THE IMPORTANCE OF THE INTERNATIONAL SPACE STATION FOR LIFE SCIENCES RESEARCH: PAST AND FUTURE

Tara M. Ruttley, Cynthia A. Evans and Julie A. Robinson
Office of the ISS Program Scientist, International Space Station Program
NASA Johnson Space Center, Houston, TX

ABSTRACT

Assembly of the International Space Station is nearing completion with the addition of its final research facilities in fall of 2010. Although assembly has been the primary objective of its first 11 years of operation, early life science research returns from the ISS have been growing at a steady pace. To date, early utilization of the ISS has fielded almost 200 experiments for international and US partner research in the life sciences disciplines. With a specific focus on life sciences research, this paper summarizes the science accomplishments from early research aboard the ISS—both applied human research for exploration and research on the effects of microgravity on life. Such accomplishments range from understanding increased virulence of various microbes during spaceflight that will aid in the development of vaccines, new methods for delivering medicine to cancer cells, and the potential development for treatments of debilitating diseases such as Duchenne’s muscular dystrophy. We will also look ahead to the full capabilities for life sciences research when assembly of ISS is complete in 2010.

INTRODUCTION AND OVERVIEW

The International Space Station (ISS) has had a continuous presence in space for the last 11 years, and has been under ongoing assembly during this time period. Representing the most complex and largest vehicle ever occupied in orbit, its importance for human space exploration includes the technologies tested, the operational tests, and experience gained from long term operation, and the knowledge gained from scientific investigations in a microgravity environment. Scientific facilities will be completed on ISS during its final year of assembly. Facilities have been designed to support investigations in basic life and physical sciences, applied investigations on participating crews on the effects of space travel on the human body, and tests of technologies and systems support for human exploration.

The first component of ISS, the Russian Zarya module, was deployed in 1998, and the U.S. Destiny laboratory followed in early 2001. During the 2008 calendar year, the laboratory space and research facilities were tripled with the addition of the European Space Agency’s (ESA’s) Columbus and Japanese Aerospace Exploration Agency’s Kibo scientific modules. Facilities within the laboratory space allow investigations within various disciplines from all over the world; a total of 24 facilities will be on ISS at assembly complete. Facilities designed to support life sciences investigations range from cell culture hardware to human physiology research as shown in Table 1, and details can be found online at www.nasa.gov/iss-science.

Throughout the ISS construction period a program of “early utilization” has allowed research investigations to take advantage of the facilities or crew already onboard. Although this early utilization has sometimes been limited in scope, science returns from the ISS have been growing steadily. Early utilization of the ISS represents the science and education activities completed during assembly, both on the ISS and on Space Shuttle assembly missions to ISS. Although mass to orbit and astronaut crew time have been very limited, these experiments provide information on the potential of ISS after assembly is complete. The ISS partnership includes the U.S. National Aeronautics and Space Administration (NASA), Canadian Space Agency (CSA), European Space Agency (ESA), and Japanese Aerospace Exploration Agency (JAXA). From September 2000 through April 2009 (Expedition 18), over 400 investigations have been conducted on the ISS, representing over 600 scientists worldwide. Of these, nearly 200 were in the life sciences disciplines. The breakdown of ISS investigations performed through Increment 18 by partner and discipline is shown in Figure 1.

The focus of NASA’s ISS research has changed strategically to support the Vision for Space Exploration that was announced by President George W. Bush on Jan 14, 2004. While still including some fundamental research in microgravity, emphasis has shifted to programs that are targeted at developing and testing new exploration technologies and reducing the risks to human explorers on missions to the moon, Mars, and beyond.

Today, NASA’s priorities for research aboard the ISS center on understanding human health during long-duration missions, researching effective countermeasures for long-duration crewmembers, and researching and testing new technologies that can be used for future Exploration crews and spacecraft.

Most research also supports new understandings, methods, or applications that are relevant to life on Earth, such as understanding effective protocols to protect against loss of bone density or better methods for producing stronger metal alloys. Designation of the US assets on ISS as a US National Laboratory increases the access to ISS for fundamental and applied research that is outside of NASA’s exploration mission (NASA, 2005).

*Correspondence to: Tara M. Ruttley
Office of the ISS Program Scientist
NASA Johnson Space Center
Houston, TX, 77058
Email: tara.m.ruttley@nasa.gov
Phone: 281-483-9615
Experiment results have already been used in applications as diverse as the manufacture of solar cell and insulation materials for new spacecraft and the verification of complex numerical models for behavior of fluids in fuel tanks.

In May of 2009, the number of crewmembers increased from three to six, thus greatly increasing the time that is available for research. The realization of the increased international scientific partnership provides new opportunities for scientific collaboration and broadens the research potential on the ISS. Engineers and scientists from around the world are working together to build from their experiences in conducting early science to ensure maximum utilization of the expanded capabilities aboard ISS.

EARLY UTILIZATION OF THE ISS FOR LIFE SCIENCES RESEARCH

The ISS laboratories were designed to accommodate a broad range of biological experiments, from cell biology to plant, small animal, and human physiology studies. NASA-sponsored microgravity research has been conducted since the first ISS expeditions; and more recently, by other U.S. government agencies and commercial partners whose use of ISS is facilitated by its designation as a U.S. National Laboratory (NASA, 2007).

Life sciences research on the ISS can be loosely categorized by the knowledge objectives it supports, and can have applications to both space exploration and life on Earth. Applied human research for exploration is aimed at understanding the effects of the space environment on human health, and on developing and proving countermeasures to allow humans to one day explore beyond Earth orbit. Basic life sciences research uses microgravity environments on ISS to better understand the function of cells, tissues, and organisms and may be focused on gravity effects on the structure and function of living systems, or may have more applied objectives related to improving life on Earth. A summary of the risk areas of spaceflight and medical investigations can be found in the Human Research Program Plan (2009) and accompanied review by the National Institute of Medicine. The following pages will present a number of life science experiments that have been implemented or ongoing on ISS, ranging from cellular biology to human physiology and space exploration.

Examples of early ISS life science research that has led to benefits to Earth as well as the knowledge gained from ISS experiments should enable future space exploration in preparation for exploring the moon, Mars, and beyond. We summarize key early research findings and facilities on ISS across partners, as well as what is planned for the future as the on-orbit labs are completed in 2010. It takes as many as 2 to 5 years for research results to be published after the flight component is completed (Tate et al., 2007), so these results serve as a prelude to the accomplishments that are to come.

CELLULAR BIOLOGY AND BIOTECHNOLOGY

An innovative biotechnology investigation that was performed on ISS in 2002 involved a novel method for encapsulation of several different anti-cancer drugs, magnetic triggering particles, and encapsulation of genetically engineered DNA (Microencapsulation Electrostatic Processing System, Principal investigator: D. Morrison, 2002). The experiment system improved on existing microencapsulation technology by using microgravity to modify the fluid mechanics, interfacial behavior, and biological processing methods as compared to the way the microcapsules would be formed in gravity (Le Pivert et al., 2009 and 2004). As a result of this ISS research, the results from the experiments have provided new insight into the best formulations and conditions required to produce microcapsules of different drugs, particularly special capsules containing diagnostic imaging materials and triggered release particles. Additionally, this research has led to the development of a ground-based technology to produce these microcapsules on Earth (Morrison, 2007). Clinical trials to directly inject microcapsules of anti-tumor drugs into tumor sites are planned at MD Anderson Cancer Center in Houston and the Mayo Cancer Center in Scottsdale, AZ.
Another life sciences experiment performed on ISS has yielded results that are contributing to advancements in medical technology. The JAXA and Roscosmos-sponsored investigation JAXA-GCF (Japan Aerospace and Exploration Agency - Granada Crystallization Facility High Quality Protein Crystallization Project. Principal investigator: H. Tanaka, 2002) was a unique collaboration between several ISS International Partners. The HQL-79 (human hematopoietic prostaglandin D₂ synthase inhibitor) protein is a candidate treatment in inhibiting the effects of Duchenne’s muscular dystrophy. Investigators used the microgravity environment of the ISS to grow larger crystals and more accurately determine the 3-dimensional structures of HQL-79 protein crystals. The findings led to the development of a more potent form of the protein, which is important for the development of a novel treatment for Duchenne’s muscular dystrophy (Okinaga, 2002). Russian investigators have collaborated internationally to grow macromolecular crystals on ISS since 2001, including genetically engineered human insulin (deposited into protein data bank in 2008), tuberculosis, and cholera-derived pyrophosphatase. The next generation of Russian-Japanese collaboration in this area is the JAXA High Quality Protein Crystal Growth experiment installed in Kibo in August 2009.

A NASA-sponsored experiment in collaboration with the Walter Reed Army Institute of Research and the US Department of Defense investigated how space flight impairs the wound repair process. The CCM- Wound Repair experiment (Cell Culture Module-Effect of Microgravity on Wound Repair: In Vitro Model of New Blood Vessel Development. Principal investigators: S. **30 ESA experiments performed since Columbus commissioning on 2/17/08**

**11 JAXA experiments performed since Kibo commissioning on 5/31/08**
Williams and J. Hoying, 2007) investigated the role of adipose-derived primary endothelial stem cells in tissue repair through a combination of revascularization, new tissue formation, and growth factor recruitment of key repair cells. In this experiment, the on-orbit wound repair model used endothelial stem cells to investigate the formation of blood vessels in microgravity, which is an important factor in tissue wound repair. Initial results indicate successful culture of the cells on orbit, and data analysis is ongoing (P. Quinn, personal communication). Results from this study will aid in the development of a potential treatment to mitigate negative effects of microgravity on wound healing and blood vessel formation during exploration missions, and its Earth-based applications can include helping prevent the loss of limbs and tissue following severe injuries, yielding benefits in both military and civilian applications.

In a related ISS study, CCM-Immune Response (Cell Culture Module- Immune Response of Human Monocytes in Microgravity. Principal investigator: W. Wiesmann, 2007), investigated the use of natural chitosan-based antibacterials in the immune response during space flight. By using a monocyte cell line and examining the gene expression as a result of bacterial based stimuli in the absence and presence of chitosan-based materials, this experiment examined the role of these materials in modulating the inflammatory responses as well as how they are connected to wound healing activity. Preliminary results from CCM-Immune Response show that the cells were successfully cultured and returned to Earth. The monocytes without chitosan treatment did not survive the bacterial infection, whereas the monocytes with chitosan were protected and survived. Preliminary analysis shows the potential for a new pharmaceutical to fight large-scale bacterial infections (P. Quinn, personal communication). Publication of the results is pending, and future flight opportunities for the CCM hardware are planned.

The radiation profile of the space environment has been a focus of the first experiments in Saibo at the fundamental cellular level, especially relative to the promotion of oncogenesis relative to radiation-induced gene damage (Ohnishi et al., 2009). JAXA has performed a series of cellular investigations in Saibo’s first year addressing genetic alterations in immature immune cells using lymphoblastoid cells to detect potential changes on the chromosome as well as p53 (tumor suppressive protein) gene expression after exposure to cosmic radiation (Detection of Changes in LOH Profile of TK mutants of Human Cultured Cells (LOH) - Gene Expression of p53-Regulated Genes in Mammalian Cultured Cells After Exposure to Space Environment. Principal investigators: F. Yatagai and T. Ohnishi, 2008). In an effort to understand how radiation damages genes, and how the genes are repaired, these experiments will detect direct evidence of gene damage and changes in repair as a result of space radiation, and contribute to our overall understanding of the effects of space radiation on our cells and its implication on the future of the radiation countermeasure development for human spaceflight. Another JAXA experiment will investigate cell differentiation and morphogenesis during culture under microgravity, and samples of the developed cells will be returned to Earth for DNA array analysis to identify any unknown genes that may have expressed in the space environment.

**MICROBIAL STUDIES**

A human presence in space is inevitably accompanied by the presence of microbes. The extent of changes to microorganisms in response to space flight conditions is not completely understood; however, spaceflight causes a dysregulation in the immune system (Sonnenfeld, 2002; Pierson, 2005; Crucian, 2009). As astronauts and cosmonauts live for longer periods in a closed environment and use recycled water and air, there is an increase in the potential for negative impacts of microbial contamination upon the health, safety, and performance of crewmembers. Therefore, understanding how the space environment affects microorganisms and their disease-causing potential is critically important for space flight missions and requires further study (Nickerson et al., 2000 and 2004).

One of the most exciting results reported from ISS research is the confirmation that common pathogens change and become more virulent during space flight (Wilson et al., 2007). The space environment has been shown to induce key changes in microbial cells that play a direct role in infectious disease, including alterations of microbial growth rates, antibiotic resistance, microbial invasion of host tissue, organism virulence (the relative ability of a microbe to cause disease), and changes in the genetic expression of the microbe (Wilson et al. 2007; 2008). The targets identified from each of these microgravity-induced alterations represent an opportunity to develop new and improved therapeutics, including vaccines, as well as biological and pharmaceutical agents aimed specifically at treating the infection.

For example, *Salmonella* infection is one of the most common forms of food poisoning in the US. Worldwide, *Salmonella* diarrhea remains one of the top three causes of infant mortality, so a vaccine has the potential to make dramatic improvements in health for developing countries (Carpenter, 2009). Several lines of research on *Salmonella* bacteria have been conducted since 2006 on space shuttle missions flown to the International Space Station. Collectively, this body of work has shown that the virulence of this organism increases in microgravity. One virulence study performed in microgravity was the Microbe experiment (Effect of Spaceflight on Microbial Gene Expression and Virulence. Principal investigator: C. Nickerson, 2006). Performed in September 2006, it allowed investigators to examine changes in *Salmonella typhimurium; Pseudomonas aeruginosa;* and, *Candida albicans.* Initial data from *Salmonella typhimurium* (a leading cause of human gastroenteritis), showed that 167 transcripts and 73 proteins were expressed differently in flight when compared with ground controls (Wilson et al., 2007). This apparent response to the microgravity
environment included widespread alterations of gene expression, particularly that of the RNA-regulatory binding protein Hfq, that increased disease-causing potential. When using a ground-based model of space flight conditions on Earth, it was possible to reproduce the Hfq regulation of some of the Salmonella responses that were observed in flight. Hfq is an RNA chaperone that binds to small regulatory RNA and mRNA molecules to facilitate mRNA translational regulation in a cell’s stress response (Wilson et al., 2007). Hfq is also involved in promoting the virulence of several pathogens (Sittka et al., 2007). Collectively, the spaceflight data suggest that Hfq is involved in globally regulating the \textit{S. typhimurium} increased virulence associated with spaceflight, and that strategies to target Hfq and related protein regulators could potentially decrease risk of infectious disease on orbit and provide novel treatment therapies for \textit{Salmonella} infections on Earth.

Subsequent \textit{Salmonella} spaceflight studies have also indicated that composition of growth media of \textit{Salmonella} plays a role in its virulence (Wilson et al., 2002). The MDRV (Microbial Drug Resistance and Virulence. Principal investigators: D. Niesel, M. McGinnis, B. Pyle, C. Nickerson, 2007) experiment had multiple objectives, including to test the hypothesis that media ion concentrations could be manipulated to prevent the enhanced microbial virulence exhibited during spaceflight. This experiment tested four microbial pathogens for spaceflight virulence: \textit{Salmonella typhimurium}; \textit{Streptococcus pneumoniae}; \textit{Saccharomyces cerevisiae}; and, \textit{Pseudomonas aeruginosa} in varying growth media conditions, including rich media, minimal media and rich media supplemented with custom salts. Initial results demonstrated that \textit{Salmonella} grown in the different media ion concentrations had a direct effect on the virulence of the spaceflight cultures, and that media ion composition can be used to prevent the enhanced \textit{Salmonella} virulence induced in spaceflight (Wilson et al., 2008). In particular, \textit{S. typhimurium} cultures grown in rich media exhibited increased virulence, whereas rich media supplemented with custom salts was sufficient to prevent increased virulence during space flight. (Wilson et al., 2008).

The 2005 NASA Authorization Act designated a portion of the ISS as a National Laboratory (Congressional Budget Office H.R. 3070, 2005). To initiate the laboratory mandate, NASA instituted the National Lab Pathfinder (NLP) missions to provide an opportunity for non-governmental entities to conduct research and development on ISS by way of each remaining space shuttle flight until the shuttle is retired. In 2008, the NLP-Vaccine experiment, sponsored by AstroGenetics, Inc., began research on its first commercial product, a vaccine for \textit{Salmonella}, which yielded progressive results surrounding virulence over a succession of four shuttle flights to ISS. The series of experiments used bacterial knock-out gene targets to progressively identify genes associated with microgravity-enhanced bacterial virulence. The genes that have been identified with this increased virulence have become the basis for formulation of a new vaccine against \textit{Salmonella enterica} which is currently in the planning stages for review and approval for commercial use.

The NLP-V bacterial virulence studies have since been extended to methicillin-resistant \textit{Staphylococcus aureus} (MRSA) on space shuttle flights to the ISS and during the Hubble Space Telescope Servicing mission between March and May 2009. MRSA is a type of bacteria that causes infections that are resistant to the typical antibiotics used to treat illness caused by this organism. According to the Center for Disease Control, it is estimated that MRSA is responsible for more than 94,000 serious infections and 19,000 deaths annually (Klevens et al., 2007). Preliminary results from the STS-119 (March 2009) MRSA experiment show that MRSA (and other microbes flown) exhibit altered phenotypical responses during spaceflight that impacted the growth of MRSA (J. Becker, personal communication). These results are highly valuable for understanding mechanisms used by pathogens to spread disease and also for designing ways to better protect humans in space. With these new insights, similar experiments will continue on the ISS with related sets of pathogens who show promise for potential new development of additional immune-therapeutic products.

The need to monitor the presence and colonization of microbes on ISS has also led to scientific studies on-orbit (Castro et al., 2004). One experiment, SWAB (\textit{Surface, Water and Air Biocharacterization. Principal investigator: D. Pierson, 2006}, initiated during ISS Expedition 13 in 2006, uses advanced molecular technologies to better understand the types of microorganisms that the crew could encounter, their sources, and assess the potential risks to the crew and changes over time in the vehicle. A particular emphasis is on those organisms that would be harmful to the crew but can only be identified by genetic analysis upon return to Earth, and not through the standard culture testing used on ISS. Previous microbial analyses of spacecraft environments only identify microorganisms that will grow in culture, omitting more than 90\% of all microorganisms including pathogens such as \textit{Legionella} (the bacterium which causes Legionnaires’ disease) and \textit{Cryptosporidium} (a parasite common in contaminated water).

A second microbial monitoring study employs the LOCAD-PTS hardware (\textit{Lab-on-a-Chip Application Development-Portable Test System. Principal investigator: N. Wainwright, 2006}, which is a handheld device that enables the crew to perform tests for endotoxins, glucan, and lipoteichoic acid on an interchangeable thumb-sized cartridge with a press of a button. The real-time data analysis provided by LOCAD-PTS hardware offers a drastic reduction in time for detection of fungi as well as gram negative and gram positive bacteria (minutes versus days), thus demonstrating an alternative to the current methods for weekly monitoring of cleanliness, such as swabbing or

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Plant growth experiments have been conducted during early ISS utilization in areas such as biomass production (Musgrave et al., 2005). Research on the ISS that is related to plant growth and plant biology requires special plant growth chambers that are capable of maintaining a healthy environment and atmosphere, delivering appropriate water and nutrients to the plants, and measuring and monitoring the test conditions for later data analysis. The BPS (Biomass Production System. Principal investigator: R. Morrow, 2001.) was an early plant facility designed to provide environmental control subsystems that would support a stress-free growing environment in microgravity in an effort to pave the way for future regenerative life support system research. Another plant growth facility, The ADVASC (Advanced Astroculture System. Principal investigator: W. Zhou, 2001) used a novel air scrubber that employs TiO\textsubscript{2} to remove ethylene from the chamber atmosphere, thus allowing longevity of the produce. The success of this technology on ISS led to the development of an air purifier that is beneficial on Earth by killing 98\% of airborne pathogens that pass through it, including Bacillus anthraci (anthrax), dust mites, molds, and harmful viruses and bacteria such as Influenza A (flu), Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, and Mycoplasma pneumonia (NASA, 2002).

The Lada-VPU-P3R experiment (Validating Vegetable Production Unit (VPU) Plants, Protocols, Procedures and Requirements (P3R) Using Currently Existing Flight Resources. Principal investigator: G. Bingham, 2008) is a jointly-sponsored on-going study between NASA and Roscosmos to advance the technology required for plant growth in microgravity and to research related food safety issues. The experiment uses the Russian Lada plant growth chamber hardware on ISS to grow various plants on orbit that can be returned to Earth and sampled for nutritional content and microbial presence, as well as to determine the psychological benefits for crewmembers in growing plants on orbit. Recent experiments performed on ISS have also shown that plant organisms are capable of normal long-duration growth, development, and reproduction in the space flight environment. Such results suggest that plants could serve as potential candidates in biological subsystem components of on-orbit life support systems (Sychev et al., 2008).

Root gravitropism experiments have also been conducted in ISS in an effort to understand fundamental plant growth in relation to the environment (Kiss et al., 2007 with spaceflight results still in work). The European Modular Cultivation System (EMCS) was first installed on the ISS in 2006, and is a key plant research facility that can also be used to study cells and invertebrate animals. This set of small centrifuges allows for a wide variety of plant growth experiments, with capabilities for microgravity and partial gravity studies. The first experiment to use the EMCS was NASA’s Tropi experiment, (Analysis of a novel sensory mechanism in root phototropism. Principal investigator: J. Kiss, 2006) which allowed for growth of Arabidopsis thaliana (thale cress) seedlings in microgravity under different wavelengths of light and levels of partial gravity. Video recordings of the plant growth were evaluated by investigators, and results yielded plant tissue samples that resulted in RNA analyzed to determine what genes are responsible for successful plant growth in microgravity (Correll et al., 2005). Insights gained from experiments like Tropi can provide fundamental information pertaining to plant gravitropism, as well as lead to sustainable agriculture for future long duration space missions. Additional European and Japanese investigators have used the EMCS facility for experiments, and results are currently pending (Table 2).

Canadian and U.S. investigators will be the first users of the Advanced Biological Research System (ABRS). Planned for launch in late 2009, the ABRS provides two independent growth volumes capable of providing illumination, thermal control, atmospheric constituent control, and relative humidity control for a variety of plants, microorganisms, or other biological specimens. The Cambium experiment (Cambium. Principal investigator: R. Savidge, 2009) seeks definitive evidence that gravity has a direct effect on cambial cells (cells located under the inner bark where secondary growth occurs) in willow Salix babylonica, and the fundamental processes by which plants produce cellulose and lignin (the two main structural materials found in plant matter). Understanding the role of cellulose and lignin production may enable researchers to control these materials in trees on Earth making those trees more suitable for paper production (more cellulose) or construction strength (more lignin).

A second experiment that will use the ABRS is TAGES (Transgenic Arabidopsis Gene Expression System. Principal investigators: R. Ferl and A. Paul), an investigation designed to provide an understanding of physiological processes such as gene expression, metabolism and general plant development that are affected in plant systems exposed to spaceflight.
Table 2. European Modular Cultivation System investigations, past and planned.

<table>
<thead>
<tr>
<th>Experiment Acronym</th>
<th>Experiment Name</th>
<th>Principal Investigator</th>
<th>Dates</th>
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<tr>
<td>GRAVI</td>
<td>Threshold Acceleration for Gravising</td>
<td>Dominique Driss-Ecole</td>
<td>2006-2007</td>
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<td>TROPI</td>
<td>Analysis of a Novel Sensory Mechanism in Root Phototropism</td>
<td>John Kiss</td>
<td>2006-2007, planned 2010</td>
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<tr>
<td>Multigen</td>
<td>Molecular and Plant Physiological Analyses of the Microgravity Effects on Multigeneration Studies of Arabidopsis thaliana</td>
<td>Tor-Henning Iversen</td>
<td>2007-2008</td>
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<tr>
<td>Genara</td>
<td>Gravity Related Genes in Arabidopsis</td>
<td>Eugenie Camer-Diaz</td>
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This experiment will use *Arabidopsis thaliana*, thale cress, with sensor promoter-reporter gene constructs that are expressed under certain environmental stresses, thus rendering the plants as biomonitors (an organism used to determine the quality of the surrounding environment) of their environment using real-time nondestructive Green Fluorescent Protein imagery and traditional postflight analyses. Such genetically modified plants and imaging tools could be used as ‘biosensors’ for characterizing spacecraft environments, and could also be used to further develop and analyze plants that could grow in either lunar or Martian bases (Paul et al., 2008 and Brown 2000).

**INVERTEBRATE STUDIES**

Early utilization invertebrate experiments included investigations focused on fundamental effects of the spaceflight environment on living systems. Scientists from France, Japan, the United States, and Canada designed the ICE-First experiment (International Caenorhabditis elegans Experiment: Physiological Study of Nematode Worms in Weightlessness). Principal investigators: Buckley et al., 2003), which investigated a variety of effects of the space environment on *C. elegans*, including microbiology, muscle protein changes, cell changes, genomics, development, ageing and apoptosis of *C. elegans* in microgravity (Szewczyk et al., 2008). *C. elegans* is a useful model organism for microgravity studies because of its ability to mate, reproduce and develop apparently normally during space flight (Szewczyk et al., 2008). It also has a short life cycle, compact size, and is used as a model organism for human medical pathologies on Earth (Higashitani et al., 2005). Previous studies of mammalian cell lines have shown an increase in apoptosis, which can have a major impact on the physiological processes of living organisms (Cubano and Lewis, 2000 and Kumei et al., 2003). While the radiation environment on the International Space Station (ISS) has been physically measured utilizing techniques and hardware from various on-orbit experiments, the ICE-FIRST study used *C. elegans* as a biological dosimeter as a means to understand the true effects of radiation on animal physiology (Zhao et al., 2005). This study was the first to investigate the effects of the space environment on DNA damage-induced checkpoint apoptosis, which is involved in the maintenance of genomic stability through elimination of cells that have failed to repair DNA lesions as in such cases as spaceflight radiation-induced damage (Higashitani et al., 2005). Results have shown that the occurrence of checkpoint apoptosis in the environment of space proceeds normally, indicating that *C. elegans*, and even humans would be able to eliminate cells that have failed to repair DNA lesions introduced by cosmic radiation during spaceflight.

The FIT investigation (Fungal Pathogenesis, Tumorigenesis, and Effects of Host Immunity in Space. Principal investigator: S. Bhattacharya, 2006), which flew roundtrip to ISS during the STS-121/ULF1.1 mission, addressed a series of human health risks that is associated with space flight. Specifically, this research examined tumor progression and the compounding effect of radiation, and the progression of an immune response in the host in response to a pathogen in space. One component of the experiment investigated changes in ground-based host (*Drosophila melanogaster*) susceptibility to space-flown fungal pathogens (*Beauveria bassiana*) in an effort to assess the effects of the
spaceflight environment (such as radiation and microgravity) on changes in fungal pathogen functions. A second component of this experiment investigated the effects of spaceflight on the growth of cancerous and benign tumors in sensitized genetic lines of *Drosophila* that show an increase in the incidence of tumor formation. Postflight *Drosophila* samples were analyzed for changes in blood cell, hematopoietic organ (lymph gland), and fat body (liver) morphologies in the space-flown *Drosophila*. Post-flight analysis is ongoing and results are expected in the near future.

**VERTEBRATE STUDIES**

In space, as on the ground, vertebrate animals have been used as a valuable component of biomedical research, and can provide insights into problems that could affect the health of long-duration crewmembers (National Research Council, 1998). Several animal facilities designed for space have flown to ISS or stayed onboard ISS during its early utilization period.

The ADF (Avian Development Facility), which is a tool for the study of embryogenesis in space, provided the support hardware that is needed for researchers to better understand and mitigate or nullify the forces of altered gravity on embryo development. The ADF provided incubation of avian eggs under controlled conditions (humidity, temperature, and gas environment) on orbit, and the fixation of the eggs for study while minimizing the effects of launch and landing. Up to 36 eggs in centrifuge carousels could be exposed to simulated gravity of zero-g to one-g in 0.1-g increments. During its flight on space shuttle mission STS-108 to the ISS, the ADF housed two investigations: the ADF-Otolith (Development and Function of the Avian Otolith System in Normal and Altered Gravity Environments. Principal investigator: J. Dickman, 2001) and the ADF-Skeletal (Skeletal Development in Embryonic Quail on the ISS. Principal investigator: S. Doty, 2001) investigations. Because many scientists have suggested that the lack of constant gravity stimulus during space flight produces significant changes in vestibular system function, the ADF-Otolith experiment was designed to study how the absence of gravity during the body's formative stage affects basic neural function in an effort to isolate the major influences on the neurovestibular system. Preliminary results of the ADF-Otolith study show that the inner ear bones and receptors in quail embryos developed differently in microgravity than on Earth, including an increase in otoconia weight by 40% when using the ADF centrifuge at 1-g compared to ground controls (Dickman et al., 2002).

The Animal Enclosure Module (AEM) hardware is used for investigations that study the influence of microgravity on rodent physiology and anatomy. It is a self-contained habitat that provides its occupants with living space, food, water, ventilation, waste removal, and lighting. To determine how microgravity is related to the bone loss that is exhibited in astronauts, the CBTM experiment used the AEM hardware to examine the effects of OPG (Osteoprotegerin, a bone metabolism regulator currently under evaluation by the FDA as a new treatment for osteoporosis) on mouse bone maintenance in microgravity (Commercial Biomedical Testing Module. Principal investigators: T. Bateman and P. Kostenuik, 2001). Mice treated with OPG and exposed to microgravity exhibited no discernable decline in femur elastic strength, and bone resorption was significantly increased as compared to in-flight mice without OPG treatment, who exhibited a 15-20% decline in femur elastic strength and 40-60% decrease in bone formation (Bateman et al., 2004). Mechanical testing data were complemented by serum, messenger ribonucleic acid (mRNA), and histological analyses that indicated a decline in bone formation and an increase in bone resorption in addition to an inhibition of mineralization. Therefore, the results of this microgravity study suggest that OPG mitigated the decline in mechanical strength by preventing increase in resorption and maintaining mineralization. Results of animal studies such as CBTM are important for the development of future human countermeasures against bone loss due to microgravity, which are critical in protecting the health of astronauts on future long duration missions. A subsequent experiment, CBTM-2, (Commercial Biomedical Test Module-2. Principal investigators: H. Han and D. Lacey 2007) examined the effectiveness of an experimental therapeutic in preventing muscle loss in mice that were exposed to microgravity. This was the first time that an experimental therapeutic for muscle loss was investigated in space; an important and significant step in developing a more effective counter-measure to microgravity induced muscle changes. Results on CBTM-2 are currently pending. Although the ADF and AEM hardware have flown as shuttle middeck experiments in the past, they could be modified for use on ISS and future transportation vehicles. A separate set of NASA-sponsored studies using the mouse model in the AEM will investigate the immune system effects of spaceflight, and are planned for 2010.

The Mice Drawer System (MDS) is an Italian Space Agency (ASI) facility which is able to support mice onboard the International Space Station during long-duration missions (from 100 to 150 days) by providing living space, food, water, ventilation and lighting (Mice Drawer System. Principal investigator: R. Canciedda, 2009). MDS will help investigators test the hypothesis that mice with an increased bone density are likely to be more protected from osteoporosis, when the increased bone mass is a direct effect of Osteoblast Stimulating Factor-1 (OSF-1), a protein involved in skeletogenesis (skeleton formation). Following return to Earth, tissue and molecular analysis will be performed by the investigator team of 20 scientists from around the world. Tissue studies will focus on characterization of the bone composition, turnover and structural changes in trabecular and cortical bones (bones with low density and strength and high surface area) of microgravity-exposed and Earth-bound mice. Molecular studies will focus on the modulation of biochemical markers at the protein level. This research is also expected to contribute data to the...
current body of research on microgravity effects on the skeletal, cardiovascular, and immune systems, liver and kidney function as well as other physiological systems through a tissue sharing program. Positive results from this research may advance our understanding of mechanistic changes that occur in various physiological systems after exposure to microgravity and support overall efforts to reduce health risks to crewmembers (Liu et al., 2009).

In 2010, the Japanese Space Agency (JAXA) will deliver a Multipurpose Small Payloads Rack (MPSR) that will house an aquatic habitat. This habitat will enable breeding experiments using medaka or zebrafish as human models for physiological countermeasure development, and can also aid in technology development for advanced life support systems. The habitat is composed of two aquariums that have automatic feeding systems, LED lights to generate day/night cycles, and video cameras for observation.

HUMAN PHYSIOLOGY STUDIES

Human research objectives during early NASA utilization of the ISS were structured by the Bioastronautics Roadmap (NASA, 2005), a peer-reviewed multidisciplinary assessment of all of the possible risks to human health from space travel. Early utilization has provided new understanding of which risks to human health are expected to have the greatest impact on space exploration. Risk-based assessments have been used to restructure NASA research objectives for human research on ISS. In 2008, the National Academies Institute of Medicine performed an external review of NASA’s evidence-based history of human spaceflight research, as well as a review of the 2005 Bioastronautics Roadmap, and provided recommendations for future human research in space (Institute of Medicine, 2008). Following the review, in 2009 the Human Research Program Plan was released (NASA, 2009), which emphasized research tasks that focus on the reduction of the most significant health risks to the crew as a result of exploration missions and increase the knowledge base to inform the development of standards for human support systems. Tasks include basic and applied research to inform crew health and medical standards and guide the development of human health countermeasures.

NASA Human Research Program (HRP) experiments aboard the ISS build from the large body of work that has been collected since the early days of the space program, including a robust set of experiments that was conducted on Skylab, Mir, and shorter-duration shuttle flights (Buckey, 2006). Clinical evidence demonstrated important physiological changes in astronauts during space flight. The HRP, together with ISS Medical Operations, sponsored experiments that study different aspects of crew health, and efficacy of countermeasures for extended-duration stays in microgravity. Since the beginning of the ISS Program, experiments have focused on the human body, including research on bone and muscle loss, the vascular system, changes in immune response, radiation studies, and research on psychosocial aspects of living in the isolation of space. Early utilization insured optimal use of each ISS crewmember as a subject for multiple physiological investigations. The HRP research now focuses on knowledge gaps in our understanding of the physiological changes that are observed during long-duration space flight and research that is aimed at ameliorating the greatest health risks (NASA, 2009). Today’s experiments are designed to provide more detail in the complex changes in crew health.

Today, an integrated set of parameters is monitored on ISS crewmembers. For example, Nutrition (Nutritional Status Assessment. Principal investigator: S. Smith, 2006 and ongoing), which is a comprehensive in-flight study of human physiologic changes during long-duration space flight (Smith and Zwart, 2008 and Smith et al., 2005) and Integrated Immune (Validation of Procedures for Monitoring Crewmember Immune Function. Principal investigator: C. Sams, 2007 and ongoing) sample and analyze participant’s blood, urine, and saliva before, during, and after space flight. These samples are used to study the changes that are related to functions such as bone metabolism, oxidative damage, and immune function. These studies are unique because of the information that they collect on the timing of changes during the course of a space mission. The European SOLO experiment (Sodium Loading in microgravity. Principal Investigator: M. Heer, 2008 and ongoing) is a metabolically-controlled study that investigates the role of fluid and salt retention relative to increases in bone resorption that occurs as a result of a rise in overall sodium balance during spaceflight. When complete, the results of this study will provide important information in the development of bone and muscle countermeasures that could be potentially implemented nutritionally on orbit.

Results of the earliest nutrition and medical monitoring missions have shown that body weight, total bone mineral content, vitamin D concentration, and bone mineral density decreased during flight (Smith et al., 2005). Additionally, antioxidant capacity decreased during flight, leading to increased susceptibility to genetic damage from radiation. These early results led to the development of an expanded ISS Nutrition study that is the most comprehensive long-duration spaceflight study to date. During this expanded study, additional markers of bone metabolism (helical peptide, OPG, RANKL, IGF-1) are being measured to better monitor bone health and countermeasure efficacy. New markers of oxidative damage are being measured (8-iso-prostaglandin F2a, protein carbonyls, oxidized and reduced glutathione) to better assess the type of oxidative insults during space flight. The array of nutritional assessment parameters previously monitored has been expanded to include serum folate, plasma pyridoxal 5'-phosphate, and homocysteine to better understand changes in folate, vitamin B6 status, and related cardiovascular risk factors during and after
flight. Additionally, stress hormones and hormones that affect bone and muscle metabolism are being measured (DHEA, DHEA-S, cortisol, testosterone, estradiol). Based on early observations of Vitamin D deficiency in space, the recommended daily amount of vitamin D has been doubled from 400 IU to 800 IU vitamin D per day for crewmembers on-orbit, and preliminary data from the in-flight blood samples demonstrates that this level of supplementation is maintaining astronauts’ serum vitamin D at optimal levels (Smith et al., unpublished data). This experiment is expected to be completed in early 2010, and another investigation founded on the effects of nutrition supplementing, called Pro-K (Dietary Intake Can Predict and Protect Against Changes in Bone Metabolism during Spaceflight and Recovery. Principal Investigator: S. Smith, planned 2009-2010).

Another health risk to long-duration crewmembers is the development of kidney stones on orbit as a result of calcium loss from bone that is combined with decreased fluid intake in flight (Whitson et al., 1999, Pietrzyk, 1997). Development of a kidney (or renal) stone in an astronaut can have serious consequences since it cannot be treated in flight as it would be on the ground. To better understand the risks to astronauts on long-duration space flights, quantification of renal stone formation potential and recovery is required. The Renal Stone experiment (Renal Stone Risk During Spaceflight: Assessment and Countermeasure Validation. Principal investigator: P. Whitson, 2001) studied the potential development of renal stones in crewmembers and the efficacy of potassium citrate (K-cit) as a pharmaceutical countermeasure, based on its proven ground-based efficacy in treatment for patients who are suffering from renal stones. Results from this investigation are expected for publication later in 2009 (Whitson et al., 2009 in press).

Several of the early experiments have led to new experiments, testing details of observations or pursuing new questions that were raised by early results (Figure 2). Early research results include characterization of astronaut bone loss under current exercise countermeasures, and the recovery profile of bone mass after return to Earth (Sibonga et al., 2007; Lang et al., 2006). The Subregional Bone (Subregional Assessment of Bone Loss in the Axial Skeleton in Long-term Space Flight. Principal investigator: T. Lang, 2001) investigation was one of the first HRP investigations to begin on ISS and studied the effect of re-exposure to Earth's gravity on the bones of crewmembers following long-duration space missions. The investigation showed that on ISS, bone mineral density was lost at an average rate of about 0.9% per month in the lumbar spine and 1.4% per month in the femoral neck (Lang et al., 2004). For comparison, a post-menopausal woman experiences loss of bone mineral on the order of 1% per year (Sirola et al., 2003). This investigation gave insight into the process of bone loss because it was the first study to differentiate the loss in the cortical bone (the outer part of the bone) and the trabecular bone (the inner parts of the bone). For example, in the hip, losses of mass in the cortical bone averaged around 1.6-1.7% per month whereas losses in the trabecular bone averaged 2.2-2.5%/month (Lang et al., 2004; Keyak et al., 2008). As potential countermeasures to such spaceflight-induced bone loss, current and future ISS research includes modification of crew diet on-orbit, as well as an investigation of the use of the bisphosphonates alendronate and zolendronic acid, antiresorptive agents that block breakdown of bone used to treat osteoporosis and other bone turnover disorders, in combination with on-orbit exercise (Bisphosphonates as a Countermeasure to Space Flight Induced Bone Loss. Principal investigators: A. LeBlanc and T. Matsumoto, 2008 and ongoing). If shown to be an effective countermeasure to space flight-induced bone loss, bisphosphonates or other antiresorptive agents could help prevent several bone-related problems for crewmembers on ISS and on future long-duration missions, including the associated possibility of developing renal stones associated with bone loss during or after space flight.

Another collaborative set of experiments measures and monitors body fluid shifts; electrocardiograms are collected to monitor the heart function and vascular health of the crew. The crewmembers periodically test their pulmonary function, and keep journals that are used to quantitatively analyze their response to isolation. A recent ISS investigation, Integrated Cardiovascular (Cardiac Atrophy and Diastolic Dysfunction During and After Long Duration Spaceflight: Functional Consequences for Orthostatic Intolerance, Exercise Capability and Risk for Cardiac Arrhythmias. Principal investigator: B. Levine and M. Bungo, 2009 and ongoing) takes advantage of the recent increase in on-orbit crew time resources to investigate long-duration spaceflight-induced cardiac atrophy and associated functional consequences in space and upon return to Earth. When combined with current on-orbit cardiovascular investigations such as the European CARD experiment (Long Term Microgravity: A Model for Investigating Mechanisms of Heart Disease with New Portable Equipment. Principal investigator: P. Norsk, 2009 and ongoing) and Canadian CCISS experiment (Cardiovascular and Cerebrovascular Control on Return from ISS. Principal investigator: R. Hughson), these studies will provide multi-national data sharing opportunities to allow the most comprehensive analysis of spaceflight-induced cardiovascular changes to date.

Future research is also ensured. Extra biological samples are collected for the Repository investigation (National Aeronautics and Space Administration Biological Specimen Repository. Principal investigator: K. McMonigal, 2007 and ongoing), which is a long-term archive of critical biological samples that are collected from ISS astronauts, for future analysis when new tools and methods can be used and new questions are posed. Because specimen collection is ongoing, solicitations for investigations using the collected samples are planned around the year 2017.
USE OF ISS AS A NATIONAL LABORATORY

Future use of the International Space Station has been expanded through the designation of the ISS US segment as a National Lab by Congress in the NASA Authorization Act of 2005 (Congressional Budget Office H.R. 3070, 2005). This opens up the ISS for use by other government agencies and commercial interests to perform scientific and engineering studies that further their objectives. Two different areas are currently being pursued under the National Lab approach related to biological sciences. First, a series of Pathfinder missions is being flown to demonstrate the benefits of ISS for biological research. These Pathfinder missions are flying a series of cell and vaccine experiments that are yielding promising results as mentioned above. The second area is a cooperative effort with the National Institutes of Health (NIH). In cooperation with NASA, NIH announced a 3-year announcement of opportunity to fly experiments that will further the NIH mission of improving life on Earth. Experiments were requested in the cellular and molecular biology areas. In order to maximize the likelihood of success for the execution of the experiments, NASA sponsored a pre-application meeting with NIH to introduce the prospective applicants to various implementation partners. These implementation partners are companies or NASA centers that have developed and flown space flight hardware geared towards cellular and molecular biology previously. By using existing hardware and experienced hardware developers teamed with the NIH investigators, the cost of experiment development is greatly reduced. This partnership between investigators and implementation partners allows the NIH investigator to focus on the science aspects of the work while the implementation partner ensures all of the processes required to certify and prepare an experiment for space flight are completed. Ideally, this model will provide a simple interface for other government agencies and commercial interests to fly experiments to the ISS. The US Department of Agriculture has also signed a Memorandum of Understanding with NASA for future use of ISS, six Space Act Agreements have been executed with commercial partners interested in using the ISS, and additional agreements are under development.
Publications of ISS scientific results, which is one metric that is used to measure success of the research program, have shown steady increases in all scientific research areas on the ISS (Figure 3). Through 2008, we have identified roughly 200 publications directly resulting from research on the ISS. These publications can be found online at www.nasa.gov/iss-science, and in NASA’s recent Technical Publication detailing all USOS ISS research through Expedition 15 (NASA, 2009). Early ISS research results have indicated that although human research investigations take longer to complete, the time to publication following completion is relatively short (less than 1 year) (Tate et al. 2007). Investigations in human research must be carried out using statistically significant numbers of human subjects in order to draw appropriate conclusions. With the exception of observational studies, crewmembers can generally participate in, at most, six different investigations per expedition (Rhatigan et al. 2005). The temporary grounding of the Space Shuttle fleet caused a decrease in the ISS crew size to two people, which limited the rate at which human research investigations were completed on the ISS. However, the increase of crew to three in July 2006 and another increase to six in May 2009 have been important factors for obtaining sufficient numbers of human subjects for investigations. Physical and biological investigations have a relatively short average completion time (less than 1 year), but may take longer to publish results; this may be due to the number of analyses and models to be completed following return to Earth. Technology development investigations on average take a little more than a year to complete but have publications in less than a year; this is due to the design of most of the investigations in this category that allows for downlink of data from the ISS with simultaneous data analysis (Tate et al., 2007).

The world’s space agencies share common goals for ISS utilization. While the various international partners may emphasize different aspects of research to achieve their goals in the use of ISS, they are unified in several important overarching goals. All partners recognize the importance of leveraging the ISS as an education platform to encourage and motivate today’s youth to pursue careers in math, science, and engineering. The ISS partners also share the goal of advancing knowledge in the areas of human physiology, biology, material and physical sciences, and translating that knowledge to health, socioeconomic, and environmental benefits on Earth. Experiments such as MEPS (Microencapsulation Electrostatic Processing System, Morrison), JAXA-GCF (Japan Aerospace and Exploration Agency - Granada Crystallization Facility High Quality Protein Crystallization Project, Tanaka et. al), Microbe (Effect of Spaceflight on Microbial Gene Expression and Virulence, Nickerson et al.), and NLP-Vaccine (National Laboratory Pathfinder-Vaccine, Hammond) are just a few success stories that will come from the use of the unique international laboratory that is the International Space Station.

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