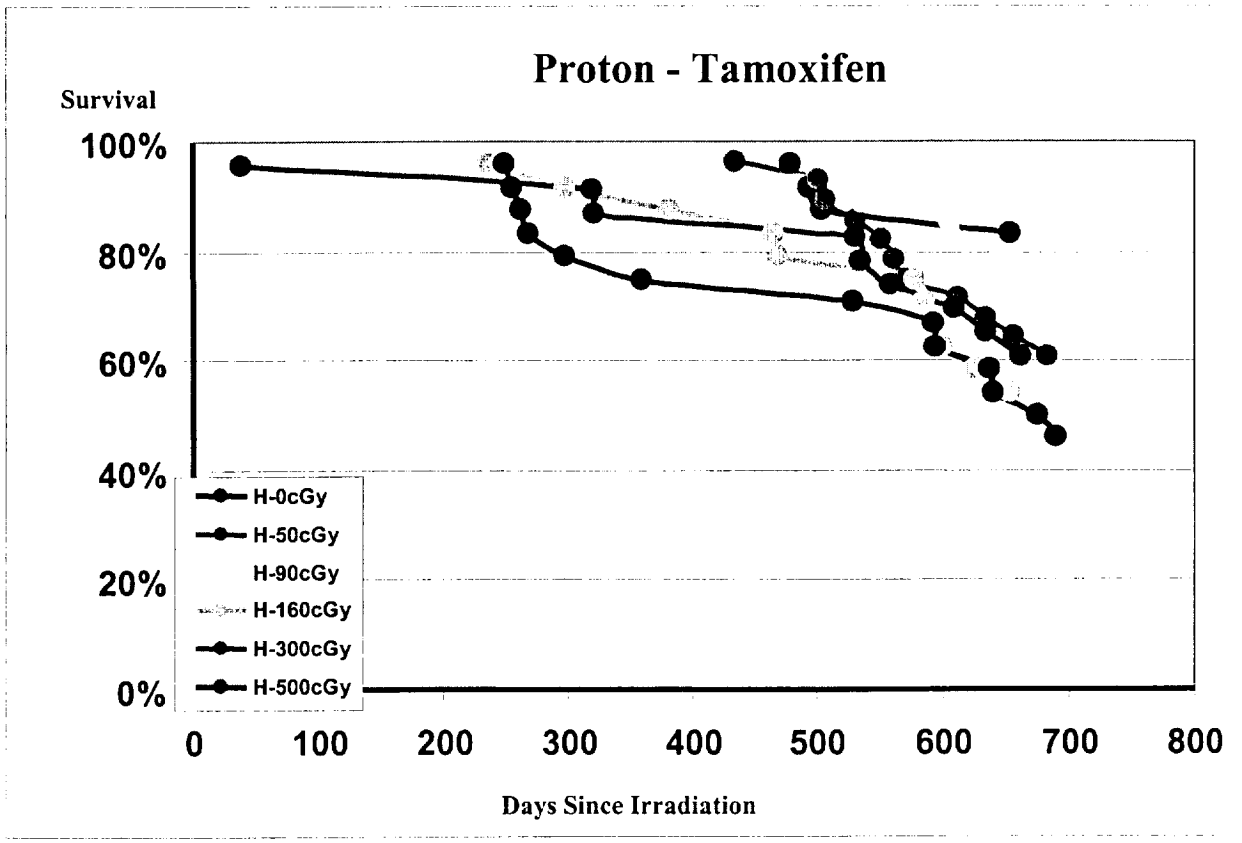
Mammary Carcinoma Incidence
Iron - Tamoxifen



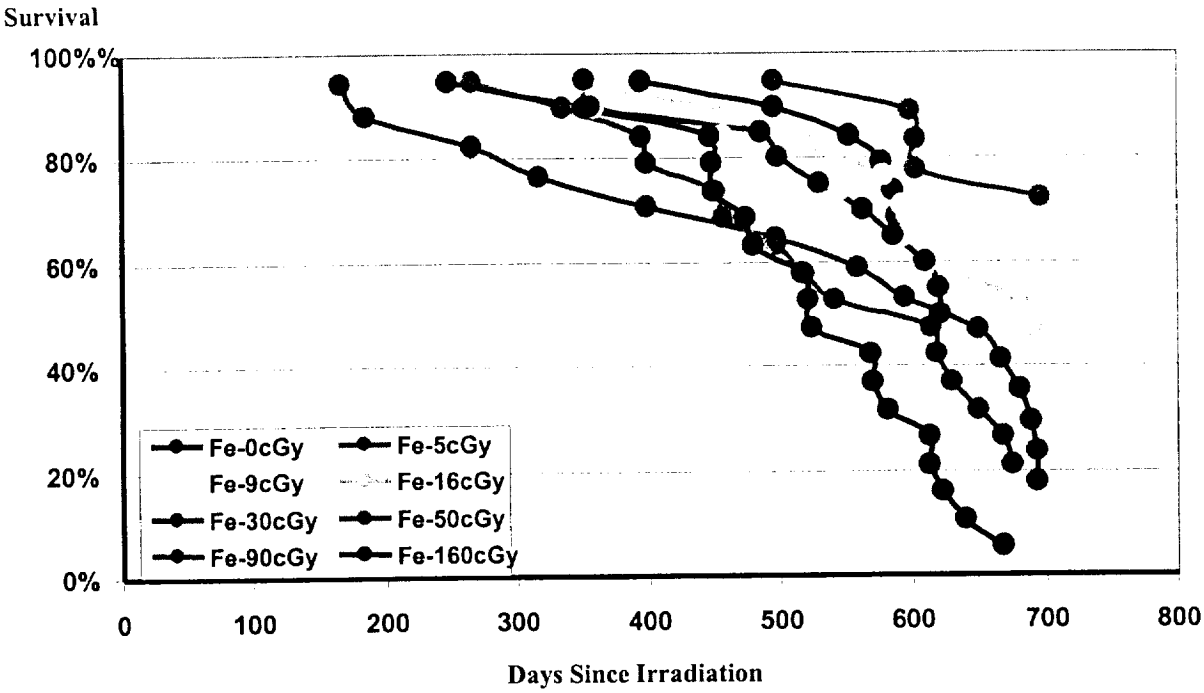

JHU Photon - No Tamoxifen
Survival
100%
80%
60%
40%
20%
0%
Cs-0cGy
Cs-50cGy
Cs-90cGy
Cs-160cGy
Cs-300cGy
Cs-500cGy
0
100
200
300
400
500
600
700
800
Days Since Irradiation


JHU Photon - Tamoxifen
Survival
100%
80%
60%
40%
20%
0%
Cs-0cGy
Cs-50cGy
Cs-90cGy
Cs-160cGy
Cs-300cGy
Cs-500cGy
0
100
200
300
400
500
600
700
800
Days Since Irradiation

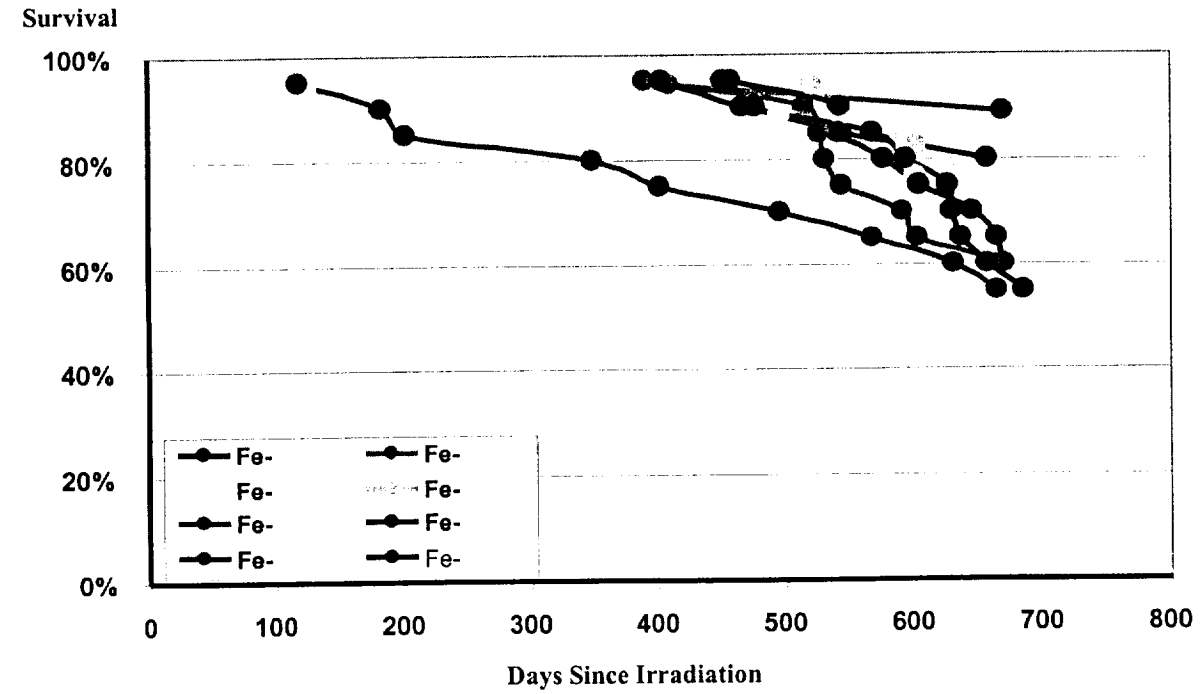

Proton - No Tamoxifen
Survival
100%
80%
60%
40%
20%
0%
H-0cGy
H-50cGy
H-90cGy
H-160cGy
H-300cGy
H-500cGy
0
100
200
300
400
500
600
700
800
Days Since Irradiation


Proton - Tamoxifen
Survival
100%
80%
60%
40%
20%
0%
H-0cGy
H-50cGy
H-90cGy
H-160cGy
H-300cGy
H-500cGy
0
100
200
300
400
500
600
700
800
Days Since Irradiation

Iron - No Tamoxifen



Iron - Tamoxifen



IV-3A. Chang's Project: Charged Particle Radiation-Induced Genetic Damage in Transgenic Mice

Four distinct populations of circulating erythrocytes are examined using flow cytometry: immature polychromatic erythrocytes (RETs), micronucleated RETs (MN-RET), mature normachromatic erythrocytes (NCE) and micronucleated NCEs (MN-NCE). Our results (Figure 1) show that the frequency of MN-RET varies as a function of time post irradiation, with a dose-dependent peak induction of up to 6-fold above control levels at 48 hrs after proton exposure. Linear increase in % MN-RETs from low doses of 0.1 Gy, reaching a plateau level at 1 – 2 Gy protons, followed by a decrease at higher (4 Gy) doses of protons. The number of circulating RET rebound at 1 week post irradiation for low doses, but remain depressed in animals exposed to high doses (4 Gy) protons, suggesting systemic toxicity. The level of MN-RET also returned to control levels within 1 week after low doses of protons. We have previously measured the kinetics of removal of MN-RET from circulating RET after a 1 Gy dose of 1 GeV iron particles. The kinetics of removal of MN-RET from circulating reticulocytes after an equi-dose of proton or iron particles exposure appears to be similar. We have, therefore, demonstrated that low doses of protons are effective in inducing elevated levels of MN-RETs in peripheral blood of mice, in an early time course after radiation. However, although the aberrant RETs are eliminated from the peripheral circulating erythrocyte populations within 1-week post-proton exposure, long-term persistent consequences of radiation exposure in tissues are not addressed in these studies.

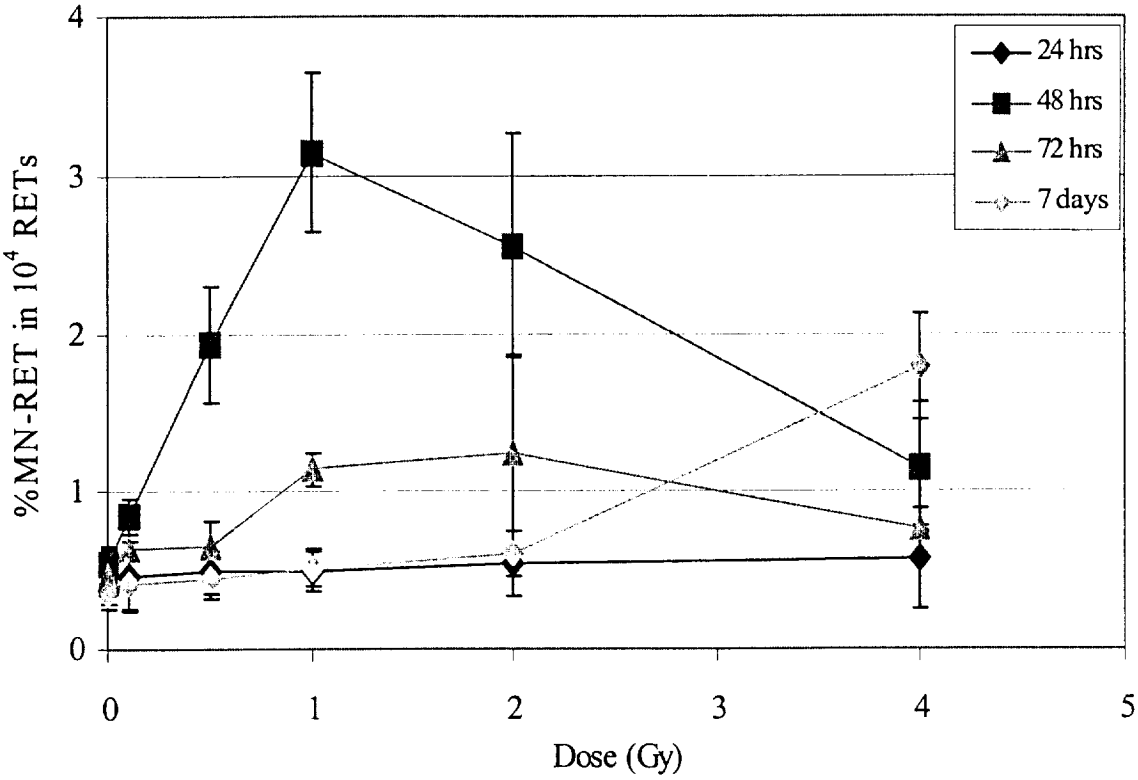


Figure 1: Dose-dependent increase in the level of circulating micronucleated reticulocytes in mice at 24-hrs, 48-hrs, 72 hrs and 7-days post proton irradiation. Error bars denote animal to animal variation within the same dose group.

In addition to the peripheral blood compartment, we measured %MN-RET in the bone marrow compartment as at 8 and 16 weeks after 4 Gy proton exposure. Our results suggest that the level of %MN-RET in bone marrow parallels those observed in the peripheral blood at 8 and 16 weeks for this dose.

Lymphocytes from peripheral blood and bone marrow were harvested at 1, 8 and 16 weeks after proton exposure, cultured and the determination of chromosome aberrations in these samples using both conventional Giemsa staining and FISH are in progress.

We examine proton radiation-induced target transgene mutation incidences in the spleen of *lacZ* animals. A minimum of 300,000 transformants was counted for each tissue sample and frequencies were calculated from the number of mutant per 10^5 transformants. We observed small increases in *lacZ* MF in the spleen at 1 week after radiation. The proton radiation induced MF was more significant at 8 weeks after radiation (Figure 2). Statistical analysis of the *lacZ* MF using the Cochran-Armitage analysis method for mutation analysis showed that proton-induced MF was significantly higher than the spontaneous *lacZ* MF for 2 and 4 Gys of protons at 1-weeks after an acute exposure. At 8 weeks after proton exposure, radiation-induced MFs were significantly above spontaneous MF for all doses $\geq$ 0.5 Gy protons. It appears that the induced MF peaked at about 1 Gy protons. We hypothesize that at higher doses, some heavily damaged cells were eliminated in the highly proliferative spleen tissue resulting in a lower induced MF.

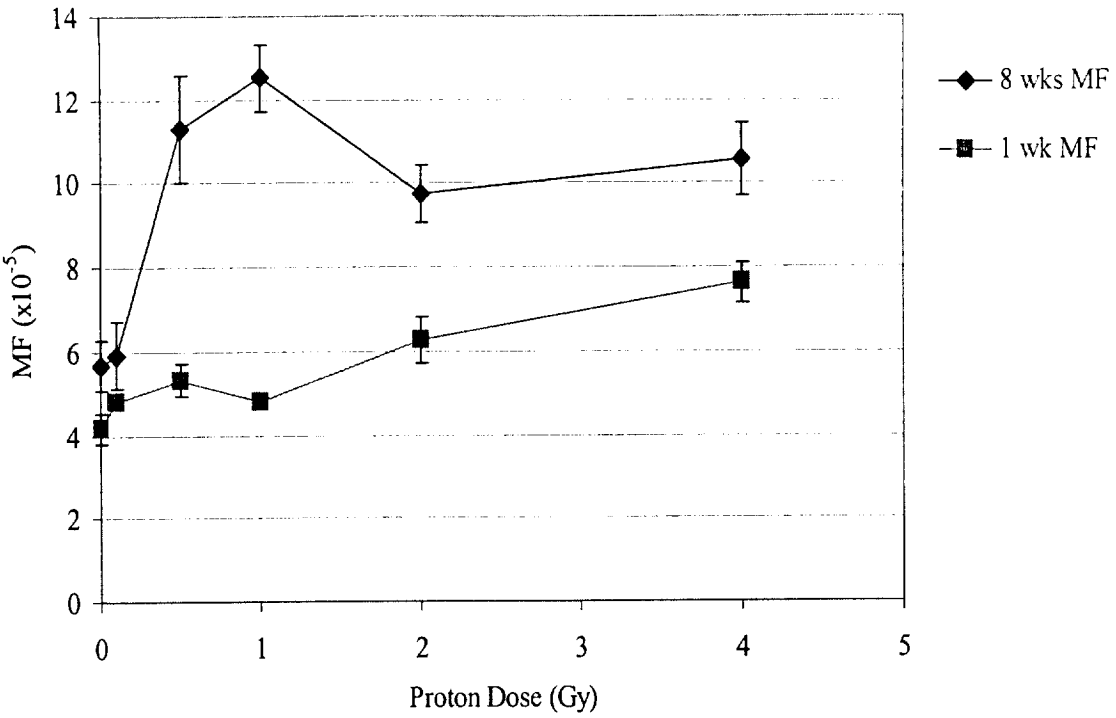


Figure 2: Mutation Frequency (MF) of *lacZ* transgene as a function of proton dose. Data from 4 – 6 animals were used in each dose point and standard errors are shown for each dose-time point.

IV-4A. Vazquez's Project: Risk Assessment and Chemoprevention of HZE Induced CNS Damage
In summary, during this award period, we studied the dose- and time-dependent radiation-induced responses in *lacZ* transgenic mice after proton radiation. We demonstrated that we can detect cytogenetic damage in circulating reticulocytes at proton doses as low as 0.1 Gy and mutagenic effects in *lacZ* transgene at doses of 0.5 Gy and above.

WHAT HAS BEEN DONE? During BNL-7 we were able to expose NT-2 cells (human neural precursor cells) to graded doses of iron ions (I GeV) in consecutive runs. Samples were exposed to 0, 0.05, 0.1, 0.25, 0.5, 0.75, 1 and 2 Gy at room temperature. Samples were processed at T0, T8, T12, T24, T48, and T72 hrs post-irradiation for the following endpoints:
1. Apoptosis induction: cells were processed for FACS analysis (detection of ANNEXIN V) and Laser Scanning Cytometry (LSC) to detect residual DNA damage (Apodirect) and cell cycle analysis.
2. Cell Toxicity: cell damage was measured by fluorescent microscopy (Live/Dead Kit), conventional microscopy (ratio of attached/floating cells) and colorimetric methods (XTT).
3. Gene expression: western blot analysis of p53 expression

RESEARCH PROGRAM ACCOMPLISHMENTS

2001 High-LET radiation experimental runs:

- January: Brookhaven National Laboratory, AGS: Fe ions (6 hrs of beam time)
- February: National Institute of Radiological Sciences, HIMAC, Chiba Japan, C, and Si ions (4 hrs of beam time).
- June: Loma Linda Medical Center, CA: protons (8 hrs of beam time)
- July: National Institute of Radiological Sciences, HIMAC, Chiba Japan: Si, Ar and Fe ions (8 hrs of beam time)

Human neural precursor cells (NT2), neurons (hNT) and rodent glial progenitor cells (CG4) were exposed to acute doses of 0.1, to 6 Gy of heavy ions (Fe, C, Si and Ar) and proton irradiation and the following endpoints were measured:

1. Apoptosis induction: cells were processed for FACS analysis (detection of ANNEXIN V) and Laser Scanning Cytometry (LSC) to detect residual DNA damage (Apodirect) and cell cycle analysis.
2. Cell Toxicity: cell damage was measured by fluorescent microscopy (Live/Dead Kit), conventional microscopy (ratio of attached/floating cells) and colorimetric methods (XTT).
3. Gene expression: western blot analysis of p53 expression

RESULTS:

Apoptosis Induction: We measured the induction of apoptosis by flow cytometry using a FACSCalibur to detect the expression of Annexin V, as an early marker in the apoptotic pathway, in NT-2 cells. The ApoAlert Annexin V assay is based on the observation that soon after initiating apoptosis, most cell types translocate phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a FITC conjugate of annexin V, a protein that has a strong natural affinity for PS. Externalization of PS occurs earlier than the nuclear changes associated with apoptosis, so the ApoAlert Assay detects apoptotic cells significantly earlier than do DNA-based assays. Data gathered during BNL-7 indicate a strong dose- and time-dependent induction of apoptosis in NT-2 cells with the peak of apoptosis appearing at 72 hours post-irradiation (Figure 1). It was

determined that Fe ion exposure were more effective to induce apoptosis (Annexin V expression) in comparison to protons and gamma rays, suggesting an high RBE values for apoptosis in exposed NT2 cells at 72 hr post-irradiation. Samples processed for LSC were shipped to Loma Linda for DNA damage and cell cycle analysis. Unfortunately, the quality of the samples was not sufficient to obtain reliable data. We expect to repeat this set of samples during BNL-8.

Cell Toxicity: Cell damage at different time points after exposure to charge particle radiation was determined by using the Live/Dead Viability/Cytotoxicity Kit on attached cells from 4 well plates. The LIVE/DEAD Kit provides a two-color fluorescence-based cell viability assay that allows the simultaneous determination of live and dead cells. The cell-permeant esterase substrate calcein AM is non-fluorescent until converted by enzymatic activity to highly fluorescent calcein, which is retained within live cells and imparts an intense green fluorescence. Ethidium homodimer-1 undergoes a fluorescence enhancement upon binding nucleic acids, producing a bright red fluorescence. This dye is excluded from cells that have intact plasma membranes but is readily able to enter dead cells. Thus, live cells fluoresce green, while dead cells fluoresce red. Results obtained from BNL-7 indicated a dose- and time dependent increase of cell damage (Figure 2). Doses as low as 0.1 Gy were able to induce a significant increase of cell damage in comparison to controls. Colorimetric (XTT) assays and the evaluation of the number of attached and floating cells confirmed this trend.

Gene Expression: Conventional western blot techniques were employed to monitor p53 and WAF-p21 gene expression. A subset of NT2 cell samples were lysed at different time points for the determination of p53 and WAFp21 expression using monoclonal antibodies against human p53 and p21. In BNL-7 we observed that p53 is over-expressed as a function of dose of particle exposure and time post-exposure. Doses as low as 0.25 Gy were able to up-regulate p53 and as early as 24 hours post-exposure. Doses of 0.75 Gy were able to up-regulate p53 as early as 12 hours post-exposure (Figure 3). Similar results were obtained when NT-2 cells were exposed to gamma or proton exposures, although higher doses were required to obtain similar degree of expression. These results appear to confirm that p53 gene is involved in the stress pathway induced by low- and high-LET radiation exposures. Up to this point we were not able to confirm changes in the expression of WAFp21 after radiation exposure in NT2 cells.

2. WHAT ARE YOU GOING TO DO?

For the upcoming run, we plan to expose NT2, hNT, CG4 cell lines and oligodendrocytes to low fluences of 1 GeV/n iron and silicon ions. We plan to organize our experiments in two runs of 7 hr for Fe ions and 3 hr for Si ions each (total beam time: 10 hr). During the first run we will expose NT2, hNT, CG4 cells and oligodendrocytes cultured in slide chambers, slide flasks and 96 well plates. For the second run, we plan to expose NT-2 and hNT cells to low fluences of Si ions (1 GeV/n). Cells will be exposed at 70% of confluence to doses of iron ions representing averaged fluences of 0, 0.5, 1, 2, 4 and 8 particles per cell. Samples will be harvested or processed for cell viability (XTT), and using fluorescent probes (Live and Dead assay). DNA damage-apoptosis will be monitored by conventional fluorescent microscopy (hoescht 33258) detection, APO-DIRECT and annexin V assays using FACS or laser scanning cytometer (LSC). Images from cultures will be acquired periodically to measure neurite outgrowth using ImageTools software for image analysis. For p53/WAFp21 gene expression studies, samples will be processed for western blot and RT-PCR. Conventional immunofluorescence microscopy will be employed for phenotypic cell characterization.

For a detailed description of the experimental design and beam time requirements, please review **Apendix I** (POWERPOINT PRESENTATION FILE)

Curtis S.B., Vazquez M.E , Wilson J.W., Atwell W., Kim M. and Capala J. Cosmic ray hit frequencies in critical sites in the central nervous system. *Adv. Space Res.* Vol. 22(2): 197-207 (1998).

Vazquez M. E. Neurobiological problems in long-term deep space flights. *Adv. Space Res.* Vol. 22(2):171-183, (1998).

Curtis S.B., Vazquez M.E , Wilson J.W., Atwell W. and Kim M., Cosmic ray hits in the central nervous system at solar maximum. *Adv. Space Res.* Vol. 25(10): 2035-2049, (2000).

Vazquez M. E. and Kirk E., In vitro neurotoxic effects of 1 GeV/n iron particles assessed in retinal explants. *Adv. Space Res.* Vol. 25(10): 2041-2049, (2000).

Vazquez M.E., Summary of the Biology Presentations and Discussions, Exploring Future Research Strategies in Space Radiation Sciences, Proceedings of the 2nd International Space Workshop 2000, Edited by H.J. Majima and K. Fujitaka, Iryokagakusha Co., Ltd., pp5-7 (2000).

Furusawa Y., Aoki M., Kanai T., Yatagai F., Yang T., Vazquez M. and Miller J., A Method to Estimate Cell Killing Induced by Heavy Ions as Function of Ion Species and LETs. Exploring Future Research Strategies in Space Radiation Sciences, Proceedings of the 2nd International Space Workshop 2000, Edited by H.J. Majima and K. Fujitaka, Iryokagakusha Co., Ltd., pp104-109 (2000).

Figure 1. Dose-response and time course of charged particle-induced apoptosis in NT-2 cells. NT-2 cells were exposed to graded doses of 1 GeV/n Fe ions and samples were taken at 0, 24, 48 and 72 hours after exposure. The percentage of apoptotic cells in culture were observed by FITC-annexin V flow cytometry. Samples from T 48 were not included due to technical problems in the cell processing.

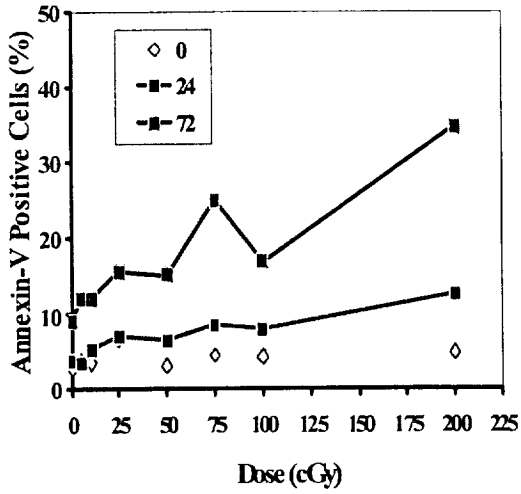


Figure 2. Cell toxicity induced by 1 GeV/n Fe ions at 72 hours post-irradiation. The percent of damaged cells at 72 hours after exposure. Cell damage was determined by using the Live/dead Viability/Cytoxicity kit (Molecular Probes) in attached cells in 4 well plates. Cells were stained by 30 minutes and 400 cells per well were differentially counted using standard a fluorescein and rhodamine filter sets.

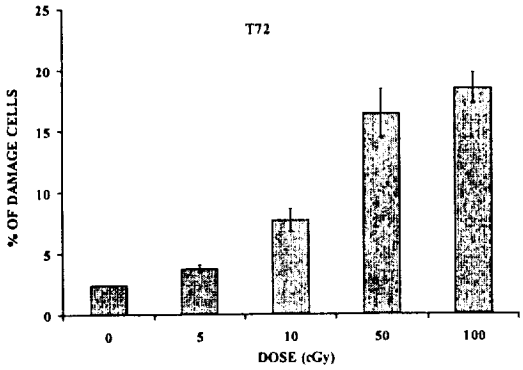
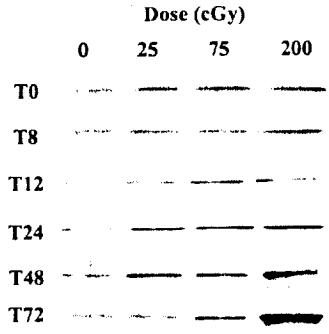


Figure 3. Western blot analysis of 1 GeV/n Fe ion-induced p53 in NT-2 cells. Cells were exposed to graded doses of iron ions and incubated up to 72 hours prior protein processing. Protein lysates of sham and exposed cell cultures were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Subsequently, the expression levels of p53 were monitored by immunoblotting.



IV-5A. Kennedy Project: Countermeasures for Space Radiation Biological Effects

This grant was funded as of 10/01/01; thus, the experiments described for this project have only recently begun (this portion of the report is being written on November 16, 2001). A discussion of research findings is not appropriate at this time. Previous relevant research was discussed in the proposal, and animal protocols have been submitted and approved at both the University of Pennsylvania and the Brookhaven National Laboratory. A proposal for beam time at the Brookhaven AGS facility for the next run was submitted and approved as well, so we are preparing for that run. The next BNL run was originally scheduled for this February, 2002 but has been postponed.

V. FUTURE DIRECTIONS

The major themes of this program are the understanding of risks and the development of effective countermeasures for the radiation-induced biological effects identified to be of major concern: radiation-induced cancer and CNS damage. It is also possible that because of the complexity of the space environment unanticipated effects may occur in organ systems other than the CNS. Thus the major aims will cover five categories:

14. Develop countermeasures for mitigating effects of radiation exposure.
15. Develop markers for determining risks and monitoring the efficacy of countermeasures.
16. Determine carcinogenic and CNS effects for space radiation.
17. Determine acute and long-term pathological responses of rapidly renewing organ systems at risk.
18. Characterize differences in cell and molecular mechanisms for pathological effects for high- versus low-LET radiation in defined model systems.

Major issues remaining to be addressed include:

19. Animal studies of other relevant tissues
20. Interspecies comparisions
21. Animal studies for protracted exposures.
22. In-vivo data for synergistic effects of mixed fields
23. Synergism with other environmental factors such as microgravity and bone loss
24. More comprehensive analysis of human responses to low-dose, protracted exposures.
25. Improved methods for extrapolating animal data to humans too imprecise.
26. Clinical trials, ground-based then flight-based.

The infrastructure for the NSBRI was designed with the research team as a mission-oriented, focused group. Because of the limited amount and high cost of beam time for radiobiological studies with HZE particles,.collaborating and sharing resources have been essential to achieve the goals of Institute and the team. The original programmatic, focused research approach of theNSBRI is ideally suited to achieving the specified goals of NASA for Radiation as exemplified by the success of the Team during the first three-year cycle. The dependency of radiation research upon two large accelerator facilities that only are available a couple of weeks per year and the large costs of assembling and executing critical experiments, necessitate close collaborations and sharing of resources. Our previous studies highlight the strengths of supporting investigators with a combination of expertise working toward a focused objective. Radiation-induced cancer and chemoprevention studies in animal models are complex and requires a variety of expertise in order to carry out meaningful experiments successfully. Providing support

for interaction between team members enabled Dr. Dicello and Dr. Huso to work closely together toward a common goal along with experts at Brookhaven National Laboratories and Loma Linda University and other researchers. This type of interaction is difficult unless a mechanism is available to support interactive project studies as NSBRI has been doing.

Our studies also point to the need to support more than 3 year scientific projects for these types of animal studies. With animal model studies, the major interest in the long-term effects of radiation during aging-often a long time after radiation exposure. NSBRI to date has done an excellent job in continuing to support such long term studies. However, there is concern that important scientific questions will be overlooked if such goals don't fit well into a 3 year, small grant format. In addition, multiple annual evaluations always carry with them a degree of uncertainty and instability, especially for trained personnel who represent a major investment on the part of senior faculty. Availability of longer-term support would provide stability for crucial support personnel which in turn would benefit the NSBRI program.

Organizational charts showing the major research sub-projects we proposed for future studies follow:

V-1 FIVE-YEAR (2001-2006) RESEARCH STRATEGY FOR THE RADIATION-EFFECTS TEAM

	2002	2003	2004	2005	2006
1. Identify Risks Accurately					
• Carcinogenesis					
• **CNS Effects**					
• **Immunological problems**					
2. Test countermeasures					
3. New Team Members					
4. Establish an Education and Outreach Program					
5. Ground Research					
• **AGS, HIMAC, Loma Linda**					
• **BAF Utilization**					

V-2 FIVE-TO-TEN-YEAR (2007-2011) RESEARCH STRATEGY

	2007	2008	2009	2010	2011
1. Validation of ground based risk prediction in space by ISS utilization					
• Carcinogenesis					
• **CNS Effects**					
• **Immunological problems**					
• **Others**					

2. Test countermeasures in space (ISS)					
3. New Team Members					
4. Test Interactions between radiation and microgravity.					
5. Recommendations for risk management and effective radiation countermeasures					

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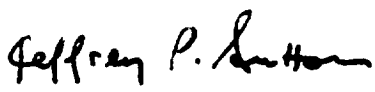
thomasj@ccf.org



Jeffrey P. Sutton, M.D., Ph.D.
October, 2001

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I. EXECUTIVE SUMMARY

The Smart Medical Systems Team (SMST) is one of four new teams of the NSBRI. At the present time, there are eight projects headed by seven principal investigators. Five projects (led by Drs. Crum, Davies, Klempner, Soller and Sutton) address research and development of novel biometric sensors that are lightweight, portable, low power, non-invasive and unobtrusive. These projects have applications for physiological and medical monitoring of astronauts, as well as for the assessment of countermeasures (CMs) that potentially diminish the deleterious effects of long duration space travel. One project (led by Dr. Putcha) develops a novel pharmacological drug delivery system for near term countermeasure administration, while another project (led by Dr. Crum) develops a revolutionary new form of non-invasive surgery. A NASA echocardiographic resource project (led by Dr. Thomas) is supported by the SMST, and is jointly supervised with the Cardiovascular Alterations Team. Three projects (led by Drs. Klempner, Sutton and Thomas) develop "smart" algorithms for minimal user evaluation and interpretation of real time physiological and medical data. All of the projects fit within the strategic plan of the SMST for NSBRI CM development (sections IIIC and IIID). Although the team is newly formed, there are already plans in place for specific flight tests of some technologies, and for applying research discoveries to enhance medical care on earth.

Research on the SMST aligns itself most closely, albeit not exclusively, with the clinical capabilities category of the Critical Path Roadmap (CPR; http://criticalpath.jsc.nasa.gov). NASA has identified six of the projects (headed by Drs. Crum, Klempner, Soller, Sutton and Thomas (×2)) as relating to Trauma and Acute Medical Problems (risk #43), which is only one of four Type I, or highest level, risk factors for long duration space missions. In the development of CMs to diminish risk #43, and other significant biomedical risks, the SMST has laid out a strategic plan for an advanced, integrated and autonomous system for astronaut health assessment, maintenance and medical care. This plan has initiated, and continues to foster, collaboration with NASA flight surgeons and other medical operations personnel and biomedical researchers affiliated with the NSBRI, Johnson Space Center (JSC), Ames Research Center (ARC), the Jet Propulsion Laboratory (JPL) and NASA Headquarters,. The focus of the plan has been at intermediate Countermeasure Readiness Levels (CRLs = 2 to 7), and linkages to NASA programs in medical systems at lower and higher CRLs. Each project within the SMST has been mapped onto the strategic plan to identify strengths and weaknesses of individual projects and the team as a whole.

Within the first year of the SMST, significant advances have been made to coordinate research projects and efforts to provide added value. New intra-team (e.g., Drs. Davies and Thomas) and inter-team (e.g., Drs. Soller and Cabrera (Integrated Human Function Team)) NSBRI collaborations have formed. New collaborations between the SMST and JSC flight surgeons (e.g., Drs. Sutton and Marshburn (JSC)) and researchers (e.g., Drs. Klempner and Pierson (JSC)) have developed. A new biotechnology company, SRU BioSystems, has become involved with the SMST through Dr. Klempner's project. The range in start dates for the projects is October 1, 2000 through September 1, 2001. During this period, the integration of projects and the team's approach to CM development has been refined, culminating in the preparation of a team wide scientific demonstration for the NSBRI External Advisory Council (EAC) meeting scheduled for October 2001.

Among the key findings and discoveries during the first year of the SMST's existence are:

- The development by Dr. Soller and colleagues to accurately and non-invasively measure muscle pH and oxygenation using multi-spectral near infrared (NIR) light in normal subjects and in critically ill surgical patients. This technology has applications for assessing and modifying individual exercise protocols as a CM. Moreover, the capacity to distinguish microvascular differences within and between subjects leads to the potential earth based spin-off to assess microvascular status in patient populations, such as diabetics.

- The derivation by Dr. Soller and colleagues of a mathematical algorithm to remove spectral variability due to human skin color variations. This has relevance to NASA since national and international space missions involve astronaut crews of various ethnic backgrounds.

- The observations by investigators on Dr. Sutton's project of an excellent correlation between diffuse optical tomography (DOT), using NIR spectroscopy (NIRS), and functional magnetic resonance imaging (fMRI) to non-invasively assess human brain activity in subjects performing simple motor tasks. DOT sensor validation is important if the technology is to be used as an objective means of assessing brain function under various cognitive loads and sleep alterations, with the aim of adjusting performance expectations as a viable CM.

- The successful use by Dr. Sutton and colleagues of anatomical and functional MRI data as a constraint on the calculation of deoxyhemoglobin and oxyhemoglobin changes in brain tissue. This finding speaks to the issue of individualized, digitized human, anatomical brain models upon which time-derivative functional data is co-registered and overlaid for automated interpretation in real time.

- The determination by Dr. Sutton and colleagues of learning curves during off-line testing of SpaceDOCK, a visuomotor task developed for the optical / MRI environment. The task emulates a space relevant task for performance assessment using behavioral and brain imaging methods.

- The ability of Dr. Klempner, his co-investigator Dr. Cunningham, and colleagues to fabricate 2D optical gratings (d»0.6mm) embossed into plastic for a novel colorimetric resonant reflectance biosensor, with high (e.g., 10 nm protein thickness) resolution detection capabilities. This revolutionary technology has CM applications relevant to not only Dr. Klempner's space biology project, but also to earth based toxic screening, pharmacology and several recent Department of Defense (DoD) initiatives.

- The successful acquisition and compression by Dr. Thomas and colleagues of ultrasound images from astronauts aboard the International Space Station (ISS), with the finding that good accuracy is maintained even at compression ratios > 100:1.

In addition to findings stemming from individual projects on the SMST, effective synergisms have been established within and between the SMST, the Technology Development Team and the Integrated Human Function Team, as well as the specific system teams within the NSBRI. It is clear that there is a need to enhance the system or platform component of the SMST in order

for the team to achieve its goals, as well as to interact better with the other NSBRI teams, and other programs within NASA (e.g., the Biosensor Group at ARC). To this end, gaps have been identified, especially with respect to the "smart" and "systems" components of the SMST. Specific needs include (a) supplemental research in decision support systems for monitoring and (b) decision support systems and knowledge bases for diagnosis and treatment. Research gaps in the treatment, or effector, modalities have also been characterized, and are deemed necessary in order to solidify, and eventually move beyond, proof-of-principle demonstrations to the actual construction and implementation of a smart medical system.

The implications of early developments within the SMST suggest that:

- Smart medical systems, as characterized in the context of the NSBRI SMST, represent potentially revolutionary advances in health monitoring and care for humans in space and on earth. A plan has been developed to convert this general statement to deliverables within five to ten years, but components of the system will be ready within the next three to five years (e.g., Dr. Soller's NIRS sensor and analyzer for tissue pH assessment and modification of exercise protocols to assess muscle performance and loss in space).

- Some of the technologies (e.g., in projects led by Drs. Putcha, Soller and Sutton) are relatively far along to be considered for flight testing in the near future to determine real time physiological changes and CM efficacy in remote, space relevant, environments (e.g., KC135)

- Some technologies have immediate applications to earth based care, and these applications are being explored (e.g., Drs. Klempner and Cunningham – colorimetric sensor for rapid detection of biological agents and organisms relevant to infectious disease monitoring in high risk environments such as intensive care units, or in the setting of biological or chemical terrorism; Dr. Soller – non-invasive assessment of diabetic microvasculature, and multiple blood and tissue components; Dr. Sutton and colleagues – non-invasive, portable means of functional brain monitoring for cognitive neuroscience and disease, such as stroke progression)

- The SMST is working as a team, where the added value of projects is apparent (a) in the synergy among technologies and (b) in the establishment of a platform upon which the vertical developments of each project can be integrated into a working system.

II. INTRODUCTION

A. Team Objectives

The SMST aims to take a leadership role in the research and development of an advanced, integrated and autonomous system for astronaut health assessment, maintenance and medical care. This includes the delivery and evaluation of medical interventions and other CMs that reduce the deleterious effects of space travel and enhance the overall well being of astronauts. In achieving this goal, it is anticipated that there will be significant impact and applications for earth-based health and medical care.

B. Health Concerns and Hazards

Health problems associated with space travel may be related to the effects of microgravity, radiation and other risks to the body that are particular to space flight, but they may also be independent of these effects. Medical problems may arise in association with a given demographic population or as a result of a toxic environmental exposure. Moreover, complex interactions may result in alterations and disorders presenting and/or responding differently in a microgravity environment relative to earth. The unique medical circumstances, requirements and limited health care resources in space pose challenges and opportunities for new strategies of physiological monitoring, medical diagnosis and treatment.

In-flight medical events are not uncommon. On STS-1 through STS-89, 98% of crew members reported medical events, excluding space motion sickness (R. Williams, NASA HQ, personal communication). In total, 1867 separate events were logged (1613 men, 254 women), with 141 (7.6%) being due to injury. It is estimated that the risk on the ISS of a significant event, equivalent to one requiring an emergency room visit or hospitalization, is between 1-3 events per annum.[1] The risks increase for long duration space flight and for older crew members. In the Russian space program, two evacuations have been precipitated by medical conditions; in both cases, the entire crews returned.

Given the importance of maintaining crew health, and since medical events can seriously impact astronauts and missions, the CPR ranks Trauma and Acute Medical Problems (risk #43) as one of the four Type I (most severe) risks. Toxic Exposure (risk #44), and Altered Pharmacodynamics and Adverse Drug Reactions (risk #45), are Type II risks. Illness and Ambulatory Health Problems (risk #46), Decompression Sickness Complicated by Microgravity (risk #47), and Post-landing Rehabilitation (risk #48) are Type III risks. A NASA mapping of the SMST projects onto the CPR risks is shown in table 1.

[1] Assuming a crew of seven rather than the current number of three astronauts when the ISS is not docked to the Shuttle.

PI	Task	Funding	CPR Risk Area	NSBRI Program Area	Risks (No.)	CQs No.	CRL Current	CRL Task Completion	CPR Congruence Score
Crum, L.A.	Guided High Intensity Focused Ultrasound (HIFU) from Mission-Critical Care	267,315	Clinical Capabilities	Smart Medical Systems	43	11.02 11.04 11.08 11.10	4	5	7
Davies, P.F.	Vascular Genomics in Gravitational Transitions	336,587	Clinical Capabilities, Cardiovascular Alterations	Smart Medical Systems	14, 48, 49	3.05 11.36 11.37 11.38 11.39 11.39 12.01	2	4	6
Klempner, M.S.	Smart Medical System for Detection of Microorganisms	362,064	Clinical Capabilities, Environmental Health, Immunology	Smart Medical Systems	43, 46, 51, 52, 22, 26	11.10 11.29 11.30 4.15 7.13	2	4	7
Soller, B.R.	Noninvasive Measurement of Blood and Tissue Chemistry	251,840	Clinical Capabilities, Environmental Health, Bone Loss, Cardiovascular Alterations	Smart Medical Systems	43, 44, 46, 47, 48, 50, 13, 10	11.02 11.12 11.08 11.13 11.22 11.29 11.33 11.38 4.16 3.04 2.14	2	4	7
Sutton, J.P.	Near Infrared Brain Imaging for Space Medicine	396,000	Clinical Capabilities	Smart Medical Systems	18, 43, 46	11.02 11.10 11.30	2	4	7
Thomas, J.D.	Diagnostic Three Dimensional Ultrasonography: Development of Novel Compression, Segmentation and Registration Techniques for Manned Space Flight Applications	314,498	Clinical Capabilities	Smart Medical Systems	43, 46	11.22 11.26 11.30 11.36	2	4	7
Thomas, J.D.	Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity	85,502	Clinical Capabilities	Smart Medical Systems	43, 46, 13	11.03 11.08 11.24	2	4	7

* Putcha, L. Not mapped in NASA info forwarded to SMST leader

Table 1. NSBRI SMST 2000 – CPR Mapping

C. Topics to Address

The SMST recognizes that to achieve its objectives (section IIA), it must (a) utilize a team approach within the context of the NSBRI CM driven mission, (b) coordinate and collaborate with other NASA efforts in space and critical care medicine, (c) emphasize research that leads to testing and monitoring of physiological functions and CM effectiveness in healthy astronauts (i.e., link the SMST to other NSBRI teams and promote CM research with broad utilization), as opposed to emphasizing trauma and acute problems only, and (d) "think outside the box" with respect to current approaches to medical care, given that resources are limited, there may be no M.D. in flight and communications to earth are limited and delayed.

The NSBRI assigned the SMST a mandate to develop innovative, possibly revolutionary, techniques for medical monitoring, diagnosis and treatment. To achieve these goals, several infrastructural needs have been identified. These include:

- New types of biometric sensors
- Novel medical and surgical techniques
- Robotic medical assistance systems
- Advanced drug synthesis and delivery systems
- Smart algorithms for medical data systems
- Automated decision support for training and care
- Systems engineered platforms for sensor, algorithm and effector integration

Additionally, it has been necessary to develop a strategic plan to link projects together, and this is described section IIIC.

III. RESEARCH PROGRAM STRUCTURE & DESIGN

A. Project Executive Summaries

Research project summaries for the SMST are accessible electronically at www.nsbri.org/research/med_sys-proj.html. The team consists of eight multi-disciplinary projects (3 animal, 1 human + animal, 2 human + computation, 1 pathogen, 1 resource) that integrate engineering, computation and biomedicine with innovation in technology and medical care. The project summaries, along with PI, updated co-PI and start date information are listed below, in alphabetical order of the PI. The CRLs of each project, along with the projected advance in CRLs over the three year span of each project is shown in Table 1. [2]

PRINCIPAL INVESTIGATOR:	**Lawrence Crum, Ph.D.**	
ORGANIZATION:	**University of Washington**	
CO-INVESTIGATORS:	**Carter, S. J.**	**U Washington**
	Bailey, M. R.	**U Washington**
	Kaczkowski, P.	**U Washington**
	Vaezy, S.	**U Washington**
PROJECT TITLE:	**Guided High Intensity Focused Ultrasound (HIFU) for Mission-Critical Care**	
FUNDING:	**$267,315 (FY 2001)**	
START DATE:	**April 1, 2001**	

Project Executive Summary

One of the most exciting new frontiers for the Manned Space Program is a long-term flight, perhaps to Mars. For such a mission, all efforts must be made to ensure that mission-critical failures do not occur. One of the most difficult medical conditions to treat, especially when operating facilities are not available, is that of blunt abdominal trauma. Indeed, it is known from studies of combat casualty care that exsanguination (uncontrolled bleeding) is the principal cause of battlefield mortality, and for those combatants who do not receive immediate hospital care, the mortality rate has not significantly improved since the civil war. Although a variety of drugs are becoming available that are intended to stop internal bleeding, they have not yet met with acceptable success. To treat a number of mission-critical medical conditions that might arise during long term space flight, we envision a lightweight, portable, smart medical device that can adequately control internal bleeding, as well as address a number of other medical conditions that require surgery. This device will use diagnostic ultrasound for guidance and High Intensity Focused Ultrasound (HIFU) for therapy. Specifically, we proposed to build an image-guided transcutaneous device for acoustic hemostasis and bloodless surgery. Because the scope of this NASA program is limited, we do not propose to deliver the flight-ready device at the conclusion of this proposed study; rather, we propose to develop an integrated ultrasound guidance and therapy engineering prototype that will be tested on large animals. Under DoD support, we are currently developing a similar instrument for use in the forward echelons of the battlefield and our experience in this area can be directly applied to the goals and objectives of this effort.

[2] The Klempner and Putcha projects are conditionally funded for one year only.

PRINCIPAL INVESTIGATOR:	**Peter Davies, Ph.D.**
ORGANIZATION:	**University of Pennsylvania**
CO-INVESTIGATORS:	**Stoeckert, C. U Penn**
PROJECT TITLE:	**Vascular Genomics in Gravitational Transitions**
FUNDING:	**$336,587 (FY 2001)**
START DATE:	**September 1, 2001**

Project Executive Summary

Orthostatic intolerance, an undesirable consequence of cardiovascular-adaptations to microgravity, frequently occurs upon the return of astronauts from prolonged space flight and requires medical management. During prolonged missions to other planets, countermeasures must be developed to prevent incapacity upon entry into remote gravity environments such as the NASA exploratory missions to Mars (approx. 3-G atmospheric entry, 0.3-G at the surface). The cardiovascular system is able to adapt to altered mechanical conditions, including gravitational changes. Reorganization of blood vessel structure and function during prolonged microgravity results in attainment of a new equilibrium state, the abrupt disturbance of which (upon re-entry) predisposes the system to orthostatic intolerance. Vascular adaptation is largely orchestrated through gene transcription. This ground-based project will address *at the level of gene expression the structural and regulatory changes in vascular tissues associated with (i) exposure to simulated microgravity, (ii) return to normal posture, and (iii) prolonged exposure to hypergravity, and its acute reversal.* Hypergravity experiments will be performed at the NASA/Ames animal centrifuge facility. We propose that the underlying mechanisms of adaptive tolerance/intolerance to gravitational shifts be studied at a fundamental but comprehensive level in blood vessels by following changes in thousands of genes in small amounts of tissue and in small numbers of vascular cells obtained from gravitationally-relevant locations in a mouse model. Linear amplification of very small amounts of vascular RNA for analysis on microarrays enables "spatio-temporal transcription profiling" of the vasculature. Changes in regions of the vascular tree known to be (a) of particular relevance to human orthostatic intolerance, and (b) of critical importance in normal blood vessel regulation, will be investigated. In addition to the use of available commercial mouse genomic arrays, customized cardiovascular microarrays and mouse-specific array building will be performed. Gene expression arrays will be constructed from clones representing both genes of known cardiovascular importance and genes of vascular cell "transcriptomes" empirically derived from subtracted, normalized libraries. In addition to regional vascular tissues, small groups of cells will be harvested from the vasculature by laser capture microscopy or mechanical microdissection, and RNA will be amplified. Hybridized arrays will be subjected to rigorous and sophisticated bioinformatics analysis and the output will be archived as raw images and as curated and annotated data. These will be made available to the space research community to provide well-defined site-specific vascular phenotypes responsive to gravitational change. The use of a murine model will later facilitate follow-up flight studies.

PRINCIPAL INVESTIGATOR:	**Mark S. Klempner, M.D.**
ORGANIZATION:	**Boston University Medical Center**
CO-INVESTIGATORS:	**Cunningham, B. SRU BioSystems**
PROJECT TITLE:	**Smart Medical System for Detection of Microorganisms**
FUNDING:	**$362,064 (FY 2001)**
START DATE:	**June 1, 2001**

Project Executive Summary

The goal of this program is to develop a revolutionary, non-culture based microbial detection, identification and quantification system that can be used as part of a Smart Medical System for exploratory space travel. Rapid detection and identification of microorganisms are critical to many military and civilian applications ranging from food and water safety monitoring, biological warfare agent detection and to diagnostic microbiology of human and other biological specimens. For long-term exploratory space travel there will be a critical need for a smart medical system to monitor the air and water supply for microbial contaminants, as well as an intermittent need for assessment of biological specimens from symptomatic astronauts.

Current microbial identification systems are based on the gold standard of *in vitro* culture or DNA/RNA fingerprinting. Both require considerable sample manipulation, delay in readout, are semiquantitative and subject to interfering substances and contamination, and require additional processing to resolve complex mixtures of microorganisms. This proposal involves the development of a novel smart medical system to detect and identify bacteria through the use of microsensors and includes three steps: 1) Development of "fingerprinting" phage display libraries which can detect, identify, quantify and discriminate bacterial species in environmental and biological specimens; 2) Application of phage displayed peptides and antibody fragments in a microarray to the surface of a microsensor to demonstrate the microarray microbial fingerprint response to selected bacterial species using optical readout and electronic MEMS resonator arrays and to characterize the sensitivity and specificity for detecting and discriminating between bacterial species using surface "fingerprints;" and 3) Development of algorithms from the microarray response for the real time identification and discrimination of bacterial species.

PRINCIPAL INVESTIGATOR:	**Lakshmi Putcha, Ph.D.**
ORGANIZATION:	**NASA-Johnson Space Center**
PROJECT TITLE:	**Microcapsule Gel Formulation of Promethazine Hydrochloride for Intranasal Administration**
FUNDING:	**$163,740 (FY 2001)**
START DATE:	**August 1, 2001**

Project Executive Summary

A continuing challenge for space medical operations at NASA is the management of pathology associated with neurovestibular adaptation during space flight. A primary manifestation of this problem, particularly in the first few flight days of shuttle missions, is space motion sickness (SMS). The current treatment of choice for symptoms associated with SMS is promethazine (PMZ). Although oral tablets and rectal suppositories have been used during space flights, the intramuscular route appears to be most effective. On the other hand, intramuscular

administration of drugs is an invasive procedure and PMZ causes irritation at the site of injection. A key research topic in the Smart Medical Systems area of the NSBRI 99-02 research announcement is development of novel therapeutic modalities for remote site medical operations such as space missions. In response to this initiative, the goal of the proposed research is to develop an intranasal dosage formulation of PMZ that will provide crewmembers with a non-invasive means of self-administering SMS medications. Accordingly, the following three aims will be addressed: 1) Develop a microencapsulated, pH-balanced gel dosage formulation and a combination form with a corticosteroid for intranasal administration of PMZ; 2) Establish the release kinetics and shelf life of the optimized dosage forms; and 3) Assess bioavailability, nasal mucosal irritability and toxicity of the selected dosage forms in rats.

The proposed formulation development will focus on tailoring the release characteristics of the dosage form to optimize therapeutic index and minimize irritability at the site of administration. Once the optimal dosage form has been identified based on release kinetics and stability characteristics, bioavailability, nasal irritability and toxicity after single and multiple dose administration will be assessed in an animal model. Development of an intranasal drug delivery system for motion sickness treatment will benefit pharmacotherapeutics in space as well as on Earth.

PRINCIPAL INVESTIGATOR:	**Babs R. Soller, Ph.D.**
ORGANIZATION:	**University of Massachusetts Medical School**
CO-INVESTIGATORS:	**Heard, S. U Mass**
	Puyana, J. U Pittsburgh
PROJECT TITLE:	**Noninvasive Measurement of Blood and Tissue Chemistry**
FUNDING:	**$251,840 (FY 2001)**
START DATE:	**March 1, 2001**

Project Executive Summary

Medical monitoring and diagnosis of acute and chronic conditions during long-duration space flight is critical to the success of these missions and must be able to be carried out by personnel with limited medical training and equipment. The most successful technologies will be those that allow noninvasive measurement of multiple parameters that can be combined for algorithm-driven decision making. Near infrared spectroscopy (NIR) has been successfully used to noninvasively assess blood and tissue for the measurement of oxygenation, pH, glucose and hematocrit and the diagnosis of cancer because NIR light can penetrate through skin and bones. Currently, this technology is limited in its ability to accurately measure these parameters for people with dark skin color and significant fat content. The hypothesis of this proposal is that NIR, in combination with unique statistical methods, can be used to noninvasively measure blood and tissue chemistry for any human subject. This project will develop new statistical methods which will enhance the processing of NIR spectral data so that medical parameters can be accurately measured on all humans, irrespective of skin color and gender. This new approach will be demonstrated by developing techniques to noninvasively measure blood hematocrit and muscle pH and oxygenation on human surgical and ICU patients. These parameters are important in diagnosing and treating hypoxia and trauma that may arise from exposure to radiation, toxic chemicals and blunt or sharp injury. They may also be useful in evaluating

exercise as a countermeasure for extended weightlessness. The measured patient data will then be used to develop algorithms to diagnose shock and hypovolemia and guide resuscitative therapies. Finally, optical specifications will be developed to build a miniaturized system to collect NIR data. This system will serve as a platform for NIR measurement of multiple parameters and the development of computerized algorithms to assist in the diagnosis and treatment of several medical conditions. The specific system demonstrated in this proposal is intended to evolve into a medical monitoring system for use during extended space flight, but will also find immediate application in terrestrial hospitals, emergency vehicles and emergency rooms.

PRINCIPAL INVESTIGATOR:	**Jeffrey Sutton, M.D. Ph.D.**	
ORGANIZATION:	**Harvard – Massachusetts General Hospital**	
CO-INVESTIGATORS:	**Boas, D.**	**Harvard**
	Koroshetz, W.	**Harvard**
	Rosen, B.	**Harvard**
	Strangman, G.	**Harvard**
PROJECT TITLE:	**Near Infrared Brain Imaging for Space Medicine**	
FUNDING:	**$396,000 (FY 2001)**	
START DATE:	**October 1, 2000**	

Project Executive Summary

This application is to the Smart Medical Systems team of the National Space Biomedical Research Institute (NSBRI). NASA's cross-risk prioritization for long duration space missions identifies four medical problems at the highest level, including (1) human performance failure because of poor psychosocial adaptation and (2) trauma and acute surgical problems. The brain is the organ central to human performance and psychosocial adaptation, and alterations in the nervous system, including those induced by trauma, can have deleterious effects to a space mission. Almost no countermeasures exist to address the possible biological and environmental impediments affecting the brain on long term human space flight. The development and implementation of a non-invasive, low power, portable, functional imaging technology for monitoring brain activity in microgravity is therefore an important advance for astronaut care. An instrument of this type could be used for ongoing monitoring, early identification of alterations, diagnosis, procedures and evaluation of countermeasures. When coupled to models of individual astronauts and a smart informatics system, it could assess brain function, and aid in countermeasure readiness, with unprecedented sophistication and autonomy.

In this proposal, the investigators plan to utilize the resources of the Massachusetts General Hospital (MGH) to engineer and apply a new non-invasive, portable imaging device capable of performing diffuse optical tomography (DOT) for space medicine. The technology performs spectroscopy with near infrared light to monitor oxyhemoglobin and deoxyhemoglobin concentrations in the brain, and is easily adapted for use in microgravity. The research builds on collaborative work among the project's investigators, and allows for validation of the instrument using other technologies, namely functional magnetic resonance imaging (fMRI) and optical coherence tomography (OCT). Specifically, DOT will be validated by imaging healthy subjects using the simultaneous, and non-interacting, methods of DOT and fMRI. Subjects will perform motor tasks of varying complexity under normal and sleep deprived conditions to assess cortical function during simulated flight tasks. DOT will also, along with OCT, be used to assess

patients with altered intracranial pressure (ICP). Changes in ICP are associated with fluid shifts, headache and performance failure, and they are amenable to countermeasures. All functional imaging data will be used to help refine a system for automated assessment, warning, and countermeasure evaluation. It is anticipated that the technologies developed in this proposal will have direct, applications for health care on Earth.

PRINCIPAL INVESTIGATOR:	**James Thomas, M.D.**	
ORGANIZATION:	**The Cleveland Clinic Foundation**	
CO-INVESTIGATORS:	**Greenberg, N.**	**Cleveland Clinic**
	Hale, J.	**Cleveland Clinic**
	Shakar, R.	**Cleveland Clinic**
	Shiota, T.	**Cleveland Clinic**
PROJECT TITLE:	**Diagnostic Three Dimensional Ultrasonography: Development of Novel Compression, Segmentation and Registration Techniques for Manned Space Flight Applications**	
FUNDING:	**$314,498 (FY 2001)**	
START DATE:	**July 1, 2001**	

Project Executive Summary

The NSBRI has identified that the efficient and automated delivery of health care in space is a key research arena for the future. Specifically, they propose to develop a "Smart Medical System" that will be able to monitor crew health, identify deviations from ground-based norms, and allow timely intervention by crew members who may have only a moderate amount of training in medicine. For the last three years, the principal investigator and colleagues have worked closely with NASA scientists, flight surgeons, and engineers to optimize research and diagnostic ultrasound aboard the International Space Station (ISS) and thus are well positioned to develop the necessary tools and techniques to integrate ultrasound into the Smart Medical System. A principal limitation of ultrasound technology is its extreme dependence on the expertise of both the acquiring examiner and the interpreting physician. This is particularly true of two-dimension ultrasound, where the examiner is required to obtain precisely oriented anatomical sections of the organ of interest.

Three-dimensional ultrasound has the advantage of acquiring a large anatomic volume from a single ultrasonic window, and thus may be less dependent upon the expertise of the examiner. Furthermore, this large volume may contain sufficient anatomic landmarks to allow unambiguous registration with previously obtained three-dimension data from either ultrasound or other modalities such as magnetic resonance imaging (MRI) or computed tomography (CT). One could thus envision a system by which whole organs or even the entire body would be imaged in three-dimensions prior to launch; data which could be used to compare with subsequently obtained three-dimensional data sets using in-flight ultrasonography. The overall purpose of this grant is therefore to perform ground-based research, development, and validation aimed at optimizing diagnostic ultrasound in manned space flight, with the following general hypothesis:

Unifying hypothesis: Serial three-dimensional ultrasound examinations will enhance diagnostic capabilities in manned space flight.

The technical aspects of this program will be pursued with the following specific aims:

1. Optimize the acquisition methods for three-dimensional sonography, utilizing reconstruction and real-time techniques.
2. Develop techniques for registering anatomical images from two- and three-dimensional ultrasound with those obtained from prior ultrasound examination and from magnetic resonance and computed tomographic imaging, considered "gold standards" for non-invasive anatomical imaging.
3. Develop tools for abstracting, in an automated fashion, anatomical changes from serial three-dimension and two-dimension ultrasound studies.
4. Develop algorithms for the optimal compression of three-dimensional ultrasound images and refine current two-dimensional compression algorithms.
5. Assess the ability of novice examiners to obtain three-dimensional sonographic data sets following minimal training.

These objectives will be pursued using data from a variety of *in vitro*, animal and clinical models. In particular, we will take advantage of a well-established collaboration with the National Institutes of Health, which permits highly sophisticated chronic animal models to be examined with a minimum of additional resources. Although the tools developed here should be applicable to any organ of the body, we will focus our efforts on the kidneys and the heart.

At the conclusion of this project, we anticipate delivering to the NSBRI and its Smart Medical System a set of algorithms and software for the non-rigid morphological registration and comparison of serial two- and three-dimensional ultrasound data sets and validated algorithms for optimal compression of four-dimensional ultrasound data. In addition to these technical deliverables, our validation work on nephrolithiasis will provide important diagnostic clues for assessing this condition in manned space flight. Similarly, the work on cardiac mass regression following unloading will be invaluable to the NASA research and medical operations community in assessing the impact of long-term space flight on cardiac atrophy and utility of prophylactic countermeasures.

PRINCIPAL INVESTIGATOR:	**James Thomas, M.D.**
ORGANIZATION:	**The Cleveland Clinic Foundation**
CO-INVESTIGATORS:	**Garcia, M. Cleveland Clinic**
	Greenberg, N. Cleveland Clinic
PROJECT TITLE:	**Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity**
FUNDING:	**$85,502 (FY 2001)**
START DATE:	**July 1, 2001**

Project Executive Summary [3]

The cardiovascular system undergoes significant changes in microgravity, including an early cephalad shift of lower extremity blood volume, loss of plasma volume over 24 to 48 hours, and long-term reduction in ventricular chamber volume and mass. In the weightless environment, these alterations generally are well tolerated, but upon return to Earth, astronauts often suffer

[3] Only key issue (specific aim) 5 regarding the establishment of an echocardiographic core facility is funded, with joint supervision from the NSBRI Cardiovascular Alterations Team.

from serious orthostatic intolerance and reduced exercise capacity, changes that may limit the long-term presence of man in space. It is essential that the mechanisms for these alterations be understood so that reliable countermeasures can be tested and implemented. Hypovolemia, cardiac atrophy, and autonomic dysfunction have each been hypothesized to contribute to this post-flight debility, but their relative importance is unclear. Furthermore, it is unknown whether actual abnormalities in the myocardium itself develop with long-term space flight. Therefore, reliable portable noninvasive methods will be needed in order to detect and quantify these changes.

Alone among such imaging modalities of radiography, magnetic resonance imaging and computerized tomography, echocardiography has the unique ability to characterize cardiovascular anatomy and physiology in ground-based models, pre- and post-flight, and most importantly during flight. Indeed, the Science Working Group (SWG) for the International Space Station (ISS) Human Research Facility (HRF) has recognized the primacy of ultrasound for medical diagnosis and physiology research, with plans to launch a specially modified commercial ultrasound instrument to the ISS in 2001. Echocardiography is similarly being used before and after shuttle flights and in a variety of bed-rest studies sponsored by NSBRI and NASA. Unfortunately, while ultrasound has the potential for high spatial and temporal resolution imaging of the heart, in the past it has been severely limited by operator inexperience and inconsistency in its subjective interpretation. Needed are new methodologies for assessing the load-independent function of the heart and consistent, objective quantification of a wide range of NASA echo studies, whether obtained on the ground, in flight or in experimental models. We propose to provide such a facility while validating novel methods for the load independent assessment of myocardial function. Our central hypothesis is that:

Microgravity affects cardiovascular function not only through changes in chamber volume and mass but also through changes in myocardial properties.

A definitive test of this hypothesis is at least several years away when dedicated life science missions are possible aboard the ISS. However, within the scope of this grant, we propose several specific aims that will be critical to the ultimate comprehensive study of the cardiovascular system in space. Key issues: 1) Assessment of the effect of chronic volume unloading in ventricular myocardial properties using a sophisticated chronic bovine model of ventricular unloading induced through the use of a left ventricular assist device (LVAD); 2) Validation of non-invasive Doppler echocardiographic indices for the assessment of left ventricular contractility and relaxation including color M-mode Doppler derived diastolic intraventricular pressure gradients (IVPG) and tissue Doppler derived myocardial systolic and diastolic strain rates (e's, e'd); 3) Validation of Doppler derived exercise cardiac output and contractile reserve and their potential utility for the early detection of myocardial dysfunction during prolonged space flight. Additional deliverables to NSBRI: 4) Development and distribution of stand-alone software and algorithms for implementing the quantitative analysis of Doppler echocardiographic data, as described above, so they may be applied to ultrasound data obtained from remote sources; 5) Establishment of an Echocardiographic Core Facility to the NASA research and clinical community, capable of applying standard and novel analysis techniques in a rigorous fashion to echocardiographic data obtained from selected ground-based experimental models, pre- and post-flight examinations, and eventually from in-flight acquisitions.

If successfully implemented, these aims will allow the cardiovascular sequellae of space flight to be studied much more rigorously, while providing consistent, objective echocardiographic interpretation to the entire NASA community.

B. Program Structure and Interactions

As stated in section IIA, the SMST objectives are broad and ambitious. However, the research program needs to be focused in order to ensure clear scientific progress towards CM development. To this end, specific synergistic relationships either exist or are being developed among team projects, as well as between SMST projects and other NSBRI and NASA projects. These relationships are summarized in fig. 1.

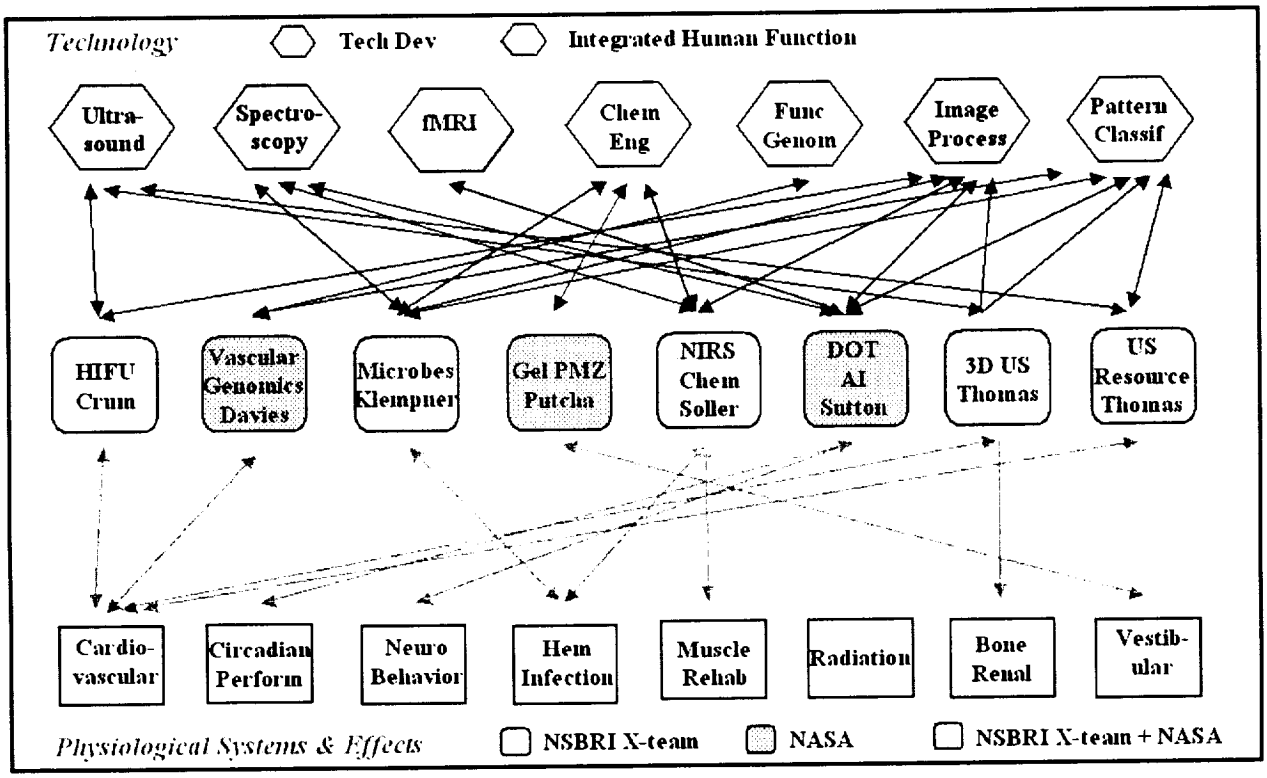


Figure 1. SMST project interactions showing relationships within and between NSBRI teams

In fig. 1, each of the eight SMST projects is represented along the middle row. The projects are coded to depict (a) NSBRI cross-team interactions (Soller with Cabrera (Integrated Human Function Team)), (b) NASA interactions (Davies with Luzod (ARC); Putcha (JSC); Sutton with Marshburn (JSC)), (c) NSBRI cross-team and NASA interactions (Klempner with Fox (Immunology, Infection and Hematology Team) and Pierson (JSC); Thomas with Cohen (Cardiovascular Alterations Team) and JSC)), and (d) none of the above (although Crum has strong ties to DoD medical technology programs).

The bidirectional arrows in fig. 1 represent relationships (a) among projects within the SMST and (b) between SMST projects and the other NSBRI teams. These relationships are broken down into two main categories: Technology; and Physiological Systems and Effects. Across the top row, interactions between SMST projects and the Technology Development and Integrated Human Function Teams are shown. These interactions correspond roughly to experimentation (Technology Development Team) and theoretical or modeling (Integrated Human Function Team) interactions. Arrows pointing to particular boxes *from* SMST projects *to* boxes in the upper row show how SMST projects contribute to NSBRI developments in specific domains on *other* teams. For example, the Klempner, Soller and Sutton projects all develop novel spectrographic devices that complement one or more projects being developed in the Technology Development Team (specifically, projects headed by Potember and by Maurer).

Arrows that originate *from* boxes in the upper row of fig. 1 and project *to* SMST projects represent links among projects *within* the SMST. The relationships are incomplete and are evolving, sometimes with added benefit to the overall NSBRI scientific program. For example, ultrasound technologies link projects by Crum and Thomas, although Thomas' projects do not develop hardware. The functional magnetic resonance imaging (fMRI) aspects of Sutton's project adds to the (non-functional) MRI developments in the Technology Development Team; hence the half shaded box in fig. 1. The chemical engineering and functional genomic and proteomic approaches on the SMST complement other core technology developments within the NSBRI program.

Bidirectional arrows between the boxes representing the SMST projects and the system teams along the bottom row of fig. 1 work similarly to those just described. There is synergy with every system team, especially the Cardiovascular Alterations Team. There is also an emerging emphasis on brain and neurobehavioral alterations within the SMST (Sutton project). At present, there is no synergy with the Radiation Effects Team, although there is scientific overlap with that team.

C. Program Strategy

While the previous section outlines the relationships among projects, it does not describe the design of the SMST to address research problems and develop countermeasures. To understand how team R&D might lead to deliverables for eventual implementation for flight, a strategic plan is required. To achieve this goal, a high level description of the system for health and medical monitoring, as well as interventions, that are currently in place was constructed. This schematic is shown in fig. 2. In this system, the astronaut and environment are handled in similar ways, since space medicine is effectively a branch of aerospace or environmental medicine. Sensors monitor the environment and astronaut, and after calibration, signal conditioning and processing, data are either stored and/or relayed to earth. Ground based personnel oversee, in coordination with the astronaut, flight surgeon and possibly the PI if appropriate, any treatment or countermeasure that is administered. There is limited autonomy and ability to assess deconditioning effects in space. Countermeasure modification and medical care delivery is severely limited.

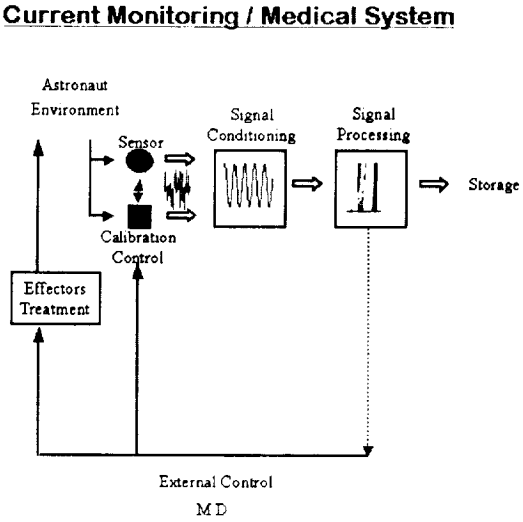


Figure 2. Current environment and physiological monitoring and medical system design

In collaboration with NSBRI, NASA JSC, ARC, JPL and other personnel, a strategic plan for the SMST was developed during the winter and spring months of 2001. It is outlined in fig. 3. The objectives were to develop a schematic that (a) characterized a "smart medical system", (b) linked NSBRI SMST research and countermeasure development to basic research, industry, space hardware and medical operations, (c) provided a format to map current projects within the SMST onto a system prototype, both at the component level and at the level of the system itself, and (d) allowed for the identification of gaps in the SMST program.

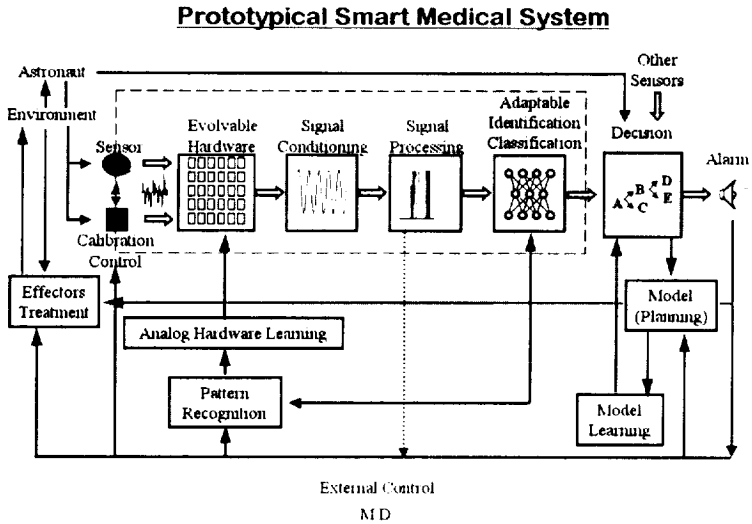


Figure 3. Prototypical smart medical system

In fig. 3, information from the environment and astronauts is sensed by a suite of small, lightweight, low power, portable, non-invasive, unobtrusive, intelligent sensors with pattern recognition capabilities. These sensors feed automatically analyzed, rather than raw, data into decision making algorithms, that also have cognitive input from the astronauts themselves. There is a model of the system, which is where the Integrated Human Function Team collaborations fit in the scheme. The model not only (a) assesses input from multiple sources, but it (b) pre-plans notification for onboard alarms and information transfer to the ground, (c) looks at contingencies and outcomes for effectors and treatments prior to the administration of CMs, (d) assesses the effectiveness of treatments and CMs, (e) monitors consequences of actions and CMs, and (f) interfaces with models for pattern recognition and analog hardware learning. Since feedback loops exist which are independent of external and M.D. control, the system is, in principle, autonomous. Moreover, the design is achievable, to varying degrees, and proposes a revolutionary new health care system for space, which is central to the initial research charge assigned to the SMST.

While there are other features of the system described in fig. 3, the main points in summary are:

- Enhanced small sensor platforms with pattern recognition and wireless capabilities
- Adaptable system of systems for sensor integration
- Algorithms and models for human assisted monitoring, CM assessment and decision making
- Common platforms for sensing and CM delivery

D. Countermeasure Development

To see that the program strategy of the SMST is aligned with the mission of the NSBRI, each project in section IIIA, along with its relationships to other projects (section IIIB), was mapped onto the strategic plan set forth in section IIIC. The CM development plan for the SMST was then identified. It includes several types of measures, as outlined below. The time line for deliverables in the CRL 2-7 range was submitted to NSBRI management as part of the formal team strategic plan of August 2001. The time line is also summarized as part of Table 1 (Putcha project estimated to have a near term pharmacological deliverable, with a start CRL = 4 and a completion CRL=7).

Specific countermeasure developments include:

1. Exercise
   - Soller: non-invasive tissue and blood chemistry measures for physiological monitoring and assessment of exercise effectiveness
     applicable across ethnic races

2. Pharmacology
   - Putcha: novel drug delivery system, with first application to intranasal promethazine HCl to reduce space motion sickness

3. Training
   - Thomas: ultrasound resource to train naïve users in medical image acquisition, with multi-systems applications (e.g., cardiovascular, bone, renal)

4. Performance Adjustments
   Sutton: non-invasive assessment of brain function under cognitive load and sleep disturbance to adjust performance expectations

5. Environmental Manipulation
   Klempner: real-time assessment of distributed microbial environment for early detection and manipulation of significant alterations
   sensors applicable for a broad range of environmental monitoring and manipulation

6. Surgery
   Crum: non-invasive use of ultrasound for diagnosis and treatment of injury

7. Gene Therapy
   Davies: functional genomic and proteomic approaches to address vascular changes in microgravity

8. Adjunctive Developments to Other CMs
   Klempner, Soller, Sutton, Thomas:
   suite of passive continuous physiological monitors and algorithms to identify the need for, and efficacy of, specific CMs, including those related to medical care

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

A. Accomplishments

As described in the previous section, one accomplishment of the first year of the SMST was to develop a strategic plan and coordinated research team that (a) successfully interfaces with other programs within the NSBRI and NASA medical operations, and (b) has high potential impact for NASA through CM development to reduce the risks associated with long duration space travel. While the start dates for projects range from October 1 2000 to September 1 2001 (see section IIIA), effective synergies have been formed and progress has been made on each of the projects. There was recruitment of intra-team resources, such as expertise on bioinformatics (Davies project), biosensors (Klempner project) and model systems of systems (Sutton project). There was a PI meeting in April 2001, regular telecons, multiple site visits among investigators, and education and evaluation of previous and current medical CMs. The team strategy was expanded from its initial focus on medical care to research on physiological monitoring, algorithms and CM effectors and assessment. The main findings and accomplishments during the past year for each project are summarized herein, with the understanding that a number of the projects are in the very earliest stages of commencement.

PRINCIPAL INVESTIGATOR:	**Lawrence Crum, Ph.D.**
PROJECT TITLE:	**Guided High Intensity Focused Ultrasound (HIFU) for Mission-Critical Care**

In vitro testing of HIFU transducers was performed for calibration in degassed water. A series of tests was undertaken to assess small pits in lesions to indicate cavitations activity. The acoustic pressure threshold for lesion production, the time required for lesion visualization and the size of the lesions were examined with a First Generation system. *In vivo* testing using transcutaneous B-mode and Doppler capabilities to target, visualize and induce homeostasis using HIFU was commenced on two swine with IRB approval. Preliminary results were presented at several talks, including the Massachusetts General Hospital and in China, where over 10,000 advanced-stage cancer patients have been treated with HIFU.

PRINCIPAL INVESTIGATOR:	**Peter Davies, Ph.D.**
PROJECT TITLE:	**Vascular Genomics in Gravitational Transitions**

This project has just started, with the plan to begin tilt experiments at U Penn before December 2001, and to begin profiling heart tissue from SMST PI Dr. Thomas as soon as Dr. Thomas can obtain it from his cardiac overload model. Dr. Davies was appointed to a NIH Delegation to Moscow in September 2001 as part of the 2001 Cardiovascular and Pulmonary Cooperation, with expertise in Biomechanics and Intracellular Signaling in the Cardiovascular System. Dr. Davies also became the PI on a new NIH Bioengineering Partnership grant entitled "Cell and Molecular Studies in Cardiovascular Engineering", awarded in September 2001, for $6.7 M over five years.

PRINCIPAL INVESTIGATOR:	**Mark S. Klempner, M.D.**
PROJECT TITLE:	**Smart Medical System for Detection of Microorganisms**

Dr. Klempner moved from New England Medical Center to Boston University Medical Center, where he was appointed Assistant Provost for Research at BU Medical Center. His NSBRI project is now at BU Medical Center and he is collaborating with Dr. Cunningham (see below) and Dr. Pierson from JSC. The latter investigator is responsible for allowing Dr. Klempner to obtain and grow fungal and bacterial species, which were isolated from environmental sites aboard Mir. Dr. Klempner's team has:

- Successfully identified, isolated and amplified peptide ligands from phage display libraries that "fingerprint" Aspergillus and Penicillium fungi which were isolated from and problematic aboard Mir
- Successfully used these phage displayed ligands to capture and distinguish Aspergillus from Penicillium in a microtiter plate format
- Successfully shown that the label free biosensor used in his project (see below) can, in real time, detect molecular interactions as small as a shift of 1 angstrom of surface thickness. This should have versatile applicability to become a useful biosensor for

microbial detection and other molecular interactions of interest. The investigators are moving to marry the biological reagents with various biosensors.

To this end, Dr. Cunningham, who was previously at Draper Laboratories where he was working on the development of a MEMS μCANARY biosensor, is now at a start-up biotechnology company called SRU BioSystems. Dr. Cunningham and colleagues have been able to fabricate 2D optical gratings (d»0.6mm) embossed into plastic for a novel colorimetric resonant reflectance biosensor, with high (e.g., 10 nm protein thickness) resolution detection capabilities (fig. 4). This revolutionary technology has space CM applications relevant to not only Dr. Klempner's project, but also to earth based toxic screening, pharmacology and several Department of Defense (DoD) initiatives.

Dr. Cunningham presented his findings at the Society for Biomolecular Screening Meeting, September 10-13 2001, in a presentation entitled "A Label-Free High Throughput Optical Technique for Detecting Biomolecular Interactions". He and his colleagues have also submitted a paper to *Biosensors and Bioelectronics* to describe their small molecule detection work. Recently, SRU Biosystems was awarded venture capital support from AGTC Funds.

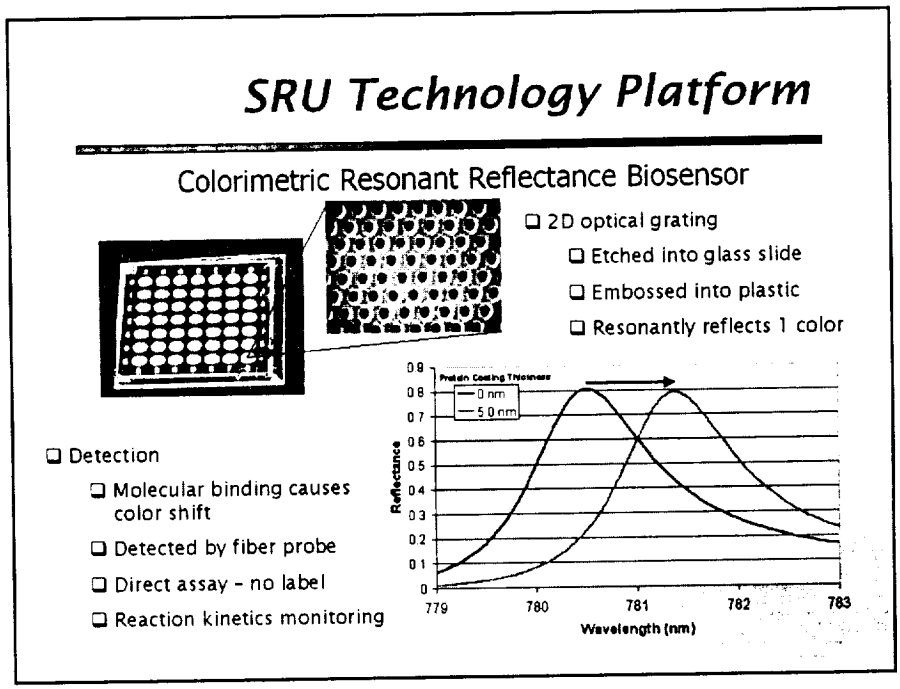


Figure 4. SRU colorimetric resonant reflectance biosensor for phage and other environmental monitoring

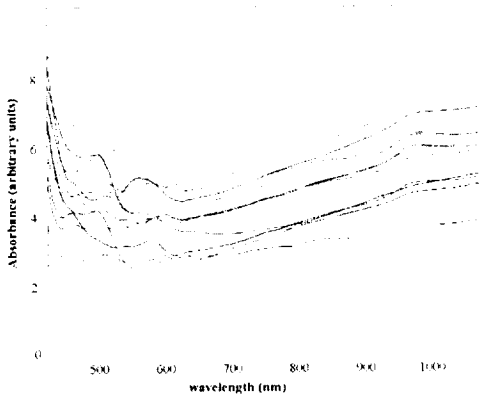
PRINCIPAL INVESTIGATOR:	Lakshmi Putcha, Ph.D.
PROJECT TITLE:	Microcapsule Gel Formulation of Promethazine Hydrochloride for Intranasal Administration

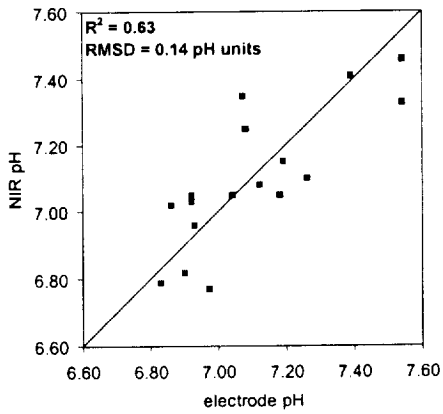
Dr. Putcha has been collaborating with Dr. Vasishtha from Southwestern Research Institute on R&D of a new microencapsulation process. They have refined their evaluation criteria of gel formulated microcapsules to include measures of non-invasive controlled delivery and space hardiness. The approach is promising for promethazine HCl in offering an alternative route of administration and minimization of CNS side effects. Dr. Putcha received a R01 NIH grant entitled "Bioavailability and Pharmacodynamics of Promethazine in Human Subjects", to commence in December 2001, for $650 K over three years.

PRINCIPAL INVESTIGATOR:	Babs R. Soller, Ph.D.
PROJECT TITLE:	Noninvasive Measurement of Blood and Tissue Chemistry

There are several new findings and discoveries on this project which include:

- The ability to noninvasively measure muscle pH and muscle oxygenation in normal and cardiac surgical patients. This measurement is sensitive enough to distinguish microvascular differences between normal and diabetic patients. It is also sensitive enough to measure perfusion difference which result in small change changes in systemic blood pressure.





	Baseline	CPB-Cold	CPB-Warm	CPB+6hr
MAP	84 ± 2	60 ± 2**	61 ± 2**	76 ± 2
Tissue temp	33.1 ± 0.5	31.5 ± 0.5	34.2 ± 0.6	34.5 ± 0.5
NIR muscle pH	7.39 ± 0.02	7.32 ± 0.02*	7.25 ± 0.02**	7.24 ± 0.02**

Mean ± SEM, * $p < 0.05$, ** $p < 0.001$ compared to baseline

Figure 5. NIR-measured muscle pH is sensitive to changes in tissue perfusion resulting from small changes in blood pressure and metabolic demand during CPB. There is excellent correlation between the invasive and non-invasive measurements.

- The successful derivation of a vector that spectroscopically describes skin color and ethnic background. The investigators have also developed a methodology for using this vector to remove spectral variability due to human skin color variations. They are currently testing the ability of this technique to improve the accuracy of noninvasive hematocrit measurements.

Dr. Soller has established a collaboration with Dr. Cabrera on the NSBRI Integrated Human Function Team. Dr. Puyana, a project co-investigator, has relocated to U Pittsburgh Medical Center and is still involved on the project. A proposal, entitled "Tissue Blood Flow Monitor for Shock and Resuscitation" has been submitted jointly with Thermal Technologies Inc. to the NSF. Dr. Soller has ongoing negotiations with three potential licensees for her hematocrit patent related to this project.

NSBRI sponsored abstracts include:

Noninvasive Assessment of Peripheral Perfusion Using NIR Spectroscopy (Early Results)
Diabetes Technology Meeting November 2001

Noninvasively Measured Muscle pH Indicates Tissue Perfusion for Cardiac Surgical Patients
Society of Critical Care Medicine Meeting January 2002

Assessing the Microcirculation by Near Infrared Spectroscopy
Cardiovascular Monitoring, Society of Critical Care Medicine Meeting January 2002

PRINCIPAL INVESTIGATOR:	**Jeffrey Sutton, M.D. Ph.D.**
PROJECT TITLE:	**Near Infrared Brain Imaging for Space Medicine**

The major accomplishments of this project are:

- Observations of an excellent correlation between diffuse optical tomography (DOT), using NIR spectroscopy (NIRS), and functional magnetic resonance imaging (fMRI) to non-invasively assess human brain activity in subjects performing simple motor tasks (fig. 6). DOT sensor validation is important if the technology is to be used as an objective means of assessing brain function under various cognitive loads and sleep alterations, with the aim of adjusting performance expectations as a viable CM.

- The successful use of anatomical and functional MRI data as a constraint on the calculation of deoxyhemoglobin and oxyhemoglobin changes in brain tissue. This finding speaks to the issue of individualized, digitized human, anatomical brain models upon which time-derivative functional data is co-registered and overlaid for automated interpretation in real time.

- The determination of learning curves during off-line testing of SpaceDOCK, a visuomotor task specifically developed as part of this project for use in the optical / MRI environment. The task emulates a space relevant task for performance assessment using behavioral and brain imaging methods. The task was developed in collaboration with Dr. Marshburn, a pilot and JSC flight surgeon.

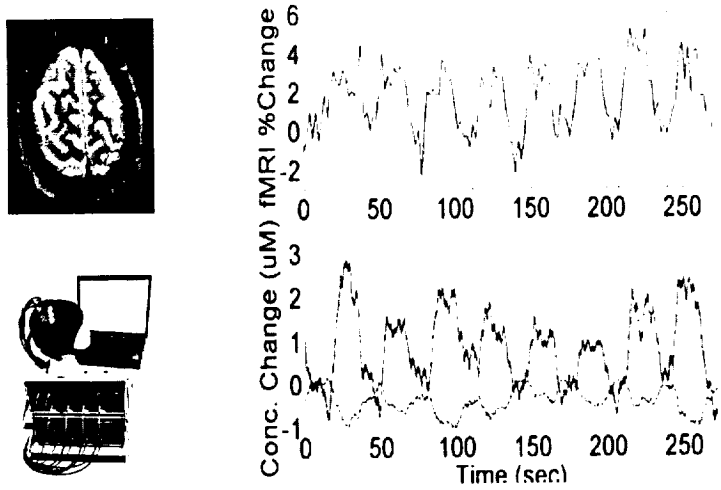


Figure 6. Simultaneous DOT and fMRI recordings

In addition to JSC flight surgeon collaborations, this project supervised a NSBRI sponsored summer intern during June - September 2001. A grant proposal, entitled "Reconfigurable Networking for Autonomous Operations" has been submitted to the Office of Naval Research. Copyright has been applied for on the SpaceDOCK task.

NSBRI sponsored publications:

D.A. Boas, M.A. Franceschini, A.K. Dunn, **G. Strangman**. Non-invasive imaging of cerebral activation with diffuse optical tomography. [Book chapter for CRC press – title TBD, Ed. R.D. Frostig]. In press.

D. Sha , D. Kennedy, **J. Sutton**. Neurocomputing for automated analysis of digital brain images. *Artificial Intelligence in Medicine.* In press.

G. Strangman, F. Halbritter, P. Groblewski, W.C. West, **T. Gaudette**, **D.A. Boas**. A high-speed, continuous-wave near-infrared spectroscopy (NIRS) system for non-invasive recording of brain activity. Submitted to *J. Biomedical Optics*.

G. Strangman, **D.A. Boas**, **J.P. Sutton**. Non-invasive neuroimaging with near-infrared light. Invited paper for *Biological Psychiatry*. In preparation.

G. Strangman, M.A. Franceschini, **D.A. Boas**. Factors affecting the accuracy of near-infrared spectroscopy (NIRS) data analysis for focal changes in cerebral hemodynamics. In preparation.

G. Strangman, J.P. Culver, **J.H. Thompson**, **D.A. Boas**, Temporal comparison of simultaneous BOLD fMRI and NIRS recordings during functional brain activation. In preparation.

NSBRI sponsored published abstracts with presentations:

J. Sutton. Micro / Nano Technologies and the Future of Medicine. *Proceedings of NanoSpace 2001*. In press.

J. Sutton, I. Jamieson. Reconfigurable Network of Neural Networks for Autonomous Sensing and Analysis. *Proceedings of the Fifth International Conference on Cognitive and Neural Systems*. 2001:64.

J. Sutton. Near infrared brain imaging for space medicine. *NASA Bioastronautics Investigators' Workshop*. 2001:433.

G. Strangman, T. Gaudette, **D.A. Boas**. Synergy from simultaneously acquired fMRI and near-infrared optical spectroscopy data. *Proc. Intl. Soc. Mag. Reson. Med* 9. 2001.

NSBRI sponsored presentations (without abstracts):

MITAC Workshop on Ultrasound
Non-invasive Functional Brain Monitoring, NIMH
Frontiers in Space Medicine, MGH Center for Innovative Minimally Invasive Therapies
National Biocomputation Center, Stanford University
NASA Ames Research Center
Jet Propulsion Laboratory
NanoSpace 2001
Neuroscience MGH
Draper Laboratories
MITAC Workshop on Wireless Communications
Human Brain Mapping

Selected Media Coverage

2000 Oct	Mars: An Adventurer's Guide. *National Geographic Adventure Magazine*
2001 Jan	The Body in Space (cover story). *National Geographic*

PRINCIPAL INVESTIGATOR:	**James Thomas, M.D.**
PROJECT TITLE:	**Diagnostic Three Dimensional Ultrasonography: Development of Novel Compression, Segmentation and Registration Techniques for Manned Space Flight Applications**

Progress on this SMST project includes:

- Initial images from ISS HRF ultrasound unit acquired on June 12, 2001
- Novice sonographer training and initial testing completed
- 3D ultrasound visualization program completed
- 3D packet wavelet transform applied to real-time 3D ultrasound images
- 100:1 compression validated with real-time 3D ultrasound images

- Successful development of registration code with application to ultrasound and nuclear images
- Acquisition of hardware to perform 3D reconstructed with ISS ultrasound equipment

Dr. Thomas is the SMST representative for the NASA – NSBRI users working group for flight experiments. This project plans to expand its artificial intelligence component and preliminary links have been made to the MIT AI Laboratory.

PRINCIPAL INVESTIGATOR:	**James Thomas, M.D.**
PROJECT TITLE:	**Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity**

The status and accomplishments of this project, which is jointly supervised by the Cardiovascular Alterations Team, are:

- Digital Echocardiographic Storage/Analysis/Database/ Capability Complete
- Bed Rest Data Analysis– Boston NSBRI Experience
- Novice Echocardiographic Training Evaluation
- Cardiovascular Functional Assessment with Color Doppler M-mode and Strain Imaging

B. Implications

The implications of early developments within the SMST are that:

- Smart medical systems, as characterized in the context of the NSBRI SMST, represent potentially revolutionary advances in health monitoring and care for humans in space and on earth. A plan has been developed to convert this general statement to deliverables within five to ten years, although components of the system will be ready within the next three to five years (e.g., Dr. Soller's NIRS sensor and analyzer for tissue pH assessment and modification of exercise protocols to assess muscle performance and loss in space).

- The SMST is working as a team, where the added value of projects is apparent in the synergy among technologies and the establishment of a platform upon which the vertical developments of each project can be integrated into a working system.

- Some of the technologies (e.g., in projects led by Drs. Putcha, Soller and Sutton) are relatively far along to be considered for flight testing in the near future to determine real time physiological changes and CM efficacy in remote, space relevant, environments (e.g., KC135)

- Some technologies have immediate applications to earth based care, and these applications are being explored (e.g., Drs. Klempner and Cunningham – colorimetric sensor for rapid detection of biological agents and organisms relevant to infectious disease monitoring in high risk environments such as intensive care units, and in the setting of biological or chemical terrorism; Dr. Soller – non-invasive assessment of diabetic microvasculature, and multiple blood and tissue components; Dr. Sutton and

colleagues – non-invasive, portable means of functional brain monitoring for cognitive neuroscience and disease, such as stroke progression)

V. FUTURE PROGRAM DIRECTIONS

A. Five-year Research Strategy (2002-2006)

The following research efforts are proposed for the SMST, given the needs of the CPR and missing or limited efforts in the prototype illustrated in fig. 3:

- Full three year support for the Klempner and Putcha projects
- Synergy support to fully integrate the SMST, and its "smart" devices, with other NSBRI efforts aimed at (a) assessing the physical and physiological deconditioning processes associated with space travel, and (b) determining whether applied CMs are effective or may require modification(s)
- Support for new projects focused on human/machine identification/classification, decision-making and informatics
- Collaborative support with the Integrated Human Function Team concerning the Digital Human Project, Distributed Networks of Networks, and decision support modeling and learning
- Enhanced program in pharmacology, especially with respect to chemical engineering for space-hard drug delivery and assessment of pharmacological agents subjected to the space environment
- Further support of non-invasive sensor/effector systems for physiological assessment and medical CMs
- Implementation of wireless communication as a component of sensor development
- Encouragement of more industry participation in the SMST, especially among biotechnology and device companies
- Support for reviewing and coordinating efforts within NASA and beyond for a national program in Smart Medical Systems

B. Five-to-Ten-year Research Strategy (2007-2011)

As the research trajectory transitions to space flight opportunities, it becomes important that the non-invasive, lightweight, portable, non-intrusive, low power sensors that comprise the front end of the SMST technology development pipeline are tested in flight as soon as possible (e.g., sooner than five years hence if possible – see section IVB). This is a top priority for proof–of-

principle demonstrations that physiological monitoring, even with low level automated interpretation, can be accomplished onboard, in real time and with minimal skill and resources required by the astronauts. Specific components of the research strategy are:

- Enhanced support for ground based development and flight testing of non-invasive sensor suite, coordinated with NASA JSC for high priority CPR and medical operations needs

- Development and flight testing of a complete smart system, including automated sensing, decision support, pharmacological intervention and evaluation of CM effectiveness, with further recommendations and passive monitoring by ground crew

- Continued support for novel, minimally or non-invasive, image guided surgery technologies for use in common, low risk medical events, as well as uncommon, high risk events

- Increased transition of functional genomic and proteomic knowledge to smart medical suite of reconfigurable effectors and CMs

- Testing of functional imaging capabilities, including data acquisition and onboard analysis, for physiological monitoring, including measurements during gravitational and pressure changes

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

TECHNOLOGY DEVELOPMENT TEAM

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FGY 2001 FINAL PROGRAM REPORT

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PROGRAM EXECUTIVE SUMMARY

The objective of the Technology Development Program of the National Space Biomedical Research Institute is to develop devices, instrument systems, and associated algorithms and software that lead to a better understanding of the barriers to long-duration space exploration and assist in the development of countermeasures to assure safe and productive space missions. The primary focus of the Technology Development Program is directed towards those technologies that support the ground-based and space-based activities of the other NSBRI Research Teams. The unique feature of this program is the opportunity to bring an integrated system engineering perspective to bear on the technological developments necessary to support basic research. Multidisciplinary development teams have been established to work on strategically focused projects that integrate individuals and institutions with vastly different capabilities into a cohesive team.

Eight development projects were selected, by independent review, for pursuit under the Technology Development Program. Three of the projects were continuing from the previous proposal cycle and these projects demonstrated excellent progress in achieving their individual goals and objectives. Five of the projects were new starts, some with staggered beginning dates, and all have made substantial progress consistent with their start dates and their stated objectives and goals. To preclude unexpected technology issues and assure that the projects address needs established by the other Research Teams, rigorous reviews of both the continuing and new projects were conducted during the year. Each project team was encouraged to work closely with one or more of the other Research Teams that would benefit from their project's development. In the continuing projects, prototype instruments and systems are already operating and have moved along the path to the establishment of definitive scientific results. For the new starts, the designs, for the most part, have been completed and some prototype development has begun, which, when complete, will have sufficient application to real problems to effectively demonstrate their utility.

The Advanced, Multiple Projection, Dual-Energy X-ray Absorptiometry (AMPDXA) Scanner System project has developed a concept for a low mass, compact, low power, highly accurate dual-energy x-ray absorptiometer that will afford NASA astronauts the ability to measure bone mineral density (BMD) and geometry of the whole human body in space. This AMPDXA supports the explicit needs of the Bone Demineralization/Calcium Metabolism Team (Bone Team). Acceleration of age-related osteoporosis is rated as Risk Rank 1 of Risk Type I and the loss of skeletal muscle is rated at Risk Rank 1 of Risk Type II in NASA's Critical Path Roadmap. The AMPDXA development is being carried out in three stages: (1) a Laboratory Test Bed used for instrument development and initial AMPDXA performance demonstration, (2) a Clinical Test System for ground-based human testing and the development of operation and testing protocols, and (3) a protoflight design for space applications that has minimum volume and mass (46 kg). Both the Test Bed and the Clinical Test System have been used to collect three-dimensional images of bones. These devices have greater precision and higher resolution than the latest commercial instruments by a least a factor of three. The AMPDXA is designed to measure BMD, decompose soft tissue into fat and muscle, and derive structural properties (cross sections, moments of inertia, etc.) over the entire body, but especially in the spine, pelvis, and upper legs. Such data permits assessment of microgravity effects on bone and muscle and the associated fracture risk upon returning to planetary gravity levels. As mentioned above, the AMPDXA has the ability to determine body composition of soft tissues important for the research of the Muscle Alteration and Atrophy Team (Muscle Team). The Clinical Test System

is operational and initial human testing trials are about to begin. Initial results of the Clinical Test System (using phantoms and cadaver parts) have shown even greater precision than the highly accurate Laboratory Test Bed results. With the AMPDXA, the loss of BMD, changes in geometry, and changes in body composition can be obtained throughout the whole body as a function of time during spaceflight. Whole body measurements are important because of the relatively poor correlation obtained between sites for age-related osteoporosis and that the bone loss in space may have even different correlations. Such measurements will provide a better understanding of the basic loss mechanisms at work, help assess the efficacy of different countermeasures, and provide a method to control the application of the selected countermeasures. The AMPDXA can also be used to provide digital radiography. The AMPDXA design can be adapted for use in bed rest studies, the Space Station, and potential planetary missions. There has been extensive collaboration with the Bone Demineralization/Calcium Metabolism Team and one of the co-investigators of this project is an investigator on that team.

The Neutron Energy Spectrometer (NES) project is developing a portable, real-time neutron spectrometer to measure the neutron energy spectrum from 20 keV to 500 MeV with a goal of 10% energy resolution and the ability to count neutrons whose energies are less than 20 keV. The NES is in direct support of the Radiation Effects/DNA Damage and Repair Research Team (Radiation Team). Carcinogenesis caused by radiation is rated as Risk Rank 1 of Risk Type I in NASA's Critical Path Roadmap. The ultimate real-time neutron spectrometer flight instrument will be the size of a briefcase with a mass of less than 10 kg. An alarm will be included to warn astronauts when preset thresholds are exceeded. The NES project is in the last stages of developing an engineering prototype that will be directly adaptable to the radiation monitoring needs of the International Space Station (TSS) and the Shuttle, and could be used on future planetary missions. To cover the required neutron energy range (20 keV to 500 MeV), the neutron energy spectrometer uses two detectors: (1) a ^{3}Helium gas tube for the low-energy neutrons, and (2) a 5 mm thick, lithium-drifted silicon detector for high-energy.

The current engineering prototype weights 27 kg. This NES prototype has flown on several F-15 aircraft flights to an altitude of 40,000 feet. Data has been obtained at or near the corona region (pressure less than 1 psi) that exists not only in the unpressurized pod of the F-15, but also will exist on the surface of Mars. In addition to the aircraft flights and a projected balloon flight to 90,000 feet, other work involves modeling and analysis of high-energy neutron experiments (50-700 MeV) conducted at the Los Alamos Neutron Science Center to verify the performance of the high-energy detector. There has been close collaboration with the Radiation/DNA Damage and Repair Research Team since an NES co-investigator is a principal investigator on the Radiation Team.

The long-term objective of the Miniature Time-of-Flight Mass Spectrometer (TOFMS) project is to design, build, and launch a flight-qualified TOFMS for use on space platforms such as the Shuttle, ISS, and a mission to Mars. The TOFMS can identify and quantitatively measure critical biomarkers associated with the deleterious effects of microgravity and long-duration spaceflight. The biomarkers can be determined from the analysis of breath, body fluids, products of infection, and, perhaps, DNA repair products and DNA mutations. As currently configured, the system appears to be of particular value to both the Bone and Muscle Teams, but biomarkers important to several other Research Teams can also be obtained.

Many of the biomarker identification procedures are complex, requiring special protocols and associated laboratory equipment. To carry the equipment and chemical supplies required to monitor the health of an astronaut would be weight prohibitive and would necessitate specialized training, and a significant fraction of the astronaut's time. The TOFMS provides a small, efficient, broadband diagnostic instrument that can rapidly identify biomarkers important for successful human space exploration.

The TOFMS system being developed is small (less than 1 cubic foot), lightweight (less than 5 kg), low power (less than 50 W), and rugged. An early prototype of the system is shown in Figure 6. This NSBRI-sponsored TOFMS is building upon technology developed for DARPA to analyze chemical and biological weapons, while being optimized for astronaut use and the identification and quantification of biomarkers. To date, the TOFMS has shown spectra of compounds ranging from under 100 to beyond 10,000 atomic mass units (amu).

The Improved Bubble Detection for Extra-Vehicular Activity (EVA) project goal is to improve current bubble detection methods. The assembly of the International Space Station requires extensive and unprecedented extra-vehicular activity. Because spacesuits operate at low internal pressures, the astronauts are highly susceptible to decompression sickness (DCS) (gas bubbles in the blood). A range of pre-breathe strategies, as well as suit gas mixtures and pressures, are employed to mitigate the risk. During ISS construction, in-suit Doppler bubble monitoring will be provided to detect conditions that increase DCS risk. Doppler bubble detection, while effective, has three primary limitations: 1) it is motion sensitive; 2) it detects only moving bubbles; and 3) it does not detect bubbles with diameters less than 80 μm.

The Improved Bubble Detection for EVA project will exploit two transcutaneous ultrasonic bubble detection and sizing instruments under development by NASA. These instruments utilize bubble resonance (not Doppler) techniques, thus allowing the instruments to measure stationary bubbles as well as bubbles of smaller size. One instrument is optimized for intravascular bubble detection in the size range of 30 to 200 μm. The other monitors extravascular bubbles in the 1 to 10 μm size range. Both instruments in *in-vitro* trials have demonstrated bubble detection at their lower range limits. The Improved Bubble Detection project will combine these instrument development teams with hypo- and hyperbaric research facilities to validate the application of these instruments for *in-vivo*, transcutaneous bubble detection. Improved Bubble Detection project results will lead to: (1) a better understanding of DCS, and (2) improved DCS monitoring and prevention techniques. The Improved Bubble Detection project has had direct interactions with both the Human Performance and Smart Medical Systems Team.

The Scanning Confocal Acoustic Diagnostic (SCAD) System project is focused at the measurement of bone loss is space. On Earth, early diagnosis and proper treatment of progressive bone loss (and/or poor bone quality) can dramatically reduce the risk of bone fracture. The principal diagnostic method for osteoporosis and microgravity-induced osteopenia is dual energy X-ray absorptiometry (DXA). Conventional DXA's provide only an index of bone mineral density, and not the bone's physical properties (the AMPDXA project described above will provide significant structural information as well). While ultrasound systems have the potential for determining the material properties of bone in a safe, repeatable and highly accurate manner, limitations in the performance of current ultrasound systems restrict their application to first-order screening, rather than the clinical standard upon which osteoporotic diagnosis and treatment regimens are based.

The research goal of the SCAD project is to continue the development and evaluation of a scanning confocal acoustic diagnostic system capable for the non-invasive generating of high-resolution ultrasonic attenuation and velocity maps of trabecular and cortical bones. The SCAD is usable not only for ground-based determination of bone's physical properties, but, because of its low weight and size, is also suitable for monitoring subtle changes in bone density and strength during extended space flights. The SCAD project is divided into four basic parts: (1) development of the SCAD system hardware, (2) correlation of SCAD-determined sound velocity and attenuation measurement with micro-CT bone BMD and structure, (3) prediction of the risk of trabecular bone failure associated with osteoporosis in the animal model, and (4) correlation of SCAD-derived BMD and structural modules with DXA measurements.

The Dynamic Exercise Countermeasures Device (DECD) is aimed at developing a countermeasure to bone and muscle loss in space. Bone demineralization (bone mass loss) is a well-documented physiologic effect of long duration space flight and microgravity. Animal experiments on Earth have clearly indicated that: (1) certain bone strains and strain rates stimulate bone deposition, and (2) repetitive loading of the lower extremity can increase osteonal bone formation even as proximally as the vertebral column. Such studies have also indicated that a relatively small number of appropriate weight-loading cycles may be sufficient to stimulate bone deposition. Based on prior research with weight-loading experiments upon the foot, a dynamic exercise countermeasure device that utilizes jumping as the mode of exercise for the astronauts is under development. The DECD project is divided into three phases: (1) develop a lightweight, vibration-isolated exercise device, suitable for use on the ISS, that will permit dynamic jumping exercise within microgravity; (2) perform system testing using zero-gravity simulation; and (3) verify DECD efficacy in true microgravity through KC-135 experiments. Currently, a prototype device is in operation. The DECD project has had direct interactions with the Bone, Muscle, and Rehabilitation Teams.

The Heavy Ion Microbeam/Detector System is aimed at studying radiation effects at the cellular level. Using microbeam irradiation facilities, it is now possible to place discrete numbers of particles in defined cellular and extracellular locations. Such facilities permit heavy-ion radiobiologists to explore the impact of signal transduction between cellular compartments as well as issues related to intercellular communication at low limiting fluences where not all cells in a population have been traversed. A high-energy, heavy-ion microbeam will allow an important unanswered question to be addressed, i.e. whether neurons that survive transversal by high-energy heavy ion (HZE) particles develop changes as a late consequence of the damage they incurred. These low-fluence studies will increase the understanding of the consequences of exposure to high, linear energy transfer (LET) radiation, such as encountered in the space radiation environment. (See the NES project above.) Currently, the microbeam detector has been designed and simulated.

The purpose of the Heavy Ion Microbeam and Micron Resolution Detector Project is to allow such radiation studies as described above to take place by developing the following tools: (1) a microbeam (diameter 10 μm) of heavy ions (e.g., iron) at energies higher than existing ion microprobes (3 GeV/nucleon), and (2) an electronic position-sensitive detector for heavy ions with a position resolution better than 1 μm. Interactions between the Heavy Ion Microbeam/Detector project and the Radiation Team have taken place.

The goal of the Space Quantifiable Magnetic Resonance Imaging (MRI) System, or Space MRI, is to develop a proof-of-concept engineering model of a space qualified MRI system for small

animals and astronaut limbs with a mass of less than 130 kg and low average power (<1 kW quiescent and <1.2 kW when scanning). An on-board processor or personal computer can be adapted to display the collected information. MRI's provide high-resolution, high-quality anatomical information without ionizing radiation, so they can be safely and repeatably used to track changes without deleterious effects.

As a result, the study of physiological alterations in space and the development, verification, and maintenance of countermeasures will be significantly enhanced. Mice and small rat models are useful surrogates to carry out in-orbit physiological studies. In-flight MR imaging of these animals will be of particular benefit to countermeasure development by several of the NSBRI Research Teams. Measurement of alterations in the limbs of the astronauts, especially the lower limbs, will provide partial confirmation of countermeasure effectiveness and of the utility of Earth-based animal models. The MRI system is particularly amenable to the study of soft tissue and bone. To date, magnet trade-off studies and initial system designs have taken place.

The Technology Development Team embodies a sense of cooperation and synergy that is unique to the Institute. There is a cohesiveness that exists between the individual project teams and researchers within the other NSBRI Research Teams. There is a strong intra-team coalition that enables free and open technology interchange. All of these attributes provide a strong basis for contribution to and support of the Institute's mission. To assure synergy, there were a dozen conference calls, meetings with the other Research Teams, a Technology Team retreat, and, during the academic year, monthly lunches for the Baltimore/Washington investigators (representing several of the NSBRI Research Teams).

II. INTRODUCTION

The Technology Development Program of the National Space Biomedical Research Institute was established to develop devices, instruments systems, and associated algorithms and software that are important to the work of the other Research Teams in the Institute and the at-large space life science research community. The activities also assist the transition of the respective technology to the civilian community to benefit society. The program's objectives are achieved through strong collaboration between the basic and applied researchers addressing the complex adaptation of humans to spaceflight and engineers, computer scientists, and physicists knowledgeable of state-of the art technologies. Multi-disciplinary teams have been established to work together on strategically focused projects that integrate individuals with vastly different capabilities into a cohesive team.

The program strategy of the Technology Development Team is to develop important systems and technologies to support the NASA Space Life Science Program with particular emphasis on working with and satisfying the needs of the other Research Teams in the Institute. It is also important that the existing technologies and capabilities of the entire research and industrial communities be fully exploited. The Technology Development Program has established and fostered cross-disciplinary, multi-institutional, multi-talented, strategically focused, and goal oriented project teams to advance technology developments to address the complex adaptation of humans to spaceflight. The specific objective of the Technology Development Program is to support the Institute Research Teams by developing systems, devices, instrumentation, algorithms, etc., necessary to:

(1) increase understanding of physiological and psychological responses to the space environment,
(2) develop countermeasures,
(3) support remote health maintenance and medical care,
(4) exploit the advances made to improve the quality of life on earth,
(5) support space life sciences educational and training programs, and
(6) promote technology transfer by collaborating with industry early in the development process.

An important NASA objective is that the Institute promotes the transfer of its technology to civilian applications. By agreement, title to the technology resides with the institution in which the technology was developed. Consequently, the Principal Investigator has been encouraged to consider establishing partnerships with industry early in the development process so the licensing institution will have a potentially ready-made commercial partner. This would reduce the time required to bring products to market and help ensure their availability to the broad research and civilian communities.

III. RESEARCH PROGRAM STRUCTURE & DESIGN

The risks associated with long-term exposure to microgravity and a high radiation environment are numerous; they represent the basis for the research program pursued by the National Space Biomedical Research Institute. Most of the ongoing NSBRI research is vertically integrated within a specific thrust area. For instance, the Research Teams typically have a core research topic that is combined with several special topic areas to form a disciplined approach to addressing a number of related issues.

The Technology Development thrust area is implemented in a different manner. The funded projects are selected, among other reasons, for their ability to provide necessary and enabling technologies for the basic research areas. Thus, the thrust area is laterally integrated with the other research areas. Figure 1 is a diagram showing the interaction between eight current Technology Development Team projects and the other eleven NSBRI Research Teams. As can be seen in Figure 1, the current Technology Development Team projects support nine of these remaining eleven research areas.

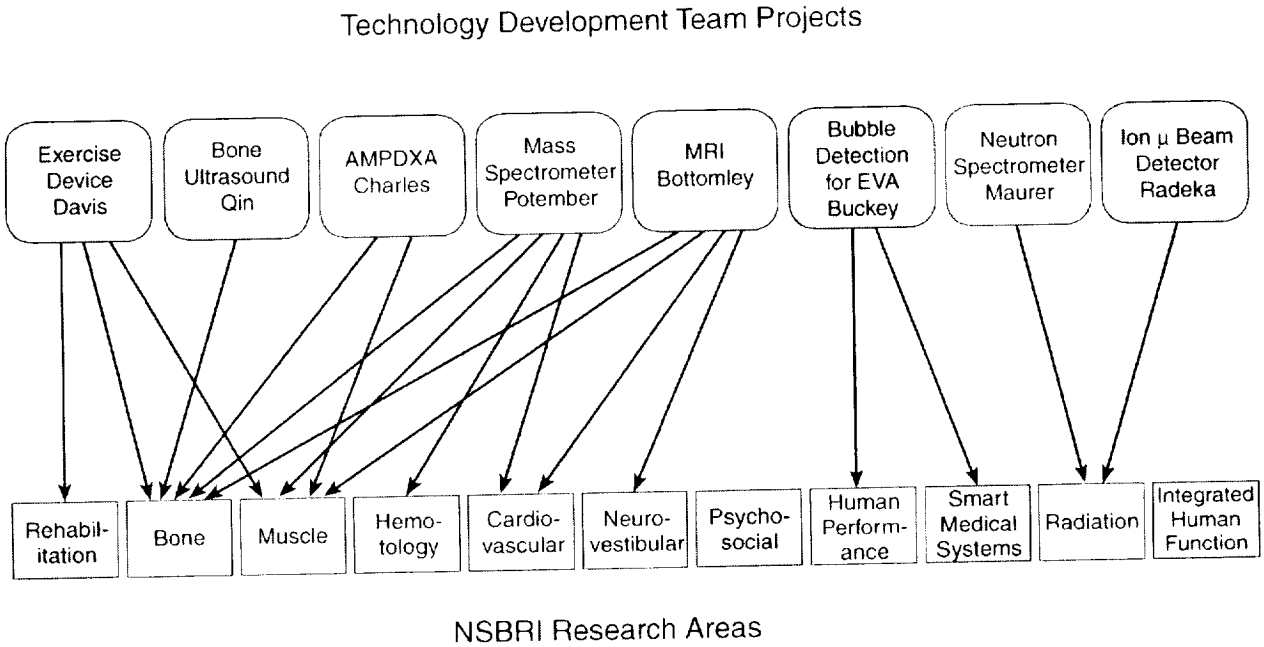

Figure 1. Mapping of current NSBRI Technology Development Team projects into the remaining eleven NSBRI Research Areas (shaded boxes indicate new projects or research areas).

Section IV below provides a detailed account of the Technology Development Team projects, including the accomplishments and the focus of their efforts to the supported basic research areas. The balance of this section describes the potential risk reduction that will be achieved by the Technology Development Team projects in terms of the supported basic research areas.

Bone Demineralization Research Risk Reduction

Two challenges associated with the development of countermeasures for bone demineralization include the understanding of the mineral loss process and being able to monitor the instantaneous

condition of the subject's bones. Technology Development Project 3 specifically addresses the process issue, while Projects 1 (AMPDXA), 5 (SCAD), and 8 (MRI) specifically address the monitor issue. Both Projects 1 and 5 have completed engineering model developments that have demonstrated the ability to provide quantitative information that is critical to the current and future research of the Bone Team.

The devices have been designed to be directly adaptable for in-space use. Size, weight, and power are currently, or will soon be, appropriate for routine launch and regular use on-orbit or in missions beyond Earth. The devices and their associated methods are highly automated and can be operated by individuals with very little training. Thus, the devices have broad utility in both space and Earth-based applications.

Historical space-based monitoring techniques have typically relied on a method of specimen sample-and-storage. Specimen analysis may, under good conditions, be completed many months after completion of the mission. This retrospective assessment may provide a limited capability to understand processes, but it certainly does not afford the ability to provide closed-loop monitoring and control of countermeasures. Projects 1, 3 (TOFMS), 5, and 8 generate data that are available in (near) real-time, thereby providing a means to achieve the closed-loop. A secondary benefit accrues by virtue of not requiring the volume and support requirements associated with specimen storage facilities.

The bone demineralization conditions that astronauts experience are similar to those that exist in clinical populations (e.g., advanced age, quadriplegia) on Earth. Thus, the research that will be supported by Projects 1, 3, 5, and 8 is expected to have a direct positive influence on a much broader population than just the astronaut community. As well, the technology itself has demonstrated better performance than commercially available devices; therefore, clinical versions of the technology will prove useful in the general clinical environment. Project 7 (DECD) is focused on a direct countermeasure for both bone and muscle loss – exercise.

Muscle Alteration Research Risk Reduction

Muscle alteration research faces the same challenges noted, above, for bone demineralization. Technology Development Projects 1, 3, and 8 provide the same armament of tools in support risk reduction for muscle as they do for bone. At this point in time though, Project 1's performance is less well developed for muscle than it is for bone, while the MRI in Project 8 will be more optimum in this regard; however, instrument development has just begun. Project 7 will directly countermeasure muscle loss in space.

Radiation Effect Research Risk Reduction

Exposure to radiation in space is a threat that can lead to an increased risk of cancer and DNA damage. A significant portion of the exposure, between 30-60%, results from neutron sources which are extremely difficult to monitor, let alone characterize, in real-time. Absence of a portable, quantitative, real-time neutron spectrometer results in an exposure safety risk for astronauts. Project 2 (NES) was proposed to develop such a neutron spectrometer and supply information on the neutron environment to the Radiation Effects Team in support of assessing radiation damage and cancer risk. The prototype of this unit is operational and has just completed several flight tests in F15 aircraft. Project 6 (Ion Microbeam) will address radiation damage at the cellular level.

Cardiovascular Alterations Research Risk Reduction

Orthostatic intolerance can result in syncope when an individual is subjected to gravitational influence after exposure to microgravity. This situation can pose severe risks to astronauts who have to execute unassisted emergency procedures or extraterrestrial landings. The ability to predict, prevent, or control orthostatic intolerance and its effects is significant to the space program. The completed cardiovascular system identification was a self-contained, automated device for measuring and characterizing alterations in cardiovascular regulation. Both Projects 3 and 8 also have the ability to provide near real-time monitoring. The TOFMS can detect various heart-related biomarkers and the MRI has potential to look at soft tissue, including vein and artery blood volumes and fluid shifts.

Human Performance Risk Reduction

While not originally a focus of the Miniature Time-of-Flight Mass Spectrometer project (Project 3), the TOFMS is capable of supporting the research of the Human Performance Factors, Sleep and Chronobiology Team by measuring melatonin in saliva as opposed to the invasive techniques now used. Similarly, Project 4 (Improved Bubble Detection) has the potential to provide real-time feedback on bubble formation in the blood and allow astronauts to avoid or modify decompression sickness.

Hemetology Risk Reduction

Project 3 can sample enzymes and other biomarkers of interest directly from blood in near real-time manner. The TOFMS can work with a small quantity of blood (finger prick) rather than the needles or catheters of normal blood sampling techniques.

Rehabilitation Risk Reduction

Project 7 is directed toward dynamic exercise and thus can play a major role in rehabilitation along with a countermeasure in bone and muscle loss.

Neurovestibular Risk Reduction

Project 8 has direct measurement capability for various neurovestibular functions on animals in space, such as the oscillatory flow in the cochlea of mice.

Smart Medical Systems

Projects 1, 3, 4, 5, and 8 are all examples of smart medical systems that will provide near real-time feedback to the astronauts. These systems will provide information in a form that can be readily assimilated and used by the astronauts. These instruments can also be used to monitor the countermeasures applied by the astronauts. While not closed loop, many of these instruments could contain decision aids to help in the countermeasure selection.

Other Research Areas

In the first four years of funded activity, the Technology Development Team did not have any directly funded projects that specifically addressed risk reduction in the Integrated Human Function and the Neurobehavioral and Psychosocial Factors Teams.

IV. RESEARCH PROGAM ACCOMPLISHMENTS

The Technology Development Team supports the needs of the other NSBRI teams and NASA. Through close communications with these groups, this team develops devices, instruments, and systems to improve research techniques and medical care on the ground and in space. Projects of the Technology Development Team focus on designing lightweight, compact research tools and on developing simple, minimally invasive and non-invasive sampling and measurement methods. Currently, there are eight active projects being pursued by the NSBRI Technology Development Team: three projects continuing from the first research cycle (1998-2000) and five new projects that were nominally started in February 2001. Some projects had slightly later starts due to funding transfer issues. One Technology Development Team project on Cardiovascular Assessment from the first cycle was completed during that period and will only be mentioned briefly in the remainder of the report. Research program accomplishments for the eight active projects are reported below.

Project 1: Advanced, Multiple Projection, Dual Energy X-ray Absorptiometry (AMPDX) Scanner System
PI: H. K. Charles, Jr.

This project used advanced sensor and detector design and fabrication techniques to develop a compact, storable, low mass, and low powered dual energy X-ray absorptiometry (AMPDXA) Scanning System that is capable of determining bone mineral density, bone cross sectional area, and bone moments of inertia at any body site. The AMPDXA has a design accuracy of one percent and has the capability to measure regional composition of muscle and fat to about five percent. The effect of a microgravity environment on bone and muscle varies from one body site to another, so the ability to measure at all body sites was critical for success. This was especially so because the correlation between sites in age-related osteoporosis is not especially high and the correlation of bone loss between sites in space may not be the same. The mass requirement for the system was less than 100 kg but the researchers had also set a non-commitment goal to reduce the mass to <60 kg. Through their research, a design with a mass of about 46 kg has been conceived. This instrument provides bone density and the structural properties of bones that are critical for understanding bone remodeling on the ground and in space. It also has the capability to measure the change in muscle mass necessary for understanding the process of muscle atrophy, but this was not part of the original proposal. This instrument can be used to investigate countermeasures for bone loss and muscle atrophy in space and then can be used to moderate the proposed countermeasure. System requirements for high spatial resolution and rotational scan geometry will permit the system to be extended to provide the additional capability of digital radiography and possibly computer tomography. The design is suitable for use in bed rest studies, the Space Station, and potential planetary missions.

This project supports the explicit needs of both the Bone Demineralization/Calcium Metabolism Research Team and the Muscle Alterations and Atrophy Team. The prototype system is capable of real-time monitoring of bone and potentially muscle loss at extremely high precision. Since the resultant measurements are patient specific, the system is useful for monitoring the effectiveness of countermeasures as well as determining the risk of fracture of individual astronauts under deployment scenarios. On Earth, the system is a natural adjunct to research on the effects of aging and disuse on bone integrity along with routine screening for osteoporosis and monitoring for efficacy of osteoporosis therapy.

The AMPDXA development is being carried out on three stages: (1) Laboratory Test Bed, (2) Clinical Test System for ground-based human testing, and (3) a prototype design for space applications.

Accomplishments:

- Laboratory Test Bed

The Laboratory Test Bed has been fully operational for the last three years. It has allowed the AMPDXA project to develop sources, detectors, and software algorithms necessary for the high-precision detection of BMD and bone structure. In this current period, the Laboratory Test Bed has allowed the refinement of our BMD and structure extraction algorithms as well as making continued progress on the high-resolution separation of soft tissue from bone. Multiple-projection analysis enables the user to evaluate bone structural properties (e.g., bending strength) independent of subject position and orientation. Empirical evaluations to date have demonstrated an average coefficient of variation in the maximum and minimum moment of inertia (I_{max} and I_{min}) of less than 3%. It is projected that further processing refinement will reduce the error in a three-projection estimate to <1%; adding more projections will also reduce the error.

Multiple-projection imaging has led to the ability to generate three-dimensional reconstructions of the imaged bones. Future adaptation will permit full modeling with mechanical properties integrated with the shapes. Thus, this will allow risk assessment of astronauts to be done on an individual basis rather than on population means as is now done with conventional DXA measurements, which can sometimes be misleading. In addition to these software and analysis improvements, the Laboratory Test Bed has allowed the development of three new antiscatter grid structures and a new, high-energy x-ray source.

- Clinical Test Unit

The clinical test unit is capable of translation and rotation of the image plane, unlike commercial DXA systems. The objective of this system, built on the chassis of a used CT scanner to save time and cost, is to provide three-dimensional bone structural information as well as directly determine magnification. In this current period, the Clinical Test Unit has become operational with a full battery of control and analysis software suitable for human testing. To facilitate human testing, a patient testing room has been refurbished and the Clinical Test Unit has been installed.

In collaboration with the bone demineralization team, a human study has been proposed in which the clinical engineering model will be used to monitor changes in bone characteristics in mobility-limited subjects. Institutional Review Board (IRB) approval has been granted for the use of the system without any conditions imposed. The FDA has granted an Investigational Device Exception for use of the Clinical Test Unit for human studies.

Due to issues associated with medical research protocols at Johns Hopkins Medical Institutions, all IRB approvals were suspended for a period of several months. Thus, previously approved IRB's had to be reapproved and no patients could be enrolled in studies until reapproval was granted. The AMPDXA IRB was just reapproved in early November and initial human test results are expected by the first of the year.

- Space Protoflight Unit/Commercialization

Launch weight and spacecraft payload size limitations are serious factors associated with the viability of a piece of flight hardware. Without these constraints, commercial DXA systems are primarily designed for subject comfort and convenience. The project team has pressed hard to achieve exceptional system performance while also accommodating ease of use, minimum size, and a significant reduction in weight. The clinical engineering model was fabricated with existing components to meet the budgetary constraints, but a design exists of a system with a mass estimate of 86 kg, which is notably lower than the originally projected weight of 100 kg. In the 1-3 year timeframe, it is expected that advancements in x-ray tube technology will further reduce the weight to approximately 60 kg. In about 3-5 years, all of the component technologies (i.e., x-ray tube, detector, power supply, electronics) will have matured to the point where a target weight of approximately 46 kg will be achievable.

An additional configuration for the AMPDXA has been conceived that promises to offer design simplicity and lower development and manufacturing costs. This unit is being considered for commercialization (spin-off company) and its ultimate implementation may lead to improvements in the current flight design concept.

Project 2: Portable Neutron Energy Spectrometer
PI: R. H. Maurer, Ph.D.

Galactic and solar cosmic rays are inordinately effective at producing secondary neutrons when they encounter spacecraft or habitat material. These neutrons can cause cellular and DNA damage to those exposed. The neutron component of radiation in a space structure is estimated to be between 30 to 60 percent of the total radiation environment when outside the Earth's magnetic field. To be able to measure the neutron spectrum, a portable brief case size, real-time neutron spectrometer prototype with a mass of less than 10 kg has been developed to support the research of the Radiation/DNA Effects Research Team. It can be used to characterize the environment for the development of a countermeasure and also can be used as a real-time monitor to control the application of countermeasures. The instrument will measure neutrons in the range from 10 KeV to 500MeV with at least 10 percent energy resolution and count the number of neutrons below 10 KeV. This portable instrument will incorporate the latest advances in energetic particle detection technology, including energy loss and total energy measurement, while building on the successful charged particle instruments built by JHU/APL for NASA/GSFC and NASA/JPL for many previous near-Earth and planetary missions. The neutron energy spectrum will be measured and an alarm will be incorporated to warn the astronaut when a safe threshold is exceeded. The devices can be ported within a space vehicle to map the local neutron environment and used in a similar manner on planetary excursions. This project is in support of an explicit need of the Radiation Effects/DNA Damage and Repair Team.

Accomplishments:

- Development of a hardware prototype device has been successfully completed.

The neutron spectrum of interest spans several orders of magnitude from the thermal (keV) to the highly energetic (MeV). There does not exist a single detector technology that will provide adequate sensitivity over this large range. The project team examined a number of detector

schemes that might be combined to provide adequate spectrum span with sufficient overlap. Initially, it was thought that three detectors would be required. However, as a result of controlled evaluation and careful design, it was possible to provide the required system performance with two detectors. The project team was successful in developing a hardware prototype device that makes use of a ^{3}He tube, a thick, solid-state lithium drifted silicon detector, and associated electronics. The use of two detectors, vice three, reduces the size, weight, and complexity of the resultant device.

- Modeling of high-energy response has been successful.

Modeling of the response of the high-energy channel from detailed cross-sections of the basic neutron-silicon interactions was undertaken using state-of-the-art computer codes. The purposes for developing the models were: to assess the accuracy of these codes for neutron-silicon interactions; to use these codes to understand the results of the energy deposition measurements; to determine whether these codes can be used to calculate the effects of packaging and instrument surroundings on the incident neutron spectrum; and, to assess the ability of the codes to supplement the experimental determination of the instrument response function. A model of the high energy channel was first developed in MCNPX code, a combination of MCNP which is widely used in nuclear engineering and LAHET which is often used in accelerator design and other high energy physics calculations. The model did not reproduce the energy deposition spectra measured at the Columbia University Radiological Research Accelerator Facility (RARAF). Investigations showed that neutron calculations in MCNPX are performed in such a way as to preclude use of this code for energy deposition calculations without a major restructuring of the code. During this process, GEANT4, a code library widely used in high-energy detector design and simulation produced at CERN, was released. The project team's first model using GEANT4 uncovered several problems with the library, which were quickly addressed by the developers. The current model using GEANT4 reproduces the energy deposition spectra measured at RARAF reasonably well, although discrepancies for the highest energy depositions remain to be resolved. One interesting result of this model is a discrepancy at low energies indicating that gamma production during exposure may not have adequately been accounted for by measuring the background spectrum when the beam is off.

- Demonstration and calibration trials have been conducted at various beam sources.

Device performance has been evaluated and demonstrated at a number of neutron energy levels. The project team conducted experiments at a variety of beam sources and compared the developmental system against qualified reference standards. Beams that were used included the ^{252}Cf and americium-beryllium sources at the National Institute for Standards and Technology (NIST), the plutonium-beryllium source at Clemson University, the Radiological Research Accelerator Facility at Columbia University, and the Los Alamos Neutron Science Center.

A response function for the silicon detector was developed for neutrons between 20 and 60 MeV. The detector has also been shown to handle flux rates up to 10,000 per second using a cesium iodide scintillator as a charged particle veto. Current work will extend the silicon detector's response function below 20 MeV.

- High-altitude aircraft testing has been conducted.

A large part of the GFY 00 effort was directed at the assembly of the integrated hardware prototype system. Before that time, the project developed and evaluated separate low energy and high-energy detectors interfaced to laboratory grade support components (i.e., power supplies, amplifiers, processors). Motivation for the development of the integrated system was the opportunity to conduct high altitude aircraft flight tests to acquire performance data in a environment that is richer in neutrons. The integrated hardware prototype included the two system detectors, support electronics, mechanical fixturing, power supplies, and data recording.

During the current period, several aircraft flights of the engineering prototype were made aboard an F15 aircraft. The prototype functioned properly, even at altitudes up to 40,000 feet. The NES project team is currently planning a balloon flight that will spend 20 hours at an altitude of 90,000 feet. At 90,000 feet, the remaining atmosphere of 20-40 g/cm^2 will create a high-energy neutron environment similar to that on the surface of Mars (the target Earth nuclei are nitrogen and oxygen, instead of the carbon and oxygen nuclei on Mars).

- Additional NASA funding was awarded for materials characterization.

The proposed research, "Development of a Neutron Spectrometer to Assess Biological Radiation Damage Behind Spacecraft Materials," was selected for funding for the period May 2000 through November 2003. The primary responsibility under this grant is to support the Lawrence Berkeley Laboratory (LBL) personnel in the evaluation of spacecraft structural and shielding materials by supplying a version of the neutron spectrometer suitable for accelerator tests. The first experiments were conducted in the first quarter of 2001 at Brookhaven National Laboratory (BNL). These experiments will collide high-energy heavy ion beams with standard and novel spacecraft materials and the spectrometer will measure the neutron energy spectrum produced as a result of these collisions. A secondary responsibility for this grant is to continue development of the modeling effort in a manner that is useful for materials science experiments as well as for assessment of astronaut biological radiation risk. Success using the GEANT4 Monte Carlo code in modeling the neutron silicon interactions will supplement the current NASA modeling efforts, which employ deterministic Boltzmann transport and FLUKA Monte Carlo approaches.

The NES project team's preliminary work on shielding materials shows that polyethylene is an effective neutron shield, but that 10 cm thicknesses or more may be necessary to significantly reduce the high-energy neutron flux. Shield designs incorporating layers or mixtures of appropriate materials may be more efficient.

Project 3: Miniature Time-of-Flight Mass Spectrometer
PI: R. S. Potember, Ph.D.

A high-resolution miniature time-of-flight mass spectrometer, already under development for other purposes, has been adapted for space flight. This instrument has the potential to identify and quantify a wide variety of biomarkers to support biomedical research and medical care. It is a rugged device that will unambiguously identify samples containing many compounds and be less than one cubic foot in size, weigh less that 5 kg, and require less than 50 W of power. Its applications include: analysis of breath, body fluids, products of infection, and perhaps DNA repair products and DNA mutations. Identification of compounds with mass ranges from under 100 to more than 10,000 amu has been demonstrated. While the instrument has a wide range of

usage, the funds available limited the initial use to analysis of a variety of compounds in fluids although the instrument will be expandable for the other identified uses. As currently configured, this instrument is of special value to the Bone Demineralization/Calcium Metabolism Team and the Muscle Alterations and Atrophy Research Team as well as being useful for gathering data on a variety of other experiments for the other Research Teams.

Accomplishments:

- Engineering model hardware has been successfully implemented.

A major objective of this project was the design and development of a mass spectrometer *system architecture* that can be utilized for diagnostics based on complex, non-volatile biomarkers species. An orthogonal extraction time-of-flight mass spectrometer (TOFMS) analyzer, incorporating a dual matrix-assisted laser desorption/ionization (MALDI) and electron ionization (EI) source, was successfully completed and demonstrated. This novel instrument greatly expands the spectrum of biomarkers that can be measured by incorporating the capability of electron impact ionization with the previously demonstrated MALDI measurements.

- Techniques for detection and analysis of urine biomarkers have been successfully developed.

Sampling from urine has been chosen as a high priority for this project. Using the TOFMS, the project team has successfully recorded full spectrum mass spectral signatures of key target biomarker analytes using the MALDI technique at physiological concentrations found in urine. Compounds investigated included: insulin-like growth factors (IGF-I), Urinary 3-methylhistidine, and estradiol. IGF-I is a potent anabolic factor that mimics most of the growth promoting actions of growth hormone (GH) *in vivo*. IGF-1 has also been identified by the Bone Demineralization/Calcium Metabolism Team as an important biomarker. Initial laboratory studies with other bone biomarkers, trivalent hydroxypyridinium crosslinks and creatinine, were completed.

Another biomarker identified to be important to the Muscle Alterations and Atrophy Team is urinary 3-methylhistidine (3-MH). MALDI techniques were applied to *quantitatively* measure 3-MH in biological fluids. Various concentrations of 3-methylhistidine in water and in urine were analyzed to determine the relationship between analyte concentration and analyte molecular ion intensity. The concentrations used in this study were based on 3-methylhistidine (3-MH) concentration typically found in urine, i.e., 20pmole – 3.5nmole. The utility of two types of internal standards, histidine, a structural analogue, and d_3-3-methylhistidine, a stable-isotope labeled analogue were examined. 3-MH samples in water and urine were prepared ranging from 5uM – 10mM, keeping the (3MH)/(histidine) ratio constant at 1:10. Protonated molecular ions for 3MH and histidine could be identified in the corresponding MALDI spectra. The ratio of relative peak intensities of (3MH)/(d_3-3-MH) verses 3-MH concentration gave a linear response with a correlation coefficient, R^2 = 0.9799 and a relative standard deviation of the slope of 4.00%.

- Techniques for detection and analysis of blood biomarkers have been baselined.

Whole blood is the biological fluid of choice for therapeutic drug monitoring and for performing pharmacokinetic studies. Spectra for whole blood were recorded in DHB matrix and in cyano-4-

hydroxycinnamic acid matrix. These spectra exhibited well-defined peaks between 100 to 400 mass units.

- Biomarkers in breath and saliva have been successfully analyzed.

A breath monitoring system was used to examine human subjects in order to select molecules that may serve as biomarkers of normal and abnormal physiology. These molecules will be used to direct the selection of molecules to be monitored with the time of flight miniature mass spectrometer.

The NSBRI Human Performance Factors, Sleep and Chronobiology Team has identified that there is a critical need for in-flight assessment of melatonin levels to develop strategies to monitor the circadian physiology of astronauts during long-duration space missions. Because the sampling of plasma melatonin is an invasive procedure, it would be desirable to have a means of measuring salivary melatonin in subjects on long-duration space missions. A preliminary analysis method of measuring salivary melatonin, using MALDI TOFMS has been developed to provide a reliable, convenient, and economical way to track melatonin during space missions.

Project 4: Improved Bubble Detection for EVA
PI: J. C. Buckey, Jr., M.D.

The objective of the Improved Bubble Detection for Extra-Vehicular Activity (EVA) project is to improve EVA efficiency and safety through the *in-vivo* validation of two unique ultrasonic bubble-sizing and detection instruments that exploit bubble resonance (not Doppler) to transcutaneously detect and size intravascular and extravascular bubbles (stationary or moving) *in vivo*.

NASA presently utilizes in-suit intravascular bubble detection based on Doppler ultrasound as an early warning for the development of decompression sickness (DCS). Doppler-based systems, however, can only detect moving, relatively large bubbles and provide little information about bubble size. The ability to size bubbles, detect stationary bubbles, and detect bubbles smaller than conventional Doppler-based systems may provide important information for early DSC detection and prevention. Creare Incorporated, under two large projects for NASA, has developed two ultrasonic bubble sizing and detection instruments intended for transcutaneous detection and sizing of: (1) intravascular bubbles in the size range of 30-200 μm, and (2) extravascular bubble detection and sizing in the size range of 1 to 10 μm. The intravascular bubble-sizing instrument has been validated extensively *in vitro* using tissue phantoms that accurately mimic transcutaneous operation, and it has been successfully applied in a preliminary *in-vivo* trial. The extravascular bubble-sizing instrument is currently under development and has already demonstrated an ability to detect bubbles down to 1 μm in size *in vitro*. The instrument is presently being tested and optimized *in vitro* using a tissue phantom to simulate transcutaneous tissue bubble detection. Although these instruments have both been tested extensively *in vitro* and some preliminary but encouraging *in-vivo* work has been conducted with the intravascular bubble detection device, the potential for *in-vivo* applications of these instruments has not been fully explored nor have they been fully validated *in vivo*. The goal of the current project is to utilize these instruments to validate and optimize their performance *in vivo* and to begin to address several important long-posed DCS research questions. The Improve Bubble Detection project has combined the hyper- and hypo-baric facilities of Dartmouth-Hitchcock with the engineering capabilities of Creare Incorporated and the operational experience of key NASA

personnel to provide a unique environment for research work on improving bubble detection techniques and DCS research. The successful completion of the project could lead to improved bubble detection, an improved understanding of bubble growth, and safer more efficient pre-breathe strategies.

The 30-200 μm device has been shown both *in vitro* and *in vivo* to detect and size bubbles in the stated size range. These were laboratory evaluations under optimal but realistic conditions. Transcutaneous operation was demonstrated *in vitro* using anatomically and acoustically accurate tissue phantom of a human thigh. Transcutaneous operation was demonstrated *in vivo* with injected bubbles. However, because of the limited scope of the preliminary *in-vivo* trials, and for the convenience of alignment on the target tissue vessel (the vena cava), the preliminary *in-vivo* studies with DSC bubbles were not performed transcutaneously. There are no fundamental reasons the instrument could not have been used transcutaneously to target the vena cava for the DSC bubble study other than the alignment issue. In the current study, a straightforward modification to the instrument will be implemented to incorporate a Doppler option to aid in alignment on the target vessel. Transducer positioning, alignment, and focusing will be essential for operational use, and these must be established through ground studies. A key parameter to be established in this effort will be the minimum-transducer input-power settings that provide adequate signal-to-noise ratio. The objective is to use transducer power settings consistent with diagnostic ultrasound levels so that the instrument can be used on humans with no concern of tissue damage. Similar operational *in-vivo* experience will be required with the extravascular bubble sizing instrument, and this work is being conducted under currently funded work for NASA.

Current Doppler systems are motion sensitive, only detect bubbles greater than approximately 80 μm in size, and can only detect moving bubbles. It is not known if the Creare instrument can: (1) detect intravascular bubbles earlier than Doppler monitoring (i.e., will the ability to detect smaller bubbles improve sensitivity?), (2) be less motion sensitive than Doppler monitoring, (3) provide histogram of bubble size distribution over time as DSC develops, and (4) detect moderately large stationary extravascular bubbles (e.g., in joints). Furthermore, it remains to be shown how this enhanced monitoring capability might be used to improve astronaut safety and improve our fundamental understanding of DCS.

One longstanding DSC concept is that gas phase nuclei exist normally in tissues before and after decompression. However, current Doppler-based monitoring techniques only allow for intravascular detection of relatively large bubbles, and as a result, little is known about the fundamental development of these bubbles. If, in fact, nitrogen bubbles normally exist in tissue and could be detected there, this instrument offers the potential to monitor DCS in a novel way, and potentially monitor the growth of disappearance of bubbles during decompression, recompression, or during oxygen pre-breathe. Such a capability could greatly enhance our ability to: (1) understand DCS, (2) detect the earliest stages of DCS, and (3) improve the efficacy and efficiency of strategies for mitigating the risk of DSC such as oxygen pre-breathing.

Accomplishments:

The Bubble Detection Project has only been underway for a few months, but has already made some definite progress. Both bubble detectors have been shown to reliably detect bubbles *in vitro*. *In vivo* signals consistent with bubbles have been identified. Such signals are site-specific, such as the hip. Studies on individuals with adynamic and decompression sickness have

been inconclusive to date, but point up the need for improved signal processing, which is currently under development.

Project 5: Scanning Confocal Acoustic Diagnostic (SCAD) System for Bone Quality Assessment
PI: Y-X. Qin, Ph.D.

The goal of this project is to develop a new technology for monitoring bone quality of humans on Earth and during long-term space missions. This will lead to a better understanding of the progressive adaptation of bone loss in both aging populations and astronauts subject to microgravity. Results of the joint Russian/US studies of the effect of microgravity on bone tissue demonstrated that bone loss proceeds at an average rate of 2% per month, ranging from no loss in the area of upper skeleton to as much as 14-20% loss in the skeleton of the lower body following a 14.5-month long mission. While these results are detected only when astronauts returned to Earth, the rate of bone loss during space mission is still unclear. The principal objective of this proposal is to develop a portable scanning confocal acoustic diagnostic (SCAD) system capable of generating non-invasive, high-resolution ultrasound (US) attenuation and velocity maps of bone, and thus determining the relationship between ultrasound parameters and bone mineral density (BMD), bone quality, and other bone physical properties (i.e., stiffness and modulus). This system is relevant not only for ground-based determination of bone's physical properties, but can effectively be used in the space environment to determine subtle changes in density and strength during extended flights. In the proposed work, we will validate the structure and density information detected by SCAD using μCT and mechanical testing methods in *ex vivo* animal models. Correlations to *in vivo* DEXA data derived from humans will also be made. The system can monitor the degree and risk of bone loss in space and on Earth, and serve as a major step towards clinic usage as an early diagnostic tool for osteoporosis. The SCAD project has a three-phase approach.

Phase 1. SCAD Instrument Development. The BMD and structural modulus of *ex vivo* bone specimens will be determined using confocal ultrasound and a three-dimensional scanning system. A series of specially designed transducers will operate in a transmitting mode at characteristic frequencies of 0.5, 1, 1.5, 5, 7.5, 10, and 15 MHz. The ultrasound beam will be focused to approximately 0.5 mm (or less) in a diameter at the focal point. We will determine the most appropriate characteristic frequency to reflect the microstructure of trabecular bone from velocity and attenuation measurements. The microstructure of bone in the central plane of interest will be identified by a digital controlled mapping system, which includes 3-D micro-movement of the sensors and 3-D movement of the specimen. The bone specimen will be scanned in increments as fine as 0.05 mm per step in each direction. We will also determine the incremental thresholds for reconstructing the velocity and attenuation maps with a certain resolution. An image array of attenuation and velocity representing the quality of bone will be obtained through the scanning.

Since the scanning confocal ultrasound concept has been demonstrated successfully, a major step towards an *in vivo* application of this technology is to build the SCAD system that can scan whole bone samples at a faster rate and demonstrate the modality suitable for use in space. The ultrasound beam will be focused to approximately 0.3 mm (or less) in diameter at the confocal point. A new broadband transmitter will be used, which will test frequency-shifting response for the bone samples. The microstructure of bone in the central plane of interested area will be identified through digital controlled mapping system, including 3-D fast micro-moving stage

(<0.1 mm step resolution) and LabView controlled stepping and recording software. Whole bone samples including cortical and trabecular components (N=32) with distinct porosity, i.e., 10%, 20%, and 30% deductions using the erosion method will be tested using the modified SCAD.

Phase 2. Ultrasound Parameters versus Bone Properties. In addition to the previously tested trabecular bone specimen of 1x1x1 cm cubes (N=17) with an experimental SCAD system, a larger number of bone samples will be utilized in the project. Total of 78 additional trabecular samples will be prepared as 1x1x1 cm cubes, which are harvested from sheep femoral distal condyle. These sheep were under a mechanical stimuli protocol and their BMD was measured using dual-energy x-ray absorptiometry (DEXA). The central plane of samples will be scanned for ultrasonic attenuation and velocity using SCAD system in three orthogonal directions. Three-dimensional bone microstructure, BMD, BMC, porosity, trabecular, trabecular space, and widths will be determined via μCT at a resolution of approximately 40 microns. All bone samples will be mechanically tested by direct force-deformation in longitudinal, medial-lateral, and anterial-posterial directions. Both ultrasonic and μCT data/images will be processed using custom software (PV-WAVE). The ultrasonic attenuation and velocity will be correlated with μCT measurements and mechanical moduli of the sample. The BMD index will be calculated through fractal analysis. The interrelationship between bone quality and SCAD determined ultrasonic parameters will be analyzed in this large-scale database, and indexes will be proposed to evaluate osteoporosis.

Phase 3. Human Testing. As a step towards potential countermeasures for bone loss in astronauts and considering the potential similarity of microgravity-induced osteopenia and age-related osteoporosis, human subjects will be measured using SCAD system in normal and osteoporotic groups. The protocol will focus on: (1) multiple sites of bone quality and property measurement to evaluate site-specific bone quality; (2) anisotropy characteristics of bone quality and properties, especially including measurements in the weight bearing direction; and (3) correlations between non-invasive ultrasonic parameters and DEXA-determined BMD and BMC. Four groups of human subjects associated with age and degree of osteopenia will be tested in the project, including control, minor, medium, and high osteoporotic conditions.

Accomplishments:

The SCAD project has been underway for only a few months, but significant progress, building on past accomplishments, has been made. The SCAD instrument has been refined and a new parameter which combines attenuation and sound velocity in such a manner as to allow correlation with animal μCT measurements. Initial cadaver test results have produced a correlation between age-related bone loss and ultrasound prediction.

Project 6: Heavy Ion Microbeam and Micron Resolution Detector
PI: V. Radeka

The use of high-energy microbeam provides a unique way to control the number of particles traversing individual cells and localizing the dose within the cell. High-energy heavy charged particles transfer their energy to biological organisms through high-density ionization and excitation along the particle track even with uniform irradiation. This characteristic of microscopically non-uniform dose delivery is expected to induce complex DNA damage and mutagenesis. This is contrasted to the relatively uniform dose delivery produced by gamma rays

or electron beam irradiation. To investigate the distinct biological effects of heavy ions, especially to determine the effects of occupational and environmental exposure of very low doses of heavy charged particles (e.g., since virtually no cells receive more than one traversal cosmic ray HZE particle in its lifetime in a spaceflight environment), one approach is to select cells with the desired exposures from a randomly irradiated population.

Using conventional track segment irradiation methods and sophisticated ion track detecting techniques, the position of the target cells and the ion tracks can be measured together. However, this conventional approach is not practical because all the responses of many cells that do not contribute to the aim of the irradiation experiment must be measured. The alternative is to control each ion hit so that irradiation experiment is not a random Poisson process. A heavy ion microbeam can be used to selectively irradiate individual cells that can be analyzed afterward to determine what changes have occurred to that cell and to its un-irradiated neighboring cells.

Although the characteristic biological effects of heavy ions are supposed to be linked to the induction of high-LET-specific DNA lesions, other pathways of radiation effects are still interesting to consider, and there have been several reports that radiation effects of heavy ions may be transmitted from irradiated cells to neighboring un-irradiated cells. A heavy ion microbeam can be used to look for pathways other than DNA damage, e.r., damage to the cell membrane or cytoplasm.

The localized dose delivery of a heavy ion microbeam can be applied to the inactivation of a microscopic region of the target organisms (called the cell surgery technique). Heavy ion beams have been applied to radiation therapy development and radiation biology. For evaluation of radiation risk on mammalian cells, proton and helium ions with energy of a few and several MeV from Van de Graff accelerators are mostly used. However, heavy ion beams from those accelerators cannot be applied to this study because of their poor penetration.

Therefore, the goal of the Heavy Ion Microbeam/Detector project is to design and test a high-energy microbeam apparatus and a micro-resolution solid-state detector for space radiobiology studies. Such a facility permits heavy-ion radiobiology to address specifically the impact of signal transduction between cellular compartments as well as issues related to intercellular communication at limiting low fluences where not all the cells in a population have been traversed by even a single particle. Moreover, a high-energy ion microbeam will permit researchers to address an important unanswered question: whether neurons that survive traversal by HZE particles develop changes as a late consequence of the damage they incurred. Therefore, these low-fluence studies promise to aid in our understanding of the consequences of exposure to high-LET radiation such as encountered in the space radiation environment. The project involves the development of two major tools:

1. A microbeam of heavy ions (e.g., iron) at energies higher than at existing microbeam facilities (up to 3 GeV/nucleon). The microbeam would have a sufficiently small diameter (about 10 micrometers) to localize the ions to a single cell.

2. An electronic position sensitive detector for heavy ions with a position resolution better than 1 micrometer, to localize the position of ion impact within a particular region of the cell.

These developments will advance significantly the state-of-the-art of high-energy heavy ion microbeams and of high-resolution heavy ion detectors. For the cell studies employing these tools, the necessary infrastructure will include a micropositioning stage with a microscope and auxiliary detectors.

Accomplishments:

Although the Heavy Ion Microbeam/Detector project has been underway for just a few months, good progress has been made. The detector simulation has been performed and an optimum set of detector parameters has been determined. Currently, the project team is working on finishing the detector's mask set design and beginning an effort on the design of the read-out electronics.

Project 7: Design of a Dynamic Exercise Countermeasures Device
PI: B. L. Davis, Ph.D.

The objective of this study is to design and develop an exercise device that primarily counteracts microgravity-induced bone loss and muscle atrophy. Secondary benefits will include alleviating some of the problems associated with vestibular and cardiovascular adaptations to microgravity. This dynamic exercise device is based on data previously collected under NASA grant NAGW-5006.

The dynamic exercise countermeasure device (DECD) project has three distinct phases:

Phase 1. Design and Construct DECD. The DECD will be designed and constructed by Foster-Miller Incorporated. The assembly is designed as an exercise machine that allows the subject to simulate jumping in a microgravity environment without subjecting the surrounding vehicle structure to any significant impact loads. The assembly configuration is based on the conservation of the momentum principle whereby the subject "jumps" on a platform with mass similar to that of the subject. Both the subject and the platform are mounted on two coaxial support rails. They are both free to move relative to each other along these rails. The subject and the platform are connected by a pair of sleeved adjustable force springs. These springs are tensioned to produce a force that approximates the subject's body weight in a gravity environment. The subject is restrained on a torso support carriage. He places his feet on the push-off plate and pushes the counterbalance assembly away by straightening his legs. This stretches the force springs and simulates standing in a gravity environment. The subject then bends his knees and "jumps" off the platform. This jumping force causes the guided subject and the guided counterbalance assembly to separate.

The two masses, traveling in opposite directions, will be decelerated by the force applied by the interconnecting adjustable force springs. The deceleration rates will be proportional to the masses. When motion has stopped, the two masses will accelerate towards each other. The subject will "land" on the platform and soften the landing force by bending his knees. Both moving masses will decelerate to zero. This is equivalent to jumping in a gravity environment. This jumping cycle and the primary forces generated and reacted within the exercise machine assembly are balanced and isolated from the exterior environment. This arrangement will not be sensitive to mass differences between the loaded carriages. When subjected to the same force, both will have the same momentum with compensating differences in accelerations and velocities.

The primary strength of our project is that we are targeting multiple systems of the body that are adversely affected by prolonged microgravity. The exercise countermeasure device that we are developing will provide physical stimuli to bones, muscles, the cardiovascular system and, most likely, the vestibular system. Another strength of the project lies in its simplicity. Astronauts will perform jumping exercises while they are tethered to a support platform. The principle of conservation of momentum dictates that when an astronaut pushes off the platform, he/she will experience a "flight" phase followed by an impact phase when "landing" occurs and there is contact with the support platform. The device is being designed with key considerations for (1) mass, and for this reason the support platform will consist of a empty chamber that will be filled once the spacecraft has reached its orbit, and (22) minimizing unbalanced forces and vibrations that are transmitted to the spacecraft.

Using the DECD: (1) astronauts will be able to exercise without the need for uncomfortable harnesses (since we generate the same forces under the feet with tether forces of 50% bodyweight as we do with 100% bodyweight tensions), (2) the hardware will be considerably lighter than the treadmill flown on previous missions (60 kg compared with 320 kg), and (3) future research will need to focus on the in-flight benefits of jumping exercises versus high frequency vibrations that are applied through the undersurface of the feet.

Phase 2. Verify DECD Performance on the Ground. In order to determine the efficacy of the DECD prior to using it on the KC-135 airplane, testing will be performed in the Biomechanics Laboratory at the Cleveland Clinic Foundation. Testing will be carried out while the DECD is suspended in the ZGS so that the efficacy of using the device in a microgravity environment can be examined. Two conditions will be examined: one in which the DECD is freely suspended and one in which it is tethered via load cells in the x, y, and z directions. In the first condition, we will ascertain how much movement would occur if the DECD was free floating. In the second conditions, we will measure the (unbalanced) forces that would be transmitted to the Shuttle if the DEDC were bolted to the structure. Under each condition, the following data wall be recorded: (1) electromyographic (EMG) recordings from lower extremity muscles, (2) kinematic data from the lower limbs, and (3) ground reactions forces under the subject's feet during the exercise protocol.

Twelve subjects will participate in the study. Subjects will be selected based on the age, height, and weight requirements of a NASA astronaut. Six men and six women will be tested. The rationale for selecting 12 subjects is based on the data shown in Figure 6a. A power analysis with a one-way ANOVA model and three levels indicated that the differences in the means could be detected at the alpha = 0.05 level and with power exceeding 80% with this number of subjects. In anticipation of a 20% dropout rate in the KC-135 due to motion sickness, we have planned to fly five subjects on each of three flights. In each of the jumping tasks, subjects will be instructed to land using three difference strategies: (1) two feet, toe-heel; (2) two feed, flat-footed; and (3) one foot, toe-heel. Subjects will perform three trials of each landing position.

Phase 3. Verify DECD Performance in Microgravity (KC-135). The experimental protocol of Phase 2 will be repeated in the KC-135 aircraft. Three flights will be performed on consecutive days in the first half of the Year 3 project. On each day, four subjects will be tested. We are planning to fly five personnel each day, such that there is a back-up subject available during the flight, and to have one subject (i.e., the Principal Investigator) overlap each day to ensure continuity of data collection and experimental procedures.

Accomplishments:

Since the DECD project has been officially underway for about six months, significant progress has been made. The preliminary design of the research tool is nearly complete. The selected dynamic exercise countermeasure device (DECD) configuration has been sized for a user population with body weights between 54 and 89 kg and standing heights between 162 and 175 cm. The device measures 305 cm in length, 89 cm wide, and 47 cm tall. Estimated empty weight is 60 kg. The general arrangement includes a pair of side rails positioned to be coplanar with the combined user/carriage center of gravity and the loaded platform center of gravity. The platform tank is being sized to achieve a platform-loaded mass of 82 kg. The DECD includes a load adjustment feature in the form of a hand crank and indicator which will allow the user to pre adjust the standing load force experienced between user and platform between 40% and 60% of the user body weight. A total of four shock cords each with a free length of approximately 460 cm long are being used to develop the standing force. Shock testing is currently underway to select the optimum length of shock cord required to satisfy the specified load range.

Project 8: Space Qualifiable MRI System
PI: P. A. Bottomley, Ph.D.

The objective of the Space Qualifiable MRI System project is to develop a proof-of-concept engineering model of a Magnetic Resonance Imaging (MRI) system for small animal models and astronaut limbs that can be space qualified. Small animal MRI systems, although commercially available, are too massive to be considered for spaceflight, with masses >1000 kg and power requirements of >5 kW. Availability of a flight qualified MRI could especially benefit the study of physiological alterations in the space environment and the development, verification, and maintenance of countermeasures. The countermeasure development efforts of the following NSBRI teams would be significantly enhanced: Bone Loss, Cardiovascular Alterations; Muscle Alterations and atrophy; Neurovestibular Adaptation; Nutrition, Physical Fitness and Rehabilitation; Radiation Effects; DNA Damage and Repair, and Smart Medical Systems. This will establish a new era in space physiology research complementing and evaluating the effectiveness of the hind limb suspended mouse and rat models and human bed rest studies that are routinely carried out on Earth. Frequent MRI scanning of mice and rats in space is especially important to many of the research teams for understanding the basic processes at work. Imaging of astronaut limbs, both lower and upper arms and legs, would also contribute significantly to the verification and assessment of countermeasures. It is intended that this project will pioneer the development of a larger MRI system that could be used for full-body imaging of humans for medical care during space exploration. A compromise in the system characteristics was made to develop a system that is consistent with Space Station resources and best meets the needs of the NSBRI Research Teams.

The major goal of this project is to develop an engineering model of an MRI system for human limbs and small animals, specifically mice and rats, to demonstrate that a flight qualified system can be fabricated with the following characteristics:

- Field strength >1 Tesla and perhaps as high as 1.5 Tesla.
- Field inhomogeneity ≤8 ppm over spherical imaging volume of 10 cm diameter and ≤10 ppm out to 15 cm.
- Imaging of small animals (mice and rats) and human limbs (arm, calf, and knee).

- Standard resolution mode giving a resolution of 234 microns over a spherical imaging diameter of 6 cm for mice and rates and 703 microns over a spherical imaging diameter of 18 cm for human limbs.
- Higher resolution mode giving a resolution of 117 microns over a spherical imaging diameter of 6 cm for mice and rats and 352 microns over a spherical imaging diameter of 18 cm for human limbs.
- Mass <130 kg.
- Average power in standby mode <1 kW and during normal use <1.2 kW.

The proposed design is a compromise between field strength, imaging volume, system mass, system average power, and the ability to image human limbs and small animals with sufficient resolution.

The engineering model will be a fully functioning MRI system to evaluate the design and performance and to provide confidence in the characteristics of a realistic flight qualified system. The system will consist of redesigned, low mass, and low power subsystems consisting of: (1) a magnet cryocooler subsystem, (2) gradient coils, (3) one gradient amplifier, and (4) a radio frequency coil. The remaining electronics consisting of: (1) additional two gradient amplifiers, (2) waveform generator, (3) radio frequency power amplifier, (4) transmitter/receiver electronics and analog-to-digital converter, and (5) processor/controller subsystem will be of conventional design. The newly developed subsystems will help confirm the mass and power estimates for the flight unit. Performance of the system will be demonstrated by: (1) imaging phantoms and animal parts and comparing to physical measurements, and (2) imaging live mice and comparing to conventional MRI systems.

Accomplishments:

The MRI Project has had a delayed start and a PI change, but progress continues to be made, including magnet selection and the identification of commercial control and analysis software.

General Team Accomplishments:

The NSBRI Technology Development Team is characterized as an integrated, multidisciplinary group chartered to develop systems, instrumentation, devices, and algorithms. The accomplishments noted above provide a clear demonstration that this objective has been achieved. In addition to this, the project teams have demonstrated unique capabilities of being able to structure and accomplish complex applied research and development. Some of the characteristics that cross project boundaries are:

- The capability to successfully conduct rapid system prototyping.

All of the Technology Development Team projects were successful in accomplishing the goal of developing and demonstrating prototype system implementations. A number of patent disclosures and/or applications have resulted from the developments. The ability to support the development of practical and useful tools in support of basic research requirements is a necessary element of a successful undertaking such as the NSBRI.

- The capability to transition developments to practical embodiments.

As an extension of the prior item, it is not sufficient to develop unique, one-of-a-kind prototypes. The developments must have practical means of supporting the basic research efforts by providing reliable and robust tools. The Technology Development Team projects have successfully demonstrated the ability to transition their developments to the real-world environment. For example, the AMPDXA scanner is ready to support human testing and consideration for a commercial spin-off are underway. The SCAD system is ready for human testing and a prototype dynamic exercise device has been built.

- The capability to network and collaborate with NASA, the medical community, etc.

All of the Technology Development Team projects have established close and on-going interactions with NASA and the medical community. The interactions were initiated during the project proposal phase to assure that the intended development addressed a current space issue and was founded in a practical medical basis. The interactions have experienced positive growth and expansion throughout the research and development cycle. The result of the networking is that the resultant development products have validated utility to the space and medical communities. And, the networking within the communities has provided very good exposure and visibility for other applications and opportunities.

- The capability to produce quantifiable results to support countermeasures research.

The basic research programs of the NSBRI are charged with developing and evaluating countermeasures to the effects of long endurance exposure to microgravity. This effort requires that cause and effect relationships be identified and characterized. Proper characterization mandates that empirical data be referenced to a standard and be quantitative in nature. All of the Technology Development Team projects have achieved a level of standardization and quantitation that is necessary to support the basic research initiatives. In fact, some of the engineering models that have resulted from the team's activities exceed the accuracy and precision found in existing clinical and commercial systems.

V. FUTURE PROGRAM DIRECTIONS

The success of the Technology Development Research Team, in working closely with the other Research Teams and in developing important systems that meet their needs, validates the utility of this program to the NSBRI. Because of its nature of development as opposed to research and the unique inter-team interests, the Technology Development Research program should be evaluated with different criteria. For example, the inter-team interactions are as significant, if not more, than the intra-team interactions. Development of instrumentation systems is not inexpensive and each of the developers was faced with severe financial constraints that limited their accomplishments. Except in software intensive projects, component costs generally consume a significant amount of the available funding leaving less than desired for labor. In many cases, the research utility of the systems will be fully demonstrated by follow-on activities not covered in this development phase, which is decidedly different from the research carried out by the more traditional Research Teams.

To help foster the creation of tools and interactions necessary for success, the NSBRI's Technology Development Team has established the following long-term goals:

1. To be a leader in developing new medical instruments and devices for both ground- and space-based research in support of human and animal studies aimed at solving the human health issues associated with long duration space flight;

2. To be a leader in identifying new technological advances with appropriate development can have a major impact on space biomedical research and astronaut health;

3. To be recognized as an important service arm to the remaining eleven NSBRI research teams;

4. To be a help agent in the transfer of NSBRI-developed technological advances to industry for the benefit of earth-based medical care.

To implement the above goals over the next few years, expansion and strengthening of the NSBRI Technology Development Program must be undertaken. Aspects of this expansion and strengthening include:

1. Increasing team's interdisciplinary membership—both in the skilled base of principal investigators and the number and diversity of participating institutions. Currently, Technology Development Team research projects apply to nine of the remaining eleven NSBRI research areas. Our goal is to have projects in all eleven areas as well as to have a more balanced program between areas. Today our projects are heavily concentrated in a few areas.

2. Fostering greater interactions with the other NSBRI research teams—through (1) more frequent Technology Working Group (TWG) meetings with expanded participation from academia, industry, and government; (2) technology team participation in the annual retreats of the other research teams; (3) development of

presentations and literature, which not only touts the progress on existing projects but also makes the entire NSBRI team aware of technological resources available at participating institutions.

3. Expanding interactions with non-NSBRI institutions that have technologic ideas and resources necessary for solving the issues that surround human space flight. Ultimately, these institutions and technology leaders should become part of the NSBRI team.

4. Establishing closer ties with the NSBRI's Industrial Forum—provide Forum members with greater insight into specific technological developments from Team Projects as well as be a conduit for conveying the assessed technological development needs to the Forum. Greater ties should also be established with the technology transfer offices of the participating institutions.

5. Structuring the NSBRI Technology Development call for proposals in such a manner as to address current critical technological research and development needs while fostering diversity among the project focus areas.

Appendix C

WORKSHOP ON
ISSUES RELATED TO THE INTEGRATED HUMAN FUNCTION TEAM
May 2, 2001

REPORT

I. Introduction

Taking note of the unfavorable remarks of the Site Visitors in their report of December 1, 2000 with respect to the Integrated Human Function (IHF) research area and the subsequent concerns expressed by the External Advisory Council (EAC) in its report to the Board of Directors on March 22, 2001, NSBRI management established a panel of seven experts to examine the present activities and recommend steps to strengthen the IHF Team. The list of members of the workshop panel and its agenda are attached (Attachments 1 and 2, respectively).

II. Background

Panel members were provided with copies of the Attachments 3–9, and these served as the foundation for the subsequent discussions. We found these documents to be effective and still timely accounts of the mission of, and the issues to be addressed by, the IHF Team. We urge that they be maintained as working documents, and as the IHF Team's charter.

We strongly support the view that integration of functions is an essential attribute of health, and that it requires attention in its own right. Understanding of integration will not emerge spontaneously from even a large set of specialized studies of physiological subsystems.

III. Current Projects of IHF Team

Strengths

The Panel examined the full proposals of the six funded, ongoing projects within the IHF Team (investigator's abstracts included as Attachment 8). We agreed that these were all meritorious and appropriate studies, and that they were aimed at producing models that, at least in principle, could contribute components to the comprehensive Digital Human model.

Shortcomings and Remedies

1. So far there is rather little evidence of synergies among the projects within the IHF Team, or between IHF and other NSBRI research teams. However, some synergistic collaborations are forming and we encourage that trend. Specifically, as examples, we note opportunities for synergism between:
   - Cabrera's project (IHF) and Soller's project (Smart Medical Systems), whose synergy, in turn, could support rehabilitation;
   - The three projects by Bers, McCulloch, and Coolahan (Winslow) (IHF) and the project by Mark and Cohen (Cardiovascular Alterations). They should and can produce a common model of the heart as a test case for merging and managing various related models, using the HLA methods of Coolahan;

- Potential synergistic contributions of the projects in the Nutrition and Bone Loss research areas to modeling important but missing integrated human function effects (e.g., metabolic acidosis dissolves bone); and
- Synergism between the projects in the Nutrition and Cardiovascular Alterations teams (e.g., lipids in the diet can affect the compliance of large vessels, with functional consequences).

Given these (and other) opportunities for synergisms within the NSBRI, the following questions arise:

- Should it be a charge to the IHF team to facilitate these synergisms and, if so, how? or
- Should the responsibility for synergisms be left to the Team leaders and, if so, how are they to be motivated to act?

2. It is the opinion of this Panel that the focus of the current IHF projects is too narrow. The existing six projects within IHF are all aimed at studies of cardiac and skeletal muscle. This concentration, though appropriate for the startup, forms too slender a physiological base for creation of a Digital Human model in the spirit of the mission of the IHF research area. We note, for example, the lack of explicit treatment of neuroendocrine systems. Therefore, we recommend the expansion of the IHF research program as described below in Section VI.

IV. Competition

We note the grand aims of the Virtual Human project based in Oak Ridge National Laboratory, and advise NSBRI to renew contact with Clay Easterly at ORNL to find out to what extent our efforts may overlap or complement each other.

V. Some Questions

The Panel discussed the following questions (among other topics) in arriving at its recommendations:

1. Should the focus of the IHF group be primarily medical, aimed at identifying potential failure modes and specifying countermeasures with respect to the health of astronauts during extended space flight? OR
2. Should the focus be on the development of a new Program Manager algorithm to manage multiple subsystem models so as to extract integrated results from the set?
3. What are the general concepts for modeling complex systems?
4. Is there an associated optimal, goal-directed strategy for such an enterprise that will lead to an effective global model?
5. What are reasonable expectations for the performance of such an effective global model?
6. How are large-scale models to be validated?
7. How do we decide what's in and what's out, to avoid overmodeling? What is a "sufficing" model and how would we recognize it?
8. Is the approach to be bottom-up or top-down?
9. Is there a preferred form for a global model of a complex, adaptive system (e.g., deterministic-mechanistic, stochastic, optimal control, adaptive....)?
10. Above all – can we assure that we are adequately informed about trends in the health of individual astronauts by using a computationally efficient model that, though global in reach, does not actually require computing every detail of every subsystem all of the time?

If so, how is such an efficient model designed? (See Special Comments on the Digital Human at the end of this report.)

As a starting point to answer these questions, the Panel notes with enthusiasm the report and presentation by Joel Leonard entitled "Whole-Body Algorithm – Early Attempts and Lessons Learned" (Attachment 9).

VI. Recommendations

1. Immediate Archival Tasks (via contracts or other means, using data archive infrastructure)

A. As an immediate task, we advise NSBRI to arrange to catalog the existing medical and research records pertaining to the health and condition of astronauts, for all of the space flights for which data were obtained during the past nearly 40 years. Later, this catalog would serve to guide the formatting of the records to make them suitable for professional "data mining."

B. Similarly, we advise that NSBRI undertake translation of leading physiological models used in the past to simulate space flight or currently under development (e.g., those of Guyton, Mark, Grodins, Stolwijk - in their most usable version) from FORTRAN (or whatever their original programming language) into some higher level domain specific language for physiological modeling. This translation will allow the models to be independent of any programming language and permit easier distribution to the community of NSBRI scientists who are doing modeling related to the Digital Human. These models should be in operational form, with documentation, so that they can be easily implemented on various computers and in various laboratories.

C. We recommend that NSBRI assign an appropriate person to contact each Team Leader and set up a modus operandi for achieving and facilitating the nascent integrations specified in Section III (Shortcomings), above, and others they may recognize. It may require a budgetary line item to expedite this recommendation– a "synergy grant."

D. A WEB-site for the IHF working scientists and others engaged in the initiative based on interest and expertise should be established. This site would be a conduit for old programs which are being translated as well as a site for scientists to communicate. The WEB master office should be supervised by a senior scientist with established experience in physiological systems modeling.

2. New Projects to be Added to the IHF Research Program

A. We recommend that a new project be solicited that provides, and demonstrates the capabilities of (using relevant models and materials), an automated or semi-automated approach to the simultaneous management and operation of multiple subsystem models in different locations.

B. We recommend that a new modeling project be solicited that deals with an important research area that is currently missing in the NSBRI (e.g., the neuroendocrine system). The new project should involve a fully integrated, hierarchical approach to neuroendocrine function in the human.

3. Long-Term Planning and the IHF Activity

A. We recommend that, as NSBRI develops its long-term strategic plan and the corresponding research program that implements that plan, the NSBRI should restructure itself so that the production of the Digital Human becomes its central (core) activity, and the rest of the research program be seen as critical and essential supporting activities.

B. We recommend creation of an Oversight Task Force within the IHF Team that will produce critical top level documents such as concept of operations and strategic plans.

VII. Commentary

Strengthening the activities of IHF can be accomplished three ways:

1. By adding specific discipline/specialty studies to increase the range of the content and scope of the IHF projects (e.g., adding a project in endocrinology).

2. By advancing methods and techniques for managing simultaneously, multiple models written in different formats, with different dynamics and a wide range of time constants, when the models reside in multiple locations/machines. This advanced program manager should "know" from "context" which subsystem models are relevant at a particular time (or state) to a chosen problem at hand. This advanced Program Manager must allow also continual development of individual models and addition of relevant analyses and programs. By using context as an operator, this program manager should be more powerful in this regard than is the "federated" approach of the High Level Architecture (HLA) utilized in the Coolahan project. However, this new approach should leverage off and be compatible with HLA, CORBA, etc. The value in expanding the modeling capability in the above fashion will be found in better addressing the issues inherent in the Critical Path that keeps the focus on the health of the astronauts.

3. By beginning development <u>now</u> of the Digital Human. Each Team Leader should be required to explain the approach his team will use for its contribution.

It will always be "too soon" to create the perfect model, and perfectionism leads to paralysis. The Digital Human will always be a work in progress, and the better for that. The development of the Digital Human should be connected to all of the research teams and it will be necessary to assign some investigators from nearly every team certain responsibilities related to the Digital Human. These investigators should draw upon the subsystem models being developed in the many projects funded by NSBRI, but more than that, they must discover the practical constraints by associations with flight surgeons, hardware engineers, and others within NASA who will have technical responsibilities for extended, manned space flights. Above all, they must facilitate a steady flow of data from all of the NSBRI research projects, and from astronauts before, during

and after all flights henceforth. To facilitate this activity, NSBRI should establish and maintain data and modeling archives that are regularly updated and made available to all interested parties.

Special comment regarding the Digital Human

It would be absurd to suppose that the Digital Human model will continuously operate subsystem models with all the detail that can possibly be included at each hierarchical level, and heterarchically as components of the same level. **The *reductio ad absurdum* of modeling is to suppose that everything is equally important, and therefore the only satisfactory model of a real system is the thing itself!** The whole point of modeling is to include just what is relevant to the question being asked. The parts and processes of the real system that are relevant will vary, depending on the context and the question addressed.

The nonlinear dynamics of complex hierarchical systems typically exhibit behaviors whose equations of change in differential equation models require inexact differentials, and in both differential and difference equation forms require nonholonomic constraints and a wide range of time domains. Furthermore, the levels in the hierarchy may sometimes overlap. A human liver with its portal triad functional unit can be treated as a lumped organ, or as N functional units. A kidney with its nephrons as (not actually identical) functional units can also be lumped, or treated (roughly) as N nephrons.

Considering all these non-idealities of human structure and function (from a mathematical perspective), it will not be practical to compute an astronaut from his or her genome! Nevertheless, it is not only rational, but essential, if we are to comprehend "health" (which is dynamic stability), to do so through a robust and adaptable dynamic model that can operate in the face of the technical difficulties mentioned above (and others). The model, given a particular situation as context, must assess health, note changing risks, sound alarms, and recommend, or even institute, countermeasures. It is a research project to find the form such a model requires. (There are hints that can be found in the physics of complex systems that can serve as a starting point.) The model must escape the "curse of high dimensionality" which leads to information overload and the loss of effective signaling. The Digital Human should be the core research effort of NSBRI, both because of its value to the Mission of the NSBRI, related as it is to extended human space flight, and its support of other teams (particularly the Smart Medical Systems Team), but also because of the potential it has to improve medical care here on Earth!

Creation of the Digital Human will certainly expose many familiar and some unexpected attributes of human health. In running the model under various perturbations of internal and external environments, we will explore sensitivities and adaptive capacities in a quantitative manner, and so discover vulnerabilities. New insights into diagnosis and therapy will surely result, as well as a better understanding of senescence and mortality.

NSBRI has its best chance for distinction through this effort. NSF, NIH, specialty Foundations, etc., with resources greater than those of NSBRI, all attend to particular diseases or subsystems. Medical practice itself has taken that specialized form, and lost the sense of integration that constitutes health. We therefore emphasize the importance to NSBRI of keeping the Digital Human as its core focus. It is the essence of the uniqueness of NSBRI.

ATTACHMENTS

1. Participants in the Workshop on Issues Related to the Integrated Human Function Team.
2. Agenda – Workshop on Issues Related to the Integrated Human Function Team.
3. Press Release (February 20, 2001) – Space research institute increases scope.
4. Section of NSBRI Research Announcement concerning Integrated Human Function.
5. NSBRI "Vision Statement" Developed in 1998 and Provided to Mr. Goldin.
6. COSPAR Article F0.1 – 0002 (2000): R. J. White, J. B. Bassingthwaighte, J. B. Charles, M. J. Kushmerick, and D. J. Newman, Issues of Exploration: Human Health and Well-being During a Mission to Mars.
7. R. Srini Srinivasan, J. I. Leonard and R. J. White, Mathematical Modeling of Physiological States, Chapter 26 in Vol. III, Book 2 (Humans in Spaceflight) of *Space Biology and Medicine* (ed. By A. E. Nicogossian, S. R. Mohler, O. G. Gazenko and A. I. Grigoriev), AIAA, Reston, VA, 1996.
8. Investigator's Abstracts for currently funded Integrated Human Function Team.
9. Presentation by J. I. Leonard "Whole-Body Algorithm: Early Attempts and Lessons Learned."

Appendix D

Workshop on Issues Related to the Nutrition, Physical Fitness and Rehabilitation Team
National Space Biomedical Research Institute
Del Lago Conference Center
Conroe, Texas
June 21-22, 2001

Members of the Nutrition, Physical Fitness Team

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Arny Ferrando, Ph.D., University of Texas At Galveston Medical Branch and Shriners Hospital for Children, Co-Investigator

Helen Lane, Ph.D., NASA (JSC), Co-Investigator

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Synopsis of the meeting

The meeting was held for one and one half days, and chaired by Joanne Lupton. Immediately preceding the workshop, the Nutrition, Physical Fitness and Rehab group had met for one day as a team to pursue areas for collaboration within the team and to discuss what the team saw as deficiencies in the overall program. All members of the team stayed on for the workshop and were active participants in the workshop.

The purpose of the workshop was to write 1-4 requests for proposals to strengthen the existing team and/or to cover new areas not already covered by the existing team. The first morning was spent hearing brief presentations from team members on their research projects, and presentations from Scott Smith and Helen Lane on existing and previous NASA sponsored nutrition projects, and from Suzanne Schneider on NASA sponsored exercise projects. The remainder of the meeting was devoted to writing four RFPs. There was extensive follow up during the week after the meeting by Email and phone to rewrite and edit the RFPs so that most points were agreed to by all participants. Each of the four RFPs is attached to this document.

In addition to writing the RFPs two other important issues were discussed:

Whether or not nutrition and physical fitness should remain together or be separated

The general consensus was that the two areas should be integrated as they each have a major impact on the other. Ideally this would be one large group with a larger number of projects than a single-focus team. Alternatively it could be two teams, with overlapping projects, whose members would meet at the same time to encourage collaboration. However, given the current economic climate it seems unlikely that either of these alternatives would come to fruition in the near future. There was concern that if the existing team were to become nutrition only, that physical fitness would not receive the funding and attention that was necessary. For all of these reasons the overall opinion of the group was to keep physical fitness with nutrition for now, and to target an RFP towards an integration of physical fitness with nutrition. Thus RFP#1 is the first priority of this group.

Capitalizing on existing projects by sharing tissues

A second real concern of the workshop participants was tissue sharing across projects. In particular they cited expensive, long term projects which would be very difficult to replicate, i.e. bed rest studies, and radiation/tumor studies. Funds should be put aside for other investigators to be able to capitalize on such studies and these resources should be widely publicized.

Request for Proposals
September 1, 2001
NSBRI
Nutrition, Physical Fitness and Rehabilitation

RFP#1 Exercise Countermeasures

Optimal human performance during space exploration requires the maintenance of cardiovascular capacity, bone mineral density, and skeletal muscle function. All of these depend on maintaining adequate nutritional status. Without routine aerobic exercise during long duration space missions, there is a decrease in intensity and endurance of aerobic capability as measured by oxygen consumption (VO2 max) and heart rate per energy exerted (watts). Resistive exercise is required for maintenance of muscle performance as measured by strength and endurance. Muscle atrophy and loss of force and power has been documented through muscle biopsies. It is proposed that resistive exercise and heal striking during aerobic exercise will promote maintenance of bone mineral density. The primary goal of this project is to study the effectiveness of exercise countermeasures to ameliorate these deleterious effects. End points should include parameters quantifying the cardiovascular response, bone metabolism, body composition, and skeletal muscle metabolism and function. The time course of selected end points is considered important. The exercise countermeasures must utilize approaches applicable or relevant to space flight (see appendix A). The study design must include strict dietary control, contain measures of energy balance, and ideally be coordinated with a currently funded bed rest and nutritional study (see appendix B). Once selected, protocols will need to be integrated along with existing protocols to maximize cross-study interpretation. The overall goal of the research is to provide a scientific basis for the design and implementation of space based exercise countermeasures. To the extent feasible, the studies should be designed to determine the optimal as well as minimal prescription for frequency, duration, and intensity of the exercise countermeasure to obtain the most time efficient method to maintain muscle and cardiovascular capacity. The project design must address one or more of the following experimental paradigms:

- Aerobic Exercise Countermeasure (For Example Supine Treadmill Running) with Bed Rest, Resistance Exercise Countermeasure with bed rest (isometric, concentric, eccentric exercise), or (preferentially) both aerobic and resistance exercise.

    - Cardiovascular Function
    - Structure-functional Relationships of Skeletal Muscle
    - Muscle metabolism: muscle protein synthesis, insulin action, fatty acid oxidative capacity
    - Markers of Bone Metabolism
    - Body Composition
    - Interaction with Nutrition

It is anticipated that one project will be funded in this area with a maximum dollar amount (including direct and indirect costs of $400,000 per year for 3 years). A 2 page preproposal containing the items listed below must be submitted by (date) to (name and address). The preproposal will be evaluated within a 2 week time period and the investigator contacted with respect to its outcome. For those proposals passing the initial preproposal stage, the Principal Investigator will be informed of the number of full proposals likely to be submitted for this award. The deadline for the submission of the full proposal will

be (give date). The full proposal will be peer reviewed by (give information). The successful project director will be notified by (give date) with anticipated funding beginning January 1, 2002.

Format for the preproposal:

The 2 page preproposal should consist of a description of the purpose, hypothesis, model proposed to be used, how the goals will be met (together with a time line) and which end points will be measured together with a description of how this will be accomplished. In addition, the principal investigator needs to mention what variables will be controlled: e.g. diet, body weight, etc. and the applicability for spaceflight. Outline form with appropriate citations is acceptable. Attached to the preproposal should be:

- A description of the bed rest facility where the research will be conducted
- A three page curriculum vitae for each investigator in NIH format, listing current funding
- Total dollar amount being requested (this includes direct and indirect costs). The budget does not need to be justified at the preproposal stage.

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RFP 1 – Exercise Countermeasures –

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Draft of Request for Proposals
September 1, 2001
NSBRI
Nutrition, Physical Fitness and Rehabilitation
RFP#2 Appetite and Thirst Controls

In spite of adequate provision of food and water, inadequate food intake is characteristic of human space flight. This reduction of food intake translates into a significant energy deficit with loss of body mass and diminution of physical fitness. Suboptimal intake of essential macro and micronutrients, and inadequate water intake also occurs. It is thought that alterations of central and peripheral appetite and thirst homeostasis underlie these perturbations.

This RFP seeks proposals aimed at understanding underlying mechanisms and designing effective nutritional countermeasures to these deficiencies in nutrient intake. Applications focusing on neuroendocrine, paracrine or redox mechanisms that may be altered by microgravity environments are encouraged, as related to changes in the regulation of appetite and thirst. Similarly, proposals addressing microgravity-induced changes in upper gastrointestinal bio-mechanical function and in the physical-chemical properties of food digestion are of interest. An additional area of interest is the influence of microgravity-induced changes on the flux of nutrients into the blood that may result in changes in central and peripheral appetite regulators. However the RFP is not limited to these approaches, other meritorious proposals are welcome.

Potential countermeasures may include but are not limited to:

- identification of appropriate patterns of meal allocation (ie. nibbling vs meal eating)
- optimal timing of nutrient intake to maximize endocrine/nutrient interaction
- identification of foods, which in the appropriate combinations, can maximize nutrient absorption and metabolism.

It is anticipated that one project will be funded in this area with a maximum dollar amount (including direct and indirect costs of $300,000 per year for 3 years). A 2 page preproposal containing the items listed below must be submitted by (date) to (name and address). The preproposal will be evaluated within a 2 week time period and the investigator contacted with respect to its outcome. For those proposals passing the initial preproposal stage, the Principal Investigator will be informed of the number of full proposals likely to be submitted for this award competition. The deadline for the submission of the full proposal will be (give date). The full proposal will be peer reviewed by (give information). The successful project director will be notified by (give date) with anticipated funding beginning January 1, 2002.

Format for the preproposal:

The 2 page preproposal should consist of a description of the purpose, hypothesis, model proposed to be used, how the goals will be met (together with a time line) and which end points will be measured together with a description of how this will be accomplished. In addition, the principal investigator needs to address the applicability for spaceflight. Outline form with appropriate citations is acceptable. Attached to the preproposal should be:

- A three page curriculum vitae for each investigator in NIH format, listing current funding
- Total dollar amount being requested (this includes direct and indirect costs). The budget does not need to be justified at the preproposal stage.

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Draft of Request for Proposals
September 1, 2001
NSBRI
Nutrition, Physical Fitness and Rehabilitation

RFP#3 Alterations in nutrient partitioning and metabolism as a function of microgravity and/or other space flight stressors

For crew members, space flight appears to increase resting metabolic rate in the presence of chronic stress and increased protein turnover. Limited data suggest insulin insensitivity and increased fat oxidation occur (2,5,6). Similar changes have been found with some ground based models (4). Examples of other changes occur with iron and calcium. With iron, red cell mass is decreased by 10-15% during space flight resulting in the release of additional iron, a strong pro-oxidant suggesting that it might be prudent to reduce dietary iron intake (1). Animal studies suggest that manipulation of the diet might be able to lessen the adverse effects of space flight on bone calcium loss and either separately or as an adjuvant to other countermeasures (3). These changes raise important questions in humans regarding the identification of nutritional countermeasures to combat the detrimental alterations in body composition and nutrient partitioning (e.g. bone, muscle, adipose tissue) as well as associated organ systems, e.g. cardiovascular. It is likely that these effects reflect alterations in systems coordination as well as individual cell function. These manifestations may relate to direct influences of microgravity or other as yet undefined space flight stressors.

This RFP solicits investigations aimed at the development of countermeasures to ameliorate detrimental alterations in nutritional physiology and biochemistry. Potential areas of investigation might include the characterization of the effects of microgravity and other space flight stressors on:

- Transport of nutrients across cell membranes.
- Oxidative stress and redox balance
- Fuel transport and metabolism, particularly potential changes in the relative importance of fat and carbohydrate substrates and the role of insulin and its counter regulatory hormones.
- Membrane chemical and structural characteristics, for example membrane fluidity, fatty acid composition, receptor expression and neural and hormonal sensitivity.

Proposals should indicate how the outcome of the work will result in the development of practically feasible countermeasures that will have a beneficial impact on recognized health problems associated with space flight.

1. Alfrey, C. P., L. Rice, M. M. Udden, and T. B. Driscoll. Neocytolysis: physiological down-regulator of red-cell mass. *Lancet* 349: 1389-90, 1997.
2. Baldwin, K. M., R. E. Herrick, and S. A. McCue. Substrate oxidation capacity in rodent skeletal muscle: effects of exposure to zero gravity. *J Appl Physiol* 75: 2466-70, 1993.
3. Fettman, M. J. Dietary instead of pharmacological management to counter the adverse effects of physiological adaptations to space flight. *Pflugers Arch* 441: R15-20., 2000.
4. Fitts, R. H., D. R. Riley, and J. J. Widrick. Physiology of a microgravity environment invited review: microgravity and skeletal muscle. *J Appl Physiol* 89: 823-39., 2000.
5. Lane, H. W., S. M. Smith, B. L. Rice, and C. T. Bourland. Nutrition in space: lessons from the past applied to the future. *American Journal of Clinical Nutrition* 60: 801S-805S, 1994.

6. Stein, T. P. *Nutrition and muscle loss in humans during spaceflight.* Advances in Space Biology & Medicine *7: 49-98, 1999.*

It is anticipated that one project will be funded in this area with a maximum dollar amount (including direct and indirect costs of $300,000 per year for 3 years). A 2 page preproposal containing the items listed below must be submitted by (date) to (name and address). The preproposal will be evaluated within a 2 week time period and the investigator contacted with respect to its outcome. For those proposals passing the initial preproposal stage, the Principal Investigator will be informed of the number of full proposals likely to be submitted for this award competition. The deadline for the submission of the full proposal will be (give date). The full proposal will be peer reviewed by (give information). The successful project director will be notified by (give date) with anticipated funding beginning January 1, 2002.

Format for the preproposal:

The 2 page preproposal should consist of a description of the purpose, hypothesis, model proposed to be used, how the goals will be met (together with a time line) and which end points will be measured together with a description of how this will be accomplished. In addition, the principal investigator needs to address the applicability for spaceflight. Outline form with appropriate citations is acceptable. Attached to the preproposal should be:

- A three page curriculum vitae for each investigator in NIH format, listing current funding
- Total dollar amount being requested (this includes direct and indirect costs). The budget does not need to be justified at the preproposal stage.

For further information contact:

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RFP #3 Microgravity effect on nutrient metabolism and partitioning

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Request for Proposals
September 1, 2001
NSBRI
Nutrition, Physical Fitness and Rehabilitation

RFP#4 Meal allocation: nibbling Vs meal eating, supplements Vs whole meals, timing of nutrient intake

The timing and frequency of meals with respect to activity, including sleep cycles and exercise and to the most effective utilization of nutrients may be a key factor in maximizing astronaut health on long duration space flights. For example, aerobic exercise has been shown to increase blood flow, which could benefit the uptake of amino acids into muscle if the amino acids were provided at the time of maximal blood flow. Similarly, after a substantial meal, there is a depression in protein synthesis. Since total body protein synthesis decreases in space flight, perhaps supplements between meals of amino acids in addition to the protein in three meals may be the most effective food pattern to enhance muscle protein synthesis and consequently maintain muscle function. For another example, extra vehicular activity (EVA) often requires 7-9 h periods of very focused activity with nothing but water. Determination of specific nutrients (both type and amount) and timing of ingestion to maximize mental and physical performance for these tasks would enhance crew safety and performance. This RFP solicits proposals on meal patterns, distribution of nutrients, and the timing of meals or supplements in relationship to maximum utilization of nutrients, physical activity and assigned tasks. Models could include divers during neutral buoyancy, those living in submergibles, some military operations, and in those working in the Antarctic. The effects of changes in sleep cycles may be included. Types of proposals considered under this RFP include, but are not limited to:

Endocrine/nutrient interactions
Optimal timing of nutrient intake with respect to
- Exercise
- Protein and other nutrient requirements
- Activities requiring optimum cognitive function (e.g., EVA)

It is anticipated that one project will be funded in this area with a maximum dollar amount (including direct and indirect costs of $300,000 per year for 3 years). A 2 page preproposal containing the items listed below must be submitted by (date) to (name and address). The preproposal will be evaluated within a 2 week time period and the investigator contacted with respect to its outcome. For those proposals passing the initial preproposal stage, the Principal Investigator will be informed of the number of full proposals likely to be submitted for this award competition. The deadline for the submission of the full proposal will be (give date). The full proposal will be peer reviewed by (give information). The successful project director will be notified by (give date) with anticipated funding beginning January 1, 2002.

Format for the preproposal:

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- A three page curriculum vitae for each investigator in NIH format, listing current funding
- Total dollar amount being requested (this includes direct and indirect costs). The budget does not need to be justified at the preproposal stage.

For further information contact:
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RFP #4 MEAL ALLOCATION

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Appendix E

National Space Biomedical Research Institute

Core Research Program Publications List

September 30, 2001

National Space Biomedical Research Institute Publications

Articles

Armoundas, A. A., N. Toshihiko, and R. J. Cohen. T wave alternans preceding torsade de pointes ventricular tachycardia. *Circulation* 101:2550, 2000.

Bassett, J. P. and J. S. Taube. Neural correlates for angular head velocity in the rat dorsal tegmental nucleus. *J Neurosci* 21: 5740-5751, 2001.

Berkowitz, D., L. Marucci, B. Winters, E. Asplund, D. Nyhan, and A. Shoukas. Impaired vascular reactivity in a rat model of micro-gravity. *J Appl Physiol*, in review.

Bloomfield, S. A., H. A. Hogan, and M. D. Delp. Decreases in bone blood flow and bone material properties in aging Fischer-344 rats. *Clin Orthop,* in press.

Bloomfield, S. A., M. R. Allen, H. A. Hogan, and M. D. Delp. Site- and compartment-specific changes in bone with hindlimb unloading in mature adult rats. *Bone,* in review.

Brooks-Asplund, E. and A. Shoukas. Baroreceptor contribution to the cardiovascular reflex responses of phenylephrine and sodium nitroprusside in the conscious rat. *Am J Physiol,* in review.

Brooks-Asplund, E. M., D. E. Berkowitz, S. L. Dunbar, and A. A. Shoukas. Hindlimb unweighting attenuates cardiac output and stroke volume responses to upright tilt in male rodents. *Am J Physiol,* in review.

Brooks-Asplund, E. M., D. E. Berkowitz, S. Y. Kim, and A. A. Shoukas. Estrogen has opposing effects on vascular reactivity in obese, insulin-resistant male zucker rats. Submitted.

Brooks-Asplund, E., D. E. Berkowitz, S. Dunbar, and A. Shoukas. Hindlimb unweighting attenuates cardiac output and stroke volume responses to upright tilt in male rodents. Submitted to *Am J Physiol.*

Colleran, P. N., M. K. Wilkerson, S. A. Bloomfield, L. J. Suva, R. T. Turner, and M. D. Delp. Alterations in skeletal perfusion with simulated microgravity: A possible mechanism for bone remodeling. *J Appl Physiol,* 89: 1046-1054, 2000.

Dai, M., T. Raphan, and B. Cohen. Adaptation to roll-tilts during constant rotation. Manuscript in preparation, 2001.

Dijk, D. J., D. F. Neri, J. K. Wyatt, J. M. Ronda, E. Riel, A. Ritz-De Cecco, R. J. Hughes, A. R. Elliott, G. K. Prisk, J. B. West, and C. A. Czeisler. Sleep, performance, circadian rhythms, and light-dark cycles during two space shuttle flights. *Am J Physiol* 281:R1647-1664, 2001.

Dinges, D. F., H. P. A. Van Dongen, et al. Cumulative sleep loss in space flight: neurobehavioral consequences and countermeasures. Submitted to *Acta Astronaut.*

Doran, S. M., H. P. A. Van Dongen, and D. F. Dinges. Sustained attention performance during sleep deprivation: Evidence of state instability. *Archives of Italian Biology: A Journal of Neuroscience* 139: 253-267, 2001.

Dunbar, S. L., D. E. Berkowitz, E. M. Brooks-Asplund, and A. A. Shoukas. The effects of HLU on the pressure-diameter relationship of rat small mesenteric veins. *J Appl Physiol* 89:2073-2077, 2000.

Dunbar, S. L., L. Tamhidi, D. E. Berkowitz, and A. A. Shoukas. Hindlimb unweighting affects rat vascular capacitance function. *Am J Physiol* 281:H1170-H1177, 2001.

Hatakana, T., K. P. McKeown, and A. A. Shoukas. Effects of pulsatile flow on extravascular fluid uptake during cardiopulmonary bypass. *J Thorac Cardiovasc Surg*, in review.

Hecht, H., J. Kavelaars, C. C. Cheung, and L. R. Young. Orientation illusions and heart-rate changes during short-radius centrifugation. *J Vestib Res*, in press.

Hegemann, S., M. Shelhamer, P. D. Kramer, and D. S. Zee. Adaptation of the phase of the human linear vestibulo-ocular reflex (LVOR) and effects on the oculomotor neural integrator. *J Vestib Res*, 10:239-247, 2000.

Hegemann, S., V. Patel, M. Shelhamer, P. D. Kramer, and D. S. Zee. Adaptation of the phase of the human linear vestibulo-ocular reflex (LVOR) and effects on the oculomotor neural integrator. *J Vestib Res*, in press.

Heldt, T., E. B. Shim, R. D. Kamm, and R. G. Mark. Computational modeling of cardiovascular response to orthostatic stress. Accepted in *J Appl Physiol.*

Hirasaki, E., S. T. Moore, T. Raphan, and B. Cohen, B. Head and body movements in the yaw and roll planes during straight walking. *Soc Neuroscience*, November 2001.

Howard, I. and G. Hu. Visually induced reorientation illusions. *Perception*, 2001.

Imai, T., S. T. Moore, T. Raphan, and B. Cohen. Interaction of the body, head and eyes during walking and turning. Submitted to *Exp Brain Res.*

Judex, S., L. R. Donahue, and C. T. Rubin. Genotypic predisposition to osteoporosis is paralleled by an enhanced sensitivity to signals anabolic to the skeleton. Submitted to *PNAS.*

Jung, A. S., D. E. Berkowitz, E. M. Brooks-Asplund, and A. A. Shoukas. Attenuated baroreflex responses in a mouse model of microgravity. *Am J Physiol,* in final review.

Khalsa, S. B., M. E. Jewett, J. F. Duffy, and C. A. Czeisler. The timing of the human circadian clock is accurately represented by the core body temperature rhythm following phase shifts to a three-cycle light stimulus near the critical zone. *J Biol Rhythms* 15:524-530, 2000.

Kim, S. Y., D. E. Berkowitz, R. Jhaveri, A. Shoukas, and D. Nyhan. Differential influence of pulmonary vasoconstrictors on milrinone induced vasorelaxation. In preparation.

Klingenheben, T., M. Zabel, R. B. D'Agostino, R. J. Cohen, and S. H. Hohnloser. Predictive value of T wave alternans for arrhythmic events in patients with congestive heart failure. *Lancet* 356:651-652, 2000.

Kourentzi, K. D., G. E. Fox, and R. C. Willson. Microbial identification by immunohybridization assay of artificial RNA labels. Submitted to *J Microbiol Methods*.

Kourentzi, K. D., G. E. Fox, and R. C. Willson. Rapid identification of microorganisms using 5S rRNA specific molecular beacons. *Curr Microbiol*, 2001, in press.

Lednicky, J. A., S. J. Halvorson, and J. S. Butel. PCR detection and DNA sequence analysis of the regulatory region of lymphotropic papovavirus in peripheral blood mononuclear cells of an immunocompromised rhesus macaque. *J Clin Microbiol*, in press.

McKeown, K. and A. Shoukas. Chronic isolation of the carotid sinus baroreceptor region in conscious normotensive and hypertensive rats. *Am J Physiol* 275(Heart and Circ Physiol 44):H322-H329, 1998.

McPartland, M. D., C. Wall, L. I. Oddsson, D. E. Krebs, and C. A. Tucker. Recovery from perturbations during paced walking. Submitted to *Gait Posture*.

McPartland, M. D., D. E. Krebs, and C. Wall III. Quantifying Ataxia: Ideal Trajectory Analysis. Accepted in *J Rehabil Res Dev*.

Meier-Ewert, H. K., P. M. Ridker, N. Rifai, N. Price, D. F. Dinges, and J. M. Mullington. Absence of diurnal variation of c-reactive protein concentrations in healthy human subjects. *Clin Chem* 47(3) 426-430, 2001.

Moore, S. T., E. Hirasaki, T. Raphan, and B. Cohen. The human vestibulo-ocular reflex during linear locomotion. *Ann NY Acad Sci*, 2001, in press.

Mukkamala, R. and R. J. Cohen. A forward model-based validation of cardiovascular system identification. Accepted in *Am J Physiol*.

Mukkamala, R., D. A. Sherman, R. J. Cohen, and R. G. Mark. A nonlinear, computational model of the pulsatile heart and circulation. Submitted for publication.

Mukkamala, R., K. Toska, and R. J. Cohen. Noninvasive identification of the total peripheral resistance baroreflex. Submitted for publication.

Murphy, J. C., G. E. Fox, and R. C. Willson. Compaction agents enhance anion-exchange adsorption of nucleic acids. *J Chromatogr A*, accepted pending minor revisions.

Murphy, J. C., G. E. Fox, and R. C. Willson. RNA isolation and fractionation with compaction agent. *Anal Biochem* 295: 143-148, 2001.

Newman, D. J., R. Wu, D. Krebs, and D. K. Jackson. Electromyographic analysis of human false platform jumping. *J Appl Physiol*, in revision.

Nyhan, D., J. Hare, and D. Berkowtiz. Modulating effects of L-arginine and age related vascular dysfunction. In preparation.

Nyhan, D., S. Kim, S. Dunbar, D. Li, A. Shoukas, and D. Berkowitz. Impaired pulmonary artery contractile responses in a rat model of microgravity: role of nitric oxide. *Am J Physiol,* in press.

O'Sullivan, C., R. S. Peng, H. Jenson, and P. D. Ling. Epstein-Barr virus genome loads in the peripheral blood and antibody titers to EBV antigens in AIDS patients before and after HAART therapy. Submitted for publication.

Oman C., W. Shebilske, J. Richards, T. Tubre, A. Beall, and A. Natapoff. Three dimensional spatial memory and learning in real and virtual environments. *J Spatial Cognition and Computation*, 2001, in revision.

Ramsdell, C. D., T. J. Mullen, G. H. Sundby, S. Rostoft, N. Sheynberg, N. Aljuri, M. Maa, R. Mukkamala, D. Sherman, K. Toska, J. Yelle, D. Bloomfield, G. H. Williams, and R. J. Cohen. Midodrine prevents orthostatic intolerance associated with simulated spaceflight. *J Appl Physiol* 90:2245-2248, 2001.

Raphan, T., T. Imai, S. T. Moore, and B. Cohen. Vestibular compensation and orientation during locomotion. *Ann NY Acad Sci*, 2001, in press.

Rogers, N. L. and D. F. Dinges. Shiftwork, circadian disruption and consequences. *The Economics of Neuroscience* 6(7) 2001, in press.

Rogers, N. L., J. McMahon, K. Lushington, and D. Dawson. Thermoregulatory changes around the time of sleep onset. Submitted to *Physiol Behav.*

Rubin, C. T, D. W. Sommerfeldt, S. Judex, and Y. X. Qin. Inhibition of osteopenia by low magnitude, high frequency mechanical stimuli. *Drug Discov Today* 6(16): 848-858, 2001.

Rubin, C. T., G. Xu, and S. Judex. The anabolic activity of bone tissue, suppressed by disuse, is normalized by brief exposure to extremely low magnitude mechanical stimuli. *FASEB J* 15: 2225-2229, 2001.

Sha, D., D. Kennedy, and J. Sutton. Neurocomputing for automated analysis of digital brain images. *Artif Intell Med,* in press.

Shapiro, R., B. Winters, M. Hales, T. Barnett, D. A. Schwinn, N. Flavahan, and D. E. Berkowitz. Endogenous circulating sympatholytic factor in orthostatic hypotension. *Hypertension* 36:553-560, 2000.

Shearer, W. T. Contamination of the spacecraft environment: Immunologic consequences. *Gravitational and Space Biology Bulletin,* 14: 7-14, 2001.

Shearer, W. T., D. J. Lugg, H. M. Rosenblatt, P. M. Nickolls, R. M. Sharp, J. M. Reuben, and H. D. Ochs. Antibody responses to phiX-174 in human subjects exposed to the Antarctic winter-over model of spaceflight. *J Allergy Clin Immunol,* 107:160-164, 2001.

Shearer, W. T., J. M. Reuben, J. M. Mullington, N. J. Price, B. N. Lee, E. O. Smith, M. P. Szuba, H. P. A. Van Dongen, and D. F. Dinges. Soluble tumor necrosis factor-alpha receptor 1 and interleukin-6 plasma levels in humans subjected to the sleep deprivation model of spaceflight. *J Allergy Clin Immunol,* 107: 165-170, 2001.

Shelhamer, M. Use of a genetic algorithm for the analysis of eye movements from the linear vestibulo-ocular reflex. *Ann Biomed Eng*, 29:510-522, 2001.

Shelhamer, M., D. C. Roberts, and D. S. Zee. Dynamics of the human linear vestibulo-ocular reflex at medium frequency and modification by short-term training. *J Vestib Res*, 10:271-282, 2000.

Strangman, G., D. A. Boas, and J. P. Sutton. Non-invasive neuroimaging with near-infrared light. Invited paper for *Biol Psychiatry,* in preparation.

Strangman, G., F. Halbritter, P. Groblewski, W. C. West, T. Gaudette, and D. A. Boas. A high-speed, continuous-wave near-infrared spectroscopy (NIRS) system for non-invasive recording of brain activity. Submitted to *J Biomed Opt.*

Strangman, G., J. P. Culver, J. H. Thompson, and D. A. Boas. Temporal comparison of simultaneous BOLD fMRI and NIRS recordings during functional brain activation. In preparation.

Strangman, G., M. A. Franceschini, and D. A. Boas. Factors affecting the accuracy of near-infrared spectroscopy (NIRS) data analysis for focal changes in cerebral hemodynamics. In preparation.

Takagi, H. Abe, S. Hasegawa, T. Usui, H. Hasebe, A. Miki, and D. S. Zee. Context-specific adaptation of pursuit initiation in humans. *Invest Ophthalmol Vis Sci*, in press.

Takagi, M., H. Abe, S. Hasegawa, T. Usui, H. Hasebe, A. Miki, and D. S. Zee. Context-specific adaptation of pursuit initiation in humans. *Invest Ophthalmol Vis Sci* 41:3763-3769, 2000.

Thurtell, M. J., M, Kunin, and T. Raphan. Role of muscle pulleys in producing eye position-dependence in the angular vestibulo-ocular reflex: A model based approach. *J Neurophysiol*, 2000, in press.

Vilchez, R. A., J. A. Lednicky, S. J. Halvorson, Z. S. White, C. A. Kozinetz, and J. S. Butel. Detection of polyomavirus SV40 tumor antigen DNA in AIDS-related systemic non-Hodgkin's lymphoma. *J Acquir Immune Defic Syndr,* in press.

Winters, B., X. Mo, E. Brooks-Asplund, S. Y. Kim, A. Shoukas, D. Nyhan, and D. E. Berkowitz. Reduction of obesity, as induced by leptin, reverses endothelial dysfunction in leptin deficient obese (lepob) mice. *J Appl Physiol* 89:2382-2390, 2000.

Wood, S., C. Ramsdell, T. Mullen, C. Oman, D. Harm, and W. Paloski. Transient cardio-respiratory to visually-induced virtual tilts. Brain Res. Bulletin 53(1), in press.

Wright, Jr., K. P., R. J. Hughes, R. E. Kronauer, D. J. Dijk, and C. A. Czeisler. Intrinsic near-24-hour pacemaker period determines limits of circadian entrainment to a weak synchronizer in humans. *Proc Natl Acad Sci*, in press.

Young, L. R., H. Hecht, L. E. Lyne, K. H. Sienko, C. C. Cheung, and J. Kavelaars. Artificial gravity: Head movements during short-radius centrifugation. *Acta Astronaut*, in press.

Young, L. R., K. H. Sienko, L. E. Lyne, H. Hecht, and A. Natapoff. Adaptation of the vestibulo-ocular reflex, subjective tilt, and motion sickness to head movements during short-radius centrifugation. Submitted to *Exp Brain Res*, 2001.

Zhang, Z., R. C. Willson, and G. E. Fox. Identification of characteristic oligonucleotides in the 16S ribosomal RNA sequence dataset. *Bioinformatics*, 2001, in press.

Zhong, Q., K. H. Ding, R. Bollag, and C. M. Isales. Glucose-dependent insulinotropic peptide-induced elevations in transforming growth factor□□ modulate proliferation in osteoblastic-like cells. Submitted to *J Bone Miner Res*, 2001.

Book and Book Chapters

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Appendix F

NIDCD-NSBRI JOINT PROGRAM FOR THE SUPPORT OF VESTIBULAR RESEARCH
Proposals Received: October 1998
Proposals Selected: September 1999

Year 1 Funding: $1,413 K
(NIH - $1,168 K; NSBRI - $245 K)

Year 2 Funding: $1,342 K
(NIH - $1,132 K; NSBRI - $210 K)

Year 3 Funding: $1,367 K
(NIH - $1,149 K; NSBRI - $218 K)

Total Funding: $4,122 K
(NIH - $3,449 K; NSBRI private - $673 K)

1. **Baylor College of Medicine:** Helen **COHEN**, Ed.D. **Funding:** **Yr. 1: $112 K**
Yr. 2: $118 K
Yr. 3: $ 88 K
Total: $318 K

MANAGEMENT OF ADAPTATION TO ALTERED SENSORIMOTOR STATES
Co-I: Jacob Bloomberg, Ph.D. (NASA JSC)

2. **Harvard University:** Daniel **MERFELD**, Ph.D. **Funding:** **Yr. 1: $299 K**
Yr. 2: $260 K
Yr. 3: $280 K
Total: $839 K

DECODING OF GRAVICEPTOR CUES, INCLUDING ADAPTIVE CHANGES
Co-Is: Conrad Wall, Ph.D.
Lionel Zupan, Ph.D.
Robert Peterka, Ph.D. (Oregon Health Sciences U.)
Mark Shelhamer, D.Sc. (Johns Hopkins Univ.)

3. **Stanford University:** Jennifer **RAYMOND**, Ph.D. **Funding:** **Yr. 1: $287 K**
Yr. 2: $294 K
Yr. 3: $319 K
Total: $900 K

VESTIBULAR AND VISUAL CONTROL OF EYE MOVEMENT

4. ***Univ. of Mississippi Medical Ctr:** W. Michael **KING**, Ph.D. **Funding:** **Yr. 1: $245 K**
Yr. 2: $210 K
Yr. 3: $218 K
Total: $673 K

SIGNAL PROCESSING AND ADAPTATION IN CENTRAL OTOLITH PATHWAYS
Co-I: Wu Zhou, Ph.D.

5. **University of Rochester**: Scott **SEIDMAN**, Ph.D. **Funding:** **Yr. 1: $241 K**
Yr. 2: $240 K
Yr. 3: $247 K
Total: $728 K

PLASTICITY IN THE VESTIBULOOCULAR REFLEXES AND PERCEPTION
Co-I: Gary Paige, M.D., Ph.D.

6. **Wash. Univ. School of Medicine**: Dora **ANGELAKI**, Ph.D. **Funding:** **Yr. 1: $229 K**
Yr. 2: $220 K
Yr. 3: $215 K
Total: $664 K

NEURAL MECHANISMS OF VESTIBULAR ADAPTATION
Co-I: J. David Dickman, Ph.D.

***NSBRI supported from private funding sources.**

Appendix G

Resuscitation, Stabilization and Critical Care
A Space Medicine Workshop

At the outset of this project the committee was charged with developing an evidence based practice of space medicine and to take the current information and develop an educational program for space medical officers.

It quickly became apparent during that the current medical care provided was based on terrestrial developed techniques and science. It also became apparent that the partial and incomplete modifications that had occurred had occurred based on experiences in micro gravity, but that this was not consistent throughout the training program. As an example, a major component is the Advanced Cardiac Life developed by the American Heart Association as the state of the art for cardiac care and the Advanced Trauma Life Support program as the state of the art in trauma care developed by the American College of Surgeons/Committee on Trauma. Although the some of the science is applicable in microgravity, none of the procedures will work in that environment. Changes have been made based on trial and error. To continue in the pathway of part micro gravity and part 1 G would produce inconsistences that would be confusing and would also not provide the best care that is available. Therefore, the committee changed directions and elected first to rewrite the protocols for care and space based on space needs and space physiology, and then to write a training program based on this knowledge.

To achieve this process a list of all potential problems that would occur in space was developed by 3 separate committees address: 1) Neuro-psychological problems, 2) general medical, cardiac and respiratory problems and 3) trauma problems.

In breakout groups these lists were made and modified for the first meeting. In time for the second meeting, and during the second meeting, protocols were developed to address each of these medical problems. The protocols were standard medical algorithms. After these were developed and agreed upon they will then be converted into the "mal" format so that this information will be presented to the space medical officers in the same way as their other troubleshooting protocols. Once the entire committee has agreed upon the details of each of the individual protocols then educational objectives, teaching format, and text material will be developed in time to be presented to the next astronaut class beginning in the Summer of 2002.

The attachments demonstrate the wide the varied background of the participants so that the protocols will be as complete as possible.

Space Medicine Workshop
Cardiorespiratory Group
Committee Recommendations
as of 8-31-01

The Cardiorespiratory Committee recommends:

1. That all groups use "General Guiding Principles in Creating and Modifying Protocols for Space Medicine" in their work for the Space Medicine Workshop.
2. That the enclosed protocols be reviewed, placed in "NASA format" and presented for full group discussion in November.
3. That in concert with adopting the Ethics protocol, that there be convened a group charged with reviewing both the Ethics protocol and the laws relevant to end – of – life issues in space. In particular, the International Partners should be polled as to their cultural and ethical input. Recommendations need to be made on policies and procedures for addressing ethical dilemmas especially in the multicultural, international environment of space medicine.
4. That the enclosed list of recommended modifications of the equipment and pharmaceuticals be reviewed and adopted as deemed appropriate.
5. That the enclosed recommendations for literature searches be implemented.
6. That NASA determine whether members of the Space Medicine Workshop are deemed to have a conflict of interest in carrying out research a member has proposed but funded by NASA.
7. That NASA consider implementing the research proposals enclosed.

Thank you.

Space Medicine Workshop
Cardiorespiratory Group
General Guiding Principles in Creating and Modifying Protocols for Space Medicine
as of 8-31-01

Strategy of the Protocols.

The following priorities govern medical care in space:

A. Survival of the crewmember
B. Return to function of the crewmember to permit mission continuation
C. Minimize impact of the medical event on the effectiveness of other crewmembers to complete the mission
D. When the crewmember cannot return to adequate function, safely evacuate the crewmember(s).
E. Pain / symptom control.
F. Making an accurate diagnosis.

Note that making a diagnosis is not essential to meeting the priorities in many clinical states. On Earth, it is often true that making a final diagnosis takes days to years, and involves the input of many physicians, results of many types of laboratory tests not available in space, and the very important diagnostic tools of watching the course of the illness and its response to treatment over time. Since the CMO may not have the luxuries of time, laboratory, or consultants, the CMO must re - prioritize medical interventions to that which can be reasonably achieved with limited resources in the environment of space.

Observation – based Protocols

The fundamental idea use to construct the protocols is that the CMO can make observations even if the CMO cannot make diagnoses, so the decision – making is based on what the CMO observes, not primarily upon making a diagnosis.

The clinical implication of any observation depends on several factors, such as timing, factors exacerbating or mitigating the observation, and whether an obvious explanation exits. For example, arm pain that has its onset immediately following a hand – crushing incident from moving a heavy object creates a scenario readily distinguished from left arm pain only occurring while running on a treadmill. Thus the protocol seeks to explore an observation enough to distinguish clinical scenarios into major and minor, emergent and less urgent.

I. General Principles of Observation and Clinical Interventions

A. What you are determining is what needs to be done, not primarily what is the diagnosis

B. Observation is the foundation of care

1. Can't treat or report anything that isn't observed
2. Must observe, think, reevaluate, communicate
3. Make the best decisions under the circumstances
4. Take appropriate action, then monitor responses

C. Accurate information is critical to decision making

1. The history
   a. What happened?
   b. The history is the key assessment element, often more important than physical examination
   c. Knowledge of disease and suspicion affect quality of observations
   d. Start with determining what happened, then follow each symptom complex sequentially.
2. The physical examination
   a. Speed should not compromise adequacy
   b. Effectiveness may be compromised by scenario
   c. Focused toward systems producing symptoms
3. Pattern recognition
   a. Compare observations with knowledge base
   b. Pattern is either recognized or identified as unrecognized
4. Assimilation
   a. Observations that match recognized patterns
      (1) A provisional plan of action can be drawn from protocols
      (2) Adapt protocol to local conditions
      (3) A provisional diagnostic impression may be achieved
      (4) Be skeptical that you have the correct diagnostic impression, and thus monitor response to treatment.
   b. Observations that do not match recognized patterns
      (1) Be skeptical. Seek additional information
      (2) If additional information does not result in recognizable pattern, choose closest protocol
      (3) Adapt protocol to local conditions
      (4) Avoid assigning a diagnosis when pattern is unrecognized
      (5) Monitor response to treatment.

D. Factors affecting quality of observations and decision making

1. Rush to judgment / tunnel vision / labelling
   a. May 'short circuit" observations
   b. May cause pattern misrecognition
   c. GIGO = garbage in - garbage out
   d. Interpersonal relationships may impair thinking
2. Uncooperative patients
   a. Distinguish willful non – cooperation from inability to cooperate

b. Inability to cooperate means risk of significant CNS injury or toxicity

3. Obvious injuries may distract both the patient and CMO from more significant injuries
4. The environment
   a. Scene chaos
   b. Violent/ dangerous situations
   c. Contact / Non – contact with Earth
   d. Illness in CMO or other crew
5. Manpower considerations
   a. Single responder
      (1) Sequential information gathering
      (2) Sequential treatment
   b. Multiple responders
      (1) History by "committee" may result in disorganized observations and intervention planning
      (2) Worse of peers comprise the committee
      (3) Pre – planning reduces error and optimizes participation of others

II. Organizing Actions

A. Calm orderly demeanor is essential
   1. Look the part
   2. Act the part
   3. "Bedside" manner is important
   4. Patients may not be able to rate medical performance but they can rate people skills and service

B. Have a "preplan" to prevent confusion and improve accuracy of the assessment
   1. While talking to the patient
      b. Active concerned dialogue
      c. Listen
   2. Take notes when acquiring the history if possible
      a. Helps prevent asking the same question repeatedly

C. Locate the essential equipment
   1. Ready to provide resuscitative care
   2. Minimizes pandemonium

D. Use the initial scene size-up to gather clues and help formulate an impression
   1. Especially useful in trauma situations
      a. Hazards
      b. Movement of the injured person
      c. Number of patients
   2 Avoid tunnel vision

E. The initial observations set the tone for the patient encounter
   3 Resuscitative approach
   4 Contemplative approach
      a. Immediate intervention not necessary

b. Generally history and physical, then interventions if required

5 Immediate evacuation may be required if

a. Assistance is needed to provide lifesaving interventions

b. Scene is too unstable/ or unsafe

c. Scene is too chaotic to allow for rational assessment

F. To find something, one must suspect it

6 During initial assessment one must actively look for life threatening problems

7 Must be systematic

8 Rapidly determine the chief complaint

9 Assess the degree of distress

10 Obtain baseline vital signs early

11 Focused on the relevant history and physical findings

G. The greater the knowledge about what is being looked for the more productive the line of questioning will be

H. Experience assists in developing the ability of "multi-tasking" or being able to ask questions and do something while listening to the answer

1 Until experienced, ask questions and just listen

2 Have partner perform necessary tasks

3 Important clues are lost by not listening

I. The patient's ability to describe symptoms and CMO's ability to listen has a great effect on the assessment

12 Pain severity does not correlate well with life-threat potential

2 Location of pain and its source also do not always correlate well; especially if it is visceral

J. Bear in mind worst case scenario but don't be overly influenced by it.

III Presenting the patient

A. Effective communication of patient information is vital

13. Patient presentation is often a weak link in care

2. Practice makes perfect.

3. Three sentence summary

B. Effective presentations

14 Are very concise, usually lasting less than one minute

15 Are usually free of extensive jargon

3 Follow the same basic information pattern, generally the SOAP format or some close variation of it

4 Includes pertinent positive observations and pertinent negatives

Space Medicine Workshop
Cardiorespiratory Group
Research Recommendations
as of 8-31-01

The Cardiorespiratory Committee recommends the following research:

8. .Studies of intermediary metabolism, hyperglycemia, hypoglycemia in space – adapted animals, then man.
9. Studies of reconstitution methodologies for delivering large volumes of crystalloid to patients in space.
10. Improved suction devices or strategies for application in space, including foot – powered devices.
11. Evaluation of LMA, Intubating LMA, Double Lumen LMA, and ETT intubation, including evaluating decay of skills over months after training in astronauts startled by a surprise drill – simulating what it would be like for an astronaut to be using airway skills in space months after being checked out on them.
12. Parallel (to # 4) evaluation of the Cook percutaneous tracheostomy device.
13. Pharmacokinetic and pharmacodynamic studies of drug delivery through LMA, ETT, mask during assisted ventilation in space. Most parenteral drugs in the Shuttle and ISS formularies should be studied.
14. Studies of chest tube placement and efficacy in space environment.
15. Studies of attitudes of astronauts / cosmonauts from all International Partners as to end of life issues.
16. Drug delivery studies for drips, like pressors, antihypertensives, insulin, glucose, volume replacement in space environment.
17. RFA for new intubation / airway management / non invasive mechanical ventilation technologies.

Space Medicine Workshop
Cardiorespiratory Group
Pending Assignments for the Group
as of 8-31-01

1. Review the updated protocols, inserting any details you believe should be there. It is particularly important to be sure that we consider at each juncture that the patient may be improving or worsening at each juncture, and we need to give the astronauts proper direction what to do with both improvement and decline. Note that at this stage we are trying to be sure the content is good, not that the format is NASA – style. However, remember that the organizational format is unitasking not multitasking. Where appropriate, insert notes or advice to the astronauts as to whether the task at hand should be performed with one or two rescuers.
2. Review the enclosed list of recommended equipment and pharmaceutical changes. Recommend additions or deletions to the list. If you recommend an exchange of one drug for another, please either provide the literature or list the entry in the list of literature searches to be performed (#3)
3. List any literature searches you feel should be performed by NASA personnel, or provide literature that you have identified.
4. Review the Clinical DataBase to make any additions or deletions appropriate to the clinical database an astronaut should be taught in order to make the observations necessary to determine which protocol to enter.
5. Re – review the protocols to be sure they are compatible with each other.
6. Review the proposals list to determine whether you support these proposals, and whether you have any to add.
7. Propose any research you think may be necessary or helpful to optimize health care for the astronauts relevant to the protocols we are proposing.
8. Review the "General Guiding Principles in Creating and Modifying Protocols for Space Medicine" for changes you feel appropriate.
9. Send all of your changes and comments to me ASAP, or sooner. We need to have our committee's work done by mid-September. Once I have your feedback, I will compatibilize your comments, and produce a composite proposal to send to Norm, Sam and David. On Sept 15.
10. It has been an honor to work with you guys.

Space Medicine Workshop
Cardiorespiratory Group
Ethics Protocol for Space Medicine
as of 8-31-01

Ethics

General Comments

1. To minimize ethical conflict based in poor communication, before flight each astronaut/cosmonaut should be presented the opportunity to provide directives / authorize a surrogate with medical power of attorney, and to designate a group of resource / notification people, such as family or specific friends.
2. Any of the choices of the patient should be documented, and the documentation should be maintained both at Mission Control and on the Space Station or Shuttle.
3. The astronaut/cosmonaut should be given a number of specific scenarios to consider, and ask for the astronaut's/cosmonaut's choice of what they direct the medical provider to do.
4. The scenarios should be quantitative, such as including questions of how long to continue resuscitation without evidence of improvement, how long to continue mechanical ventilation without improvement.
5. To the extent possible, an attempt should be made to gain the directives of the astronaut/cosmonaut prior to flight, but the crew should be given some specific instruction on what the limits are in providing prolonged care in space.
6. The crew should be given information but the crew should not be left without support and direction by the physician team.
7. Similarly, the Medical Officers should maintain a "General Limits of Care" list.
8. This list should be presented to the crew so they can know what general recommendations the medical staff have for end - of - life or similar extreme situations.
9. The information provided to the crew pre - flight should include the General Limits of Care list.

Recommendations for the General Limits of Care list:

1. 30 minutes of CPR, with no return to spontaneous pulse and breathing without chest compression during the last 10 minutes
2. 10 minutes of CPR performed as well as can be achieved under circumstances with Capnography showing CO2 less than 10 torr
3. Exhaustion of drug supply for the condition in question
4. Exhaustion of the crew providing medical care
5. Extreme risk of injury, contamination or infection in crew members in continuing the medical care in question
6. Extreme risk of injury in crew members in de- orbiting
7. High probability that medical care during de - orbit and pre- hospital terrestrial care would be impractical or unsuccessful
8. If the patient is severely hypothermic (less than 96 degrees) and the equipment and medications are available, attempt to re- warm patient prior to deciding to stop resuscitation.
9. If the patient is in the Airway – Breathing or Life Support Protocols, and is not at 1 ATM, consider increasing cabin pressure to 1 ATM before stopping resuscitation unless another General Limit of Care is achieved, such as exhaustion of the drug supply for the condition at hand.

Recommendations

Review what laws govern provision of medical care, especially end - of - life care in space.
Review any agreements among the International Partners that bear on end - of - life decisions
Review any customs or expectations that apply to each of the International Partners
If no written laws or agreements set policy, produce a written policy on end - of - life decisions and medical care in space

1. Unsure what action to take, is the matter an ethics issue?	[patient rights, truthfulness, adherence to religious or legal paradigms, balancing one person's rights against another person's] Go to #2	[Unwillingness or hesitancy to take action known to be the correct action, where the hesitation is not based on ethical uncertainty] Do not use this protocol. Issue is to be resolved using communication with the involved people.
2. Can the patient now speak for him or herself?	Go to #3	Go to #4
3. Is the patient competent to make decisions (the degree of impaired judgment from the clinical state, including medications, is minor relative to the magnitude and urgency of the decision to be made)	Inform the patient about the implications of the decision to the extent known, including risks and benefits of both the recommended procedure and its alternative. Then ask the patient to make his or her own decision.	Inform patient generally, but decision making is managed as if patient was unavailable for self - determination
4. Patient is physically incapable of self - determination or not competent (using definition in # 3). Has the patient established instructions in writing that apply to this clinical scenario?	Implement the decision - making process authorized in writing by the patient, if the designated decision-makers are available.	Go to 5
5. If no written instructions from the patient as in #4, has an individual been designated with medical power of attorney and is such documentation confirmed?	Go to #6	go to # 7

6. Is communication available with the surrogate with medical power of attorney	contact the surrogate with medical power of attorney, inform about the clinical scenario, ask for consent / decision.	If timeliness requires action, person providing care to proceed with decision - making. If timeliness allows, contact the surrogate with medical power of attorney. Go to # 6
7. Has the patient given directions as to choices by verbal, written, or surrogate mechanisms?	Go to #8	go to # 2
8. Is the choice of the patient congruent with the recommendation of the person providing medical care?	Go to # 9	Go to # 10
9. Is there conflict between the choice of the patient+medical provider versus the Mission goals/Mission Control	Go to # 10	Proceed to implement the patient's choice
10. Is the probability of success or the practicality of proceeding with the medical process clearly prohibitive of continuing medical care or implementing the choice of the patient?	Do not implement the choice of the patient or continue the impractical medical care. Inform the patient and others predesignated by the patient, Inform Mission Control.	Implement or continue any non - conflictual measures. For the conflictual issues, explore / discuss to attempt resolution or consensus. Utilize the patient's pre - authorized resource personnel and documentation to resolve what can be resolved. Go to # 11
11. Is the probability of success or practicality of proceeding not clearly prohibitive of continuing care or implementing the patient's choice?	Go to # 12	Go to # 9
12. Is the care in question is included in the pre - flight General Limits of Care list?	Implement the decision - making process as in the General Limits of Care list	If timeliness requires action, person providing care to proceed with decision - making. If timeliness allows, contact the surrogate with medical power of attorney and Mission Control.

Appendix H

REPORT OF THE FIRST MEETING OF THE CREW STATUS EVALUATION WORKING GROUP

Purpose

The CSE (clinical status evaluation) working group met for the first time from July 31 to August 2, 2001 at the Del Lago Conference Center in Conroe, Texas. A list of the attendees, representing NASA and the NSBRI is attached (appendix 1). The motivation driving the formation of this group stemmed from a concept that there should exist "...a standardized, integrated physiological/medical data set on all persons who fly in space as part of the U.S. space program." It was emphasized that the CSE should be cost-effective ("a minimal data set"), consistent with evolving medical requirements for space flight, evidence-based, and initially directed toward the landing, entry, and postflight period. Three specific tasks were promulgated in the charge to the working group:

1. Develop recommended CSE components for entry, landing, and the immediate postflight period.
2. Define those research tasks required to develop an evidence base for CSE components.
3. Identify the specific knowledge, skills, and judgment required of physicians, crewmembers, or other health providers to enable them to utilize the CSE.

Methodology

Most of the first day was spent in a review of historical data, current shuttle/station medical capabilities, evidence-based medicine methodology, and critical path analysis. During these presentations the Integrated Testing Regimen (ITR) was discussed. It was noted that the ITR bears some similarities to the proposed CSE; however, it is designed to evaluate countermeasures rather than to provide a biomedical knowledge base characterizing individual crewmembers. As such, the two programs are seen as complementary, rather than redundant. It was recognized that there will be many areas in which the data gathered by the ITR can be shared by the CSE, but the focus of the latter will stress clinical rather than research issues and, in general, will be broader in scope than the former. At the end of these introductory sessions the group met to develop a process through which the requirements of the CSE could be met. It was noted that the following categories of problems exist about which a knowledge base must be developed:

1. Problems not unique to spaceflight which have an equal likelihood of occurrence whether in space or on earth. Examples include routine viral and bacterial infections. For these types of problems, there is a great deal of evidence in well-established and general biomedical data bases such as Pub Med, the Cochrane Collaboration, and standard reference books. These sources should be adequate to provide appropriate recommendations.

2. Problems not unique to spaceflight but exacerbated by the environment. Conditions such as cancer, nephrolithiasis, and anemia fit into this category. For these types of problems, one may utilize the general body of biomedical knowledge, but it must be augmented by research and experience reported in the space medicine literature.
3. Problems unique to spaceflight such as Space Adaptation Syndrome. In this group of problems the evidence base must rely on the limited data available from manned missions.

The following approach evolved from the above discussion to achieve the goal of building an appropriate knowledge base:

1. Using the results of the critical path analysis, address each of the 55 identified risks. It is recognized that some of these will not be clinically relevant, so it will be necessary to establish a subset of the risks that will be addressed in the CSE.
2. For each clinically relevant risk utilize the format of the evidence-based US Preventive Services Task Force (USPSTF):

    a) Provide a short statement of the frequency and impact of occurrence of the risk.
    b) Ensure that a viable intervention exists or can be developed to make testing useful. If no intervention exists, knowledge of a condition becomes a research task, not a clinical one.
    c) List currently available or evolving tests or evaluations that could prove useful in ameliorating the risk. As a start, the current ITR components assigned to the risk and the Medical Requirements Categorization document are to be examined; however, the current biomedical literature should be scrutinized to construct a more exhaustive list of possible testing. Evaluate the proposed test in terms of:

        1) Is the test valid, reliable, and does it possess a good positive predictive value?
        2) What is the quality of the evidence supporting the test? The following USPSTF scale can be used:
            a. Level I: Evidence obtained from at least one properly randomized controlled trial.
            b. Level II-1: Evidence obtained from well-designed controlled trials without randomization.
            c. Level II-2: Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one center or research group.
            d. Level II-3: Evidence obtained from multiple times series with or without the intervention.

e. Level III: Opinions of respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees.

f. In addition to this traditional system, a designator of S (space-based) and/or G (ground-based) may be used describe the source of the evidence. This recognizes the environment of space as unique and should attach additional significance to evidence obtained from space research. For example, III-S could characterize a case report based on flight experience.

3) Make a summary of the body of accumulated evidence. This document should include citations from the related biomedical literature following a critical evaluation of the available knowledge base.

4) Based on the evidence considered, provide a succinct summary along with a strength of recommendation for the proposed testing and its scheduling (e.g., on a periodic or as required basis):
   a. There is good evidence to support the recommendation that the evaluation be included.
   b. There is fair evidence to support the recommendation that the evaluation be included.
   c. There is insufficient evidence to recommend for or against the inclusion of the evaluation.
   d. There is fair evidence to support the recommendation that the evaluation be excluded from consideration.
   e. There is good evidence to support the recommendation that the evaluation be excluded from consideration.

5) Determine when the evaluation could be accomplished; i.e., preselection, preflight, pre-entry, during entry or postflight.

6) Determine if the identified test requires new capabilities, resources, or research.

7) Determine if the evaluation requires new crew or health care provider skills.

With the above format in mind, the group was divided into three smaller teams to examine three critical risks. The risks chosen for initial scrutiny were based upon their high level of impact to the crew and also upon their natural history. Thus, one long-term postflight problem was chosen (carcinogenesis), an intermediate-term risk (osteoporosis), and an immediate postflight risk (orthostasis). The teams were divided so that individuals with expertise in the related fields were

included, as well as a NASA flight surgeon, a current or former astronaut, and a member of NASA management. The list of teams is delineated in Appendix 2.

The results of the small groups were presented in summary during a subsequent plenary session. Although the approaches of the groups were somewhat different, the same general format was followed by each. One group (osteoporosis) proposed using a scale to grade risk; another team (orthostasis) developed a set of decision rules to use and presented its findings in outline form; the third (carcinogenesis) produced a more formal statement based on the USPSTF template. It may well be that each identified risk requires a slightly different thought process although it is felt that the general methodology will remain the same. The goal will be to produce a rational, cost-effective, thorough, and evidence-based approach to building the knowledge base. The products of the groups are attached as Appendices (3 to 5). In the short period of time available to these groups, it was not possible to construct a polished document, explore the literature, or make a comprehensive list of possible evaluations. However, the process by which this can be accomplished has hopefully been established.

Recommendations

In order to systematically generate the required knowledge base that will serve as the foundation for the selection of CSE components and the ultimate definition of a standardized, integrated, clinical data set for all U.S. crewmembers, the following long-term plan is recommended for implementation:

1. NASA management, in conjunction with the technical management of the NSBRI, will review, modify as needed, and approve the general approach and format as outlined above. Once approved, NASA will assign the discipline-specific Integrated Product Teams (IPT's) to address each of the clinically relevant risks within their respective discipline areas using the described evidence-based approach. This will involve a comprehensive literature review and systematic assessment of available evidence surrounding each risk with its attendant clinical correlates. For each of the risks, the individual IPT's will include, in addition to their overall review and knowledge synthesis, their recommendations for candidate CSE components.

2. In order to insure a balanced evaluation of the risks and issues addressed, the IPT membership will include a NASA flight surgeon(s), NASA discipline expert(s), NSBRI clinical expert(s), NSBRI discipline expert(s), and at least one biostatistician and/or expert in evidence-based medicine practices. The IPT membership will number anywhere from 8 to 12 members and will be guided by the general principles described above. It is anticipated that each IPT will develop its own format appropriate to the individual subject matter but will maintain the prescribed common framework.

3. The IPT's will meet on a periodic basis with the CSE Working Group and present the results of their evidence-based assessments for the critical risks in their particular discipline. They will also submit recommendations for inclusion of CSE components to the Working Group. It is anticipated that a subset of the IPT's will present to the Working Group at any given meeting, rotating the multi-disciplinary IPT's throughout the course of this entire activity until all critical risks are evaluated. The CSE Working Group plenary meetings will be one day in length consisting of focused reviews and discussions and will meet approximately 3 to 4 times in the coming year. The first follow-up Working Group meeting is tentatively scheduled for January, 2002.

4. Once all critical risks have been reviewed and presented to the CSE Working Group, the final meeting will focus on collating all the CSE component candidates recommended by the IPT's and by the Working Group throughout the course of the reviews. The recommendations will be scrutinized by the collective group to establish a minimal, integrated, clinical data set. This knowledge base will provide a health status assessment on crewmembers during all phases of space flight missions. A comprehensive list of biomedical research questions that are required to enhance the CSE evolution and broaden the evidence supporting its composition will also be generated by the Working Group. All reviews, evidence, and supporting discussions will be documented and organized into a final, comprehensive report with relevant appendices on each critical risk as provided by the IPT's. This CSE document will be submitted to NASA and the NSBRI for review and approval.

5. Finally, many of the committee members voiced strong recommendations to improve communication between the operational and research communities and to provide greater access to biomedical data. While these concerns were not directly related to the task assigned to the committee, it is felt that the ultimate success of our efforts can only be enhanced by greater cooperation between the two groups. Appendix 6 calls attention to some of the concerns expressed during the meeting.

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Orthostasis Working Group Report

Risk: Postflight Orthostatic Intolerance

Recommendation

Postflight orthostatic intolerance should be monitored in crewmembers returning from long-duration space flight until such time as they are able to return to general activities of daily living.

Burden of Suffering (Statement of Impact of Risk)

Frequency and Impact of Occurrence
Orthostatic intolerance (OI) is a clinically relevant consequence of space flight, which, in some crewmembers, may interfere with rapid egress from the vehicle should an emergency occur at or after landing. OI may also delay return to general activities of daily living (ADL) in affected crewmembers.

Clinically significant OI (inability to complete a 10 minute stand test 1–2 hours after landing) occurs in about 20% of crewmembers returning from short-duration (4–10 day) missions (n = 200; refs #n-m; Quality of Evidence = II-2). It also occurs in about 83% (6/7 crewmembers tested) returning from long-duration (4–6 month) missions (n = 7; refs #p-q; Quality of Evidence = II-2).

Detectable changes in OI persist for 3–10 days after landing in short-duration crewmembers (n = 200; refs #p-q; Quality of Evidence = II-2); however, functionally relevant changes limiting return to ADL persist only for hours to days (unpublished clinical assessment data from J. Clark; Quality of Evidence = II-2). Detectable changes in OI persist for an unknown period after landing in long-duration crewmembers; however, functionally relevant changes limiting return to ADL persist for days to weeks (Quality of Evidence = III).

Postflight OI may result from multiple etiologies. Primary among these are cardiovascular system deconditioning and neurological adaptation that occur during space flight and resolve gradually without intervention after return to Earth.

Efficacy of Possible Interventions

1) Fluid Loading (pre-entry):
   a) n = 34; ref Bungo
   b) Quality of Evidence = II-3
   c) Strength of Recommendation = B
2) Liquid Cooling Garment (entry/immediate post-landing):
   a) n = ?; ARC
   b) Quality of Evidence = III

c) Strength of Recommendation = ?

3) G-Suit (entry/landing/egress/postflight):
   a) n = ?; refs large literature indirect relevance
   b) Quality of Evidence = ?
   c) Strength of Recommendation = ?
4) Kentavr Suit (entry/landing/egress/postflight):
   a) n = ?; refs large literature indirect relevance
   b) Quality of Evidence = ?
   c) Strength of Recommendation = ?
5) Intravenous Fluid Loading (postflight):
   a) n = ?; refs #?
   b) Quality of Evidence = ?
   c) Strength of Recommendation = ?
6) Midodrine (postflight): flight validation trials in progress
   a) n = ?; refs bedrest study
   b) Quality of Evidence = ?
   c) Strength of Recommendation = ?
7) Promethazine, Meclazine, other anti-emetics w/wo amphetamines (pre-entry, postflight):
   a) n = ?; refs ?
   b) Quality of Evidence = ?
   c) Strength of Recommendation = ?

Accuracy of Screening Tests

Extant Medical Requirements

1) Operational Tilt Test: quantifies the HR and BP responses 5 minutes before and 10 minutes after a rapid, whole body, upright tilt from a supine resting condition
   a) Test timing: L-10, R+0, R+3
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test: normative populations: ?, clinical populations: ?, space flight populations: II-2
2) Computerized Dynamic Posturography (Functional Neurological Assessment): quantifies the contributions of vestibular, visual, and proprioceptive sensory cues to upright balance control (equilibrium).

a) Test timing: L-60, R+3, R+8, ... as req'd clinically

b) Validity/Reliability/PPV of test: MR performs only SOT trials. MCT trials and or voluntary head movements may be required to accurately assess vestibulo-spinal dysfunction. To be effective in early postflight screening, an R+0 test session should be added.

c) Quality of Evidence supporting test: normative populations: ?, clinical populations: ?, space flight populations: II-2

Other Useful Tests to be Considered (TBD)

1) Enhanced Tilt Test:
   a) Test timing: preflight, postflight
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
2) Early Tilt Test:
   a) Test timing: preflight, ctv
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
3) Plasma Volume Measurement:
   a) Test timing: preflight, postflight
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
4) Entry Monitoring of BP/ECG (SMO):
   a) Test timing: preflight, entry
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
5) LBNP Stress Test:
   a) Test timing: preflight, pre-entry
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:

6) EEG Monitoring:
   a) Test timing: preflight, postflight
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
7) Trans-Cranial Doppler Monitoring:
   a) Test timing: preflight, postflight
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
8) Vestibular Evoked Myogenic Potential (VEMP) Test of Vestibulo-Spinal Function:
   a) Test timing: preflight, postflight
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
9) Oculo-Motor Control Testing:
   a) Test timing: preflight, postflight
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
10) Dynamic Visual Acuity (DVA) and/or Locomotor Coordination Test:
   a) Test timing: preflight, postflight
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
11) Echo-Cardiographic Assessment of Cardiac Muscle Atrophy:
   a) Test timing: preflight, postflight
   b) Validity/Reliability/PPV of test:
   c) Quality of Evidence supporting test:
   d) New capabilities required:
12) Pulmonary Function (RR/End-Tidal CO_2):
   a) Test timing: preflight, postflight

b) Validity/Reliability/PPV of test:

c) Quality of Evidence supporting test:

d) New capabilities required:

13) Fatigue Monitoring (SCAT?):

a) Test timing: preflight, postflight

b) Validity/Reliability/PPV of test:

c) Quality of Evidence supporting test:

d) New capabilities required:

Carcinogenesis Working Group Report

Statement of Risk

Because of the unique environment of space, including factors such as the high levels of natural radiation, exposure to potentially toxic materials, and potential compromise of immune defenses, the risk of development of neoplastic disease is increased over the lifetime of individual astronauts. Because there is much to be understood about the complex mechanisms involved in carcinogenesis, it is probable that there will be many new discoveries linking the space environment to malignancies. Limited data exists to define these risks in the context of long-duration space flight. As a result, increased surveillance of astronauts for the development of cancer is appropriate.

Frequency and Impact of Occurrence

At least five deaths from neoplastic disease have occurred in the astronaut population, including one while on active duty with NASA. In addition, approximately 10 other cases of cancers have been diagnosed although death has not resulted from the condition. These cases have included blood dyscrasias (lymphoma and leukemia), solid organ tumors (gall bladder, brain, thyroid, renal, prostate, etc) and skin cancers. The impact ranges from relatively benign conditions to death. While it is most likely that this disease will be manifested later in life, it is probable that some cases will occur while an astronaut is in an active status, perhaps even while in flight. The likelihood of the latter possibility is increased by greater levels of exposure through multiple long-term spaceflights. This may be especially true of rapidly-growing neoplasms such as leukemias, which can occur within two years of radiation exposure.

Potential Interventions

If clinical testing is utilized to detect an increased risk for cancer, possible interventions do exist which include:

1. Chemoprevention. These might include vitamin, mineral, trace element, and antioxidant therapy for prevention of disease. Another example could be the use of agents such as tamoxifen to lessen the risk for development of breast cancer.
2. Increased surveillance. If a crewmember had been exposed to a condition predisposing him/her to a neoplastic condition, additional testing such as following tumor markers or regular diagnostic imaging might improve the probability of early detection of the disease.
3. Operational maneuvers. In-flight monitoring of radiation levels may lead to a real-time decision to relocate the crew to an area inside the spacecraft that has a higher level of shielding. The detection of a toxic hazard could lead to a purging of the cabin, decontamination, donning of protective gear, or a return to earth.

Evaluation

1. Chromosomal aberrations. Chromosomal aberrations in lymphocytes are already measured as a biodosimeter and as a general indicator for the amount of radiation-induced DNA damage. As this is a correlation between the amount of DNA damage produced by radiation and the risk of cancer development, the level of chromosomal aberrations is considered to be a surrogate endpoint biomarker of carcinogenesis.
Level of evidence: TBD
Strength of recommendation: TBD
New capability required: None. Already being performed.
New skills required: None.

2. Genetic analysis. This is a highly controversial area of testing that is currently in its infancy. Certain genetic mutations exist which are thought to predispose individuals to the development of cancer later in life. Examples include mutations in the p53 gene, the BRCA 1 and 2 genes, the retinoblastoma gene and the ataxia-telangiectasia gene. The presence of such genetic abnormalities could put a crewmember at high risk when placed in the environment of space and may be a possible factor for consideration in the selection process as an astronaut, in assignment of long-duration crewmembers, or in retention as an astronaut. Early detection, while fraught with ethical and legal difficulties, could have a significant impact on longevity, whether the individual flew in space or not.
Level of evidence: II-2G
Strength of recommendation: C
New capabilities required: Testing facilities and partnering with academic institutions doing active research in this field.
New skills required: None

3. Immunologic testing. Evidence exists that establishes the loss of immune function (anergy) as a marker that indicates an increased risk for the presence of neoplasm. A simple skin test such as a panel of routine delayed hypersensitivity reaction agents could provide an inexpensive, early clue to the development of anergy. It is envisioned that this test could be performed upon selection and yearly after flight. A change in the test results would lead to a more extensive evaluation.
Level of evidence: II-3S/G
Strength of recommendation: A
New capabilities required: Annual testing of flown astronauts.
New skills required: None

4. Antioxidant/Vitamin Status. There is a body of literature that supports the hypothesis that deficiencies in certain antioxidants and vitamins (selenium, vitamin A, and glutathione, etc.) may lead to the development of neoplastic disease. It is also thought that exposure to radiation can further deplete the levels of those substances. A deficient state can lead to a decrement in the body's ability

to scavenge free-radicals and to repair damage to DNA caused by radiation. A known deficiency preflight would dictate supplementation, and the development of low levels in-flight might precipitate the use of therapeutic doses. Testing would consist of periodic serum level evaluation.
Level of evidence: II-1G
Strength of recommendation: A
New capability required: Development of standards.
New skills required: None

5. Tumor markers. Numerous associations have been made between levels of certain antigens (PSA, CA-125, CEA, AFP, CA 19-9) and various tumors (prostate, ovarian, colon, liver and testicular, and pancreatic). With certain exceptions, there has been no evidence that use of these tests leads to a lowering of mortality from these diseases in the general population. However, the use of these tests in high-risk populations in which an increased prevalence of the disease exists has become common in clinical practice. An example is the annual evaluation of alpha fetoprotein levels in patients with hepatitis B and C, given their greatly increased risk for the development of hepatocellular carcinoma. Given the unknown risks associated with long-duration spaceflight, the use of these tests at baseline and periodically postflight may be reasonably considered.
Level of evidence: II-CG
Strength of recommendation: D
New capability required: None
New skills required: None

6. H. pylori testing. There is a strong association established between the presence of *H. pylori* and the development of several types of stomach cancers including MALTOMAs and gastric cancer. Additionally, this organism predisposes individuals to duodenal ulcers and, to a lesser degree, to gastric ulcers. Relatively inexpensive and non-invasive means for detection exist, such as serologic evaluation and urease breath tests. A highly effective means of eradication of the organism is well-known and affordable. In addition to the prevention of cancer, treatment would prevent the spread of the organism to fellow crewmembers and would eliminate the development of *H. pylori*-associated ulcers, which could manifest first while in flight. Evaluation for the presence of *H. pylori* infection meets most of the criteria for an effective screening test.
Level of evidence: II-2G
Strength of recommendation: A
New capability required: None
New skills required: None

7. Diagnostic Imaging. There are many new imaging modalities being developed of malignancies. These include CT, MRI, PET, and SPECT. While costly, these could provide fairly sensitive baseline and longitudinal data for the development

of neoplastic disease. Testing could have the additional benefit of detection of a wide range of benign conditions such as coronary artery disease, biliary and urinary tract calculi, polyps, soft tissue adenomas, among many others. Coupled with on-orbit ultrasound capability, the existence of baseline scans would provide real-time analysis of newly discovered masses whether benign or malignant. The training associated with the task of ultrasonography could be minimized through the development of procedures akin to other tracking skills such as manipulation of the RMS.
Level of evidence: III G
Strength of recommendation: C
New capability required: Development of a 3-dimensional body coordinate system.
New skills required: Ultrasonography crew training and flight surgeon interpretative skills.

Note: There are many other potential surveillance and screening tests that should be considered. Time did not allow the group to discuss additional possibilities. The "strength of recommendation" and "level of evidence" listed in each category were based on the opinions of the group and not upon an investigation of the extant literature. Included below is one example of how the literature might be applied in this area of risk. The abstract cited describes a prospective cohort study (level of evidence II-2G) that evaluated the efficacy of an expanded surveillance program of a group (oil refinery workers) at potentially high risk for the development of hematologic malignancy. The authors conclude that a program such as this has "limited utility," even in this high-risk group (Recommendation: D). Other studies evaluating similar programs in other high-risk populations may reach a different conclusion. The task of a group using this evidence-based approach will to scrutinize the available literature, and based on the findings, to make appropriate recommendations for inclusion of the test as part of the test suite.

Medical surveillance for hematological disorders among active and retired oil refinery workers.

Tsai SP - *J Occup Environ Med* - 1998 May; 40(5): 475-80 From NIH/NLM MEDLINE, HealthSTAR
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Abstract:
Ten-year (1985-1995) results of an expanded medical surveillance program of 2475 active employees and retirees of an oil refinery and petrochemical complex

in Illinois are presented. At the end of the program, 116 participants with persistent abnormalities of complete blood cell count had been referred for hematologic evaluation, and most were found to have benign conditions. Fifteen of the 116 were referred for bone marrow and cytogenetic studies. All of the referred active employees (seven) were found to have completely normal bone marrows with no evidence of any myelopathic process. Among the eight retirees, two had normal bone marrows, one was diagnosed with Philadelphia chromosome-positive chronic myelogenous leukemia, one declined to participate, and four were diagnosed to have myelodysplastic syndrome (MDS) of various subtypes. A total of eight cases of MDS were identified, including six cases among program participants and two cases among nonparticipants. The MDS standardized incidence ratio of 1.26 (95% confidence interval = 0.54-2.47) was not statistically significant, and there was virtually no increase of MDS in persons less than 80 years of age (4 observed and 3.8 expected). This MDS increase was entirely from program participants, probably because of intensive follow-up and diagnostic screening. Routine surveillance of complete blood cell count information did not identify any new cases of leukemia or MDS in active employees. These findings suggest that the utility of expanded medical surveillance program in this population is very limited.

Risk: Osteoporosis

Recommendation

Overall clinical assessment of bone and associated mineral and metabolic status should be monitored in all crewmembers of long-duration space flight missions throughout all phases of a mission, throughout the entire course of their active astronaut career, and beyond retirement.

Statement of Risk

Microgravity-induced bone loss is believed to be primarily a result of skeletal unloading in which bone strains remain below a defined modeling threshold resulting in cessation of normal modeling. The complex interplay among biomechanical factors, hormonal and metabolic balances, and dietary status results in a sustained condition in which bone formation is either unchanged or decreased and bone resorption is increased. This accelerated bone loss and the consequent potential of osteoporosis (critical. path risk #9) poses to crewmembers the increased clinical risk of stress/traumatic fractures and impaired fracture healing (risk #10), soft tissue injury (risk # II), and renal stone formation (risk #12) during and most particularly on return to Earth and throughout their remaining lifespan. The current evidence-based evaluation addresses critical path risks #9 and # 10 and their attendant clinical consequences of functional impairment due to pain, decrease mobility and the secondary complications of fracture or immobilization.

Frequency and Impact of Occurrence

Loss of bone during exposure to microgravity, as measured by alterations in bone mineral density (BMD), occurs in 100% of astronauts at a rate of 0.4 to 1% a month depending on the skeletal site measured and varying with the characteristics of an individual (e.g. gender, race, genetic background, age, body weight, smoking history); (Quality of evidence = 11-2). This bone loss appears to be related to length of space flight. Accumulating follow-up data from long-duration crewmembers suggest that over a period of 2 to 3 years postflight bone recovery occurs in most but not all individuals (Quality of evidence = 11-2).

Bone loss increases risk of significant osteoporosis and fractures. From extensive ground-based studies it has been shown that decreases of one standard deviation in femoral neck BMD and lumbar spine BMD are associated with a 2.5 -fold increase in hip fracture risk and a 2 -fold increase in vertebral fracture risk (Quality of evidence = 11-1/ 11-2 ?). However among individuals, like bone loss itself, the risk of fractures is highly variable. This most likely reflects the influence on fracture risk of other factors other than bone mass such as characteristics of the individual and bone geometry/quality. The impact of the increased risk therefore can range from minimal with mild acceleration of aymptomatic, age-related osteoporosis to severe with premature stress and/or traumatic fractures causing functional impairment and increased morbidity. For missions of six month durations, this impact is anticipated to occur at an intermediate time (> 10 y) after a mission. For missions beyond one year duration, although unknown, the impact is anticipated to occur prematurely during the immediate postflight period or shortly thereafter (2-5 y) a mission.

Potential Interventions

Potential mitigation strategies to eliminate or reduce the increased risk of significant osteoporosis and fractures in long-duration crewmembers focus on maintaining skeletal loading, preserving muscle mass and strength, increasing bone formation, and decreasing bone resorption. They include:

1) **Resistive Exercise.** Hypothesized to prevent the decreased strain which results from disuse and to provide mitigation to regional bone losses.
   a) References: bedrest, space flight
   b) Quality of evidence: 11-3 (?)
   c) Strength of recommendation: B
2) **Biphosphonates.** Shown to decrease bone resorption in ground-based clinical trials in post-menopausal women and increase bone mass. In bedrest trials simulating microgravity.
   a) References: large literature on post-menopausal women; limited in bedrest in men/women
   b) Quality of evidence: II-1 (clinical trials); 11-2 (bedrest)?
   c) Strength of recommendation: C (?)
3) **PTH and other investigational agents.** Shown in ground-based studies with diseased subjects (hypoparathyroidism) to increase bone remodeling.
   a) References : limited, none in microgravity simulations
   b) Quality of evidence: ?
   c) Strength of recommendation: ?
4) **Adequate nutrition/diet.** Diet in it of itself will not provide effective countermeasures for bone loss, however, an adequate intake of calcium, vitamin D, magnesium, and phosphorus will be essential in combination with other countermeasures to preserve bone mass and architecture.
   a) References: extensive in ground-based clinical trials on Earth; limited in space (Scott et al.)
   b) Quality of evidence: 11-2 (?) on ground; III (?) in space
   c) Strength of recommendation: B (?)
5) **Artificial Gravity.** If other interventions prove minimally successful in preventing bone loss, a centripetal- induced artificial gravity concept may require development for exploration class missions such as those to Mars.
   a) References: limited (?)
   b) Quality of evidence: ?
   c) Strength of recommendation: ?

Evaluation

The utility of clinical testing lies in the ability of individual or collective sets of parameters/measurements to assist in the establishment of a diagnosis, prognosis, or likelihood of response to treatment in an individual. In the case of space flight -induced osteoporosis, the diagnosis is based on the already established guidelines and criteria set forth for ground-based

clinical scenarios here on Earth. The critical questions in this intermediate term risk focuses principally on the clinical testing that provides predictive power towards establishing the level of risk for fracture. A useful tool in this regard is the development of clinical decision rules (CDRs) and is a potential approach for critical risks where multiple clinical components of the history, physical exam, and clinical testing collectively contribute towards increased accuracy of a diagnostic and/or prognostic assessment (JAMA. 2000; 284:79-84). Specifically for bone loss and the increased risk of fractures, there are a number of tests/parameters with potential predictive power of the outcome of interest (fractures) that could be used for development, validation, and impact analysis of a CDR (Appendix A). Whether through the development of such a clinical tool or as individual components of the CSE, the available or evolving clinical tests of relevance to osteoporosis and fracture risk are delineated below. These include, in addition to tests useful for prediction of fracture risk, clinical tests that also address assessment of the effectiveness of countermeasures and response to interventions.

1) **DEXA Bone Scan.** Measures bone mineral content and bone mineral density with sufficient precision and accuracy to measure longitudinal changes of the magnitude of approximately 1% to 2%. Currently is the gold standard as surrogate marker for bone breaking strength and is used to predict risk of fracture in the general population. Testing should be pre and postflight in intermediate length missions (< 1 y) with long term follow-up measurements throughout crewmembers' career. In extended missions exceeding a year, the potential for inflight measurements should be considered for monitoring the rate and extent of bone loss and for implementing interventions if required.
   Quality of evidence: normative populations: II-I(?); clinical populations: II-I(?); space flight: 11-2
   Strength of recommendation: A

   New capability required: only for inflight DEXA monitoring if available
   New skills required: DEXA training for inflight use

2) **Physical Fitness Evaluation.** A series of functional fitness tests preformed pre- and postflight to assess skeletal muscle strength and endurance.
   Quality of evidence: ?
   Strength of recommendation: C
   New capability required: none
   New skills required: none

3) **Metabolic and Hormonal Markers.** Subset of parameters that reflect bone and calcium status and may provide clinical indicators of bone formation, bone resorption, nutritional status, and fracture propensity. Importantly, they may contribute significantly to monitoring of the effectiveness and adequacy of response to specific countermeasures. Measurements conducted principally pre- and postflight with possible inflight analysis on missions of longer than 1 year.
   In blood:
   a) Vitamin D metabolites : 25-OH D Vitamin, 1,25-OH D
   b) PTH

c) Osteocalcin. Index of bone formation and turnover.
d) Calcium (Total and Ionized)
e) Alkaline phosphatase (Total and Bone-specific). Bone formation marker.
f) Thyroid hormone
In urine:
a) Collagen crosslinks. N-telopeptide, pyridinoline, deoxypyridinoline.
b) Calcium
Quality of evidence: ?
Strength of recommendation: ?
New capability required: None for pre/postflight evaluation; on-board analysis capability for select parameters
New skills required: inflight sample collection, processing and analysis

4) **Measures of Bone Quality.** Bone geometry in addition to bone mass is related to bone strength and therefore, a relevant parameter contributing to fracture risk. The cross-sectional moment of inertia, determined from a cross section of the femoral neck using DEXA scans, may be useful in assessing bone architecture.
Quality of evidence: ?
Strength of recommendation: ?
New capability required : None
New skills required: None

Clinical Research Directions

Areas of clinical research identified as needed to enhance the CSE evolution and broaden the evidence supporting its composition in the risk area of osteoporosis and fracture risk are:

1) Re-define fracture risk estimates vs. DEXA (e.g. current estimate is that fracture risk increases 2.5 fold for each decrease in standard deviation. How does this apply to persons in an extended weightless setting ?)
2) Determine fracture incidence in Astronauts/Cosmonauts : this data is available.
   Data is required about:
   a) Fractures which have occurred during flight
   b) Fracture incidence postflight : duration of follow up, method of follow up.
3) Define markers of bone quality vs. data available on bone quantity.
   a) Correlate bone biomarkers used inflight to bone quantity and quality
   b) Assess data existing (archival) data on bone loss by cosmonauts after multiple long-duration flights
4) Assess longitudinal data on bone changes during Astronaut Conditioning Program (pre-/postflight)
   a) Evaluate the adequacy of the current BMD/biomarkers database
   b) Does the magnitude of bone loss or change in biomarkers during flight predict the rate of return? Implications for conditioning program.

Additional Recommendations

Any minimal dataset collected as part of the standardized flight data collection will need to include data that is collected immediately after touchdown and egress from the Shuttle or Soyuz after long-duration space flight. This will be necessary because some of the most common medical problems experienced by astronauts after space flight present acutely after landing, especially cardiovascular deconditioning and neurovestibular dysfunction. Extremely important data is being collected by the flight surgeons during this critical period as is necessary to make clinical decisions as part of their care of the astronauts. It is not clear if the data is being or can be collected in a way that optimizes the quality of the clinical evidence for subsequent analysis. The methods of collection of data in this acute period may be best determined by collaboration between the clinical investigators of NSBRI and the flight surgeons.

This collaboration would include general discussions of the state of the astronauts as they first leave the shuttle upon return to Earth, the clinical decisions facing flight surgeons at that point, and the current approach to treating these problems. This type of collaboration may result in a refinement of the data being collected by the flight surgeons in so far as it helps them make clinical decisions. A more formal collaboration may help preserve this data in a manner that is suitable for generating observational, epidemiological, and empirical evidence that would be useful in the future. Finally, this type of collaboration may refine issues that require further research.

This collaboration should not result in the violation of privacy or confidentiality, or discussion or circulation of attributable data. If access to specific medical records is desired, it must be done with the informed consent of the astronaut. A more formal collaboration between the flight surgeons and the clinical investigators from NSBRI may be highly beneficial. It is recommended that NASA and NSBRI set up a mechanism by which coordination between the NSBRI clinical investigators and the flight surgeons can take place on a regular basis.

Appendix I

NSBRI Peer Review

Special Announcement 00-02
Education and Public Outreach Panel

November 14-16, 2000

Wayne M. Sukow, Ph.D. (Panel Chair)
Teacher Enhancement
Advanced Technological Education Programs
National Science Foundation
Arlington, VA

Jane Fisher
SETI Institute
Mountain View, CA

Mary M. Frasier, Ph.D.
University of Georgia
Department of Educational Psychology
Athens, GA

Bruce A. Jackson, Ph.D.
Boston University
School of Medicine, Department of Biochemistry
Boston, MA

J. Lawrence Katz, Ph.D.
Case Western Reserve University
School of Engineering
Cleveland, OH

Sandra K. Kolb
Poulsbo, WA

Gail M. Nordmoe, Ed.D.
Richard R. Green Institute
Augsburg College
Minneapolis, MN

Anne Tweed
Eaglecrest High School
Aurora, CO

Thomas Yulsman
Center for Environmental Journalism and Mass Communication
University of Colorado at Boulder
Boulder, CO

Appendix J

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

Annual Program Report: *Education and Public Outreach Team*

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EDUCATION AND PUBLIC OUTREACH TEAM PROJECTS

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I. EXECUTIVE SUMMARY

Mission. The mission of the Education and Public Outreach Team is to communicate the significance and excitement of space life sciences to local, national and international audiences, while transferring and disseminating knowledge gained by the biomedical advances achieved by other NSBRI Research Teams. This mission currently is being accomplished through an integrated array of programs that focus on students and educators at all grade levels, as well as the general public.

Basic Approach. The NSBRI Education and Public Outreach Team is comprised of seven primary partners: Baylor College of Medicine (BCM) in Houston, Texas; Mount Sinai School of Medicine (MSSM) in New York, New York; Massachusetts Institute of Technology (MIT) in Cambridge, Massachusetts; Morehouse School of Medicine (MSM) in Atlanta, Georgia; Rice University and The University of Texas Medical Branch (RU/UTMB) in Houston and Galveston, Texas; Texas A&M University (TAMU) in College Station, Texas; and the University of Washington (UW) in Seattle, Washington. Additionally, 26 other organizations and institutions are working with NSBRI's Education and Public Outreach Team to enhance its mission and to ensure the widest possible dissemination of its products and programs. These collaborations include state public school systems, public television and radio stations, state space grant programs and museums.

The Education and Public Outreach Team develops and implements activities that address four major programmatic themes. These are: Teacher Professional Development, Curriculum Development, Science Literacy and Public Awareness, and Career Awareness and Access.

- Teacher Professional Development. Teachers are the critical link between curricula, students and their parents. NSBRI teacher professional development activities are designed to help teachers understand space life sciences and change their practices and behaviors to improve the learning experiences they provide students. Teacher Professional Development activities include workshops, summer institutes and research experiences.
- Curriculum Development. NSBRI curriculum development activities are occurring across the educational continuum from primary grades through graduate preparation. At the K-12 levels, materials are being developed that are aligned to the national science standards. These materials are addressing the need for accurate, balanced, effective and inquiry-based materials for the nation's classrooms. At the undergraduate and graduate levels, courses are being developed to expand students' understanding of on-going NSBRI research.
- Science Literacy and Public Awareness. Promoting greater understanding and awareness of NSBRI space life sciences research is essential for public support. Numerous activities are underway. They include television and radio news programs, informal science activities at museums, direct mailings of informational posters to schools and magazine stories designed to expand public understanding of how NSBRI research will impact long-term space exploration and the everyday world.
- Access and Career Awareness. There are many barriers to promoting diversity and access to careers in the space life sciences. Activities within this theme include research experiences for high school and undergraduate students as well as high school teachers in NSBRI laboratories. Courses focusing on NSBRI research areas will assist in promoting undergraduate and graduate students' interest in spaces life sciences research careers.

Strengths and Major Accomplishments. The Educational and Public Outreach Team is comprised of experienced educators and scientists from some of the most noted research institutions in the nation. Baylor College of Medicine, recognized for its research in biomedical sciences, also is one of the nation's leaders in K-8 curriculum development. It also has a 30-year history of developing programs to promote access to careers in medicine and science, especially for underrepresented populations. Morehouse School of Medicine has an established track record in developing innovative, problem-based curriculum materials for secondary and undergraduate students, and is a national leader in creating pathways for underrepresented students to access life science research careers. Massachusetts Institute of Technology is one of the nation's premier institutions of higher education that is now marshalling its talented faculty in the development of undergraduate and graduate courses focusing on NSBRI research areas. Mount Sinai School of Medicine is at the forefront in biomedical science research and is translating NSBRI research into meaningful curriculum materials for 9th grade classrooms. Two institutions of academic excellence, Rice University and The University of Texas Medical Branch in Galveston have developed a yearlong program for high school teachers to develop curriculum materials for high school students and a 10-week research program for high school students in NSBRI sponsored laboratories. Texas A&M University has one of most recognized colleges of education in the country. It has established the NSBRI Teacher Academy Program that brings together master science teachers from across the country to learn about NSBRI research and subsequently transfer that knowledge to teachers and students in their home states. The University of Washington, known for world-class technology and research, brings an established publishing program to NSBRI, including the *Northwest Science & Technology* magazine, with a subscription of close to 28,000 readers, to produce NSBRI stories.

Collectively, the Education and Public Outreach Team has begun to establish NSBRI as a leading resource in the preparation of teachers and the development of quality materials bring the excitement and importance of NSBRI space life science research into the nation's classrooms and homes. Since January 2001, hundreds of teachers and thousands of students have benefited from NSBRI sponsored programs. The public has been reached through television and radio news programs and national magazine articles.

Gaps. The NSBRI External Advisory Council and the 2000 Site-Visit Team made five recommendations for improvement. These recommendations were: (1) improve dissemination, feedback and assessment methodologies; (2) establish a coordinated development plan; (3) establish university level education programs; (4) articulate team's unique abilities to contribute to national education; (5) promote diversity. The addition of MIT to the Education and Outreach Team will help to address the recommendation of establishing university level education. MIT will develop and test two graduate level courses, one of which will be offered to undergraduate students. The remaining recommendations are being addressed in the strategic objectives outlined in the Education and Public Outreach Team Five- to Ten-Year Strategic Plan.

Implications for Future Direction. The following objectives will guide the development of the Education and Public Outreach Team over the next ten years:

Five-Year Strategic Objectives

- Establish a strong publication record with respect to space research curricular supplements and programs.
- Increase scientific literacy by involving scientists in community education and bringing NSBRI and space-based science into classrooms and homes.

- Attract more young students (especially those from underrepresented groups) to careers in space life sciences, engineering and technology-based fields.
- Promote excellence, achievement and systemic change in education through the development and implementation of high-quality space-based science, mathematics, reading/language arts instructional materials designed to facilitate measurable success for all students, apply best understandings of how students learn, and incorporate assessment as an integral component.
- Enhance the space-based science and technological readiness, skill and teaching impact of educators by providing professional development focusing on partnerships with scientists and pedagogical strategies utilizing NSBRI-generated resources that empower educators to (1) teach all students more effectively, and (2) communicate these new instructional resources to peers in education.
- Create and support stimulating, informal space life sciences education programs outside of school to develop and maintain public interest in, and awareness of, NSBRI scientific and technological developments.
- Establish partnerships with external groups that bring additional funding support to NSBRI activities and assist the Education and Public Outreach Team to disseminate and promote space-life science education programs.
- Develop and implement a media plan to include, but not be limited to: public affairs announcements and programs for radio and television, brochures, posters, video-documents and Web sites, and a national writer-in-residence program.
- Work with state educational agencies to integrate NSBRI space life sciences content into required curricula.

Ten-Year Strategic Objectives

- Establish NSBRI as a national leader in the development and deployment of K-16 Internet-based distance education.
- Design and implement research studies that examine the effectiveness of NSBRI-sponsored Internet-based curriculum materials, as compared to traditionally formatted materials.
- Offer online graduate programs in space life science education for elementary, middle and secondary school science, mathematics, physical education and language arts teachers.
- Promote NSBRI research through educational demonstrations conducted on the International Space Station and broadcast worldwide.
- Establish an International Society of Space Life Sciences Educators, including a fellowship program for its members.
- Create a Center for Research in Space Life Science Education in Houston to infuse NSBRI research into educational practice in schools and study ways to increase student motivation in science education and career pursuits.
- Enter into commercial partnerships with publishers, software manufacturers and broadcast media corporations to disseminate information and materials nationally that describe and promote application of NSBRI educational activities.
- Work with state educational agencies to integrate NSBRI space life sciences content into required curricula.

II. INTRODUCTION

The mission of the Education and Public Outreach Team is to communicate the significance and excitement of space life sciences to local, national and international audiences, while transferring and disseminating knowledge gained by the biomedical advances achieved by other NSBRI Research Teams. This mission currently is being accomplished through an integrated array of programs that focus on students and educators at all grade levels, as well as the general public. The major program themes are explained below.

- Teacher Professional Development. Teachers are the critical link between curricula, students and their parents. NSBRI teacher professional development activities are designed to help teachers understand space life sciences and change their practices and behaviors to improve the learning experiences they provide students. Teacher Professional Development activities include workshops, summer institutes and research experiences.
- Curriculum Development. NSBRI curriculum development activities are occurring across the educational continuum from primary grades through graduate preparation. At the K-12 levels, materials are being developed that are aligned to the national science standards. These materials are addressing the need for accurate, balanced, effective and inquiry-based materials for the nation's classrooms. At the undergraduate and graduate levels, courses are being developed to expand students' understanding of on-going NSBRI research.
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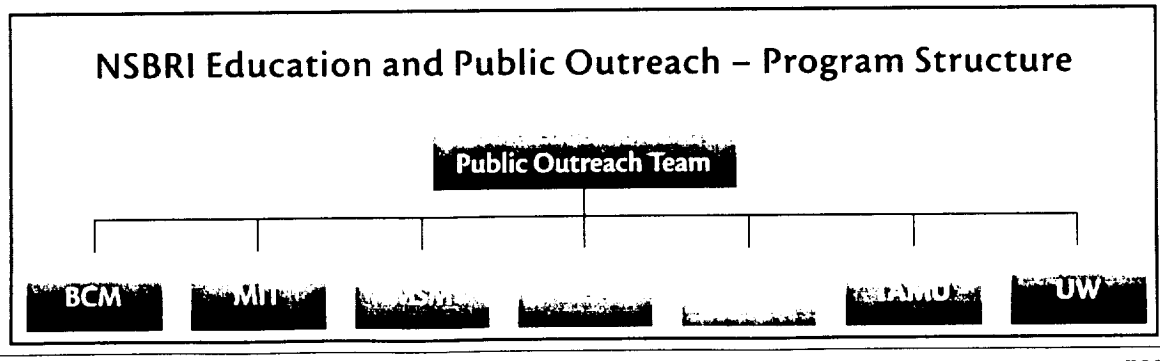
- Access and Career Awareness. There are many barriers to promoting diversity and access to careers in the space life sciences. Activities within this theme include research experiences for high school and undergraduate students as well as high school teachers in NSBRI laboratories. Courses focusing on NSBRI research areas will assist in promoting undergraduate and graduate students' interest in spaces life sciences research careers.

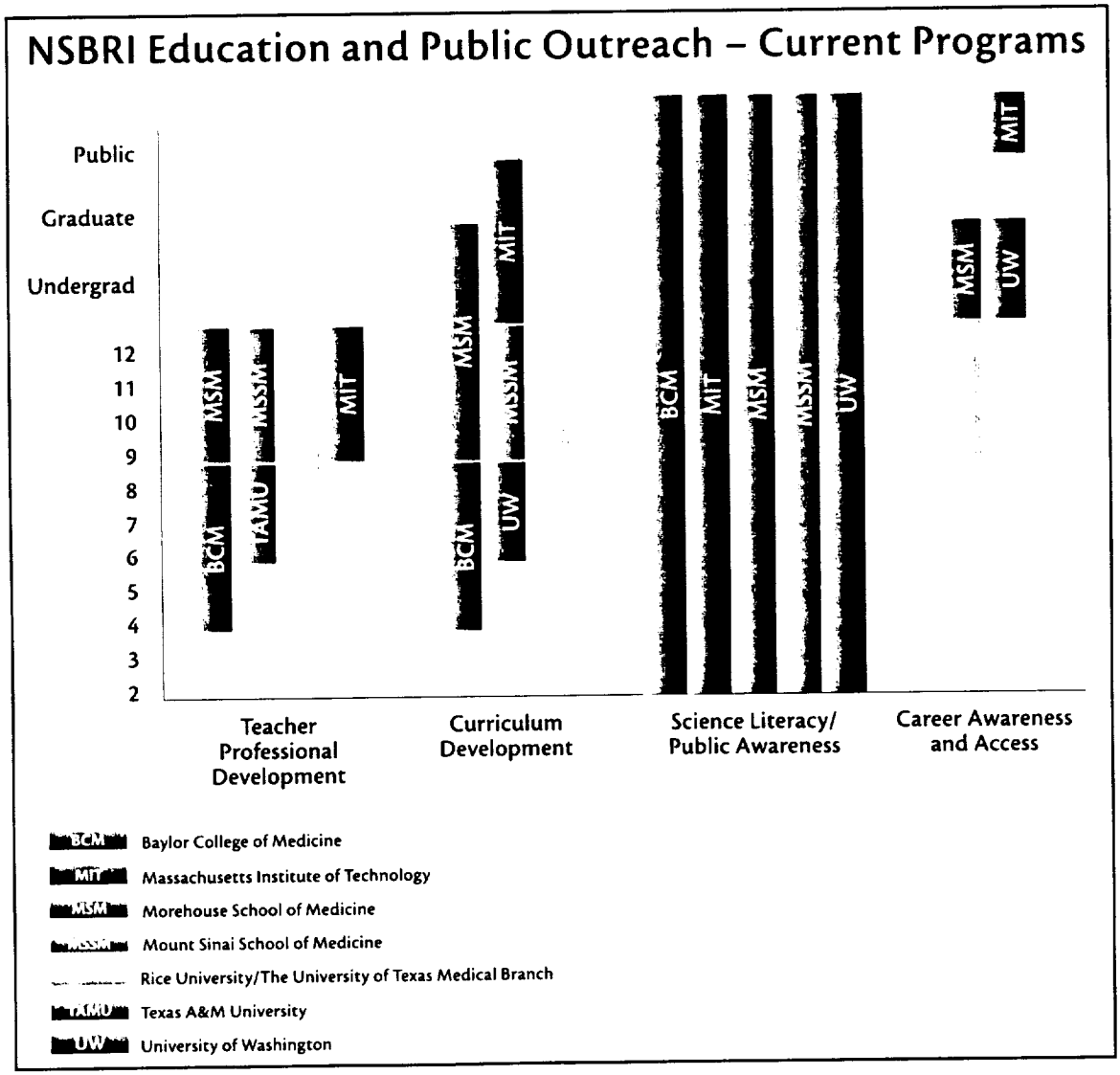
III. PROGRAM STRUCTURE AND DESIGN

Program Structure. The Education and Public Outreach Team's mission is being accomplished through an integrated array of programs administered by eight partner institutions: Baylor College of Medicine (BCM), Houston, Texas; Massachusetts Institute of Technology (MIT) in Cambridge, Massachusetts; Morehouse School of Medicine (MSM), Atlanta, Georgia; Mount Sinai School of Medicine (MSSM), New York, New York; Rice University/The University of Texas Medical Branch (RU/UTMB), Houston and Galveston, Texas; Texas A&M University (TAMU), College Station, Texas; and the University of Washington (UW), Seattle, Washington.



Many organizations and institutions are working with NSBRI's Education and Public Outreach Team. Partners include Atlanta Public Schools, Atlanta Public Television and Radio, DeKalb Public Schools, Emory University, Fernbank Science Center Museum, nine Galveston County school districts, Georgia Institute of Technology-SECME Program, Georgia State Partnership for Excellence in Education, Harvard Medical School, Houston Independent School District, Houston Public Television, Johnson Space Center, New York Public Schools, New York Hall of Science, North Forest (Houston, Texas) Independent School District, Space Center Houston, Spelman College, Texas Alliance for Science, Mathematics and Technology, Texas Rural Systemic Initiative, the Texas Statewide Systemic Initiative, and the Washington Space Grant.

Synergy among individual Education and Public Outreach institutions and goals is achieved through the following four themes: teacher professional development; curriculum development; science literacy and public awareness; and career awareness and access. Specific foci and activities of NSBRI's Education and Public Outreach partner institutions are described in the graphic and narrative below.



Baylor College of Medicine (BCM)—*From Outer Space to Inner Space: Sharing NSBRI Progress with the Community*. BCM, Space Center Houston, Houston Public Television and the Houston Independent School District are collaborating to convey the excitement and promise of NSBRI space life sciences research to students, teachers and the general public through coordinated formal and informal educational opportunities that will be embedded within local and state science education reform programs.

This partnership engages scientists and educators in the production, evaluation and dissemination of a planned series of elementary and middle school curriculum materials based on NSBRI research themes. It also produces bimonthly, nationally distributed radio stories on NSBRI research areas. The partnership reaches thousands of students, teachers and members of the general public each year. It also generates public awareness and appreciation of the benefits of NSBRI research. Project activities are aimed at middle school (grades 5-8), which has been identified as a particularly weak link in the K-12 science/mathematics education continuum.

Measurable project objectives are: (1) collaboratively create, evaluate and disseminate three interdisciplinary teaching units (one per year) on NSBRI research themes for middle school students; (2) improve teacher practice and content knowledge through multiple professional development opportunities conducted in formal and informal educational settings; (3) develop an online workshop resource for NSBRI scientists to use for outreach to teachers, students and the community-at-large; and (4) create and implement cost-effective models for communicating NSBRI research to local and national populations through television and radio short-format news and newsmagazine stories.

Massachusetts Institute of Technology (MIT)—*Space Biomedical Sciences and Engineering Curriculum and Outreach Project*. MIT will develop curricular materials to educate a generation of scholars in space life sciences by transferring NSBRI space life sciences research into undergraduate courses and to younger students and the public. Two graduate courses will be developed: *Space Biomedical Engineering and Life Support* and *Sensori-Neural Systems: From the Vestibular Periphery to Motor Responses, Perception and Adaptation.* The modular materials will cover eight of twelve NSBRI research areas and will be designed for adoption among NSBRI consortium institutions.

The MIT team includes academia and two small businesses in partnership with the national non-profit organization, "For Inspiration and Recognition of Science and Technology." MIT will design a knowledge station that allows learners to interact with curricular materials via state-of-the-art information technology and a physical platform designed specifically to facilitate human interaction and learning.

Intended outcomes are to: (1) provide multi-level space life sciences curriculum; (2) excite and educate the public about the wonders of science, engineering and medicine by disseminating knowledge gained through NSBRI research; and (3) develop a set of innovative pedagogical strategies that represent the application of tested learning principles as a basis for comprehensive educational evaluation tools. In addition, the project will develop multimedia tools that are particularly suited to active learning accessible through the Internet. The evaluation plan will assess learning and knowledge transfer of curriculum that makes use of these technological advances as well as assessment of the new student (or 'learner') population.

Morehouse School of Medicine (MSM)—*Secondary and College Education for the Next Generation of Space Life Scientists*. The MSM program is multi-faceted. *The MSM-Fernbank Museum Space Station Teacher Institute* admits two science teachers into a yearlong residency at MSM to develop, test and disseminate secondary problem-based curriculum supplements. Teachers work with scientists and physicians to write a cardiovascular case, *Bobby's Beat.* They attend the Texas A&M Teacher Academy to learn this method and develop leadership skills.

The *MSM-Georgia Institute of Technology SECME Program* will deliver a teacher professional development module on the Human Body in Space at the annual SECME national meeting, and also sponsor a noted space scientist lecture to address the estimated one thousand attendees. The *Summer Research Program* enrolls four undergraduate students, selected from a national applicant pool, to engage in a research-intensive internship at MSM. One MSM medical student is sponsored to undertake clinical research in the Harvard Medical School Sleep and Circadian laboratory headed by an NSBRI scientist. A longitudinal database is maintained to measure the outcome of the program.

The *NSBRI Film Archive* contains more than 150 hours of video relating to NASA's Neurolab mission, NSBRI team science, and the Human Body in Space course. This one-of-a-kind repository will be used to develop interactive Internet accompaniments to the proposed textbook and the problem-based cases written by the teacher fellows. It also will support the outreach and pubic affairs of the entire NSBRI enterprise. An electronic, undergraduate curriculum on the human body and weightlessness will use a multidisciplinary perspective to support national undergraduate, science education standards and space life sciences at the college level.

Mount Sinai School of Medicine (MSSM)—*Defying Gravity: Enduring Life in Space*. MSSM is developing a 9th grade, space-based science and mathematics curriculum that links human health in Earth's gravity and in space's microgravity. It explores scientific knowledge essential to formulate countermeasures; provides working models of scientific and mathematical principles; and includes hands-on laboratory sessions, with group discussion to demonstrate current paradigms and unifying principles that relate research to space biomedicine and hypothesis testing. The curriculum integrates mathematical principles to concepts in the biological and physical sciences and technology, and to data collection, organization, analysis and graph design.

Defying Gravity is being developed by educators from MSSM Teacher's Summer Institute 2001: A Space Research Odyssey. It will be field tested at the New York City Life Sciences Secondary School, among an underrepresented and academically challenged student population. Products derived will include: a hard copy of the curriculum; an interactive Internet version of the stand-alone curriculum with downloadable text, images and digital video/audio sessions; a live scientist discussion room; teacher's lounge email FAQ and questions; interactive CD ROMs of selected curricular components; a *High School Teaching for Biomedical Scientists handbook*; a hands-on exhibit at the New York Hall of Science; and National multi-media outreach and dissemination via MSSM and NSBRI/Public Broadcasting Services (PBS) television channels.

Rice University/University of Texas Medical Branch (RU/UTMB)—*Outreach Program for the Professional Development of Students and Teachers on Studies Related to Biomedicine in Outer Space*. This collaboration attracts young people to space-related enrichment programs, promotes excellence and innovation in America's science education system, and enhances the scientific background of teachers, students, their families and the community as a whole. It consists of the

Academic Development of High School Students (Student Research) and the Teacher Institute for the Advancement of Space Science Education (Teacher Institute).

Students and teachers are partnered with ongoing space biomedicine research projects conducted at Rice and UTMB. The *Teacher Institute* selects 20 secondary school teachers in a yearlong program to enhance their knowledge of space biomedicine through interactive discussions with researchers; a one-day, hands-on research experience; and special tours of NASA Johnson Space Center and Space Center Houston. Teachers use their knowledge to design a space biomedicine mini-module/unit plan to be taught in class and refined for publication on the Rice, UTMB and NSBRI educational resources web sites. The *Student Research component* enrolls 12 high school summer students to conduct research projects in Rice and UTMB science labs, participate in a field trip, and meet researchers engaged in a wide variety of space biomedicine research.

Texas A&M University (TAMU)—*Teacher Academy Project*. The NSBRI *Teacher Academy Project (NSBRI TAP)* prepares Master Teachers to assist their peers in infusing cutting-edge, space-based science activities into middle school. The specific objectives are to: (1) establish a national cadre of 90 middle level science teachers and prepare them to provide staff development that will reach 1,800 middle level science teachers; (2) identify and provide access to extant teaching resources for middle level science educators; and (3) develop supportive partnerships to access and utilize the resources and skills of key organizations, and work collaboratively with other NSBRI member institutions to improve the quality of middle level science in the classrooms of teachers who participate in NSBRI activities.

NSBRI TAP will select a cadre of master teachers to help develop a summer institute. These teachers will utilize recent NSBRI scientific discoveries to create curricular supplements, attend a leadership and staff development training module, and engage in follow-up activities and conferences to obtain certification as master teachers and Fellows of the Academy. Academy Fellows will form a national professional development staff that trains all middle level science teachers to implement space-based science. It is anticipated that extensive collaborations with other Education and Outreach teams will occur with respect to resource sharing and support with the identification of master teachers. *TAP* also will produce a national cadre of 90 master teachers who are successful in helping at least 1,800 of their peer space science teachers to implement space-based science in their classrooms. Both qualitative and quantitative data will be collected, with on-going analysis of the data shared with the Director.

The University of Washington (UW)—*Northwest Outreach Program on Space Biomedicine Research*. The UW program leverages an existing communication/education program, *Northwest Science & Technology*, at UW to: (1) transfer/disseminate space biomedical knowledge to homes and classrooms throughout the Northwest; (2) increase literacy about science in general, and about space biomedical research and terrestrial applications in particular, among the general public, teachers and students; (3) prepare scientists and future reporters and public information officers to communicate more effectively about science and space biomedicine issues to general audiences; and (4) attract young people to careers in NSBRI space biomedical research.

The program will develop and disseminate articles on space biomedical research via *Northwest Science & Technology (NWS&T)* magazine, a new regional science publication with a circulation of almost 30,000 in the Pacific Northwest region and beyond. Student writers will write, adapt and disseminate special materials on space biomedical research for middle school students and their parents and teachers via an insert in *NWS&T*. In addition, the UW program will deliver a

series of three summer science writing workshop for NSBRI consortium members to: (1) improve the ability of scientists and public information officers to communicate with general audiences; (2) conduct a high school summer research program; produce a series of five NSBRI news or feature articles over three years; and (3) develop a national writer-in-residence program for in-service journalists focusing on space biomedical research.

Education and Public Outreach Team institutions also have a significant amount of synergistic interaction with other NSBRI Research Teams, as delineated in the table below.

2001 Activities by NSBRI Research Area/Education and Public Outreach Theme

	EDUCATION AND PUBLIC OUTREACH THEMES			
NSBRI RESEARCH AREAS	**Teacher Professional Development**	**Curriculum Development**	**Science Literacy/Public Awareness**	**Career Awareness and Access**
Bone Loss	BCM, TAMU, RU/UTMB	BCM, MIT, MSSM	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Cardiovascular Alterations	TAMU	MIT, MSM	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Human Performance Factors, Sleep and Chronobiology	BCM, TAMU	BCM, MSSM	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Immunology, Infection and Hematology	RU/UTMB	MSSM	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Integrated Human Function			BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Muscle Alterations and Atrophy	BCM, TAMU, RU/UTMB	BCM, MIT	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Neurobehavioral and Psychosocial Factors	TAMU, RU/UTMB	MIT, MSSM	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Neurobehavioral Adaptation	TAMU, RU/UTMB	MIT, MSSM	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Nutrition, Physical Fitness, and Rehabilitation	BCM, TAMU	BCM, MIT	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Radiation Effects	TAMU, RU/UTMB	MSSM	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Smart Medical Systems		MIT	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW
Technology Development	TAMU	MIT	BCM, MIT, MSM, MSSM, RU/UTMB, TAMU, UW	MIT, MSM, RU/UTMB, UW

BCM: *Baylor College of Medicine;* ***MIT:*** *Massachusetts Institute of Technology;* ***MSM:*** *Morehouse School of Medicine;* ***MSSM:*** *Mount Sinai School of Medicine;* ***RU/UTMB:*** *Rice University/The University of Texas Medical Branch;* ***TAMU:*** *Texas A&M University;* ***UW:*** *University of Washington*

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

Baylor College of Medicine

Teacher Professional Development and Dissemination of Existing Curriculum Materials. To reach as many teachers as possible in the most cost-effective manner, alliances have been formed with a number of systemic, ongoing teacher professional development programs in Texas and throughout the US. This approach has proven to be more effective than isolated, "stand-alone" workshops in ensuring that NSBRI space life sciences content is integrated into science instructional programs and that teachers are supported in their applications of space life sciences activities with students in classrooms. The following professional development activities have been conducted in 2001.

- *The Challenge Within: Microgravity Effects on the Human Body*. Space life sciences summer institute for Houston-area teachers at project partner, Space Center Houston, June 18-20. 17 teachers participated. Evaluations indicated a very high level of satisfaction with the institute. On a scale of 1 to 5 (in which 5=excellent and 1=poor), the overall effectiveness of the program received a mean rating of 4.7; instructor knowledge was rated 4.9; and individual inquiry activities were rated from 3.6 to 5.0.

- *Teacher "Sleep-over" workshop at Space Center Houston*. July 19-20, 60 teachers spent the night at the museum and participated in professional development on space life science topics. Space Center Houstons also is serving as a distribution center for BCM-developed NSBRI teaching materials. To date, Space Center Houston has disseminated more than 500 each of *Muscles and Bones* and *Sleep and Daily Rhythms* directly to interested teachers.

- *Unit Workshops on existing educational units developed for NSBRI* by BCM (to date in Year 2001—702 teachers, representing more than 13,000 students; since March—484 teachers, more than 8,500 students).

  Texas Council for Elementary Science, 1/13/01, Annual Symposium, Texarkana, 75 teachers (1,800 students), *Muscles and Bones*, *Sleep and Daily Rhythms*

  Texas Council for Elementary Science, 1/14/01, Annual Retreat, Port Aransas, 90 teachers (2,160 students), *Muscles and Bones*, *Sleep and Daily Rhythms*

  Tackling the Tough-to-Teach Topics, BCM/Exxon Mobil Workshop Series, 1/18/01, 20 teachers (480 students), *Sleep and Daily Rhythms*

  Tackling the Tough-to-Teach Topics, BCM/Exxon Mobil Workshop Series, 1/15/01, 20 teachers (480 students), *Muscles and Bones*

  National Science Teachers Association, National Meeting, St. Louis, MO, 3/24/01, 27 teachers (2,573 students), *Muscles and Bones*

  North Forest ISD, 8/22/01, 13 elementary teachers (312 students), *Sleep and Daily Rhythms*

  Baylor College of Medicine, Science Education Leadership Fellows Program, 4/07/01, 8 scientists, 16 elementary teachers (384 students), *Muscles and Bones*

  Space Center Houston, International Space Station Educations Conference, 2/09/01, 23 middle school teachers (1423 students), *Muscles and Bones*

  Baylor Science Leadership Program, conducted in collaboration with the Houston Urban Systemic Initiative, 6/18-29 and 7/16-27, 160 Houston Independent School District

elementary science lead teachers (3,840 students), *Muscles and Bones*, *Sleep and Daily Rhythms*

Aerospace Education Service Program, 9/10/01, 50 teachers, *Muscles and Bones*, *Sleep and Daily Rhythms*

Metropolitan Association of Teachers of Science, Fall Conference, 200 K-12 teachers, 10/13/01, *Muscles and Bones*, *Sleep and Daily Rhythms*

Council for the Advancement of Science Teaching, 11/1/01, Austin, TX
National Association of Biology Teachers, Annual Meeting, Montreal, 11/08/01

In addition, BCM has promoted the use of existing educational units (*Sleep and Daily Rhythms* and/or *Muscles and Bones*) for adoption and use within whole districts, schools and grade levels as part of the ongoing science curriculum.

North Forest ISD (Houston, Texas) fourth grade students in Hilliard and Rogers Elementary Schools (13 teachers, 312 students), *Sleep and Daily Rhythms*

Canton City Schools, Canton, Ohio (100 elementary teachers, 2,400 students), *Muscles and Bones*, *Sleep and Daily Rhythms*

Guides and NSBRI educational posters also have been requested for use in the 27 following schools: Buckman Heights Elementary School, Rochester, NY ; Sylvan Avenue Elementary, Bayport NY; Hirsh Elementary, Spring, TX; Shenango Elementary, New Castle, PA; Bridgeport Elementary; Transfiguration School, New York, NY; Kingsboro Elementary, Gloversville, NY; Alden Primary School, Alden, NY; Union Elementary, Reading, PA; Brewer Middle School, Oakland, CA; Betsy Ross Elementary, Houston, TX; West Laurens High School, Dublin, GA; St. Michael's School, Dublin, Ohio; Georgetown High School, Georgetown, TX; Sky View High School, Sky View, CO; Bishop Garriga Middle School, Corpus Christi, TX; Jacobs Jr. High, Stevens Point, WI; Lee Middle School, Portland, OR; Sprout Jr. High, Springs, CO; Church Hill County High School; Fallon, NV; North Middle School, Colorado Springs, CO; Kiley Middle School; Springfield, MS; Deady Middle School, Houston, TX; Revere Middle School, Houston, TX; Harman Middle School, Houston, TX; The Rice School, Houston, TX; Messiah College, Granham, PA

Public Awareness and Science/Health Education. The BCM team is communicating important health concepts related to NSBRI research to the general public through radio and TV stories. During 2001, three radio broadcasts were created and disseminated by BCM Public Affairs as part of the Radio Healthline series. Radio Healthline is distributed to approximately 2,900 radio stations nationwide. The stories aired were: How will loss of heart mass affect astronauts in space; Helping astronauts keep their balance; Helping astronauts adjust to gravity.

In addition, BCM is collaborating with KUHT-TV, Houston Channel 8, Public Broadcasting affiliate (University of Houston) in the production of in-depth news-format stories on NSBRI topics. These stories are aired as live interviews or taped stories as part of the ongoing Weekday Edition program. Fifteen stories to date have been aired. These include two live segments on family science and health (sleep and children's health, bone health and calcium), two in-depth stories on applications of stem cell research and 11 additional taped short format stories on osteoporosis, cancer prevention, balance, nutrition and aging. To emphasize the connection to NSBRI research, Channel 8 also airs the NSBRI logo at the beginning of each Weekday Edition broadcast.

Curriculum Development. BCM is completing a draft teachers guide (*Food and Fitness*) containing sequenced activities on cardiovascular fitness, nutrition and the importance of diet and exercise. The draft, which will be the third in the *From Outer Space to Inner Space* series for teachers, will be field tested in January 2002 in Houston middle schools. Hands-on activities include testing for fat content in common snack foods, estimating students' cardio-vascular fitness levels, and comparing levels of anti-oxidant vitamins in common foods. Activities use nutrition and fitness concerns related to astronauts' health to introduce important topics related to diet, obesity and exercise for students and their families.

Massachusetts Institute of Technology
MIT received first NSBRI funding in October 2001, so they have no results to report at this time.

Morehouse School of Medicine
MSM-NSBRI Teacher Institute. The MSM-NSBRI Teacher Institute is a partnership with Georgia Institute of Technology SECME Program, DeKalb school system, Fernbank Science Museum, the Atlanta Educational Telecommunications Collaborative Inc., and NSBRI's Teacher Academy Program at Texas A&M University. The project aims to support the professional development of secondary teachers by providing fellowships and workshops that foster collaborations with university scientists, connections to the scientific community, participation in hands-on, inquiry-based workshops, access to the latest science information, and mentors to develop their ability to review educational materials for accuracy and usefulness. To date, this program has delivered four space science modules to 95 teachers, disseminated over 300 units of NSBRI education materials to teachers from 17 states, and is supporting two teachers in a year-long fellowship at MSM. The following activities have occurred during 2001.

- Two Teaching Fellows received release time to spend approximately three hours per week at MSM.
- A full-time, temporary consultant (Ph.D./M.D) with expertise in Problem-Based Learning (PBL) was recruited to head PBL case writing team - program director, 2 MSM scientists, multi-media Technologist, NSBRI cardiovascular team representative (to be named).
- Fellows' Orientation completed, including meeting with newly appointed Director of Fernbank Science Museum.
- First draft of cardiovascular case scenario, background content completed.
- Teacher Fellowship Manual - seminars, lectures, references, is being compiled.
- Beta testing of Bobby's Beat - 6 teachers/30-60students scheduled for November 2001.
- Reports from TAMU Teacher Academy Program completed.
- Leadership Module prepared and delivered at TAMU -TAP.
- Vestibular lesson and activity prepared and delivered at TAMU-TAP
- Collaboration established with SEEMA. Glen Research Center.
- Draft MSM-SECME Memorandum of Understanding under discussion.
- MSM team (3 educators /scientists)prepared and delivered 4 modules to 95 SECME teachers at Arizona State University.
- Distributed 300 units of NSBRI education products - tapes, film, instructional materials.
- Sponsored Astronaut Bernard Harris to address 800 SECME teachers (Arizona).

MSM-NSBRI Undergraduate Summer Research Program. This program provides research opportunities for four undergraduate students, who engage in a research-intensive internship at MSM, learn about space biomedical research from eminent space sciences guest lecturers, and meet role models involved in space biomedical research. The 2001 summer research program

schedule, taught by MSM scientists, covered laboratory safety, biohazard and radiation, animal care and use, tissue culture methods, cellular proteins, molecular biology techniques/PCR, molecular methods, and ethics in science.

In addition, each student was assigned a mentor and participated in laboratory research activities. Students also were required to read and present a scientific article for a weekly journal club, attend a science writing seminar, and prepare a college-wide poster session. The NSBRI multimedia archive staff supported students during their presentation preparation. 2001 Summer Research Program students and research topics/mentors are listed below.

Jason Patrickson, Biology Major, Oakwood College, Alabama
Title: Cell Proliferation in Tissue Culture in a Rotating Wall Vessel
Mentor: Dr. Brenda Klement

Carl Jones, Pre-med Major, Cornell University, New York
Title: *Efferent Projections of the Medial Preoptic Area Involved in Circadian Locomotor Rhythm*
Mentor: Dr. John Patrickson

Jared McShall, Psychology Major, Wesleyan University, Connecticut
Title: *Possible Pathway for Circadian Input to Nucleus Accumbens and the Amygdala*
Mentor: Dr. John Patrickson

Maxime Madhere: Biology Major: Xavier University, Louisiana
Title: *Melatonin Modulates Ionic Conductances in Dopamine Neurons*
Mentor: Dr. Joseph Whittaker

One MSM medical student completed a clinical research internship in NSBRI's Sleep Human Performance Factors, Sleep and Chronobiology laboratory at Harvard Medical School. He did his research under the direction of Dr.Woodrow Weiss. His research focused on sleep apnea and hypertension, with specific reference to how varying levels of carbon dioxide change firing rates of the sympathetic nervous system. The student shared his findings with the MSM summer students at the final presentation session. A longitudinal database will be maintained to measure outcomes of the program.

The 2001 summer program also hosted 22 National Youth Leadership Forum high school merit scholars and 15 MSM Summer Science Institute high school students. Students attended a series of lectures on space biomedical research and visited MSM space science research laboratories. Summer research students served as role models and guides for the high school students. The following activities occurred in association with the Undergraduate Summer Research Program.

- Five undergraduate students recruited for summer internship.
- Mentors appointed and students assigned.
- Orientation, program lectures, graduation, assembled and managed.
- Four students completed ten week-intensive research program.
- One student completed a clinical internship with the NSBRI Sleep team at Harvard Medical School.
- Exit interviews completed for database.
- Internet site to facilitate continued communication underway.
- National merit scholars and local high school students hosted.

MSM-NSBRI Multimedia Archive. This Archive contains over 150 hours of video relating to NASA's Neurolab mission, NSBRI team science and the Human Body in Space course. Film and design footage is made available to NSBRI consortium member schools and other organizations. The Discovery Channel, ZDF-German TV, RDF Television-London, Atlanta and DeKalb public schools, and the SciTrek and Fernbank museums have used materials from the archive. This one-of-a-kind repository will be used to develop interactive Internet accompaniments to proposed textbook and the problem-based cases written by the teacher fellows. The following activities were conducted in 2001.

- Maintained and expanded the existing MSM multimedia archives.
- Responded to requests for multimedia material.
- Expanded archives to ensure inclusion of up-to-date space biomedical research findings.
- Provided technology support for summer program presentations.
- Established Internet "chat room" to maintain contact with summer school participants.
- Researched technology requirements for cardiovascular case.
- Formatted lectures from an undergraduate level pilot course, The Human Body and Weightlessness.

Mount Sinai School of Medicine

A summer institute resulted in a program product that exceeded our expectations by submission to us on August 3rd, of eight well-conceived and masterfully constructed Beta-level lesson plans. Further, science journalism graduate students were recruited to publish a weekly newsletter that kept the *Defying Gravity* program and institute participants, schools, teachers associations, and District Superintendents Office, among many others, informed of MSSM activities and educational outreach intent. The newsletter is available on a dedicated MSSM website to share program progress with a wider audience at no additional cost. Subsequently, in collaboration with teachers, MSSM has been refining products for delivery during the beta-testing phase, commencing November 7, 2001, at Manhattan Center for Science & Mathematics.

PHASE I - Summer Institute Activities. A Summer 2001 institute involved a five-week program in which a number of curriculum development and fine-tuning activities were conducted and educational outreach components were tested by high school teachers and students.

- On June 15th, an inaugural reception was attended by in-house program participants and all teachers and students who were recruited (and their parents). Presentations were made by members of NSBRI research teams such as Dr. Bernard Cohen and Mitchell Schaffler, as well as MSSM Dean, Dr. Nathan Kase; NSBRI Board of Directors, Dr. Miki Rifkin; and *Defying Gravity's* Dr. Patrick Gannon. A delegate from the *Manhattan High School Superintendents Office* was present to represent the local *New York City Board of Education.*

- The program inaugurated on July 2nd, when MSSM/NSBRI scientists presented their space biomedicine research projects and were introduced to the teachers and students who would work with them over the summer.

- During week one, field trips to: (1) the American Museum of Natural History - Rose Center for Earth & Space (private tour provided by Astrophysicist Dr. James Sweitzer); and (2) the New York Hall of Science, where Martin Weiss, Ph.D. (NYHS Director of Biology) spoke about formulation and testing of science museum exhibits. Post-visit discussions involved relevance of AMNH-RC & NYHS hands-on exhibits for use by *Defying Gravity* program.

- Also during week one, the following curriculum module formulation and production classes were led by MSSM staff and others: (1) Intro to Web and Internet searching techniques and databases and Web Page Evaluation (2) Intro to HTML; (3) PowerPoint; (4) Intro to MS EXCEL; (5) Intro to HTML and Netscape Composer; and (6) Adobe Photoshop.

- In weeks two to four, teachers and students worked with scientists every day as teams to produce curriculum products that would be compelling and workable. Students were usually involved with production of PowerPoint presentations and teachers with construction of lesson plans with space biomedicine information provided by the scientist.

  Other tasks included discussion and breakout sessions on: (1) Presentation of Defying Gravity at high school Science Teacher's Association meetings; (2) NYC & NYS Science Education Standards; (3) *Defying Gravity's* link to National Science Education Standards; (4) educational CD ROM evaluation; (5) review structure of teacher/scientist partnerships; (6) development of a scientist teaching handbook; (7) Internet science education products and assessment; (8) beta testing and school test-site mechanisms; (9) production of *Defying Gravity* museum/NY Hall of Science exhibit; (10) mechanisms and incentives for recruiting teachers for ß-testing at NYC and State test sites; and (11) evaluation of the *DG* curriculum.

  Although the original plan for the summer institute did not involve creation and production of formal lesson plans, most of the teachers strongly advised that we pursue this strategy up-front. This was the format they were most familiar with and considered to be the most useful for a more meaningful future utilization. Although the teachers did not utilize a standardized lesson plan configuration, the information provided should allow for transformation between platforms to attain a standardized structure for the final product.

- The eight completed Beta Lesson Plans (LP) include the following.

  LP 1) *Bones in Space: If You Don't Use Them, You Lose Them*
  (Schaffler & Brandriss)
  LP 2) *Neurovestibular Adaptation in Space: Use of Countermeasures*
  (Moore, Cohen, Wearne & Bassin)
  LP 3) *Concepts of Magnitude: Powers of 10*
  (Good, Schweitzer, Shemmer & Singh)
  LP 4) *Radiation in Space: Solutions for Long Voyages*
  (Rosenstein & Bassin)
  LP 5) *Psychosocial Stress: Mental Well-Being in Long Distance Space Travel*
  (Brook & Bassin)
  LP 6) *Sleep in Space: Rest and Performance*
  (Genden & Singh)
  LP 7) *Breathing in Space: Head Down Tilt Models of Microgravity*
  (Gannon, Kheck &Chan)
  LP 8) *Smell & Taste: Importance of Quality of Life in Space*
  (Gannon, Kheck & Chan)

- MSSM published five, four-page newsletters with color illustrations over the five-week period of summer institute. Newsletters were printed, mailed out and published on the *Defying Gravity* Intranet site. More monthly issues are planned as the program proceeds.

Perspectives, based on interviews and other journalistic techniques were provided on cross spectrum and user-friendly subjects such as the following.

Vol. I. "Mount Sinai Launches Program"; "Defying Gravity Aims to Improve Science Education Standards"; and "Science and the City - DG Hits the Rose Center"
Vol. II. "Neurovestibular Research at Mount Sinai"; DG Explores New York Hall of Science"; "Q & A Sessions with Drs. Steven Moore and Susan Wearne" and "Curriculum Development"
Vol. III. "A Word from Our Director"; Studying Radiation and Genetic Mutations"; "Interview With Dr. Martin Weiss, NY Hall of Science"; and "Applied Technology and New Research at NSBRI"; DG is making Great Strides in Utilizing WebEd to its Full Potential"
Vol. IV. "Talking With Dr. Bernard Cohen"; "Tobie Brandriss and Students Create Curriculum Module in Dr Mitchell Schaffler's Bone Research Laboratory"; "Students Adding Their Own Perspectives to Curriculum"; "Curriculum Development Speeds Ahead"; and "A Note From Our Co-Director"
Vol. V. "Ear, Nose and Throat: Central to the NSBRI Mission" and "A Long Collaboration of Research"; Dr. David Brook, Dennis Bassin and Students Study Psychosocial Stress in Space"; "Dr. Morton Slater of the Gateway Institute"; "Dr. Patricia McArdle, Associate Dean for Curriculum Support at MSSM" and "Retrospective Opinions by Students Nelson Shih and Sarah Walters"

- The Web-Ed Intranet mechanism was used to coordinate the activities of summer institute participants and provide an easily accessed route for transmission of information and the tools to create a comprehensive information system. Similar to a standard www page, the site could be accessed from MSSM and at home. Many teachers and students utilized the website over the summer.

  The website was an effective communications tool. For example, when participants were asked to report their daily activities, their responses were recorded for printout by the program director and others on the Intranet web page. Similarly, all stages of lesson plan formulations were recorded on this page for editorial access and feedback from scientists, teachers and students. When asked to identify and critique www sites that may serve as models (similar to the originally proposed NASA NeurOn [Neurolab Online] site) for the *Defying Gravity* product in Year Three, sample URLs were posted to the page for review and commentary. Adobe Acrobat versions of the weekly newsletter also were posted to the site and may be accessed, with password, by any person within the NSBRI arena and can be linked to the NSBRI web site.

PHASE II - Beta Testing of Eight Curriculum Modules. The Beta-test phase (Phase II) will commence with recruitment of 30, 9th grade students and six teachers who will be involved with the MMSM program, for twice monthly after school sessions from November 2001 to June 2002.

Curriculum Student Associates
On October 16th, Drs. Gannon and Kheck presented a program recruitment overview to the entire 9th grade freshmen of Manhattan Center for Science and Mathematics. Five students will be selected from Life Sciences Secondary School. A two-page recruitment flyer was distributed to students one week prior to presentation. Formal applications, with parental consents, are being collected and assessed by DG associates, and 25 students will be selected by criteria formulated

for selection of DG summer institute participants (which includes a written statement of interest). Students selected will be notified by the end of October. The first module class "*Concepts of Magnitude: Powers of 10*" will be conducted on November 14th and 28th.

Each module will comprise the following three elements: (1) teacher and scientist meet to discuss strategy and format of pre-lesson plan; (2) presentation of lesson plan by teacher; (3a) PowerPoint-based overview presentation (10-15 minutes) of space biomedicine research project by scientist, within same session; and (3b) hands-on, inquiry-based lab session with formulation of ideas, conduct of study, data collection, analysis and interpretation, followed by open discussion. During discussion sessions, important concepts (e.g., countermeasures development and quality of life versus life-threatening issues) will be promoted. In particular, the alluring concept of how quality of life amalgamations may give rise to life-threatening situations and outcomes will be explored.

Curriculum Teaching Associates
Teachers will attend all sessions and act as curriculum associates to refine and assess each of the eight modules in the curriculum as they are delivered to students in the school classroom. This committee will report to Dr. McArdle, who will evaluate overall success of program products within the high school setting. Dr. McArdle will formulate an evaluation strategy to determine follow up for what did and did not work, what needs to be done, and the application context for curriculum application. Master teacher Dennis Bassin (Assistant Principal for Sciences, Art and Design High School) will preside over this team of six teachers including: (1) Ms. SaoLing Chan, Manhattan Center for Science & Mathematics (MCSM); (2) Dr. Roopali Singh, Life Sciences Secondary School; (3) Tobie Brandriss, Hunter College High School; (4) Ben Shemer, Math teacher, Upper East Side Academy; and (5) a second MCSM teacher to be recruited at a school staff meeting.

Rice University/University of Texas Medical Branch
Teacher Institute. Twenty teachers were selected for this two-week institute, and a total of 16 participated. Teachers were grouped into four groups. They developed the following units, to be used in their high school science classrooms.

- One unit highlighted the use of a clinostat, an apparatus that simulates a microgravity environment. For this unit, the team designed and constructed a version of the high-tech clinostat instrument with materials readily available to high school teachers at a very low cost. Accompanying these design plans are experiments for high school biology classrooms in which students investigate the effects of microgravity on the phototactic abilities of one-celled organisms, such as *Euglena* and *Eudorina.*
- A second unit focused on investigating the effects of microgravity on major body systems.
- A third unit used a game format to teach the physiological effects of radiation.
- A fourth unit highlights how measurements in simulated space environments are used during the evaluation of countermeasures.

The instructional units developed by participating teachers will be posted on the NSBRI Rice University/UTMB web site (http://nsbri_utmb.rice.edu) for national dissemination and use on the projects' websites. To supplement widespread dissemination to the K-12 science education community, teachers will serve as local, district, regional, state and national trainers and presenters in conferences and departmental inservice sessions in their respective schools.

Student Research Accomplishments. Twelve student participants in the seminar series gained exposure to number of different scientific research projects through the following experiences.

- Participating in a weekly research seminar conducted by faculty who are not preceptors.
- Reporting on their projects, either via slide presentation or a poster presentations.
- Participating in a weekly brown bag lunch discussion of career opportunities.
- Engaging in a mentoring relationship as part of the brown bag lunch discussions.
- Participating in discussions about the philosophy and ethics of science related to space biology and medicine.

In their work with the research teams, participants became familiar with the background of their teammates and faculty seminar presenters. Concomitantly, students increased their awareness of the academic preparation necessary for scientific careers as expressed through their articulation of research methodologies in individual laboratory settings, and their interaction with colleagues and fellow team members regarding the research going on in their respective laboratories.

In the university environment, the students become acquainted with the university and college settings through participation in the opening reception, personnel department, program and library orientation, and also in the campus tours and concluding reception after the poster session for the students, staff, preceptors, parents and other community members.

Texas A&M University

Providing NSBRI outreach to 500,000 middle level science teachers with the latest in research and materials is a major challenge. The NSBRI Teacher Academy is building a model to accomplish this objective through establishment of a national cadre of experienced and trained space science teachers. In 2001, 30 teachers are providing staff development to 600 peers.

Teacher Academy Project. Care was taken to ensure that Teacher Academy participants were drawn from diverse geographic, ethnic and gender groups. Participating school districts were asked to nominate teachers who represent a variety of gender and ethnic groups. Academy staff personally invited nominated teachers to apply. All project partners, especially key school districts, were asked to nominate Master Teachers to participate in the Academy. Out of the 31 teacher participants, six are African American, one is Hispanic, and 24 are white. Seven are male and 24 are female. 2001 participants were drawn from 20 states, from Alaska to Texas and California to New York.

The first eight days of the Summer Institute were conducted at Texas A&M University, College Station. This gave participants access to the research of NSBRI scientists working on campus. Participants also were exposed to leadership and staff development training and space resource identification, and were able to share their own space-based teaching ideas with each other.

The Institute then moved to Houston for five days, based at Ramada Inn NASA. This provided easy access to NASA, Space Center Houston, The Houston Museum of Natural Science (with its planetarium and Challenger Center), and the telescopes at the Brazos Bend State Park for viewing Mars. The amenities at these locations were put to good use in furthering the teachers' understanding of long duration space flight and adding to their fund of teaching activities and ideas. The Houston location also facilitated access to a range of informative speakers. The teachers met with and listened to presentations by astronauts Dr. Ellen Baker and Dr. Joe Kerwin; Dr. John Charles, Code U Mission Scientist, on *Life Sciences and Supporting*

Engineering Overview; Dr. Mike Greenisen, NASA Scientist, on the Countermeasures labs; Jerry Woodville, NASA Scientist, on *The Space Educators' Handbook* and Apollo 13; and Dr. Chris Flynn, NASA Flight Surgeon, on *Key Psychological Challenges of Long Duration Space Flight.*

Teacher feedback during the Summer Institute was overwhelmingly positive. Despite already being well versed in space science activities, they had assimilated new knowledge focused on a curriculum supplement for secondary schools, *Human Physiology in Space* (White & Lujan, 1995); collected additional space science resources; made useful contacts (e.g., NSBRI scientists and institute speakers); and initiated networking with each other.

This network has shown immediate value, as teachers share ideas with each other at the start of a new school year on the listserv set up during the institute. The listserv has allowed teachers to create a joint presentation to be given during the Civil Air Patrol Conference held in conjunction with a proposed Shuttle launch on November 29, 2001. This conference will be the venue for the first follow-up meeting for the 2001 Teacher Academy members. At this time, Teacher Academy staff will review progress toward achieving project objectives with the teachers.

The teachers report that they are using Summer Institute activities in their classrooms. In fact, the participant from Alaska, who works at a Challenger Center, reported that he had already used the *Puffy-Head, Bird-Legs* activity that simulates fluid shifts with students at his summer camp. This demonstrates that Teacher Academy participants are not only classroom teachers, but also resources to disseminate NSBRI information to different audiences. Two other participants who work with the Houston Urban Systemic Initiative will hold workshops for science teachers throughout that school district, thereby broadening NSBRI and Teacher Academy impact.

While still early in the school calendar, Teacher Academy teachers already have made plans within their school districts to present hands-on, inquiry-based workshops (Level 2 Training) that will disseminate NSBRI research and findings to other teachers. For example, one of the two participants from California arranged two workshops on *Mars or Bust.* One was at UC Irvine's *Future of Science* Conference in October; the other will be at the Orange County Science Educator's Association Conference in November. The two teachers from Alabama will make a joint presentation on NSBRI at the Alabama State Science Teachers Conference next March, while the teacher from Pennsylvania has arranged a series of four professional development workshops within her district based on NSBRI Teacher Academy Project activities.

This is an encouraging beginning to ensure that NSBRI research and findings are disseminated to a wider audience of both teachers and their students. To further this goal, a project web page is being developed (http://coe.tamu.edu/lburlbaw/nsbri/TAPHomePage.htm). At the moment, this site includes links to people, resources and evaluations. It will be expanded greatly during the coming year.

University of Washington

Development and Publication of Materials in *Northwest Science & Technology* Magazine.

NSBRI funds have supported a graduate research assistant, Holli Riebeek, to research and write an article announcing the UW's joining the NSBRI consortium. The article was published in the Autumn 2001 issue of *Northwest Science & Technology*, which reaches some 28,000 readers throughout the greater Pacific Northwest.

Also appearing in that issue was an advertisement submitted by Baylor College of Medicine (BCM) about its NSBRI teaching series *From Outerspace to Innerspace*. Positioning the ad on the inside front cover gave it maximum visibility to the 4,500 middle school teachers and administrators added to the UW distribution list as a result of the outreach effort. This display is helping BCM reach teachers throughout the Northwest about its teaching materials.

Furthermore, Ms. Riebeek—a master's student in the UW Department of Technical Communication whose career goal is to become a science news reporter—has nearly completed a feature article on virtual reality technologies and their application to space biomedical research. This article tentatively is scheduled as the cover story of the Spring 2002 issue of *NWS&T*.

With UW's Principal Investigator for NSBRI (Illman), Ms. Riebeek also is developing an article about Astronaut Susan Helms. The article is slated for the cover story in the Winter 2002 issue of *NWS&T*. Astronaut Helms, who hails from Portland, Oregon, returned in August 2001 from a 167-day space station mission. UW requested permission through the NASA press office to follow astronaut Helms through the six-week rehab process that astronauts undergo upon returning from space. Although that request was denied, UW did succeed in securing an interview in September, and work is underway to develop a profile article about Helms and the rehab process. Covering this subject in a profile of Helms (who is both an astronaut and Northwesterner), will help to promote the subject of space biomedical research for readers in the Northwest, and to demonstrate in a vivid way, by getting to know a real astronaut, why development of countermeasures to the effects of space travel is vitally important. This material also is slated for adaptation to the middle school insert.

Middle School Insert. UW will produce two middle school inserts under this three-year NSBRI outreach program. Because of the timing of the BCM ad, the first NSBRI-funded middle school insert to appear in the Spring 2002 issue. The overall objective is to attract young readers to science news, cultivating a life-long interest in reading about advances in science and technology, and specifically, to increase awareness of, and interest in space and space biomedical science and technology.

During the summer of 2001, UW developed the insert's conceptual design with the assistance of education specialists and science writers with expertise in writing for young audiences. The layout of the first insert is being developed by UW's magazine design firm, Gable Design Group of Seattle. The approach is modeled after some of the newsstand magazines for this age group, such as *Current Science* and *Science World*. UW team members have developed a format, name, length, types of articles and evaluation approach. This concept provides the general framework for any theme that the insert may treat in future issues.

The concept will be piloted in the Winter 2002 issue, focusing on marine biotechnology (funded by the Washington Sea Grant program). Feedback obtained from that pilot test will be incorporated into the design of the NSBRI-sponsored insert on the theme of space biomedical research in the Spring 2003.

The insert is called *NWS&T SciScape*. It is a four-page, full-color, tear-out piece appearing at the inside back of the magazine. It is positioned as "a companion to *Northwest Science & Technology* for the young and young-at-heart." The flap of paper used to secure the insert into the binding, which appears inside the front cover, will be utilized both to draw attention to the

insert as well as to provide a tear-out feedback card for readers to send in their comments about how the insert was used and their reaction to its contents.

SciScape will contain feature articles as well as what might be termed departments, including: "Fun Facts"—interesting tidbits presented in a lively way around the selected theme of the insert; "Meet the Scientist"—profiles of key scientists (or astronauts) to give young people insights into careers in science and technology; "Try It At Home"—activities relating to the theme of the insert that can be done with common household supplies; and "Puzzlers"—enjoyable games or other brain teasers that challenge young readers to apply what they have learned and refine their problem-solving skills.

SciScape must appeal in its design and content to adults in addition to youngsters. Adults are the first marketing target: they are the gatekeepers, the ones who receive the magazine and pass the insert on to relatives, friends, colleagues and/or students. They may read the insert to evaluate its content and, in this process, many adults may learn a few science basics along the way.

During fall quarter, Ms. Riebeek is drafting the content for the NSBRI space biomedical insert with input and assistance from Illman, Kushmerick, Nancy Moreno (BCM) and Janice DeCosmo (Washington Space Grant). The draft content will be evaluated with a group of middle school age students and feedback incorporated into the final insert and feedback card to be disseminated spring of 2002.

Improving Communication with General Audiences. Development of a Science Communication Workshop for NSBRI scientists and public information officers is underway. The events of September 2001 have complicated and delayed planning of the venue and date of the first workshop. These details will be worked out in the coming weeks in conjunction with the NSBRI Outreach Team members.

As a direct result of a visit by Astronaut Dave Williams, M.D., to UW in spring 2001, a planning effort has begun to develop a National Space Science Writer-In-Residence Program that would provide special writing opportunities for in-service journalists on topics in space biomedical science and engineering. The goal of this effort is to enhance media coverage of space biomedical science and engineering specifically and issues relating to space exploration more broadly. The program would be coordinated by UW and Texas A&M, with participation by Johns Hopkins University and MIT. A planning workshop is anticipated for early 2002.

In addition, with funding from the UW College of Engineering for UW School of Communications Ph.D. student Fiona Clark, UW initiated a content analysis of recent *New York Times* coverage of space-related events and issues. The objective is to characterize current journalistic practices with regard to space exploration and space biomedical research, and to provide a benchmark against which future developments in coverage can be assessed. The outcome of this study is expected to be an article for submission to one of the principal scholarly journals in the field of communication (e.g., *Journalism Quarterly*).

For most Americans, the news media are the predominant source of information about space. It was decided to address the question of whether or not coverage of space is keeping up with the many new developments in the field of space exploration. A content analysis is being conducted of *New York Times* coverage of space issues for the year 2000.

The *New York Times* was chosen because of its large readership, its widely acknowledged ability to set news agendas for local and regional papers, and also because its readership tends to include the political elite. UW has obtained copies of all articles listed under the headings "space" and "satellites" in the *New York Times* index for the year 2000. UW also is conducting searches on space-related keywords in the Lexis-Nexis database, to find additional materials that appeared in the *New York Times* but which are not listed in the printed index. Analysis focuses on the amount and type of coverage of the following themes.

- space exploration: human and non-human
- space science (origins, structure, and evolution of the universe)
- new space technologies
- commercial applications of space technologies
- national security issues
- financial considerations
- legal issues (treaties, laws, property rights in space, etc.)
- impact of space issues on international relations

UW has reviewed existing research literature on media coverage of space. Most studies focus on either Cold War rhetoric of the early days of the space race, or on press-NASA relations. A small number of studies address the importance of space coverage to the development of science journalism as a profession, and in advancing scientific literacy among the general public. Others address topics such as news diffusion in the wake of the Challenger accident. None, however, address the question of characterizing current coverage as described above. Therefore, this study targets an important research question.

Summer Experience for High School Students. Because UW's outreach project began in April 2001, and since the NSBRI research projects at UW were just gearing up and were experiencing some personnel changes, the UW outreach team decided to bring in the first group of high school students during summer quarter 2002. Working with Washington Space Grant director Janice DeCosmo during this project period, UW initiated a recruiting plan, described below, to identify four to six students during the 2001-02 academic year who will come to campus during summer 2002 to work in laboratories of UW-NSBRI scientists.

UW plans to leverage the small number of student research assistantships available through the NSBRI outreach grant to reach a larger group of student applicants by collaborating with the Washington NASA Space Grant Consortium's student scholarship and research programs. The UW outreach effort can benefit from the highly visible recruitment of high school seniors conducted annually by Washington Space Grant. In this way, UW will identify four to six promising young students to participate in space biomedical research the summer before their freshman year in college. In addition, the work of these students will serve to attract additional interest in the field through the Space Grant seminars and poster sessions.

The Washington NASA Space Grant Consortium annually offers scholarships for incoming UW freshmen. Each year some of Washington State's best high school seniors vie for a chance to study science or engineering at UW. These competitive scholarships are based on high school academic records, aptitude for science or engineering studies, and students' essays about their career plans to contribute to space-related science and engineering fields.

The UW Space Grant scholarship program is designed to create a small college atmosphere within the larger university, building a community of students with similar interests in space and related topics. Along with financial support and other programmatic elements, Space Grant scholars benefit from invaluable hands-on experience doing research in one of the many science or engineering laboratories on campus in the Summer Undergraduate Research Program (SURP).

As part of the selection process for Space Grant scholarships, approximately 40 finalists are invited to tour UW and interview with the selection committee. The tours highlight opportunities for students to become involved in faculty research the summer before they begin their UW studies, as part of SURP.

During winter quarter 2002, UW-NSBRI scientists will offer tours of their laboratory programs as part of these UW tours. After the tours, students will be invited to apply for SURP to work with UW-NSBRI faculty. Four to six opportunities will be held specifically for these students.

Students will benefit from the mentoring provided by NSBRI scientists, and also will participate in the SURP seminars and poster session. SURP typically serves 60-75 students each summer, and all participants spend some time learning about each other's projects. This will be a useful forum for a larger group of high-achieving students interested in scientific research to learn about opportunities in space biomedicine.

V. FUTURE PROGRAM DIRECTIONS

Team members have collaboratively established the five-to-ten year strategic goals for NSBRI Education and Outreach. Through retreats and conference calls, team members identified the challenges of building a national identity for NSBRI through educational outreach. It was agreed that the quality of materials and activities and the extent to which they are disseminated would be initial defining factors for all NSBRI sponsored educational activities. Once planned and implemented, presentations and publications will document the programmatic impacts.

The guiding principals for *Curriculum Development* include the production of accurate, balanced, relevant, scientifically-vetted and field-tested educational materials. Research will be undertaken to determine if NSBRI materials are easily integrated into existing curricula and if they are effective in advancing teaching and learning and increasing interest in the space life sciences amongst students.

Teacher Professional Development will be guided by demonstrated increases in teacher knowledge and skills of NSBRI research areas and changes in teaching behaviors in classroom settings. NSBRI sponsored professional development of teachers will include opportunities for continuous skill and knowledge acquisition needed to create effective learning opportunities for students. Outcome studies will be conducted to determine if teachers have translated new knowledge into practice.

Science Literacy and Public Awareness recognizes the importance of communicating the scientific principles and advances NSBRI research activities to the public. The effectiveness of planned media and informal science activities that occur at museums and through television and radio productions will be evaluated.

Career Awareness and Access activities will include undergraduate and graduate level courses and research experiences to share the contributions of NSBRI activities with high-ability students. These students will be exposed to career opportunities available to them. Additional activities will target K-12 students demonstrating the excitement of space life science research and that careers are attainable. Ultimately, the numbers of students attracted into space life science, engineering and technology-based careers will be used to evaluate the effectiveness of career awareness and access activities.

Five-year Strategic Goals of the Education and Public Outreach Team

- Establish a strong publication record with respect to space research curricular supplements and programs.
- Increase scientific literacy by involving scientists in community education and bringing NSBRI and space-based science into classrooms and homes.
- Foster healthy behaviors and attitudes among students and families, and increase opportunities for families to become more involved in their children's learning through family/school/community partnerships.
- Attract more young students (especially those from underrepresented groups) to careers in space life sciences, engineering and technology-based fields.
- Promote excellence, achievement and systemic change in education through the development and implementation of high-quality space-based science, mathematics, reading/language arts instructional materials designed to facilitate measurable success for all students, apply best understandings of how students learn, and incorporate assessment as an integral component.
- Enhance the space-based science and technological readiness, skill and teaching impact of educators by providing professional development focusing on partnerships with scientists and pedagogical strategies utilizing NSBRI-generated resources that empower educators to (1) teach all students more effectively, and (2) communicate these new instructional resources to peers in education.
- Create and support stimulating, informal space life sciences education programs outside of school to develop and maintain public interest in, and awareness of, NSBRI scientific and technological developments.
- Establish partnerships with external groups that bring additional funding support to NSBRI activities and assist the Education and Public Outreach Team to disseminate and promote space-life science education programs.
- Develop and implement a media plan to include, but not be limited to: public affairs announcements and programs for radio and television, brochures, posters, video-documents and websites, and a national writer-in-residence program.
- Work with state educational agencies to integrate NSBRI space life sciences content into required curricula.

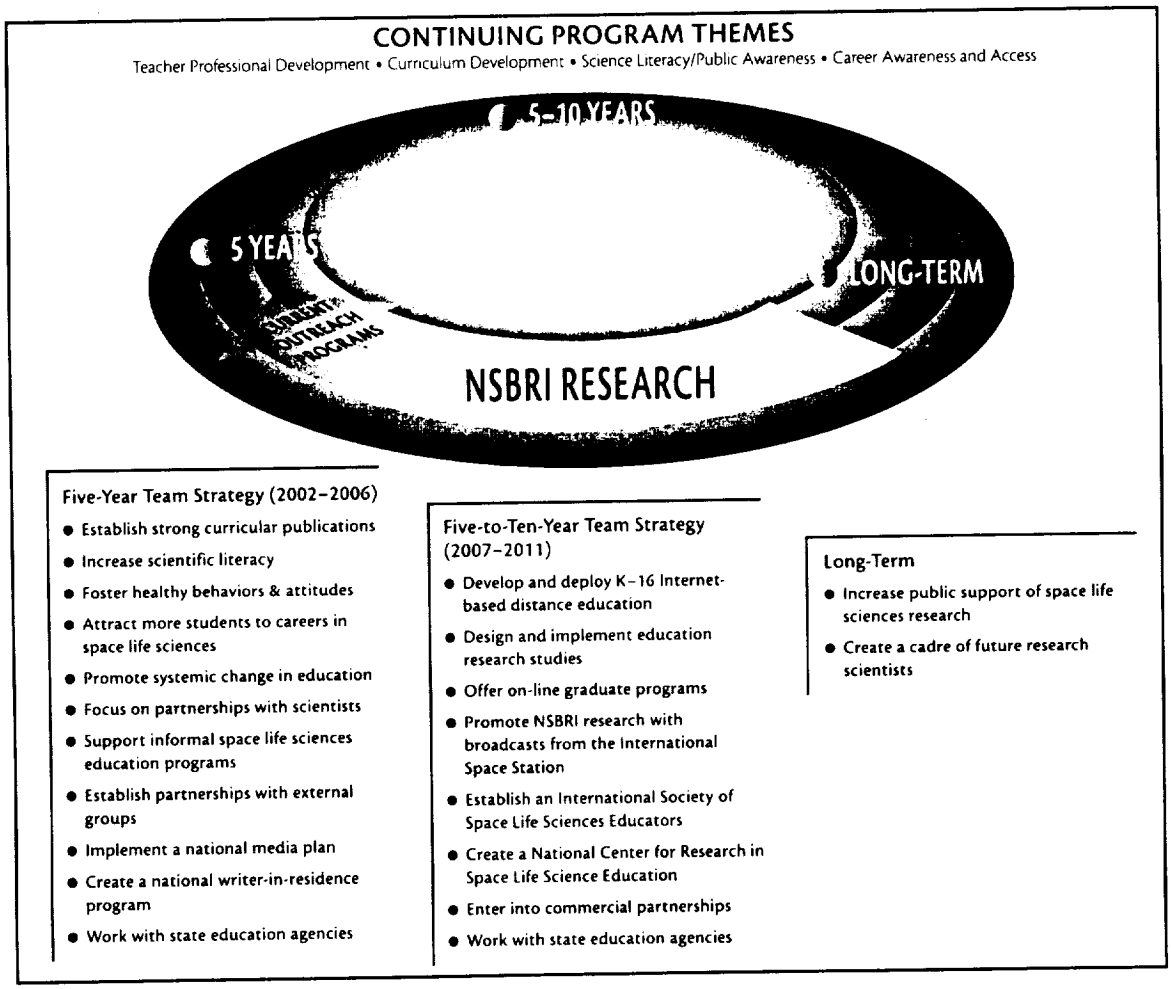
Five-to-Ten-Year NSBRI Education and Outreach Strategy (2007-2011)

The five-to-ten-year (2007-2011) strategic goals of NSBRI's Education and Public Outreach Team include goals listed under the Five-Year Research Strategy as well as those listed below.

- Establish NSBRI as a national leader in the development and deployment of K-16 Internet-based distance education.
- Design and implement research studies that examine the effectiveness of NSBRI-sponsored Internet-based curriculum materials, as compared to traditionally formatted materials.
- Offer online graduate programs in space life science education for elementary, middle and secondary school science, mathematics, physical education and language arts teachers.

- Promote NSBRI research through educational demonstrations conducted on the International Space Station and broadcast worldwide.
- Establish an International Society of Space Life Sciences Educators, including a fellowship program for its members.
- Create a Center for Research in Space Life Science Education in Houston to infuse NSBRI research into educational practice in schools and study ways to increase student motivation in science education and career pursuits.
- Enter into commercial partnerships with publishers, software manufacturers and broadcast media corporations to disseminate information and materials nationally that describe and promote application of NSBRI educational activities.
- Work with state educational agencies to integrate NSBRI space life sciences content into required curricula.

Please refer to the figure below, Continuing Program Themes, for a display of the five-year (2002-2006) and five-to-ten-year strategic goals of the Education and Public Outreach Team.



Appendix K

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

EDUCATION AND PUBLIC OUTREACH PROGRAM YEAR 4 - FY 2001

September 25, 2001

NSBRI EDUCATION AND PUBLIC OUTREACH PROGRAM FUNDED PROJECTS

Team Leader: MacLeish, M. T. — Morehouse School of Medicine

Gannon, P. J.	**PI**	**Mount Sinai**	**Defying Gravity: Enduring Life in Space**	**2**
Illman, D. L.	**PI**	**Washington**	**Northwest Outreach Program on Space Biomedical Research (in Association with *Northwest Science and Technology* Magazine)**	**4**
Kushmerick, M. J.	CO-I	Washington		
James, R. K.	**PI**	**Texas A&M**	**National Space Biomedical Research Institute Teacher Academy Project**	**6**
MacLeish, M. T.	**PI**	**Morehouse**	**Secondary and College Education for the Next Generation of Space Life Scientists**	**8**
Newman, D. J.	**PI**	**MIT**	**Space Biomedical Sciences and Engineering Curriculum and Outreach Program**	**10**
Merfeld, D.	CO-I	Harvard		
Smith, R. B.	**PI**	**Rice**	**Outreach Program for the Professional Development of Students And Teachers on Studies Related to Biomedicine in Outer Space**	**11**
Houston, C. W.	CO-I	UTMB		
Thomson, W. A.	**PI**	**Baylor**	**From Outer Space to Inner Space: Sharing NSBRI Progress with the Community**	**13**
Moreno, N. P.	CO-I	Baylor		

NSBRI AREA:	**Education and Public Outreach**
PRINCIPAL INVESTIGATOR:	**Patrick J. Gannon, Ph.D.**
ORGANIZATION:	**Mount Sinai School of Medicine**
PROJECT:	**Defying Gravity: Enduring Life in Space**

Project Executive Summary

The 1995 National Science Education Standards (NSES) reported that scientific literacy has become increasingly important in the US workplace where people are more often required to learn, reason, think creatively, make informed decisions and solve problems. However, results of the 3rd International Mathematics and Science Survey reported a marked decline in science and mathematical knowledge from 8th-12th grades in the US compared to an international average of 41 countries, many of which have invested in creation of a scientifically literate workforce. As such, we have chosen to focus our science educational outreach efforts on the 9th grade, which we consider to represent a potentially pivotal, formative educational stage at which to confront this underperformance and to help them prepare for the new NY Regents Living Environment exam.

NSES also recommended that inquiry-based curricula should be the new educational model applied for generation of enthusiasm, learning, understanding and a respect of science. We propose to extend the NSES strategy by generation of a genuine enthusiasm for learning science and math, via presentation in the classroom by MSSM scientists and mathematicians of "working models" of space biomedical research. Subsequently, development of a stand-alone curriculum will allow science teachers to utilize the theme in various courses of study. In order to address issues related to goals stated within NSBRI's educational outreach mission, we plan to found fundamental concepts of biomedicine, basic science and mathematics within the rich and enticing framework of space exploration. We propose a novel research-based educational outreach program entitled "DEFYING GRAVITY: Enduring Life in Space" to symbolize how a basic body plan, that evolved over many millions of years for life on earth within "Normal Gravity," may adapt to endure and solve any problems encountered in "Microgravity" and function efficiently. Goals for exploration of this compelling concept are to provide: 1) a "unifying theme" that will afford meaningful links between the wide breadth of issues related to human health in Earth's "normal" gravity and in space's microgravity; 2) insight into the scientific knowledge essential for formulation of countermeasures, and how they will be implemented to ensure the well being of humans who spend long periods of time in space, and 3) working models of scientific and mathematical principles.

The basic *Defying Gravity* curriculum will be fine-tuned, primarily assessed, and then adjusted as considered necessary within partnership/teams of MSSM scientists/educators, high school science teachers and students of the "MSSM Teacher's Summer Institute 2001: A Space Research Odyssey" (TSI). This will represent the penultimate formulation phase of the program's curriculum.

A fully formed first-run curriculum, presented as semi-formal "research lab meeting" style seminars/workshops followed by hands-on laboratory sessions with group discussion, will demonstrate: 1) current paradigms and unifying principles that relate research to space biomedicine; 2) approaches used to devise and test hypotheses (i.e., the Scientific Method); 3)

integration of mathematical principles as they relate to concepts and common themes with the biological and physical sciences and technology; and 4) how to formulate components of, collect, organize, analyze, graph, and interpret, data. The new Life Sciences Secondary School, with an "underrepresented" and academically challenged, minority (>95%) student population, will represent a first level test site over the second and third years. Selected curriculum components will also be applied during the third year by MSSM-TSI participant 'lab mentor' teachers, as a stand-alone product to a wide and more diverse group of 9th-11th grade students and to other teachers and students within the well-established Gateway Program (Slater and Iler, 1991; Iler and Slater, 1998). These schools will represent second level, broader spectrum, test sites. Additional curriculum components will be incorporated within these test sites as the program progresses. Both formative and summative measures will be used for example: to determine mid point progress, clarify program strengths and weaknesses and sum up overall program impact.

Products derived from the *Defying Gravity* program will include: 1) A hard copy of the stand-alone curriculum with lesson plans and hands-on laboratory experiences; 2) An interactive www-site version of the stand-alone curriculum with downloadable text, images and digital video/audio sessions; live (scheduled) scientist discussion room; teacher's lounge email FAQ and questions (modeled on the well designed and tested "NEURON NEUROLAB ONLINE" www page; 3) Interactive CD Rom's of selected curriculum components, produced by NSBRI's Morehouse School of Medicine with Public Broadcasting Atlanta's studio (Dr. Marlene MacLeish with Milton Clipper and Wayne C. Sharpe, in years 2 and 3); 4) "High School Teaching for Biomedical Scientists" handbook; 5) An interactive, hands-on exhibit at the New York Hall of Science, of selected curriculum components; and 6) National multi-media outreach/dissemination via MSSM and NSBRI/PBA channels.

NSBRI AREA:	**Education and Public Outreach**
PRINCIPAL INVESTIGATOR:	**Deborah L. Illman, Ph.D.**
ORGANIZATION:	**University of Washington - *Northwest Science and Technology* Magazine**
PROJECT:	**Northwest Outreach Program on Space Biomedical Research**

Project Executive Summary

Needs addressed: The University of Washington, a new addition to the National Space Biomedical Research Institute, is the only consortium member in the Northwest corner of the country and as such, is poised to make a significant contribution toward NSBRI's goal of communicating the significance and excitement of space life sciences to the public.

Project Goals: We would leverage an existing communication/education program at the UW to:

- transfer/disseminate the space biomedical knowledge to homes and classrooms throughout the Northwest;
- increase literacy about science in general, and about space biomedical research and terrestrial applications in particular, among the general public, K8-20 teachers, and students;
- prepare scientists and future reporters and public information officers to communicate more effectively about science to general audiences, particularly about space biomedical issues; and
- attract young people to careers in space biomedical research.

Proposed Activities:

- Develop and disseminate articles on space biomedical research via *Northwest Science & Technology (NWS&T)* magazine, a new regional science publication with a circulation of 23,000 in the Pacific Northwest region and beyond;
- Involve student writers (both undergraduate and graduate) in our science writing curriculum and in development of these articles;
- Develop/adapt and disseminate special materials on space biomedical research for middle school students and their parents and teachers by means of an insert in *NWS&T;*
- Improve the ability of scientists and public information officers to communicate with general audiences by developing and delivering a summer science writing workshop for NSBRI consortium members; and
- Attract students in the pipeline to careers in space biomedical research by means of a summer experience for high school students in the laboratories of NSBRI projects at the UW.

Nature of Partnerships involved: This is a collaboration between Illman in the Department of Technical Communication and Martin Kushmerick, NSBRI team leader, integrated human function, and other NSBRI investigators at the UW. The project will be advised by a planning group including Nancy Moreno, Associate Director, Center for Educational Outreach, Baylor College of Medicine, and Janice DeCosmo, Director, Washington Space Grant.

Intended Outcomes: A series of 5 news/feature articles over 3 years, covering the addition of the UW to the NSBRI consortium and results of NSBRI projects funded at the UW would reach an audience of some 23,000. Middle school science teachers would be added to our distribution list and two middle school inserts would be developed and disseminated. A series of three

summer workshops on science writing would improve the preparation of NSBRI personnel to communicate with lay audiences. Finally, a summer research experience for high school students, organized by Kushmerick, would bring students in after their junior year to the UW to spend a summer working in the laboratories of NSBRI projects.

Evaluation Plan: Development and testing of the insert would be done in conjunction with Moreno at Baylor College. A reader survey would be used to gather feedback on *NWS&T* articles. Course evaluations and exit surveys would gather feedback from student writers. Exit surveys of high school students would provide feedback on the summer research experiences. The progress of these students would be followed during their senior years and beyond.

NSBRI AREA:	**Education and Public Outreach**
PRINCIPAL INVESTIGATOR:	**Robert K. James, Ph.D.**
ORGANIZATION:	**Texas A&M University**
PROJECT:	**NSBRI Teacher Academy Project**

Project Executive Summary

Needs Addressed. The need to reform science education in this country is well documented in the research literature (e.g. the National Assessment of Educational Progress, the Third International Mathematics and Science Study). Letters from 103 middle level science teachers described the value of this project. They spoke of how space science study motivated their students and how disappointed their students were when they had to move on to other units of study. There is a significant population of teachers ready to take the leadership role in disseminating space-based science in the middle school. By their nature middle school students need exciting, creative, inclusive and current instructional activities that space-based science can provide.

Project Goals/Objectives. The purpose of the NSBRI Teacher Academy Project (NSBRI TAP) is to prepare Master Teachers to assist their peers in infusing cutting-edge, space-based science activities in middle school. We will work with other NSBRI research institutions in their outreach to teachers. The specific objectives are to:

1. Establish a national cadre of 90 middle level science teachers and prepare them to provide staff development to reach 1800 middle level science teachers.
2. Identify and provide access to extant teaching resources for middle level science.
3. Develop supportive partnerships with key organizations in order to access and utilize their resources and skills.
4. Work collaboratively with other NSBRI member institutions.

Improve the quality of middle level science in the classrooms of teachers who participate in this project.

Proposed Activities. Major components include: the selection of a strong cadre of master teachers; a summer institute with updates on recent NSBRI scientific discoveries, leadership and staff development training and resource identification; local space-based peer teacher training by the master teachers; follow-up activities and conferences; and certification of master teachers as Fellows of the Academy.

Nature of Partnerships Involved. Partnership development will be led by the staff of the Texas Alliance. Partnerships will include the NSBRI Education and Public Outreach Team, NSBRI scientists, the National Science Teachers Association, key school districts and teachers, National Space Grant and NASA Education.

Intended Outcomes. The intended outcomes of the NSBRI TAP are to provide a national cadre of 90 master teachers who are successful in helping at least 1800 of their peer space science teachers to implement space-based science in their classrooms. We expect to demonstrate the efficacy of this model as a strategy for reaching large numbers of middle level science classrooms and hence, the next generation of young adults so that they might make informed

political decisions about manned space flight. Beyond that, they will know and be able to profit from the technology transfer that will enhance their current quality of life.

Evaluation Plan. The evaluation plan is designed to address progress, achievement, and impact of the primary activities of the project and the achievement of the overall goal of producing a cadre of teachers who will conduct staff development activities for their peer space science teachers. Both qualitative and quantitative data will be collected, with on-going analysis of the data shared with the Director. Discrete evaluation protocols will provide data on the achievement of each objective.

NSBRI AREA:	**Education and Public Outreach**
PRINCIPAL INVESTIGATOR:	**Marlene Y. MacLeish, Ed.D.**
ORGANIZATION:	**Morehouse School of Medicine**
PROJECT:	**Secondary and College Education for the Next Generation of Space Life Scientists**

Project Executive Summary

Three schools – Baylor College of Medicine (BCM), Morehouse School of Medicine (MSM), and Texas A&M University (TAMU) – have strategic responsibility to effect the National Space Biomedical Research Institute's (NSBRI) education and pubic outreach mission. MSM has been funded by the NSBRI for the past three years to support national science education reform through the production of innovative secondary education curriculum supplements and the development of a pipeline of minority group science students. MSM is requesting continued funding for four ongoing projects and one new initiative to support this mission.

The four ongoing project are: the NSBRI Teacher Fellowship Program, a collaboration with the DeKalb school system - Fernbank SpaceStation and Georgia State University SECME programs; the Summer Research Program which enrolls college students to do summer research in a MSM science laboratory; an undergraduate level course, The Human Body in Space, taught at Spelman College; and the NSBRI Film Archive. MSM is proposing a fifth initiative - the production of an undergraduate textbook on the human body and weightlessness.

The Teacher Fellowship Program aims to support the professional development of secondary teachers by engaging them in the production of innovative problem-based science cases. Fernbank Museum SpaceStation Program is providing release time for the first NSBRI Teacher Fellow. She is using the Harvard problem-based case model to write a cardiovascular case, *Bobby's Beat*. She will attend the TAMU Teacher Academy Program to introduce this method to science teachers enrolled in that program and to learn leadership skills to effect change when she returns to her home system. Georgia State University SECME will sponsor the 2001 teacher fellow. This Program also enrolls one student intern. The 2000 student intern produced the video, *Immortal Heavens*. A second intern will be selected in Spring 2001 to work on *Bobby's Beat*.

The Summer Research Program provides research opportunities for four undergraduate students to engage in a research intensive internship at MSM, learn about space biomedical research from an eminent space sciences guest lecturer, and meet role models involved in space biomedical research. To date, 13 students, selected from a competitive applicant pool, have participated in the intensive 12-week research program. A longitudinal database exists on the students to measure the outcome of the program.

The NSBRI Film Archive contains over 150 hours of video relating to NASA's Neurolab mission, NSBRI team science, and the Human Body in Space course. Film and design footage are made available to NSBRI consortium member schools as well as other organizations. The Discovery Channel, ZDF-German TV, RDF Television-London, the Atlanta and DeKalb public schools, and the SciTrek and Fernbank museums have used materials from the archive. This

one-of-a-kind repository will be used to develop interactive Internet accompaniments to the proposed textbook and the problem-based cases written by the teacher fellows.

The proposed textbook on the human body and weightlessness will enrich teaching of space biological sciences at the undergraduate level. A well-designed textbook that uses a multidisciplinary perspective to elicit their understanding of the world is timely. The need for such a text is amply demonstrated by the Spelman College, Colorado State University and Johns Hopkins University courses that rely on collated materials from disparate sources. Existing course materials do not adequately meet national undergraduate science education standards and are not uniformly appropriate for an undergraduate audience.

NSBRI AREA:	**Education and Public Outreach**
PRINCIPAL INVESTIGATOR:	**Dava J. Newman, Ph.D.**
ORGANIZATION:	**Massachusetts Institute of Technology**
PROJECT:	**Space Biomedical Sciences and Engineering Curriculum and Outreach Program**

Project Executive Summary

The National Space Biomedical Research Institute (NSBRI) has a need to develop graduate-level curriculum for students at all NSBRI consortium institutions to educate a generation of scholars in space life sciences. There are also crucial needs to transfer advanced space life sciences knowledge into material appropriate for undergraduate courses as well as to disseminate the material to younger students and the public. The objectives of our proposed curriculum and outreach efforts are to develop two graduate courses, namely, *Space Biomedical Engineering and Life Support* and *Sensori-Neural Systems: From the Vestibular Periphery to Motor Responses, Perception and Adaptation.* The innovative modular curriculum covers eight of the twelve NSBRI research areas and proposes a qualitative-to-quantitative progression of information and is designed for adoption throughout NSBRI consortium institutions. A second objective is for selected topics to be adapted for use in undergraduate courses and disseminated to thousands of younger students and the public through our proposed global outreach effort.

The proposing team includes academia and two small businesses in partnership with the national non-profit organization FIRST, "For Inspiration and Recognition of Science and Technology" for our outreach program. We propose the design of a knowledge station that allows learners to interact with curricular materials via state-of-the-art information technology and a physical platform that is designed specifically to facilitate human interaction and learning.

The intended outcomes from our proposed program in space biomedical sciences and engineering curriculum and outreach are to: 1) Provide multi-level space life sciences curriculum. 2) Excite and educate the public about the wonders of science, engineering and medicine by disseminating knowledge gained through NSBRI research. 3) Develop a set of innovative pedagogical strategies that represent the application of tested learning principles as a basis for comprehensive educational evaluation tools. To maximize accessibility to educational material, we propose the development of multimedia tools that are particularly suited to active learning accessible through the Internet.

The proposed evaluation plan provides assessment of the education and outreach project by utilizing learning theory as a basis. Continuous evaluation will provide feedback for necessary curricular enhancement and improved outreach efforts during the course of the project. Traditional methods of science and engineering education can no longer be the pedagogical model to meet the needs of a generation of students raised in an increasingly visual, interactive information technology world. The evaluation plan will assess learning and knowledge transfer of curriculum that makes use of these technological advances as well as assessment of the new student (or 'learner') population.

NSBRI AREA:	**Education and Public Outreach**
PRINCIPAL INVESTIGATOR:	**Roland B. Smith, Jr., Ed.D.**
ORGANIZATION:	**Rice University**
PROJECT:	**Outreach Program for the Professional Development of Students and Teachers on Studies Related to Biomedicine in Outer Space**

Project Executive Summary

A unique, collaborative partnership between the University of Texas Medical Branch, Galveston (UTMB) and Rice University (Rice) is dedicated to furthering the NSBRI mission to communicate the significance of space life sciences and microgravity biotechnology to local and national audiences, while disseminating the biomedical knowledge gained through research programs to the classroom and community.

Our goals are to attract young people to space-related enrichment programs, promote excellence and innovation in America's science education system, and enhance the scientific background among teachers, students, their families, and the community as a whole. This will be achieved through a program consisting of two parts: 1) the Academic Development of High School Students (Student Research) and 2) the Teacher Institute for the Advancement of Space Science Education (Teacher Institute). Students and teachers will be involved in the ongoing space biomedicine research projects conducted at Rice and UTMB, including those in the areas of i) bone and musculoskeletal loss, ii) integrated human function, iii) immunology, infection, and hematology, iv) neurovestibular adaptation, and v) technology development.

For the Student Research component, twelve high school students each year will conduct laboratory research from June to August, taking advantage of the academic environment at both Rice and UTMB. Their interactions will extend beyond those in their own laboratory through a series of workshops designed to expose young scientists to researchers and the wide variety of research being conducted in the field of space biomedicine. The research experiences will also be supplemented by field trips designed to motivate students to pursue scientific research as a career.

The Teacher Institute will be conducted throughout the year beginning with an intensive, two-week session held in June. Each year, twenty secondary teachers will enhance their scientific knowledge of space biomedicine through interactive discussions with space biomedical researchers, a one-day, hands-on research experience, performing activities in some of the existing NSBRI and NASA educational modules, and special tours of NASA Johnson Space Center and Space Center Houston. Teachers will apply their knowledge to design a space biomedicine mini-module/unit plan to be taught in class and refined for publishing on the educational resources web sites of Rice, UTMB and the NSBRI. Both student and teacher components will be monitored and evaluated by an external evaluator.

Rice University has a long-standing commitment to the greater Houston area through its 54 K-12 outreach initiatives, many of which are programs for student enrichment and professional development of teachers focused in the area of science and mathematics education. UTMB has a long-term interactive partnership with the nine Galveston County school districts through a

variety of K-12 programs including summer science research camps for 7-12 grade students, science teacher workshops, and an annual Regional Science Teacher Conference. The synergy created by the combined scientific and educational expertise at Rice and UTMB will culminate in an outstanding program that will not only benefit the participating students and teachers, but also the community at-large.

NSBRI AREA:	**Education and Public Outreach**
PRINCIPAL INVESTIGATOR:	**William A. Thomson, Ph.D.**
ORGANIZATION:	**Baylor College of Medicine**
PROJECT:	**From Outer Space to Inner Space: Sharing NSBRI Progress with the Community**

Project Executive Summary

Baylor College of Medicine (BCM), Space Center Houston, Houston Public Television and the Houston Independent School District propose to convey the excitement and promise of NSBRI space life sciences research to students, teachers and the general public through coordinated formal and informal educational opportunities that will be embedded within local and state science education reform programs. The proposed partnership builds on the NSBRI-funded outreach program conducted by BCM during 1998-2000. This effort partnered scientists and educators in the production, evaluation and dissemination of the first two units of a planned series of elementary and middle school curriculum materials based on space biomedical themes. It also produced bimonthly, nationally distributed radio stories on NSBRI research areas.

The new partnership will reach thousands of students, teachers and members of the general public each year with significant science/health knowledge and skills for living. It also will generate public awareness and appreciation of the benefits of NSBRI research. Project activities will be aimed at middle school (grades 5-8), which has been identified as a particularly weak link in the K-12 science/mathematics education continuum.

In addition to the primary partners, the proposed project will involve: Texas A&M University, Texas Rural Systemic Initiative, Texas Statewide Systemic Initiative and University of Washington. Measurable project objectives are: (1) Collaboratively create, evaluate and disseminate three interdisciplinary teaching units (one per year) on NSBRI research themes for middle school students; (2) Improve teacher practice and content knowledge through multiple professional development opportunities conducted in formal and informal educational settings; (3) Develop an online workshop resource for NSBRI scientists to use for outreach on space life sciences themes to teachers, students and the community-at-large; (4) Create and implement cost-effective models for communicating NSBRI research to local and national populations through television and radio short-format news and newsmagazine stories.

Appendix L

Fax received this date

National Aeronautics and
Space Administration
Office of the Administrator
Washington, DC 20546-0001

MAR 16 2001

Bobby Alford, M.D., CEO
National Space Biomedical Research Institute
One Baylor Plaza, NA-425
Houston, TX 77030-3498

Dear Dr. Alford:

As you know from our conversations, Mr. Goldin has requested that NASA and the NSBRI work together to develop a NASA response to the important observations, findings, and recommendations of the "Site Visit Review Report of the NSBRI" that was conducted on November 28 through December 1, 2000. I promised the review team members that I would inform them of NASA's response to, and plans for implementing suggestions arising from their review on current and future plans for how the NSBRI's work should continue. I have received numerous inquiries about the response to the report. It is critical for our success that we have a mutually agreed response prior to delivering Congressional testimony on the FY 2002 budget and preparations for the FY 2003 budget. This process is beginning now and will continue for the next 6 to 8 weeks.

I would like NSBRI to take the lead in drafting a response that identifies the main issues in the report, and lays out the response and any plans and timelines for corrective actions. Please coordinate your draft response with the Biomedical Research and Countermeasures Program at Johnson Space Center and with the Bioastronautics Research Division at Headquarters. Chuck Sawin at JSC, and Guy Fogleman and David Tomko at Headquarters are ready to work with you. The Office of the Chief Scientist will settle any differences, and produce a final NASA response using your inputs. If there is any difficulty with preparing such a response by April 1, 2001, please let me know.

Sincerely yours,

Kathie L. Olsen, Ph.D.
Chief Scientist

cc:
UB/Dr. Fogleman, Dr. Tomko
JSC/SA/Dr. Sawin, Dr. Williams

Bobby R. Alford, M.D.
Chairman of the Board and CEO
Friedkin Chair for Research in Sensory System Integration and Space Medicine

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March 16, 2001

Kathie Olsen, Ph.D.
NASA Headquarters
Chief Scientist
300 E. Street, S.W.
Mail Code: AS
Washington, DC 20546

Dear Dr. Olsen:

Thank you for putting together the engaging Site Visit Review Team for the NSBRI three-year review. I appreciated the leadership that you provided. Your pre-site visit planning with us was very helpful.

Since receiving the Draft Site Visit Review Report of the National Space Biomedical Research Institute (NSBRI) submitted by the Review Team, I have given it a lot of thought and have had a great deal of input from our board members, members of the External Advisory Council and Team Leaders. We are gratified that the Executive Summary contained key elements that impressed the Review Team: "the scientific strengths of the NSBRI; its progress in developing countermeasures; its innovative scientific leadership; and, its outstanding student trainees." We were also pleased that the Review Team recommended that the NSBRI continue for its second five year funding period and commended the NSBRI for providing added value. There are a few points in the report about which there is concern.

Below I have listed those matters that I believe to be significant:

1) No one on the site visit team had been part of the original Institute competitive selection process and thus there was little point of reference to measure the NSBRI after three years against expectations that led to its creation and selection or operation;

2) There were only four physicians on the fifteen member site visit team, which is not reflective of the leadership of the NSBRI or of the leadership of our Integrated Research Teams, or the emphasis on translational "bench to bedside" research required for the development of countermeasures and understanding the special clinical biomedical requirements for solutions to the risks identified in the Critical Path Roadmap;

3) The statement in the Draft Site Visit Review Report that says "They flunk from top to bottom, side to side, they are obviously disregarding diversity" is a serious indictment and words to that effect are misleading and serve to discredit (in fact, there are currently four members of our Board who reflect ethnic and gender diversity, the External Advisory Council has gender diversity, there have been and are several PI's and Co-PI's, Team Leaders, or Associate Team Leaders that reflect ethnic, cultural and gender diversity, and the NSBRI headquarters staff reflects diversity);

4) The Site Visit Review Report does not focus on the "new way of doing business" NSBRI accomplishments and value but stresses instead much more education and outreach than originally proposed or that the available resources permit.

I have served eight years on 2 National Institutes of Health institute National Advisory Councils, eight years as a member of NIH review committees (chairing one of them for years), six years on the Residency Review Committee for the Accreditation Council on Graduate Medical Education, and made and chaired more site visits for NIH program reviews than I can remember. Consequently, I believe I understand the site visit review process and the associated responsibilities. I suppose that explains some of my concern over this report. I have never been identified with a report like this one.

There is one other matter that I wish to bring to your attention. The face page of the final report that we received containing the signatures of all of the Site Visit Review Team members has the wrong title. We are not the National **"Science"** Biomedical Research Institute.

With every good personal wish I am,

Sincerely yours,

Bob

Bobby R. Alford, M.D.

BRA:bgr

cc: Mr. Daniel S. Goldin

bcc: Ronald J. White, Ph.D.
Laurence R. Young, Sc.D.

National Aeronautics and
Space Administration
Office of the Administrator
Washington, DC 20546-0001

APR 11 2001

Bobby R. Alford, M.D.
Chairman
National Space Biomedical Research Institute
One Baylor Plaza, NA-425
Houston, TX 77030-3498

Dear Dr. Alford:

Thank you very much for your letter of March 16, 2001, in response to my letter of the same day. I would have expected that many of the issues that you raised would have been discussed with us long ago. I am glad that you have recognized and embraced the complimentary aspects of the review. As you know from your long service on NIH Advisory Councils and Review Panels, reviews conducted by independent panels of peers serve two purposes. First, peer-panels identify the strengths of proposed research and of the scientists managing and conducting the work. Second, they identify weaknesses that when corrected will result in the improvement of the research effort.

In the case of the Chief Scientist's Third year review of the NSBRI, the review panel identified key areas for improvement that need to be addressed to optimize the effectiveness of the NSBRI's research contributions. Some require corrective action on the part of the NSBRI, some require corrective action on the part of NASA, and some will require a collaborative NASA/NSBRI response. Some of the key issues that were identified by the reviewers from my perspective are as follows:

- Additional resources have been provided to the NSBRI in the absence of what the review panel called a "forward-looking strategic plan" or proposal that will critically evaluate the priority and resources allocated to each area of its activities, including the research teams, the administrative infrastructure, and its educational programs. This is a standard requirement for Federal agencies in funding research, and is even more important given the magnitude of the NSBRI's budget and increases. The NSBRI must develop such a research plan, to include a detailed operating budget that can be evaluated by peer-review to insure that current and future allocations are appropriate and adequate

- The reviewers recommended that NASA continue to fund the NSBRI as a team-based organization, whose mission is tightly-focused and targeted to the development of mechanism-based countermeasures. In this context, the reviewers expressed concern that the team leaders, who are critical and integral to the focused team approach, must be protected from accusation of real or apparent conflict of interest or cronyism. Team leader selection must be made more transparent to protect NASA and NSBRI from any real or perceived conflict of interest in selection of research or disbursement of Federal funds. They recommended that the selection process for building teams using individual research grants

(following the peer review) needs to be reexamined and reformulated to provide assurance that conflicts of interest or cronyism are avoided.

- The reviewers recommended that each NSBRI Research Team develop and publicize a long-term strategic plan for future guidance. Such a plan will materially help the grant application process by providing direction to the research community on the NASA's unique research requirements for each team's research discipline. The strategic plan must address issues relating to the in-flight condition and post-flight recovery of the astronauts.

- The review panel was concerned that the NASA NSBRI currently gives equal resource priority to each team. The NSBRI strategic plan should address how NASA's priorities are used to guide prioritization of team funding.

- Regarding NSBRI education and outreach activity, the reviewers did *not* recommend that NASA spend more money on these activities. They were very complimentary about the quality of education and outreach work being done by NSBRI. I believe that their intent is that the NSBRI strive to make Education and Outreach a more integral part of all NSBRI's daily activities, as opposed to a separate activity. They also recommended that NSBRI start a university-level education program. With the already-existing strong group of students and fellows associated with the research tasks, I think that making education and outreach a part of each institute member's daily activities is an achievable goal and should be described in the NSBRI strategic plan.

- The panel recommended that NSBRI consider the discontinuation or the reorganization of the new team for integrated human systems, as the reviewers were not convinced that it could contribute to the development of countermeasures in the near term. Since you have made research selections and awards for this team, you obviously disagreed with the panel, and I would like to have an explanation of your rationale for disregarding this piece of advice.

- The panel recommended that NASA conduct an external site-visit review for each individual NSBRI team in year 5, and every 3-5 years thereafter.

- The panel recommended that the NSBRI not be used by NASA as a "funding agency", and that NASA Headquarters, in close cooperation with the NSBRI, the NASA Johnson Space Center, and the Ames Research Center should extend and expand NASA's NRA grant program to fund individual research grants that will address biomedical countermeasure research needs.

- It was recommended that the NSBRI continue to make use of outside advisors, including its Board of Directors, its Board of Scientific Counselors, and its External Advisory Council. However, the reviewers pointed out a need for increasing the human diversity of these boards. The panel pointed out that increased attention is needed to human resource issues at all levels of the NSBRI employment, especially at upper levels.

- The reviewers advised that NSBRI, JSC, and NASA HQ should find ways of increasing the accessibility of relevant performance and medical data to the scientific community. Database development should be a priority, with appropriate access to investigators outside NSBRI.

- The panel recommended that NSBRI establish policies and guidelines for technology transfer, intellectual property rights, and industrial collaborations.

Many of these items are easily addressed, as procedures have already been implemented to answer the suggestions. Others will require additional work before they can be reported back to the committee.

NASA takes seriously its responsibility to insure that all segments of the American population have the opportunity to participate in the *all* parts of the NASA experience and dreams. Mr. Goldin and I take that responsibility personally and seriously as well. I interpret the report's strong language about our need for improved diversity as an exclamation point about that need rather than an indictment or an attempt to discredit us. I suggest that we put into place a plan to insure that we have done our utmost to insure that all components of American society are robust participants in all aspects of NSBRI activity.

Lastly, even though I had already left when the signature page was typed and signed, I take full responsibility for the typographical error found only on the signature page that incorrectly identifies NSBRI as the National Science Biomedical Research Institute. No malice was intended, just a human error by my helpers in striving to help the review team to achieve my goal of completing and signing the site visit report before leaving the site visit. Indeed, the President of Baylor College of Medicine on April 5 made the same error in his discussions with Senators Hutchison and Gramm. I will see that the error is corrected on the original document so that the permanent record of the review is accurate.

I look forward to hearing about your plans to address substantive concerns identified by the review team as potential improvements in an already good operation.

Sincerely yours,

Kathie L.

Kathie L. Olsen, Ph.D.
Chief Scientist

cc:
UB/Dr. Fogleman
Dr. Tomko
JSC/SA/Dr. Sawin
Dr. Williams
ULCA/Dr. Tobin
NSBRI/Dr. Young

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April 12, 2001

Kathie L. Olsen, Ph.D.
Chief Scientist
NASA Headquarters
300 E. Street SW
Mail Code: AS
Washington, DC 20546

Dear Dr. Olsen:

Enclosed with this letter is our response to the findings and recommendations of the "Site Visit Review Report of the National Space Biomedical Research Institute." As you requested in your letter of March 16, we have coordinated our response with the appropriate programs at Johnson Space Center and NASA Headquarters. We appreciate your extending the time available for us to prepare and coordinate our response.

We are delighted that the mutual relationship among the three parties involved in planning and implementing the Bioastronautics Program seems to be strengthening as a result of the meetings and telecons that have been set up to increase our communication and cooperation. Drs. Fogleman, Rummel and White have undertaken serious discussions on ways to improve all aspects of our programs and we hope that the results of these discussions will be real progress in mutually beneficial ways.

Sincerely,

Bob

Bobby R. Alford, M.D.

Enclosure

cc: Guy Fogleman, Ph.D.
John A. Rummel, Ph.D.
Ronald J. White, Ph.D.
Laurence R. Young, Sc.D.

RESPONSE TO THE SITE VISIT REVIEW REPORT OF THE NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

April 5, 2001

This response was prepared by the National Space Biomedical Research Institute (NSBRI) in coordination with the Biomedical Research and Countermeasures (Bioastronautics) Program at Johnson Space Center, represented by John Rummel, and the Bioastronautics Research Division at NASA Headquarters, represented by Guy Fogleman.

The NSBRI is pleased with the strong support provided in the final report of the Site Visit Review team and is particularly delighted that the review team found that the NSBRI provided "added value both for the institute as a whole and for individual teams" and recommended "that NSBRI continue for its second five-year funding period." Among the many other positive aspects of the report are the following samples of extracted comments:

- The committee was impressed by the scientific strengths of the NSBRI, by its progress in developing countermeasures, by its innovative scientific leadership and by its outstanding student trainees.
- We recommend that NSBRI continue to serve as a team-based organization, whose targeted mission is the development of mechanism-based countermeasures. ... The management plans and strategies are effective overall for developing countermeasures. Interactions with JSC are outstanding. ... The development of the critical path roadmap is also evidence of effective, focused interaction (*with JSC*).
- Adequate checks, balances, and corrections are provided by the Board of Directors, External Advisory Council, and Board of Scientific Counselors. ... We recommend that NSBRI continue to make use of its extensive network of outside advisors, including its Board of Directors, its Board of Scientific Counselors, and its External Advisory Council.
- We were pleased by the ability of the NSBRI leadership to make "mid-course corrections" in its research agenda. ... Priority setting and development of schedules by NSBRI have been impressive ... . Management did not wait but made corrections when the occasion called, which took courage. We viewed turnover among Team Leads positively, an indication that the NSBRI management did not hesitate to change the status quo.
- The NSBRI bone microgravity research activities have the potential to integrate and synthesize the science relevant to understanding the risk levels associated with space travel. Interactions with the muscle team are particularly useful.
- ... the existing and planned activities (*of the muscle team*) are sound, their approach is innovative and unique, and the science meets the expectations of the community. ... their vision to collaborate with members of other teams to explore models intended to study the effects of proposed countermeasures on more than one tissue, muscle plus bone, is commendable.
- The individual projects (*of the neurovestibular adaptation team*) are related to one another and the interactions between and among projects will certainly improve the likelihood of successfully developing countermeasures. These projects also have a definite potential to impact several health issues on Earth, particularly balance disorders.

- Research efforts of the Human Performance group provide an important added value to the biomedical research community at large. Many of these same problems facing astronauts are also faced by ground personnel, pilots, shift workers, and individuals suffering from jet lag and affective disorders related to circadian disruption. Thus, insights and countermeasures derived from this group are likely to have significant impact on the broader biomedical community and workforce.
- The activity of the Technology Development Team is an excellent example of the value added by synergistic interactions between the various components of the NSBRI. It exemplifies the value of the NSBRI structure in facilitating a complex mission-orientated research and development program. Much of the work performed by this team would not have been undertaken without the motivation and facilitation of interactions provided by the NSBRI.
- Creation of the new (*neurobehavioral and psychosocial factors*) team constitutes a response to a recommendation of very high priority by the National Research Council (NRC) Strategy Report (1998) and Review of NASA's Biomedical Research Program (2000), and it corrects a major deficit in the overall biomedical research and countermeasures effort. The selected projects are appropriately focused on ultimate development of countermeasures. The proposed program appears to be well-balanced and responsive to NSBRI mission objectives.
- This (*nutrition, physical fitness and rehabilitation*) research group represents a much-needed new research direction for the NSBRI. Exercise-based countermeasures have not been adequately validated in space and have not proved effective in preventing cardiovascular, bone, and muscle integrity. ... In general, the review team was impressed with the direction the group has taken.
- The added value (*of the smart medical systems team*) to this work is enormous. Every single project has potential clinical significance in important medical and environmental areas including field trauma, intensive care units, and hospital based infection monitoring and prevention. Further applications may include minimally invasive robotic surgery. This team has an exciting approach to important medical problems in space. They will be able to interact with other teams to enhance their work and they will contribute to important land-based applications. The projects have both short term and long term goals that have been appropriately set.
- NSBRI's activities enhance ongoing biomedical research in the participating institutions. New technology and new therapeutic approaches may be widely beneficial.
- Interaction with JSC is moving along well. The strong desirability of such interaction was the reason for establishing NSBRI in the first place, and the collaboration is strong in spite of budget cuts at JSC. The NSBRI/JSC interaction has allowed the reduced JSC staff to focus their efforts on the higher CRL ratings.
- NSBRI has formed important alliances with the NIH, the Department of Energy, and the Brookhaven National Laboratory, especially with its Booster Applications Facility, and with the National Institute of Deafness and Communication Disorders (NIDCD). ... There are also affiliations with foreign research centers in Germany (Institute of Aerospace Medicine), Italy (Politecnico di Milano), France (Institute for Space Physiology and Medicine), and Russia (Institute for Biomedical Problems). ... These domestic and foreign affiliations should enhance the NSBRI mission significantly. Obviously the relationship to NASA and JSC is critical and NSBRI needs greater access to the JSC database and to astronaut medical information. NIH funding of NSBRI investigators should allow more research productivity. The

Brookhaven National Laboratory has outstanding research programs in human brain imaging and radiation biology. Finally, the foreign affiliations will enhance NSBRI outreach and education missions as well as provide unique scientific opportunities.

- The current strength of the NSBRI education activities rests on the enthusiasm and commitment of the core team led by Dr. Marlene MacLeish and the efforts of individual scientists. ... The committee agreed that both the commitment of the team and the quality of their activities is excellent. ... NSBRI has done an excellent job creating grades 5-12 curriculum materials that stimulate interest in science and strengthen students' inquiry methods. Materials shown were evidence of committed collaboration of persons who believe strongly in the education/outreach mission.
- The synergy grants are great. They promote interdisciplinary projects and contribute to the value-added aspect of NSBRI.
- The fact that everybody must recompete at the end of grant funding is applauded.

We fully recognize that several of the recommendations of the Site Visit Review committee require further action on the part of the NSBRI and/or NASA and we have already begun to develop and implement plans for corrective actions, where appropriate. These include the following:

- Whether or not the additional funding is provided, we strongly recommend that NSBRI, under its new Director, promptly prepare a forward-looking strategic plan, which can then be properly reviewed. The new plan should critically evaluate the priority and resources allocated to each area of its activities, including the research teams, the administrative infrastructure, and its educational programs.
  - Although it clearly was not obvious to the review committee prior to their site visit, the NSBRI had developed a forward-looking strategic plan and coordinated that plan with the NASA Administrator, the former Associate Administrator for Life and Microgravity Sciences, the former Life Sciences Division at NASA Headquarters, the Director of Johnson Space Center, and the Director of Space and Life Sciences at JSC. The competitive expansion of the Institute consortium, the development of management infrastructure, and the release of two competitive research announcements and one competitive education and outreach announcement were all carried out as part of that plan. The NSBRI certainly expects to continually evaluate its strategic plan and to involve the Director in that evaluation.

- We recommend that the leadership make the process of team leader selection more transparent, in order to avoid any appearance of cronyism. NSBRI's credibility and its effectiveness in recruiting new researchers will depend on management's ability to manage apparent or potential conflicts of interests, especially at the level of team leaders.
  - Real or apparent conflict of interest and cronyism have been among the concerns of the Institute since its inception. Management has engaged several groups in active discussion of this issue, including our External Advisory Council and most panels reviewing proposals under the leadership of the Board of Scientific Counselors. With the assistance of these groups, we are in the process of formulating a plan to select team leaders using a process that removes them completely from the standard project competition and bases their selection on both scientific and management criteria. In a certain sense, team leaders are analogous to NIH intramural laboratory heads and the new process will recognize that fact.

- Because of the increasing potential for conflict of interest, we recommend that the selection process (following peer review) should be reexamined and reformulated.
  - ➢ This issue is tied closely to the preceding one. Reformulation of the team leader selection process to remove them fully from the standard competition will address this issue as well.

- We strongly recommend the establishment of a university-level education program-especially at consortium institutions-that takes advantage of the ability of space research to excite student interest in NASA' s and NSBRI's long-term goals.
  - ➢ The NSBRI is aware of the ability of space research to excite student interest in NASA's and its own long-term goals. Plans to aggressively pursue this aspect of the Institute's program were not included in the original, competitively selected proposal and have not competed well for the limited funds that the Institute has obtained to this point because of other high priority activities more directly related to the Institute's research agenda. This topic will be discussed with our investigators at our next full Institute retreat in January 2002.

- To increase the pool of future space researchers, we strongly recommend increased support and coordination of NSBRI's educational programs. Additional funding should track the increase in NSBRI's budget. Rather than increasing educational activities *in vacuo,* we recommend significant integration of educational and research efforts, and we suggest future reviews include the evaluation of the plan and the accomplishments of this effort.
  - ➢ Support for the Institute's educational programs has already "tracked the increase in NSBRI's budget." As the Institute's funding for research doubled this past year, so has the funding for education. In addition, over $1,000,000 in other selectable project proposals are on "hold," pending identification of a funding source. With the appropriate increase in our budget for next year, the education program could grow to over three times its original size. The Education and Outreach group has been elevated to team status and will be managed accordingly.

- We strongly recommend increased attention to human resource issues at all levels of NSBRI employment, especially at upper levels.
  - ➢ Although the NSBRI has been and will continue to be concerned about human resource issues, the force with which this issue is expressed in the review report is surely a result of misinformation. There are currently four members of our Board of Directors who reflect ethnic and gender diversity, three members of our External Advisory Council are female as are five of our Team Leaders or Associate Team Leaders, and our Principal and Co-Investigators reflect ethnic, cultural and gender diversity. Of the thirteen members of the NSBRI Headquarters staff, only two are white male.

- In establishing future priorities, we urge NSBRI to consider the recent determination by NASA that radiation is the number one health-and-safety issue associated with deep space flight.
  - ➢ The NSBRI understands and will make appropriate use of this relative importance in determining future program growth.

- We recommend that NSBRI consider the discontinuation or the reorganization of the new team for integrated human systems.
  - ➢ This team is being restructured. An advisory group, chaired by F. Eugene Yates of UCLA, will meet in May 2001 to make recommendations concerning the optimal approach to take in that restructuring.

- NSBRI, JSC, and NASA Headquarters should find ways of increasing the accessibility of relevant performance and medical data. ... Database development should be a priority, with appropriate access to investigators outside NSBRI.
  - ➢ All parties agree that this is an issue whose time has come to settle. A new working group is being established to deal with this matter. Given the appropriate increase in the budget for FY 2002, funding to this area will increase significantly.

- We recommend that a NASA external review be scheduled for each individual team in year 5, and every 3-5 years thereafter.
  - ➢ We do not agree with this recommendation. The timing is inappropriate. Year 5 is FY 2002 and teams are just beginning their new research program now, in mid-2001. Team Reviews such as these should be coupled to the five-year review of the Institute and should precede that full review by two-four months. Then, the full review could focus on Institute-wide issues rather than team-related issues.