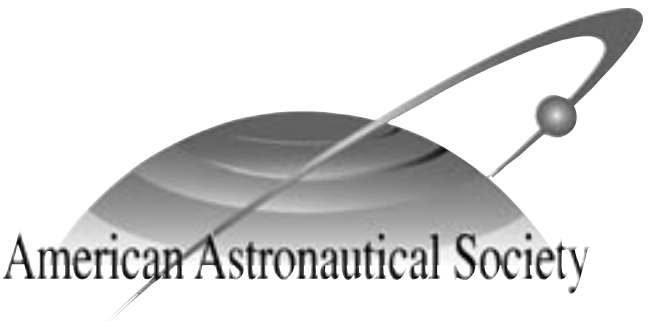

Isolation

NASA Experiments in Closed-Environment Living

Advanced Human Life Support Enclosed System

Edited by: Helen W. Lane, Richard L. Sauer, and Daniel L. Feeback

Volume 104

SCIENCE AND TECHNOLOGY SERIES

A Supplement to Advances in the Astronautical Sciences

Published for the American Astronautical Society by
Univelt, Incorporated, P.O. Box 28130, San Diego, California 92198

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Acknowledgements

The editors of this volume would like to acknowledge many people for their contributions, including all of the scientists and engineers which made the chambers studies possible with the support of the Office of Bioastronautics. This list includes Leonard Nicholson, George Abbey, John Rummel, Chuck Sawin, and Will Ellis. Additionally, the editors would like to recognize Al Behrend for his contributions as the director of the Advanced Life Support Program. The crewmembers of course made an invaluable contribution in time and effort for these studies. We also wish to thank those who made this book possible. Hai Nguyen and Jennifer Muslin for editorial and organizational support, Donna Baumer and Betty Conaway for production capabilities, and Sue McDonald for her publishing expertise.

Note: Each study independently assigned the crewmembers subject numbers. A single crewmember is not the same subject number throughout this book, but rather may be referred to differently throughout the publication.

Front cover: Artist's conception of the Space Shuttle docked at the International Space Station orbiting the Earth. Image of the Shuttle and Space Station created by Marco Zambetti. Photos of the Earth, the Moon, and Mars were compiled from the NASA archives, arrangement done by Sean Collins.

Back cover photo: S96-10208 (05/15/96) – Portraits of the Early Human Testing test subjects outside of the building 7 20-foot chamber – standing on the catwalk outside of the chamber are the Phase II prime crew of Doug Ming, Katy Hurlbert, Patrick O'Rear and John Lewis as well as the backup crew of Nigel Packham, Stephanie Ayers, Terry Tri and Fred Smith.

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1.1

Isolation and Integrated Testing: an Introduction to the Lunar-Mars Life Support Test Project

Dave R. Williams, M.D., F.R.C.P.

"Present technologies on the shuttle allow for stays in space of only about two weeks. We do not limit medical researchers to only a few hours in the laboratory and expect cures for cancer. We need much longer missions in space – in months to years – to obtain research results that may lead to the development of new knowledge and breakthroughs."

– Dr. Michael DeBakey, Chancellor Emeritus, Baylor College of Medicine, U.S. House of Representatives, June 22, 1993

"One test result is worth one thousand expert opinions."

– Wernher von Braun

Spectacular advancements in life on Earth can be made with the knowledge gained through research on long-term space flight. In order to achieve long-term space flight, however, there is much we need to determine. We began these chamber studies to develop technologies, methodologies, techniques, and the knowledge needed to make such flight possible. Before efficient long-term stays in space can occur, NASA must determine how to best solve the issues related to a closed living environment; these chambers studies were a test bed for such potential solutions.

Space flight has progressed rapidly from the territory of dreams, to tentative steps of exploration, and now to an established endeavor and pursuit. We have experienced great success sending humans into space, and we have currently made substantial headway toward building and utilizing the International Space Station (ISS), where humans can remain on orbit for months at a time. This platform in space will be instrumental in gathering information on the human body and its response to the

microgravity environment. Important studies of materials and physical sciences will be conducted, allowing us to examine how matter behaves in the absence of a dominant gravity vector. Implicit in the Space Station's viability, and of paramount importance, is the testing of technologies that ensure the health, safety, and well being of the crew. These technologies create the conditions which allow humans to survive in space – the provision of clean air, water, food, and waste removal.

The primary goal of the Lunar-Mars Life Support Test Project (LMLSTP), conducted from 1995 through 1997 at the NASA Johnson Space Center, was to test an integrated, closed-loop system that employed biological and physicochemical techniques for water recycling, waste processing, and air revitalization for human habitation. As an analogue environment for long-duration missions, the conditions of isolation and confinement enabled studies of human factors, medical sciences (both physiology and psychology), and crew training. The results of these studies provide a wealth of important data not just for Space Shuttle and ISS missions into space, but also for other populations who experience similar conditions – Arctic and Antarctic expeditioners, submariners and crews of other submersibles, and other ground-based test beds – as stressed by the following:

> *"If large numbers of people are to spend extended periods of time isolated and confined in space, the goal must be to discover or to establish positive conditions under which psychological functioning and social life can prosper and flourish."*
>
> – Philip Robert Harris, *Living and Working in Space* (3)

Research on closed-loop human life support began in the 1950s, with studies of oxygen regeneration using algae. Interest became more focused in the 1970s when the success of the emerging space program called for support of future long-term missions. NASA has since developed plant-based systems to yield food, regenerate oxygen, and process waste into usable products. The primary goal of ground-based test beds such as the LMLSTP is to test integrated, complex systems that support life and to qualify them for life support during space flight.

The LMLSTP studies were a major accomplishment and met the goals of NASA's Advanced Life Support (ALS) Program. Air and water systems were monitored for efficiency of function and for microbiological content, crew members were monitored for health and performance, and medical systems and technology were tested. In conducting these studies, the LMLSTP met the ALS Program goals of 1) providing self-sufficiency in advanced life support for

productive research and exploration in space, for benefits on Earth and 2) providing a basis for planetary exploration. More specifically, the data also met the research goals of the Space Human Factors Program (1):

1) to expand knowledge of human psychological and physical capabilities and limitations in space through basic and applied research tests and evaluations
2) to develop cost-effective technologies that support integrating the human and system elements of space flight
3) to ensure that mission planners use space human factors research results and technology developments to increase the probability of mission success and crew safety, and
4) to make NASA technology available to the private sector for Earth applications and to use new technologies developed by private industry where appropriate.

Indeed, the information gathered from these studies may have far-reaching applications for other populations. Factors that overlap between space crews and analogous populations (4) are workload, exercise, medical support, personal hygiene, food and provisions, group interaction, habitability of the 20-foot chamber environment, external communications, privacy and personal space, and recreational activities, to name a few. Submarines serve as both a platform for closed-environment living and an environment most likely to benefit from the chambers studies (note: submarines use only physicochemical life support systems). Nuclear-powered submarines can operate submerged for months at a time. Even more than in conventional submarines, the physical and psychological stamina of the crew on nuclear-powered submarines becomes a crucial factor. They must also deal, while on patrol, with being largely isolated from the outside world, including their families, for long periods of time. Similarly, Arctic and Antarctic personnel are isolated for nine to 12 months. Their means of living are self-sustaining, and they are dependent on technology for survival. Moreover, personnel at military outposts and remote oil rigs are also populations where group interactions and confinement adaptations play a crucial role in the success of the project.

The initial phase of this project began as a study of air regeneration using wheat plants, and enough oxygen was generated to support one experimental subject. As Phases II, IIa, and III of the LMLSTP continued, the systems grew increasingly more complex and interdependent. These later phases achieved success in providing life support systems for four crewmembers. The crewmembers provided plentiful data on the human factors evaluated in the project. As a result, some generalizable lessons were learned, such as the kinds of personalities that compose a good crew, complex dynamics that affect group interaction, the kinds of problems (for example, stress) that can be prevented or mitigated, and the kind of countermeasures that would make life easier for people in isolated environments. However, there are some issues unique to space travel which must be addressed.

While in space, the human body experiences a multitude of adaptations in microgravity. There are many systemic responses to the reduced gravity, such as decreases in bone and muscle mass and shifts in the cardiovascular system. The body also experiences changes in the neurosensory and neuromotor systems. In addition, the stresses of the mission and conditions of isolation elicit behavioral changes in crewmembers. Research is ongoing to better understand these adaptations and to mitigate these changes.

Since the chamber studies did not have microgravity conditions that the space crews experience, research focused on the parameters of isolation and confinement. For example, sleep studies were performed as the crew completed their chamber stay to evaluate adaptation to a situation of confinement with its accompanying stresses. The immune system was also monitored, specifically for the occurrence of reactivation of latent viruses. Previous research in space and analogous crews has shown that the stress of confinement can affect the immune system, and the results of this LMLSTP experiment confirm this. This emphasizes the important point that these studies provide data that are useful for space flight crews as well as for populations that experience similar conditions.

In addition to challenges to the human body and mind, the space flight environment poses challenges to an exploration mission; the spacecraft system is a tool to overcome those challenges and allow humans to carry out their mission safely and efficiently. To ensure the safety, productivity, and success of an exploration mission, designers will have to facilitate human performance by creating a system that responds effectively to the challenges of the space flight environment. In the LMLSTP, the standard research and technology advances were validated as an integrated system, well beyond the simplicity of isolated experiments. The support technologies involved were mature enough for integrated testing, and the following tenet emphasizes the importance of this state:

> *"Never underestimate the complexity of closed systems, or the importance of testing in closed systems. Integration and interaction with other systems cannot be ignored in the design and operation of spacecraft life support systems. To badly quote Newton, "for every action there is an equal and opposite reaction." For example, coatings, off-gas products, and trace metabolic products have triggered entirely unexpected responses in a flight environment.*"
>
> – John Graf et al., *Basic Tenets for Designers of Life Support Systems for the Space Environment* (2)

These series of integrated tests – human and systems – have demonstrated the quality of data that can result from a test bed such as this. Research is ongoing to find better, more efficient, and self-sufficient systems for advanced life support,

such as the growth of foods in space, bioregenerative systems (which provide food and oxygen, remove carbon dioxide, and generate clean water), further research on physicochemical systems, and further studies in habitability and human factors. There is a strong need for a dedicated, long-term facility in which to test and study large-scale bioregenerative planetary life support systems and to integrate more disciplines and components of space flight – training, mission operations, automation and robots, etc. These long-term test beds will continue to produce a wealth of information that will benefit not only the space explorers who depend on these technologies, but also Earth-bound populations who experience similar isolated conditions. In partnership with research conducted in space, the test bed research will yield knowledge that can be applied to further advance our viability in space. With this perspective, the vision that we conceived decades ago of long-term space flight becomes an even greater probability – and soon perhaps even a reality.

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1.2

Introduction Overview of the Report

Helen W. Lane, Ph.D.

SUMMARY

The United States' National Aeronautics and Space Administration (NASA) is dedicated to research and exploration, utilizing the unique qualities of space flight. Specifically, a major mission of NASA is "to open the space frontier by exploring, using, and enabling the development of space and to expand the human experience" (4). As flight time increases and we build outposts such as the International Space Station (ISS), it becomes imperative for both safety and cost to minimize consumables and increase the autonomy of the life support system. By recycling air, water, and other consumables, a closed system can be developed that will increase productivity by reducing mass, power, and volume necessary for human support. This requires NASA to invest in high-leverage technologies.

Technologies emerging from this effort also have wide-ranging applications on Earth. These same technologies can potentially help urban planners faced with increased development and a limited water supply. Earth needs include improved technologies to decrease air and water pollution, an ability to recycle urban water, and improved energy efficiency. Additionally, maintaining good air quality in today's airtight homes, office buildings, and industrial sites is of great concern to public health. There is a continuing need for new technologies to mitigate polluting emissions outdoors from a wide array of sources. The air revitalization technologies developed by NASA life support for extended duration space flight have already been applied to ground-based systems.

As part of this effort, NASA's Advanced Life Support Project develops and tests life support technologies and systems to enhance the success of human space exploration. This volume provides a summary of the engineering, life sciences, human factors and performance, and medical accomplishments during the four closed-chamber tests conducted at NASA/Johnson Space Center, Houston, TX, between 1995 and 1997. This introduction will provide a description and history of the tests. For the purposes of NASA's discussion, the term "closed chamber tests"

refers to studies involving a well-trained team who live and work in an enclosed limited volume, or chamber. The only interactions with the outside of the chamber are through communication tools such as telephones, computers, and video. In all these studies, there is a serious attempt to reduce replenishment of supplies from outside the chamber and to recycle air, water, and other consumables and wastes to the greatest extent possible. This provided a step toward having a high fidelity analog to an autonomous operation necessary for space flight beyond low-Earth orbit. There is a limited exchange of personal items and no exchange of crew members. The crewmembers are responsible for all internal maintenance and repairs.

The term " life support" refers to the sum of the engineering, medical sciences, and technology utilized to provide air, water, food, thermal control, trash and solid waste management efficiently and effectively. To significantly reduce outside replenishment, NASA has used these chamber tests to focus on finding ways to close off life support systems from the general population, and amenities, and allow the crews to be more autonomous. This promotes development of recycling technologies especially for air and water.

Both the former Soviet Union and the United States utilized closed chamber tests to develop their spacecraft life support systems. These tests included ground-based simulations of the Apollo missions (personal communication, Dr. Joe Kerwin) and Skylab missions (1). General ground-based closed-chamber tests similar to the ones discussed in this volume were completed in 1970 by McDonnell Douglas Astronautics Company, Western Division, Long Beach, California (2). The former Soviet Union had similar ground-based simulations as well as a biologically-based system (3).

Early Ground-Based Chamber Tests – Regenerative Life Support Study by NASA Langley Research Center

An operational 90-day manned test of a regenerative life support system, completed in September 1970, was conducted by the McDonnell Douglas Astronautics Company, Huntington Beach, CA. The focus of that test was an integrated life support system and built on a previous 60-day test in the same chamber. The test chamber was 12 feet in diameter and 40 feet long, with 4,100 cubic feet, a 160 cubic foot airlock, and 2 smaller airlocks which were 18 inches in diameter. The chamber operated at 10 psi with 4 male volunteers. The tested life support systems included air, water, waste, and food. The water and oxygen were totally recycled with no resupply. All expendables, food, chemicals, filters, and spare parts were stored at the beginning of the study with no pass-ins. Complete mass balance was determined and each system and its hardware were evaluated. Environmental monitoring assessment

included organic, microbiological and inorganic monitoring of the chamber and water, along with air quality including trace contaminents. Water was analyzed for chemical and microbiological content. Interestingly, $^{238}PuO_2$ was used to produce heat for the water recovery subsystem, and crewmembers routinely handled this system so crew radiation exposure was monitored. Total iodine at 6 ppm was used to prevent microbiological growth in the recycled potable water. All the various monitoring hardware was evaluated for performance. Aspects such as power usage and maintenance were studied. There were several biomedical studies including sleep, exercise-metabolic, medical crew status such as vital signs, body water pools and plasma volume changes, lean body mass, and psychomotor performance with short-term memory studies. Also, the acceptability of the habitat, crew training evaluations, and computer assistance scheduling were evaluated.

A complete report was provided (5) giving the details of all the evaluations. This was a successful study that provided a great deal of understanding of life support systems performance at a total atmospheric pressure of 10 psi, along with important information about crew health in such an enclosed life support system. This study provided confidence that these types of studies could be conducted with safety and reliability of the engineering for life support.

Apollo Ground Based Tests

In order to prepare to fly to the moon, two-closed chamber tests were completed. Dr. Joe Kerwin, a Skylab astronaut, served as a simulation crewmember during these Apollo tests. The following is an excerpt from his report of those tests.

> ***Personal Report by Dr. Kerwin , Skylab Astronaut***
>
> *Manned vacuum chamber tests were carried out at Johnson Space Center on two Apollo Command/Service Modules (CSM's), Spacecraft 008 and 2TV-1, in preparation for the actual flights. These tests were operational; that is, their purpose was to test under realistic conditions fully developed systems, rather than to advance the state of the art. The first version of the Apollo spacecraft had a pressure of 5 psi with 100 percent oxygen. The "Achilles' heel" of this system was that before launch the cabin was to be purged with pure oxygen at slightly higher than sea-level pressure (about 15 psi), because the spacecraft reduced its pressure to 5 psi during launch, and air at 5 psi has insufficient oxygen to support human life. But pure oxygen at 15 psi provides an environment conducive to fire, which was the cause of the Apollo fire. The initial or "launch" atmosphere was changed for the second test, which had 60% oxygen and 40% nitrogen at 15 psi. The cabin pressure was allowed to equilibrate to 5 psi once the test began. Replenishment during flight was with oxygen.*
>
> *The CSM was one of two manned spacecraft required by the lunar land-*

ing program. In it the crew launched, traveled to lunar orbit, and returned to earth. The Lunar Module carried two of the three crewmembers to the lunar surface and back into lunar orbit. The CSM life support system did not attempt to recycle oxygen, water or any consumable. Although there was no possibility of resupply on flights to the moon, and it was imperative to keep system weight to the absolute minimum, the missions were too short (less than two weeks) to make recycling systems feasible. The CSM was small, with only 310 ft^3 of pressurized volume.

The first full up test took place October 26 until November 1, 1966. In the first major test there were numerous and significant failures, both large and small. The urine dump system froze, the suit leaked cabin air into the suits, coolant pumps failed repeatedly, excessive moisture condensed on the inside of what became a very cold spacecraft, and numerous valves failed and/or broke. All of the fuel cells flooded and two failed immediately, the third after four days. The test finished using ground power. During this test the crewmembers wore a biomedical vest and exercised with an ergometer.

Pump down for second test commenced at 1430 hours on June 16, 1968, and the spacecraft was, as the crew invitation said, launched into the world's first constant-latitude orbit at 58 feet mean sea level, the altitude of Johnson Space Center, Houston, Chamber A, Building 32. Repress was complete at 0630 on June 24, 184 hours later. This test was very successful and gave data to support the Apollo program. Both cabin and suits provided an acceptable environment, with a robustness that later helped save the crew's life when called upon in Apollo 13.

Some biomedical procedures were completed including before and after flight cardiovascular assessment (exercise tolerance) and body fluid analysis; atmosphere sampling for trace contaminants; potable water servicing and testing to assess the adequacy of the chlorination scheme; dietary assessment; and those portions of the hematology, microbial monitoring and immunology tests which were approved for subsequent flights. These were done to gather baseline data against which to assess flight changes. It is noteworthy that, in the 33 years subsequent to this test, in both ground based and flight experiences, it has never been concluded that the reduced cabin pressure had any significant effect on the crew's physiological response or other biological systems such as microbiological treatment of water.

The Skylab Medical Experiments Altitude Test (SMEAT)

By the 1970s, NASA was committed to an orbiting space laboratory, Skylab. In order to prepare for these intense biomedically-focused space flights, a ground-based simulation was completed. The Skylab Medical Experiments Altitude Test (SMEAT), conducted at NASA/Johnson Space Center, was a 56-day ground-base simulation of a Skylab mission. This test provided a "full-up dress rehearsal" for the 3 Skylab biomedical complement of studies. Unlike the 90-day Langley Research Center test, the three crewmembers were astronauts. The atmosphere was similar to Skylab at 5 psi, 70 percent oxygen and 30 percent nitrogen. The goal of this test was to simulate as much as possible a Skylab 56-day mission including science activities, training, data collection, crew issues, flight equipment, and learning about medical operations and crew health. This test was conducted with two-floor configuration in the same 20-foot diameter and height chamber utilized in three of the four tests reported in this volume. The SMEAT chamber had similar water and waste management systems as Skylab, including a collapsible shower, and ability to collect urine and stool samples. The lighting and food systems were identical to those on the Skylab station. Air monitoring hardware was tested including a carbon dioxide/dew point monitor. A cryotrap system was used to sample gas returning to the air conditioning return duct. This provided data on 25 compounds found in the atmosphere. Tests of the urine system demonstrated specific problems, such as being too small, resulting in redesign for Skylab flight systems. Life sciences experiments conducted were also similar to those for Skylab: lower body negative pressure, vectorcardiogram, bone mineral levels, metabolic activities, blood and urine parameters, crew microbiology, oral health, habitability/crew quarters, crew training, as well as specific hardware tests. With this test, there was increased confidence that the Skylab biomedical research could be completed successfully: A correct conclusion.

The Next Generation of Life Support Studies

This volume provides a summary of the results of individual projects from the four chamber studies conducted from 1995 through 1997. The outcomes from the many chamber tests conducted provide a good model for future long-duration space flight and operational experiences with the technologies and protocols that will be used in space flight. Furthermore, these operational tests demonstrate technologies that may reach the terrestrial commercial market. The four tests described in this volume had very different specific advanced life support objectives as described in the overview chapter of this report. In general, these tests were focused on

engineering life support systems with humans-in-the-loop. Although some aspects of human activity can be simulated by metabolic simulators, the integration with humans eventually must be tested. Metabolic simulators are not able to fully test the advanced life support systems.

The book begins with the crewmembers' and medical officer's observations. It is clear that the crewmembers were committed to providing a very successful set of tests. These crewmembers were selected from volunteers, and represented engineering and life sciences specialties needed to conduct the tests. A medical officer was assigned to evaluate the crewmember's health before, during and after the test. As seen by the description, there was constant interface with the crewmembers and their families throughout the test to ensure their physical and psychological health.

Dr. Henninger, the chief scientist for these 4 tests, provides an overview to the life support studies. This is followed by a section on human factors/behavior and performance. Within the section is a description of the internal configuration of the 20-foot chamber to make it an effective tool for life support studies, and to provide the crew with a safe habitable environment. Under the environmental section, there is a description of air, water, and microbiological monitoring results. The food system was different for each test and it is described also. The two last sections summarize the biomedical experiments and training studies completed. These four tests involved many disciplines, aerospace companies, and university support.

Phase I

The goal of the Phase I test was to demonstrate the use of higher plants to provide the air revitalization to meet the oxygen requirements of a single test subject. A primary objective of the test was to demonstrate how a wheat crop could continuously provide the CO_2 removal and O_2 generation required for a single human test subject for 15 days. Air quality was determined through out the study including trace contaminant control. The test also demonstrated that plants could be utilized to control the O_2 and CO_2 concentrations in human-habited systems.

Phase II

The Phase II test was a 30-day, four-person test completed in the 20-foot diameter chamber. The purpose of the test was to verify performance of integrated physicochemical (P/C) life support system technologies for air revitalization, water recovery, and thermal control as an integrated life support system, capable of sustaining a crew of four for 30 days. This required demonstrating the air revitalization system, a water recovery system to successfully produce potable water from hygiene water (shower, hand wash, laundry), urine, and humidity condensate, and an effective active thermal control subsystem. Psychological, human factors, and studies on the microbiological environment were conducted.

Phase IIa

The Phase IIa test was a 60-day, four-person test completed with U.S.-provided life support subsystems functionally similar to that on the International Space Station. The purpose of the test was to verify integrated performance of baselined ISS life support technologies for air revitalization and water recovery and to provide additional integrated test data to the Advanced Life Support Test Project. ISS-like hardware representing significant advances in state-of-the-art life support capabilities emulated the flight hardware and provided integrated data to the ISS Program. All life support systems were monitored and biomedical tests were completed with the four crewmembers.

Phase III

The final test was the Phase III test which incorporated the use of biological systems and physicochemical life support system technologies to continuously recycle air, water, and part of the solid waste stream generated by a four-person crew for 91 days. In the chamber, lettuce was grown and bread from wheat grown in a nearby chamber was baked into bread. These additional activities taxed the air revitalization system. This 91-day test had the most complete set of biomedical studies with a closed ground-based system, of any closed chamber tests. Data from these tests have been used to provide better habitability and health to the crew systems for ISS.

These four tests successfully accomplished many engineering, medical, and scientific goals: those accomplishments are reported in this volume (see Table 1.2-1).

Table 1.2-1 *Timing of the experiments which took place during the Lunar-Mars Life Support Test Project*

Chapter	Phase I (15-Day)	Phase II (30-Day)	Phase IIa (60-Day)	Phase III (91-Day)
2.1 Test Phases and Major Findings	x	x	x	x
2.2 Chamber Studies Medical Care Overview: Officer's Report	x	x	x	x
3.1 Architecture		x	x	x
3.2 Habitability: an Evaluation		x	x	x
3.3 Acoustic Noise			x	x
3.4 Assessment of Sleep Dynamics in a Simulated Space Station Environment			x	x
3.5 Operational Psychology		x	x	x
3.6 Spaceflight Cognitive Assessment Tool				x
3.7 Sociokinetic Analysis as a Tool for Optimization of Environmental Design			x	x
4.1 Air Quality	x	x	x	x
4.2 Water Chemistry Monitoring	x	x	x	x
4.3 Microbiology	x	x	x	x
4.4 Crew Food Systems		x	x	x
5.1 Nutritional Status Assessment			x	x
5.2 Exercise Countermeasures Demonstration Project			x	x
5.3 Reactivation of Latent Viruses			x	x
5.4 The Influence of Environmental Stress on Cell-Mediated Immune Function				x
5.5 Physiological Effects of Iodinated Water on Thyroid Function		x	x	x
6.1 Telemedicine				x
6.2 In Situ Training Project			x	x

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1.3

The Lunar-Mars Life Support Test Project: the Crew Perspective

Nigel J. Packham, Ph.D.
Commander, 91-Day Test

SUMMARY

A series of four tests of advanced life support (ALS) systems were performed from 1995 to 1997 at the National Aeronautics and Space Administration's (NASA) Johnson Space Center. Human crews of up to four persons spent up to 91 days inside closed environmental chambers. Originally called the Early Human Testing Initiative (EHTI), the project was later renamed the Lunar-Mars Life Support Test Project (LMLSTP). A total of over two person-years of confined testing were performed during EHTI and the three phases of LMLSTP. The first test (EHTI Phase I) was designed to evaluate the performance of plants and their ability to provide the air revitalization function of an advanced life support system. In 1995, one crewmember spent 15 days in a small chamber (approximately 2.5 by 2.5 by 2.0 meters) breathing the oxygen produced by 22,000 wheat plants growing in an adjacent chamber. In later years, the three phases of LMLSTP were performed in a larger (6-meter diameter) chamber housing four crewmembers at a time. With each phase, the complexity of the systems under test was increased. Water recycling, air revitalization, and waste processing technologies were investigated, employing both biological and physicochemical approaches. As the duration of the tests increased, finally approaching projected mission durations for individual crews on board the International Space Station (ISS), the crewmembers also became a resource for investigators in the areas of human factors, medical sciences (both physiology and psychology), and crew training. Rather than going into detail about the life support or experiment approaches of each test, this report will focus on the unique aspect of the test from within the closed chambers from the crew's perspective.

General Background

Crewmember volunteers were solicited from within the ALS community, initially focusing on scientists and engineers within the Johnson Space Center. For later tests, the entire ALS community (the four prime NASA centers engaged in

ALS activities, academia, and industry) was seen as a source of viable crew members. Although not a primary objective of the series of tests, one of the benefits of using scientists and engineers rather than flight-certified astronauts was that it provided those selected individuals with valuable insight in how to design, build, and test better life support system components and subsystems. Science disciplines ranged from chemistry to food science, microbiology, and soil science. Several engineering disciplines were represented including mechanical, electrical, aerospace, and chemical.

Crews were of mixed gender (except the single crewmember in EHTI Phase I). In the LMLSTP, crews were made up of a commander, two life support systems technical experts, and an individual who coordinated science activities within the chamber. The commander's duties involved representing the crew with outside entities, including management, visitors, and education/outreach groups. One of the more challenging tasks for the commander was to relay the fact that the test was more than just four people stuck in a chamber. The science crewmember acted as the sole point of contact between principal investigators from the science disciplines and the crew, setting schedules for each experiment activity. The life support systems experts, together with the systems test control room personnel, maintained, repaired, and optimized/evaluated all aspects of the life support systems within the chamber.

Crew selection was loosely based around the astronaut selection process. A crew selection committee evaluated the individuals who applied based upon the skills mix anticipated for that particular test. Medical testing (equivalent to an Air Force Flying Class III medical), coupled with in-depth psychological testing, provided a short-list of qualified individuals from which to build a crew. Back-up crewmembers were always selected in case one of the prime crew could not participate further.

Crew training prior to the test depended upon the exact objectives of the specific test in question. For example, microbiological sampling was taught to the crewmember in EHTI Phase I so that plant, atmosphere, and surface sampling could be performed. By the time LMLSTP occurred, two crewmembers had been trained as phlebotomists and as crew medical officers, all crewmembers had undergone extensive isolation training at an underwater facility in Key Largo, and the entire test team had participated in a crew/control room resource management class to minimize the "us versus them" potential.

Crew Selection

From the crew selection viewpoint, it may be surprising to most to find that the potential crewmembers went through very similar experiences as potential flight crewmembers might. Having personally been through both processes, the emotions experienced throughout each of the waiting periods between interview and

selection announcements were very similar. However, the same camaraderie that exists between astronaut candidate interview groups was also plainly evident during the chamber crew selection process.

One important factor that must be carefully considered is the effect of any change in selected crew configuration at any time after selection. Moreover, the closer to test start any change occurs will correspondingly increase the impact to crew cohesion during the test itself. Therefore, unless absolute necessity demands a crew change, all efforts should be focused around a way to include, rather than preclude, specific crewmembers from participation.

Test Preparation

Different crewmembers prepared for each of the tests in widely different ways, dependent mainly upon their role during the test. The life support specialist crewmembers spent the majority of their time prior to the start of the test getting the systems ready for test. Similarly, the science specialist crewmembers spent the majority of their time understanding the objectives and procedures necessary to successfully complete each of the science projects. The commander's role was primarily one of a support person during the pretest planning phase, spending as much time as possible with both fellow crewmembers and test management personnel.

A portion of pretest planning involved getting one's affairs in order both at home and at work. Obviously, this involved an increased dependence upon other people (family, friends, and co-workers). No attempt was made to make the chamber living quarters like a home, but the bare essentials for living were provided.

There is nothing that can be done to prepare the crewmember for the instantaneous change that occurs on the morning of the test start. Bidding farewell to family and friends seems an almost comical process since the crews are the ones going nowhere while friends and family are the ones departing for home.

The feeling that the test is actually a "mission" is an important concept to realize. Indeed, although the chamber and its inhabitants are going nowhere, most crews took on the test as a mission, especially as test durations approached the types of lengths of missions aboard the Mir or the ISS.

The First Few Days

Settling in to chamber life took several days. The stark contrast between the pretest preparations, specifically the chamber entry celebrations, and the relative quiet of the chamber took some getting used to. However, once a daily routine was established (a few days), the crew settled in for the long haul. Again, although pretest planning helped a great deal in terms of individual roles and responsibilities, the first few days were used to further refine these roles so that each crewmember

knew exactly who was responsible for what. The benefit of starting the test on a Friday was also evident in that the weekend was available for arranging the chamber to prepare for the duration of the test. Several audiovisual system problems and procedures were also worked on during this time.

Life Support Systems Maintenance

The two life support systems specialists fell rapidly into their roles, responding to various problems with the life support systems and subsystems. It should be noted that failures of systems or components of systems were expected and in fact welcomed. Should the test have proceeded with completely nominal performance, its value would have been significantly less than it actually was. Only by experiencing hardware and software failures can the team learn how to avoid mistakes and build better systems.

Of course, the systems within the chamber experienced failures at all times of the day or night requiring both systems specialists and, on occasion, other crewmembers, to be awakened during sleep periods. Because of this, consideration must be given to allow crewmembers additional sleep periods during normal work time to ensure sufficient rest is obtained.

Finally, a word on schedules. It is an impossible task to timeline events surrounding the life support systems or any other tasks to any reasonable degree. It became obvious very quickly that a shopping list approach to tasks would provide the best solution. Using this approach, the crewmembers could allocate time themselves during the workday. Of course, if there was a time-critical task to be performed, this could be easily scheduled, but it is suggested that for tests and/or missions of this duration, scheduling to a timeline to the same degree as shuttle missions is both impractical and impossible.

Involvement With Science

In general, excellent compliance with all planned scientific experiments was obtained for all phases of the LMLSTP. Compliance with certain experiments such as the exercise protocol was near 100 percent since they represented a valuable tool to the crewmember for both psychological as well as physiological benefits. The involvement of all crewmembers pretest was invaluable, not only from the crew training perspective, but also in failure investigations during the test phase.

One of the main reasons that the science aspect of the test proceeded so smoothly was that the crewmembers themselves arranged the pretest, during test, and posttest schedules for all science activities with the principal investigators for each experiment. Hence, no surprises arose during the test phase. Of course, adjustments to the schedules were made to accommodate other activities, but once again, these were coordinated by the crewmembers and the principal investigators real-time.

One comment from all crews was that the science experiments should be designed so that the method of collection of data should not influence the data itself. The most obvious case here was the experiment designed to evaluate crew sleep patterns. The hardware involved in this experiment interfered with the normal sleep patterns of the crew, hence giving rise to spurious data in terms of actual crew sleep patterns when the hardware was not being used. This is not meant to single out this specific experiment, but rather to suggest that careful consideration be given to methods of data collection.

Time Shifting

Interestingly, had the crew been left to settle into their own time cycles, it is certain that they would have shifted towards a 28- to 30-hour day/night cycle. The major reason they didn't was the continuous contact with the control room. This, coupled with the fact that the crew was always aware of the time of day, kept them to a regular 24-hour day. However, it is interesting to note that most crews spent a considerable amount of time awake, compared to pre- or posttest times. On a personal note, my workday approached 20 hours, followed by 1 to 2 hours of personal time presleep. The two- to three-hour sleep period did not seem to affect my performance during the test phase. All crews have reported similar shifts in work/sleep cycles compared to pre- or posttest periods.

Environmental Effects of the Chamber on the Crew

One of the interesting phenomena of spending three months inside a 6-meter diameter chamber was the effect on the senses of the crew. Eyesight in particular was affected in several crewmembers. Since items in the chamber were no more than 6 meters away, it took a learning period of several hours posttest to acclimatize to focusing on objects further than 6-meters distant.

Hearing was another sense that was affected. Since the chamber provided a near constant, and relatively higher, ambient noise environment, upon exiting the chamber normal functions such as sleeping proved to be more difficult than previously expected. In general terms, the relative quiet of the external environment was a stark contrast to the constant noise within the chamber. Also, the constant nature of the noise inside the chamber afforded the crew an invaluable tool to assess problems with a variety of hardware. For example, when pumps or motors were experiencing problems, the crew often reported the problem long before any effect was noticed by the control systems.

Although as noted, the noise levels were generally higher than the external environment, the crew spent a considerable amount of time during the first few days of the test isolating particularly noisy areas and components. In most cases, the crew was successful in abating such noisy areas/components.

The acuteness of the sense of smell was evidenced by all crewmembers being instantly aware of new items transferred into the chamber through the transfer lock. This was additionally enhanced by the constant nature of the chamber odors, i.e., differences were very easily detected.

The lack of variable-intensity lighting inside the chamber also affected the crew. Normal circadian rhythms outside the chamber environment are influenced by a gradual darkening of the sky at night and a gradual lightening during the waking hours. Inside the chamber, conditions represent instant day or instant night, depending on whether lights are on or off. This manifested itself in a longer period of "waking up" in the morning, and a difficulty in falling asleep at night. Although the crew attempted to mitigate this effect by introducing lamps in their quarters that slowly increased in intensity in the early morning, the benefits were not obvious, and the use of the lamps in this manner was discontinued.

A Question of Confinement

One of the most common questions asked of any of the chamber crews is how they were able to cope with long periods of confinement in a small chamber. The answer from all of the crewmembers has been consistent. One very rapidly adapts to the surrounding environment. Therefore, as soon as the chamber door was closed (and remembering all of the pretest preparations), the limitations on the environment switched instantaneously. Indeed, perhaps the more challenging task was exiting the chamber after having become accustomed to the small volume.

If you couple the small volume of the chamber with the addition of three other crewmembers, the logical question now changes to "How did you cope with being around three other people in such a small space?" Apart from long-duration space flight, there really is no analogous situation that you could conceive whereby you would spend three months in the company of the same individuals for every waking hour.

The answer to the above question for all crews has been the same. There were no problems at all inside the chamber. There were perhaps some miscommunications between the inside crew and management which led to some issues in the early phases of the project. However, as lessons were learned, and as test durations increased, more emphasis was placed on an overall team-integration approach including resource management with all team members, including management.

One aspect of crew life that has to be accepted is the lack of privacy inside the chamber. Although each crewmember had his or her own crew quarters, the doors were rarely closed. Also, unless one was alone on the first floor of the chamber, there was absolutely no audible privacy, i.e., all conversations, whether work or family-related, were heard by all. What this drove was a complete requirement for trust within the chamber, which was complied with in every case. This respect of

privacy needs to be continued to the outside crew. Communications by electronic or other means should not be forwarded, referenced, or communicated in any fashion to personnel who do not need to know, unless the release of such information is granted by the initiating crewmember.

Unexpected Happenings

Other than the expected problems with the life support system hardware and controls software, several unexpected events occurred during the 91-day test. The first was a complete power outage across the entire Johnson Space Center. Although back-up generators were supposed to automatically start-up, for some reason, the crew was left (literally) in the dark for a short period. The only lighting came from the glow of our laptop computer screens, running on battery power. Since all of the life support systems lost power, the quiet of the chamber became deafening, leading to the thought that perhaps the living breathing chamber had indeed taken its last breath. Of course, the crews both inside and outside set about the task of safing the system for the return of power. When power was reestablished, a quick survey of the systems and the chamber revealed no damage from the short outage.

Alarms were actually few and far between throughout the 91 days, and all were caused by faults in the alarm systems (i.e., false alarms). Whenever an alarm did go off, the crew (per test rules) immediately made their way to the airlock door in case evacuation was necessary.

One constant throughout the test series was the ability of the chamber to "know" when the last day of the test was. This may seem to be a strange comment, but in all four phases the last day proved to be one when alarms were rife. For example, on the ninety-first day of the final test, I happened to be taking a long shower, having been cleared to use as much as I wanted since all water recycling operations had ceased. In the middle of my glorious five-gallon shower, the alarm (false) sounded. During an alarm, all water supply to the chamber ceases. Therefore, still covered in soap, I managed to make my way out of the shower to the first floor waiting area. Power to the systems was eventually reestablished, but I can only guess what a sight I must have been with a towel wrapped around me trying desperately to reconfigure the tanks and valves using the computer mouse with soapy hands. Having finally managed to turn the water supply back on, I recommenced my shower. One minute later, it happened again.

The Absolute Need for Humor

One very important characteristic of long-duration crewmembers and the people who sit at the console day after day is to have a sense of humor. Without one, you may just as well shut your door and let three months pass you by. Humor

provides a release of tension and is an invaluable tool to defuse critical situations. This is not to say that practical jokes need to be a part of everyday life inside, but to be able to laugh something off rather than let it fester was beneficial in every sense.

A variety of planned events maintained the level of humor both inside and outside the chamber. Movie nights were held each Wednesday evening with all of the test crew participating in a "Science Theatre 2000" show. Almost always, a theme was chosen for movie night such as science fiction, spaghetti westerns, or Elvis movies. The volume of singing during the Elvis night almost proved fatal to the communications system, and only through extended efforts by our audiovisual gurus did we manage to reconfigure the system just in time for our planned link-up with the STS-87 crew the next morning.

Other planned celebrations included a birthday for one crewmember (with a paper candle on her cake), Thanksgiving dinner (eaten at the same time as the control room crew), celebrations of the 15-, 30-, and 60-day marks, and the halfway point (over-the-hump day). Events such as these were beneficial in maintaining a healthy test team.

Posttest Blues

Different crewmembers have reacted to the end of the test in different ways. Some decide to leave for two weeks' vacation; some tend to stay around the chamber (some actually carried on their work after the test within the, now open, chamber). All crews were sorry to see the test end. Whether that is from the lack of being "in the limelight," or whether it is due to the loss of a feeling of camaraderie, it is difficult to say. From my personal point of view, I certainly experienced a mild form of depression in the weeks following test completion. Perhaps some of these feelings were related to not knowing the future of such tests. Whatever the true reason, it is important to provide a mechanism for discussions of feelings even after the test/mission has been completed.

Lessons Learned

Apart from the invaluable lessons learned regarding the operations and designs of the life support systems, other lessons were learned which should be examined closely for any planned long-duration testing. The first is to avoid crew changes late in the preparation phase. Although the 91-day test proceeded smoothly even with a change in crew at the test start minus 10-day mark, it was an unknown that should not have appeared that late in the flow. Crewmembers (both potential and selected), should be made aware ahead of time of specific items that could cause them to be deselected prior to the start of the test.

During this series of tests, significant medical data was collected on each crewmember. This data represents important scientific material and should therefore be used by the scientific community to help solve issues for long-duration space flight. Such data, however, can be extremely sensitive in nature. All scientific teams have been extremely conscientious in terms of maintaining the confidentiality of such data. Yet, a problem still exists. That is, the use of such data in the selection or deselection of a crewmember for future chamber tests or during application for astronaut candidacy. Unless definitive explanations about the use of collected data are provided to the crewmembers before experiments commence, what will inevitably happen is that crewmembers will either be noncompliant during the test, or will decide not to participate (an option that is always available to any test subject or flight crewmember). Of course, if the data shows there to be a life-threatening condition, then such data should obviously be included in any review for future selection.

An important aspect of communications from inside to outside was the direct contact between the system specialists on the outside with the crew on the inside, i.e., there was no single point of contact through which all communications were made (such as the "Capcom" during Shuttle/ISS missions). It was felt that the people who knew the systems should be the ones giving advice on how to maintain or fix problems with hardware or software within the chamber. Although a small amount of such contact is taking place on ISS (particularly on the Russian side), it is recommended that more leeway for one-on-one discussions be provided even for flight.

One of the disappointments during the test was the lack of public awareness. The press conference held at the halfway point was attended by one member of the local press. Ironically, since the test was completed, media from around the world have included the test series in programs in several different languages. The one common question asked by these media representatives is "Why didn't we know about this during the testing?"

The final lesson learned is that testing with humans in the loop is an absolute necessity to understand the intricacies of life support systems. Real metabolic profiles, real-time problem diagnoses, and instantaneous feedback are just some of the benefits of human crews.

So. What's Next?

It would be foolish of me to suggest that we are ready to take the big step of going outside low-Earth orbit (LEO) back to the Moon or on to Mars. We are, however, in a much better position to design, build, and test a life support system that will get us there and back safely. We are dependent upon the ISS to act as our proving ground for the Mars transit vehicle, since we have little to no understanding of how our systems will react to a long-duration microgravity environment. ISS will give us the benefit of a long-duration test bed, without the added risk of being a year away from home.

Although the advanced life support project has accumulated 195 days of closed-chamber testing, it is certainly insufficient to be able to design the final flight hardware.

The BIO-Plex will be our ultimate test bed before we commit to flight designs. This closed-loop test bed will provide scientists and engineers with a high-fidelity environment to develop the reliable and optimized system to keep our crews alive for long durations (up to one and a half years).

Whether or not the global community decides to venture beyond LEO and to take the next giant step, the LMLSTP series of tests has taken the first small step to make it possible to do so. I truly believe that the benefits that LMLSTP has given the agency will prove to be invaluable when and if the call is made.

FURTHER INFORMATION

Photographs of each of the crews follow. However, many more photographs, including ones taken by the crew, are available on the Advanced Life Support Project Web site: http://advlifesupport.jsc.nasa.gov.

If, after visiting this site, you still have questions, the Advanced Life Support Project at the Johnson Space Center would be happy to hear from you:

Advanced Life Support Project
Mail Code EC
National Aeronautics and Space Administration
Lyndon B. Johnson Space Center
2101 NASA Road 1
Houston, TX 77058
U.S.A.

Figure 1.3-1
This group portrait shows the EHTI Phase I team, with the in-chamber crewmember visible through the window

Figure 1.3-2
The entire EHTI Phase I team poses for a group portrait as the crewmember joins in the back row

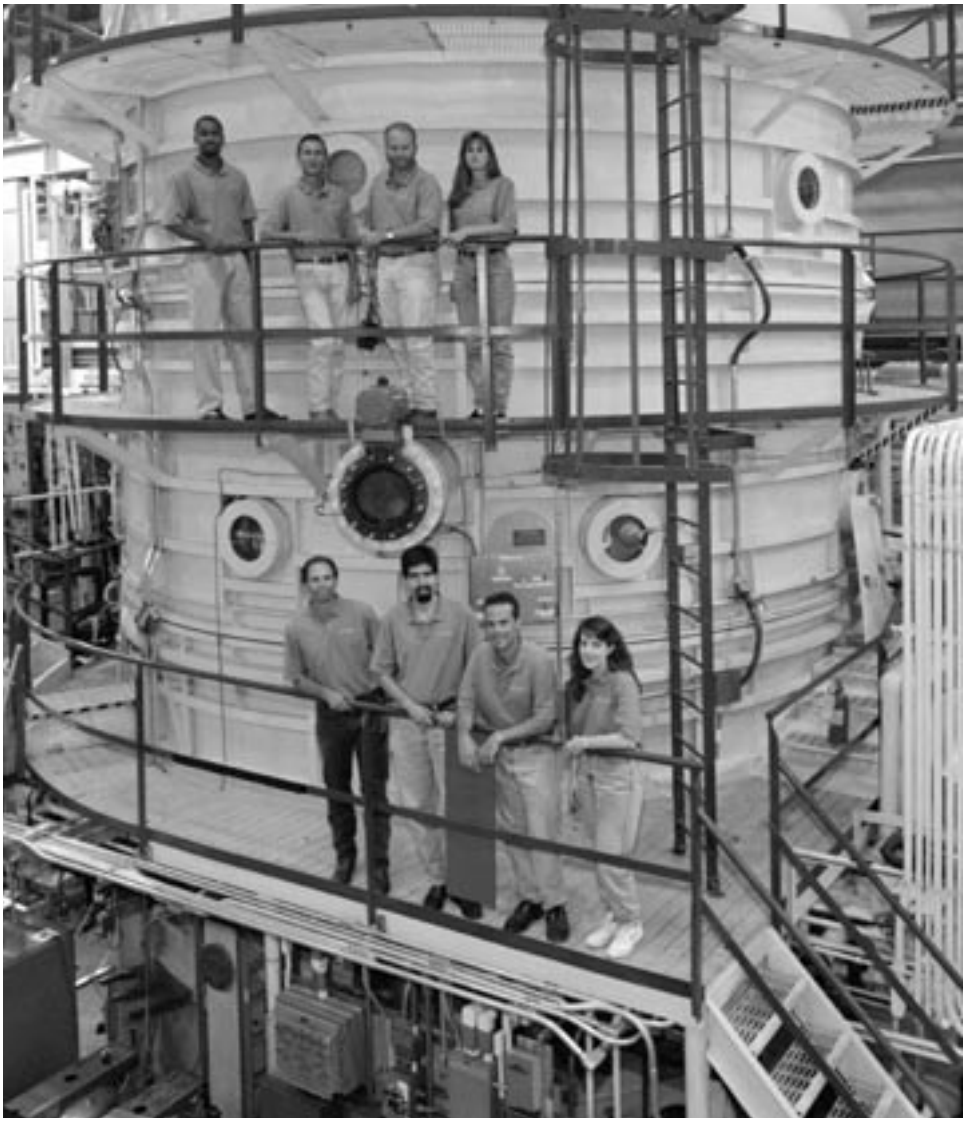


Figure 1.3-3
The LMLSTP Phase II crews stand outside of the testing chamber, including the primary crew (bottom row) as well as the back-up crew (top row)

Figure 1.3-4
The 20-foot test chamber is surrounded by the test team and crewmembers for LMLSTP Phase II

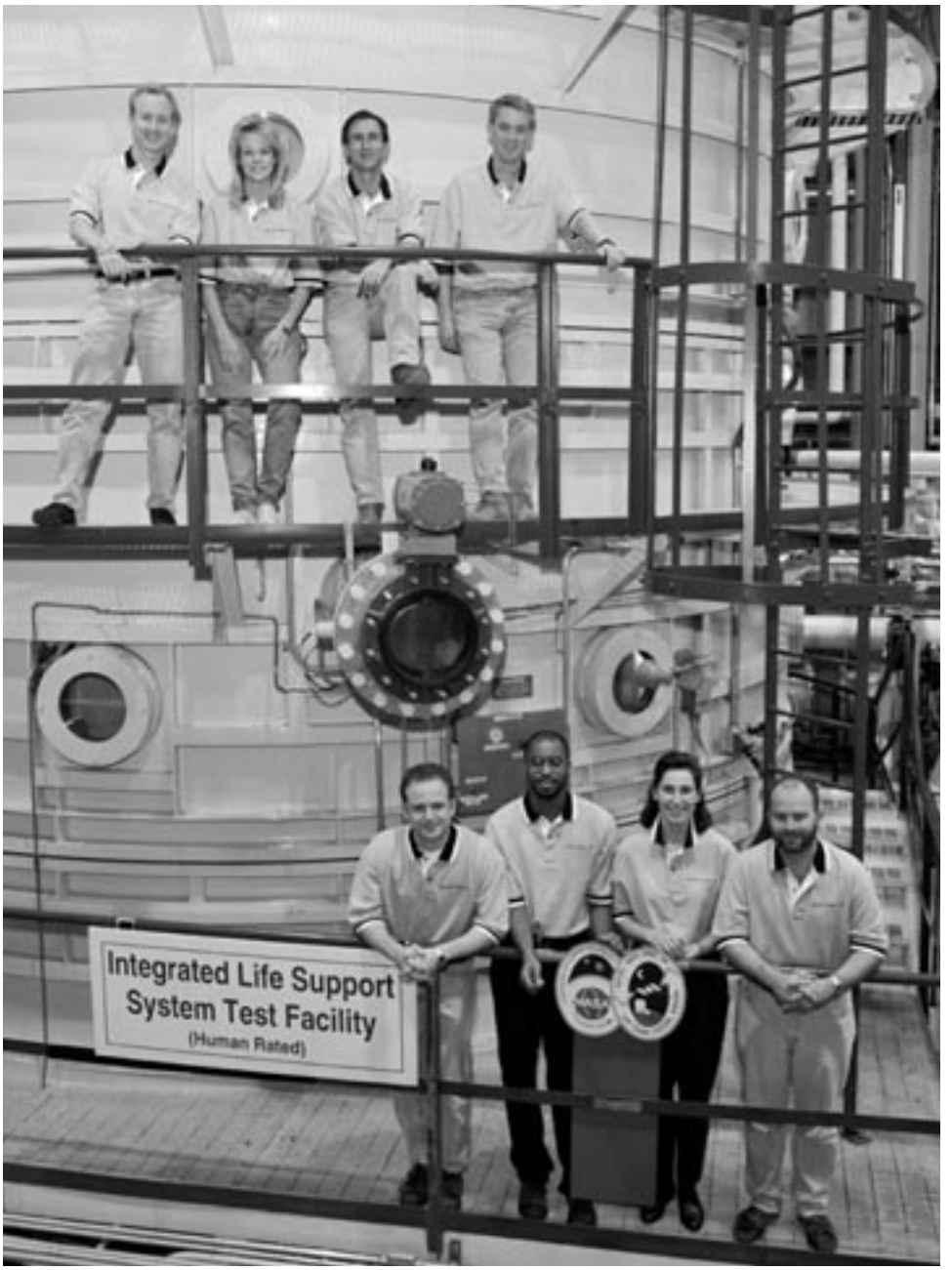


Figure 1.3-5
With the 20-foot chamber serving as the backdrop, the Phase IIa primary crewmembers stand on the lower balcony and the back-up crewmembers pose on the upper balcony

Figure 1.3-6
In this Phase IIa group photograph, the primary and back-up crewmembers gather on the lower balcony while surrounded by other Advanced Life Support personnel

Figure 1.3-7
The Phase III primary and back-up crews pose in front of the testing chamber

Figure 1.3-8
Before the start of the test, the entire LMLSTP Phase III staff poses for a group portrait

2.1

Test Phases and Major Findings

Donald L. Henninger, Ph.D.

SUMMARY

NASA' s (National Aeronautics and Space Administration's) Advanced Life Support Project life support systems are an enabling technology and are integral to the success of human space exploration. As NASA embarks on the Human Exploration and Development of Space (HEDS) Mission it becomes imperative, for considerations of both safety and cost, to minimize consumables and increase the autonomy of the life support system. Utilizing advanced life support technologies provides this autonomy and increases productivity of the mission by reducing mass, power, and volume necessary for human support, thus permitting larger payloads for science and exploration. Two basic classes of life support systems must be developed, those directed toward applications on a transportation/habitation vehicle and those directed toward applications on the planetary surfaces. In general, it can be viewed as those systems compatible with microgravity and those compatible with hypogravity environments. The goal of the Advanced Life Support Project is to provide life support self-sufficiency for human beings to carry out research and exploration productively in space and for benefits on Earth. To accomplish this goal, five major technical objectives have been identfied as follows:

1. Provide Advanced Life Support technologies that significantly reduce life cycle costs, improve operational performance, promote self-sufficiency, and minimize expenditure of resources for missions of long duration

Supporting Objectives

- Fully closed (i.e., no additions of water or air from outside the chamber) air and water loops in a manner that minimizes expendables
- Develop and integrate resource recycling/processing from solid wastes and contaminant control systems that increase the level of self-sufficiency
- Optimize food loop closure, with concomitant air and water regeneration, based on the growth of crop plants

- Provide efficient, reliable active thermal control (heat acquisition, transport, and rejection)
- Develop fully regenerative integrated systems technologies that provide air, water, food, and resource recovery from waste. Note: The term "regenerative" used here refers to technologies which can perform the desired function without significant replacement of any component of the technology – with minimum use of expendables. (This is usually accomplished at the expense of energy input but is a favorable trade for long-duration space missions where high resupply rates are prohibitive.)

2. Develop and apply methods of systems analysis and engineering to guide investments in technology, resolve and integrate competing needs, and guide evolution of technologies

Supporting Objectives:

- Refine existing procedures for systems assessment to allow consideration of the whole spacecraft or mission – including medical and scientific needs – to obtain synergism with life support systems, resolve incompatibilities, and evaluate options
- Conduct ongoing cost/benefit trades to guide technology investments
- Conduct advanced mission studies to guide definition of technology requirements, long-term investments, and evolution
- Develop methods for concurrent engineering of technologies through subsystems to integrated systems
- Develop system models and maintain an archival database of lessons learned, operational results, and key design information

3. Resolve issues of microgravity and hypogravity performance through space flight research and evaluation

Supporting Objectives:

- Develop predictive models of fluid and fluid/gas behavior and interactions in both microgravity and hypogravity that can be used as a basis for design of new life support hardware
- Achieve equivalent productivity, control, and predictability of bioregenerative life support components in microgravity as on Earth and characterize performance of bioregenerative systems at lunar and Martian gravities
- Demonstrate microgravity and hypogravity performance of gravity-sensitive life support hardware components and subsystems (e.g., membrane behavior)

4. Ensure timely transfer of new life support technologies to missions
Supporting Objectives:

- Develop and maintain effective relationships between technology developer and mission user to establish needs or requirements for mission technology
- Conduct definitive (ground and in-space) testing and verification
- Conduct regular discussions between mission users and technology providers on technology development status and transfer protocols
- Disseminate scientific and technological information through journals, the Internet, electronic and video media, workshops, and special programs
- Work in partnership with intermediaries such as the entertainment industry, media, museums, etc. to bring the space experience to our nation's citizens
- Participate in preparation of instructional materials reflecting the discoveries and adventure inherent in space exploration through partnerships with educators, providing access to facilities and supporting classroom instruction
- Cooperate with other nations to design an international strategy for exploring the Moon and Mars

5. Transfer technologies to industrial and residential sectors for national benefit
Supporting Objectives:

- Identify and initiate dual-use development early in the technology development cycle
- Establish rapid response solicitation and funding mechanisms to maintain the national "market edge"
- Identify and provide incentives to NASA personnel that promote technology

To accomplish these objectives, the Advanced Life Support Project is conducting focused research and development to advance technology readiness of regenerative life support and thermal control components, validate regenerative life support technologies integration through long-term testing with humans, and identify terrestrial applications for life support technologies.

Integrated testing of life support technologies with humans allows for evaluations of their efficacy to provide sustenance to humans. Such tests allow for demonstration of technology-to-technology interface compatibility and end-to-end functionality and operability of life support hardware and software. Conducting integrated testing and verification of technologies on the ground greatly increases our confidence in successful in-space operations and greatly reduces risk to human crews.

Finally, integrated testing is an extremely useful tool to identify weaknesses in technologies and in turn allows better focusing of future research and technology development resources.

The Lunar-Mars Life Support Test Project's four tests (Phases I, II, IIa, and III) conducted in 1995-1997 were the beginning of the long-term testing with humans. All tests were conducted at the National Aeronautics and Space Administration's (NASA) Johnson Space Center (JSC), in the Crew and Thermal Systems Division's Variable Pressure Growth Chamber (VPGC) and its attached airlock (Figure 2.1-1). Future testing will include integration of all functional elements of a space-based life support system and will entail progressively longer testing durations.

Phase I

The goal of the Phase I test was to demonstrate the use of higher plants to provide the air revitalization requirements of a single test subject for 15 days. The primary objectives of the Phase I test performed in July and August 1995 were to: 1) demonstrate the ability of a wheat crop to continuously provide the CO_2 removal and O_2 supply functions for the air revitalization needs of a single human test subject for 15 days, 2) demonstrate three different methods of control of the O_2 and CO_2 concentrations for the human/plant system, 3) monitor populations of microorganisms important to human and plant health, and 4) determine ethylene and other significant trace gas contaminants generated during the test.

Plants were grown in the plant growth chamber, and the airlock was outfitted for human habitation. Air was transferred between the airlock and plant growth chamber through an interchamber ventilation system so that CO_2 produced by the test subject could be removed by the plants and O_2 produced by the plants could be used by the test subject (Figure 2.1-2). Three different methods of control were demonstrated. The first method optimized conditions for the plants so that they provided maximum photosynthetic output. The use of integrated physicochemical systems to complement the biological air revitalization was demonstrated. The second method demonstrated actively controlling the level of biological air revitalization by modulating the photosynthetic photon flux to control the rate of photosynthesis. The third method demonstrated passively controlling the level of biological air revitalization by limiting the amount of available CO_2 to control the rate of photosynthesis.

Comparison of plant performance before and after the human entry showed there was no effect of the human on the plants' photosynthetic rate. All three control meth-

ods were successfully demonstrated in the test. Microorganism populations in the human habitat increased over the course of the test but did not reach steady state. No microorganisms were identified which would be of concern to either human or plant health at the levels measured. Trace gas contaminants observed were those expected based on past spacecraft measurements. The test successfully demonstrated the use of higher plants for air revitalization for humans and the robustness of the plant systems as part of a human life support system. Also, the test demonstrated that plants can be integrated into regenerative life support systems and can be controlled to provide a specific desired performance.

Figure 2.1-1 *The Variable Pressure Growth Chamber shown positioned behind the Phase I support crew*

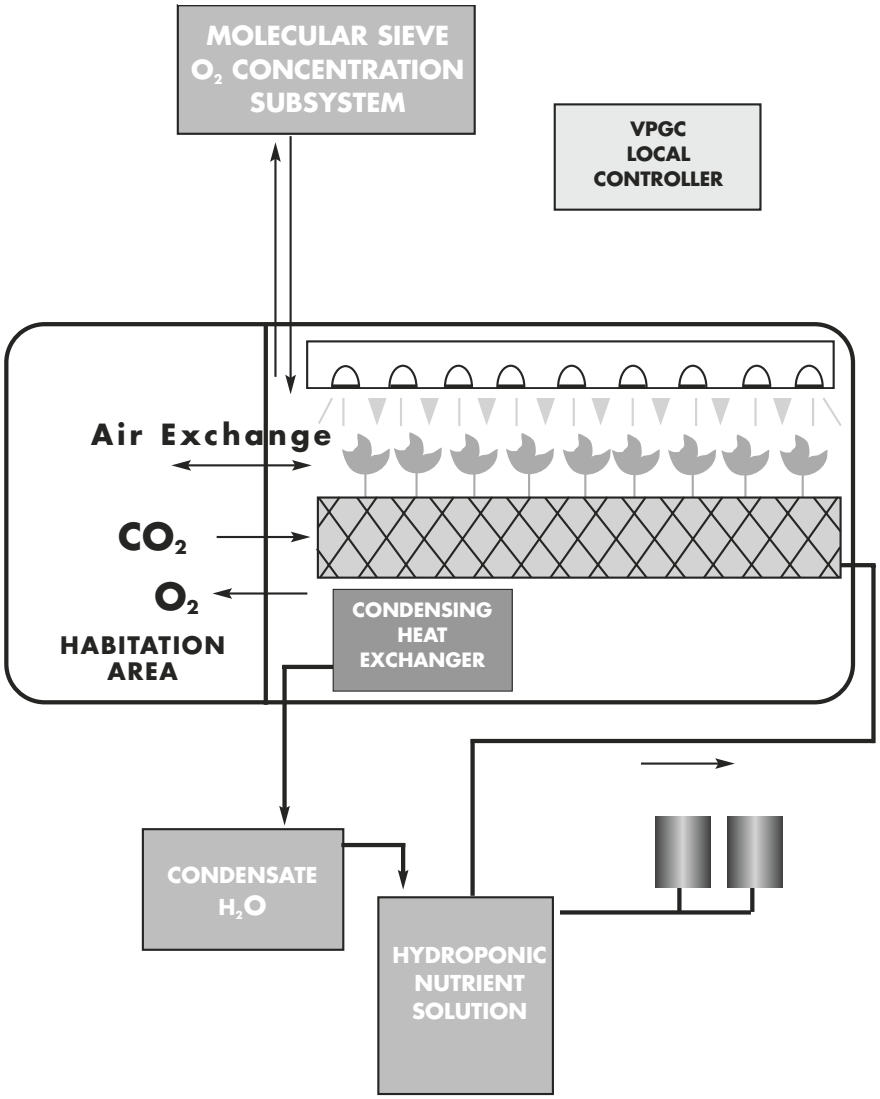


Figure 2.1-2 *The Phase I, 15-day test functional schematic*

Phase II

The Phase II test was a 30-day, 4-person test completed on July 12, 1996. The purpose of the test was to verify performance of integrated physicochemical life support system technologies for air revitalization, water recovery, and thermal control. Testing began with a human metabolic simulator and culminated in a continuous 30-day human test. The specific objectives were as follows:

Primary Objective:

- Develop and test an integrated human life support system capable of sustaining a crew of four for 30 days in a closed chamber

Secondary Objectives:

- Provide a regenerative air revitalization subsystem capable of removing carbon dioxide from the internal atmosphere of a sealed chamber, recovering oxygen from the carbon dioxide, and controlling trace gas contaminants for a crew of four for 30 days
- Provide a regenerative water recovery subsystem capable of recovering potable water from hygiene water (shower, hand wash, laundry), urine, and humidity condensate for a crew of four for 30 days
- Evaluate an active thermal control subsystem capable of acquiring heat from the chamber interior, transporting the heat to the exterior, and simulate the capability of rejecting the heat in a lunar day environment (107˚C surface temperature)

Tertiary Objective:

- Evaluate a computer monitoring and control system for operation of the air, water, and thermal subsystems

Cooperative Research Objectives:

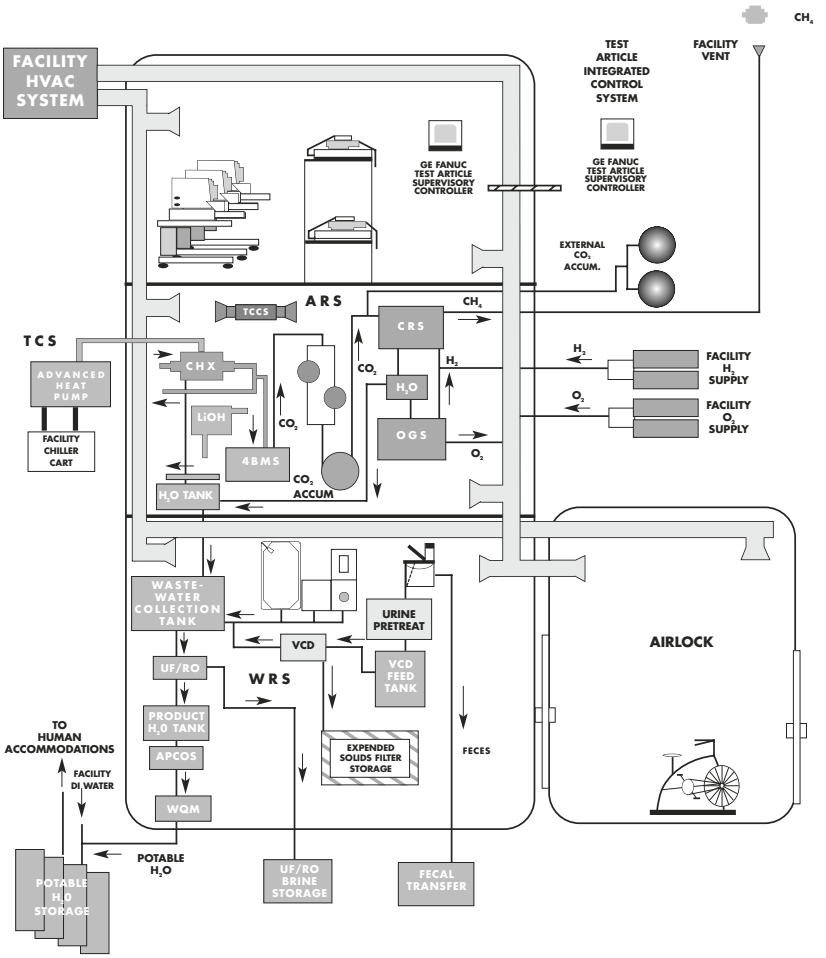
- Psychology: Evaluate test subject productivity as a function of time in the test chamber using a computer survey system
- Microbiology: Evaluate changes in the human microbiological population as a function of time in the test chamber
- Human Factors: Evaluate the perceived effects of sound on the human test subjects as a function of time and type of sound

The test was carried out in the Life Support System Integrated Test Facility (LSSIF) (Figure 2.1-3). The LSSIF is a modification of an existing vacuum chamber with a diameter of 6.1 meters and a height of 8.4 meters separated into three

Figure 2.1-3 *The LSSIF standing over the shoulders of the Phase III crew*

working levels. The LSSIF is outfitted with an emergency monitoring system (e.g., fire detection-suppression-warning, low oxygen monitoring-warning, etc.) and was outfitted with an air revitalization system, water recovery system, habitation areas, and all other associated hardware and subsystems (Figure 2.1-4).

The air revitalization system maintained an acceptable chamber atmosphere during the entire 30-day test with normal CO_2 levels between 0.30 and 0.55%. The CO_2 removal system (4-bed molecular sieve) was operated for 700 hours and removed 112 kg of CO_2. The Sabatier CO_2 reduction system performed satisfactorily, operating 600 hours reducing the CO_2 to water and methane. The O_2 generation system (electrolysis unit) operated for 700 hours and processed 100 kg of water (69% from the Sabatier unit and 31% from the water recovery system) to produce 86 kg of O_2. Oxygen levels were maintained between 20.3 and 21.4% during the 30-day test. Trace gas contaminants were controlled by passing air through an activated charcoal canister which maintained air quality within acceptable limits for U.S. space vehicles.



KEY

APCOS = aqueous phase catalytic oxidation subsystem
ARS = air revitalization subsystem
4BMS = four-bed molecular sieve
CHX = condensing heat exchanger
CRS = carbon dioxide reduction subsystem
DI = deionized
GE FANUC = programmable logic controller used for the LMLSTP
HVAC = heating ventilation, and air conditioning
OGS = oxygen generation subsystem
TCCS = trace contaminant control subsystem
TCS = thermal control subsystem
UF/RO = ultrafiltration/reverse osmosis
VCD = vapor compression distillation
WQM = water quality monitor
WRS = water recovery subsystem

Figure 2.1-4 *The Phase II, 30-day test functional schematic*

The water recovery system treated all wastewater originating from the shower, hand wash units, galley sink, laundry, and urinal as well as humidity condensate water. Pretest verifications of the water recovery system were carried out, including

hardware functional tests, two donor-mode tests where volunteers provided the life support loads to the hardware but were not restricted to the chamber, and a viral challenge test of the water recovery system to ensure the proper functioning of the system prior to the 30-day test. The Vapor Compression Distillation (VCD) urine processor operated nominally for the first 27 days of the test when a motor controller failed. However, enough urine had been processed to complete the 30-day test. The VCD processed 182 kg of urine and flush water, recovering 179 kg of processed water (98% recovery rate). The Ultrafiltration/Reverse Osmosis (UF/RO) system operated nominally and processed 3089 kg of waste water, recovering 2957 kg of water (95% recovery rate). The postprocessing subsystem operated in the modified state as described in a later section and produced potable water for consumption by the crew during the 30-day test. (The tests described herein are the first times NASA has ever recycled water for potable use.)

The thermal control system included a high-temperature life heat pump which was to be evaluated as a technique for rejecting heat on the Earth's moon. This component of the thermal control system developed a leak just prior to the 30-day test and could not be repaired in time to start the test. Consequently, cooling was provided by a facility cooling cart and facility-chilled water during the 30-day test.

The controls system consisted of three main components: 1) the regenerative systems control and data acquisition component for controlling the air revitalization and water recovery system; 2) the facility emergency matrix component which managed all devices critical to ensuring human safety within the test facility such as fire detection and suppressions systems; and 3) the basic facility systems control and monitoring component which supervised the external test chamber equipment such as the chamber heating, ventilation, and air conditioning system. The controls system operated nominally during the test with relatively minor modifications during the test in terms of hardware replacement and software changes.

Phase IIa

The Phase IIa test was a 60-day, 4-person test completed on March 14, 1997, with life support subsystems functionally similar to those on the International Space Station (ISS). The purpose of the test was to verify integrated performance of baselined ISS life support technologies for air revitalization and water recovery and to provide additional integrated test data to the Advanced Life Support

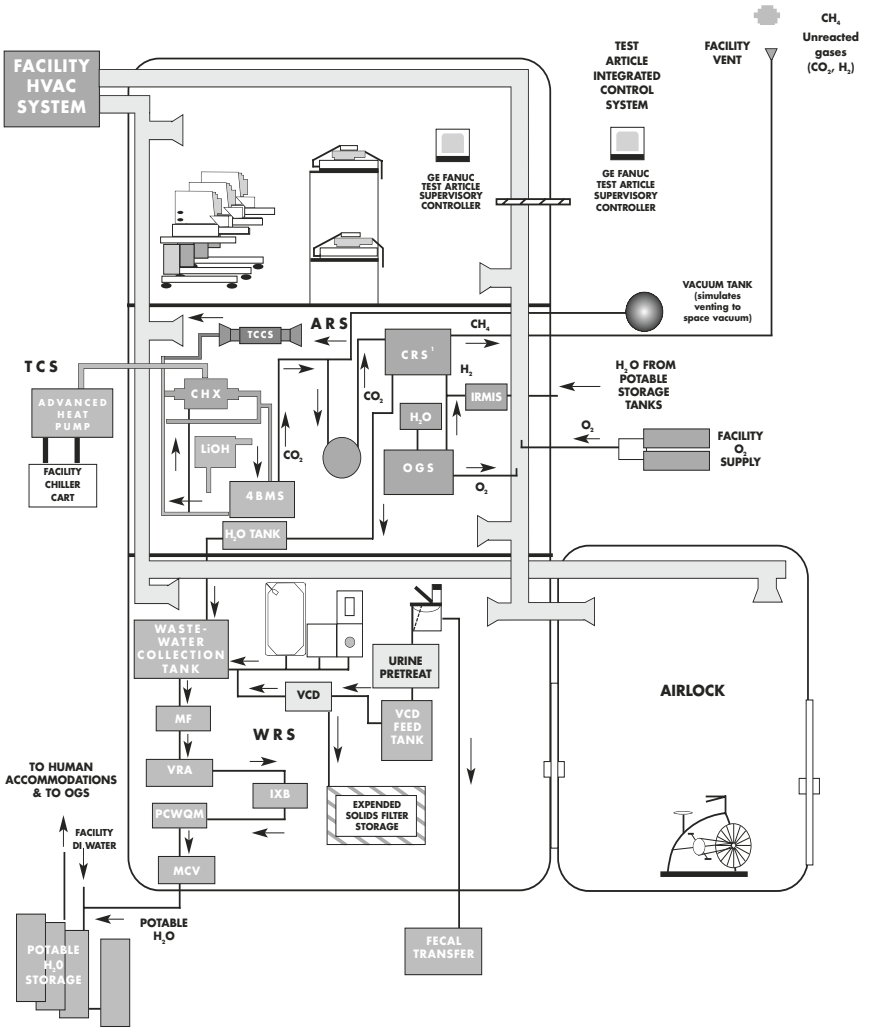
Project. ISS-like hardware representing significant advances in state-of-the-art life support capabilities emulated the flight hardware and provided integrated data to the ISS Program.

The test was carried out in the LSSIF (Figure 2.1-3). The Phase IIa LSSIF was outfitted with an emergency monitoring system, air revitalization system, water recovery system, habitation areas, and all other associated hardware and subsystems (Figure 2.1-5).

The air revitalization system maintained an acceptable chamber atmosphere during the entire 60-day test with normal CO_2 levels between 0.22 and 0.60%, while O_2 concentrations were maintained between 20.05 and 21.85%. Two air revitalization system configurations were evaluated during the test. The first 30 days of the test consisted of CO_2 removal as in Phase II with the CO_2 vented (to a vacuum tank simulating space vacuum), oxygen generation with an electrolysis unit, and operation of a catalytic oxidation trace gas contaminant control unit. The second 30 days of the test consisted of CO_2 removal as in Phase II with the CO_2 fed to a carbon dioxide reduction unit, oxygen generation with an electrolysis unit, and operation of a catalytic oxidation trace gas contaminant control unit. The first segment was representative of initial ISS operations, and the second segment was representative of enhanced ISS Earth orbital operations. Additionally, the air revitalization system was controlled in a cyclic manner simulating orbital day/night cycles of the 90-minute orbit of the ISS (53 minutes of day and 37 minutes of night).

With the exception of formaldehyde, all trace gas contaminants were kept within acceptable spacecraft maximum allowable concentrations (SMAC). The formaldehyde level was approximately 0.16 ppm throughout the test. The 7-day SMAC is 0.04 ppm, and the threshold limit value for industrial workers is 0.30 ppm. The sources of formaldehyde were later identified to be the acoustic tile used throughout the chamber walls and ceilings, while the carpeting was a secondary source.

The water recovery system treated all waste water originating from the shower, hand wash units, galley sink, and urinal as well as humidity condensate water. The VCD urine processor operated nominally but required servicing of both the vacuum pump and fluids pump. The Multifiltration (MF) unibed was changed on day 45 after processing 2858 L (755 gal) of waste water. The 0.5-micron filter was changed five times during the 60-day test. The ion exchange bed in the Volatile Removal Assembly (VRA) was changed on day 28. The two Microbial Check Valves (MCV) were changed on day 50 after iodine levels in the recovered water declined.



KEY

ARS = air revitalization subsystem
4BMS = four-bed molecular sieve
CHX = condensing heat exchanger
CRS = carbon dioxide reduction subsystem
DI = deionized
GE FANUC = programmable logic controller used for the LMLSTP
HVAC = heating ventilation, and air conditioning
IRMIS = iodine removal and mineral injection system
IXB = ion-exchange bed
MCV = microbial check valve
MF = multifiltration unit
OGS = oxygen generation subsystem
PCWQM = process control water quality monitor
TCCS = trace contaminant control subsystem
TCS = thermal control subsystem
VCD = vapor compression distillation
VRA = volatile removal assembly
WRS = water recovery subsystem
[1]Operated during second half of test

Figure 2.1-5 *The Phase IIa, 60-day test functional schematic*

Phase III

The final test was the Phase III test, which incorporated the use of biological systems in concert with physicochemical (P/C) life support system technologies to continuously recycle air, water, and part of the solid waste stream generated by a 4-person crew for 91 days.

The Phase III test was conducted using two environmental test chambers at JSC. The Life Support Systems Integrated Test Facility (LSSIF) (Figure 2.1-3) housed the crew as well as most of the life support systems. This chamber was integrated with the Variable Pressure Growth Chamber (VPGC) (Figure 2.1-1) in which wheat was grown to provide supplemental food and air revitalization for the crew during the test. The human portion of the test began on September 19, 1997, and ended on December 19, 1997, for a duration of 91 days. The wheat crop was initially planted on July 23, 1997, and the final harvest was on January 9, 1998.

The Phase III test was the first test conducted by NASA to integrate human test subjects with combined biological and P/C life support systems (Figure 2.1-6). This integration was accomplished in four distinct ways. First, the CO_2 generated by the crew in the LSSIF was separated from the atmosphere, concentrated, and used by wheat in the VPGC as the major source of CO_2 for photosynthesis. In tandem with this process, 95% of the O_2 produced by the wheat plants was separated, concentrated, and used by the crew for respiration. On average, the plants consumed CO_2 and generated O_2 equal to that required by one crew person over the course of the test. The remaining three person-equivalent's worth of CO_2 removal and reduction and O_2 production was accomplished with P/C systems.

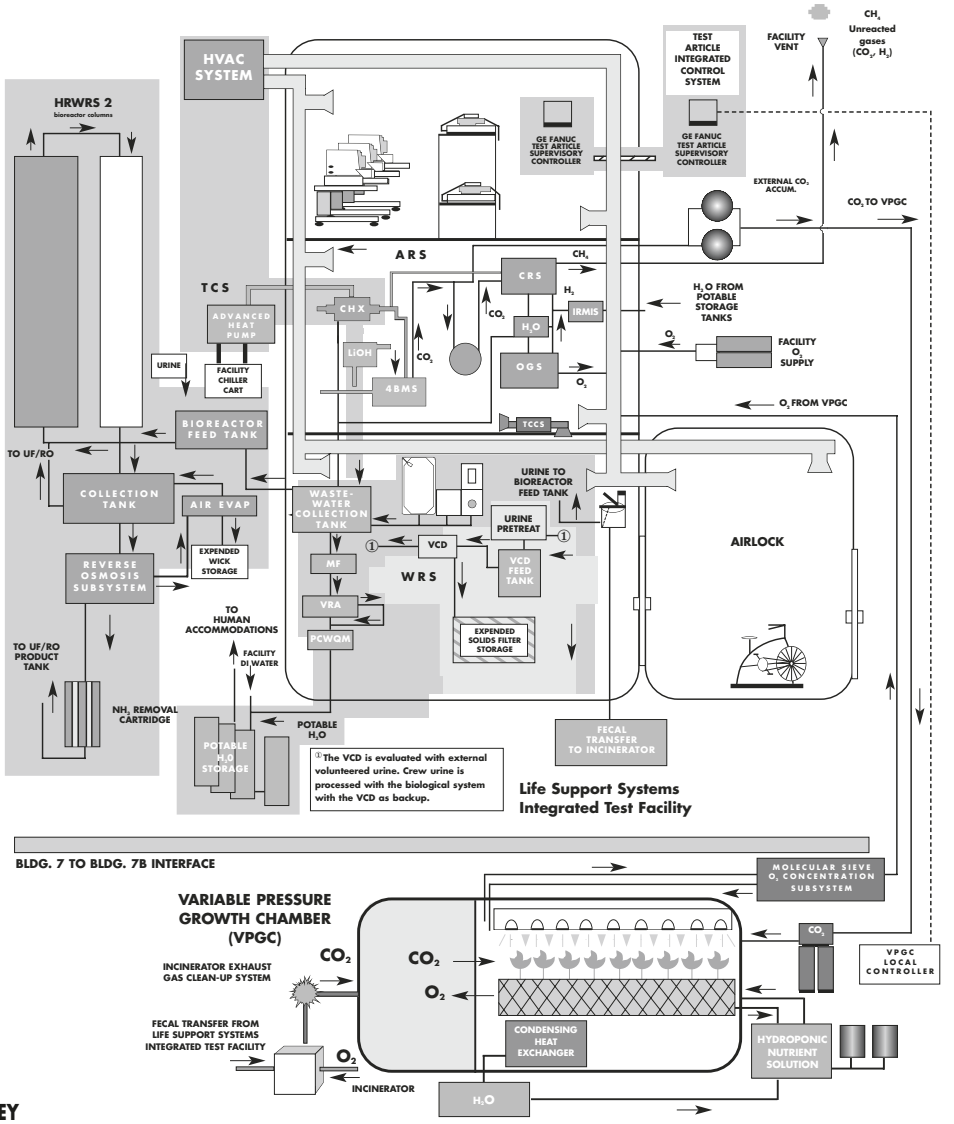
The second biological and P/C integration involved the Water Recovery System (WRS). The WRS processed 110.6 L (29.2 gal) of wastewater each day, equivalent to the daily requirement for a crew of four. Bioreactors (aerobic digesters) were used as the primary treatment step for the combined wastewater stream generated by the crew's showering, hand washing, clothes washing, and urination as well as humidity condensate from the chamber. These bioreactors depended on microbial species to oxidize organic carbonaceous and nitrogenous materials in the wastewater. The bioreactors were integrated with P/C subsystems, which removed inorganic salts and performed final polishing of the water before being reused by the crew. The initial eight-day supply of water cycled though the chamber and the crew 10 times. No additional water was required during the test.

The third biological and P/C integration method pertained to the Solid Waste Incineration System (SWIS) and the wheat plants. The crew's fecal material was

incinerated in a fluidized bed incinerator. Oxygen required for the combustion of the fecal material was provided from the O_2 produced by the wheat plants. The CO_2 produced as a result of the incineration reaction was used as a second source of CO_2 for wheat photosynthesis. The test utilized a hierarchical control system for handling the competition for resources. This competition is inevitable when biological systems, which operate continuously, are used to provide the life support function for a crew. Wheat was harvested periodically throughout the test and after drying, threshing, and milling, the wheat flour was provided to the crew to bake bread in the LSSIF. The wheat provided less than 5% of the crew's caloric intake during the course of the test.

The final biological and P/C integration method was the incorporation of a small chamber to grow lettuce within the LSSIF. This chamber was able to provide four heads of lettuce for the crew approximately every 11 days.

The Phase III test was very successful in integrating biological and P/C life support system technologies for long-duration life support. The use of a biologically-based WRS demonstrated the operation of a system that recovered essentially 100% of the influent wastewater for reuse. In addition, the first step in recovering useful materials from the crew's fecal material was demonstrated in an integrated system. These capabilities are critical for all of NASA's future, long-duration human exploration missions.



KEY

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PCWQM = process control water quality monitor
TCCS = trace contaminant control subsystem
UF/RO = ultrafiltration/reverse osmosis
TCS = thermal control subsystem
VCD = vapor compression distillation
VRA = volatile removal assembly
WRS = water recovery subsystem

Figure 2.1-6 *The Phase III, 91-day test functional schematic*

2.2

Chamber Study Medical Care Overview: Medical Officer's Report

Kathleen A. McMonigal, M.D., Terrence J. Pattinson, M.D.

SUMMARY

Primary medical and health responsibilities for the Lunar-Mars Life Support Test Project (LMLSTP) were assigned to the Medical Operations Branch at Johnson Space Center (JSC). The prime medical officer for Phases I, II, and IIa, John F. Zieglschmid, M.D., and the prime medical officer for Phase III, Kathleen A. McMonigal, M.D., were designated to carry out these responsibilities, which included medical evaluation and health care of the test subjects. The medical officer was responsible for all medical aspects including pre- and posttest crew medical examinations, ensurance of in-chamber water, and atmospheric gas and food quality. The medical officer also coordinated with principal investigators, demonstration project investigators, the management of the crew and thermal systems division, and the management of the life sciences division. Terrence J. Pattinson, M.D. served in the capacity of Institutional Review Board medical monitor and was deputy medical officer for Phases IIa and III.

Pretest Activities

This medical officer's (KAM) participation in LMLSTP activities began with assignment to the project approximately six weeks prior to the test. This period was occupied with introductions to the investigators and members of the management team, review of the organization of the various supporting functions, crew testing, and examination of prime and back-up test subject crews.

During the pretest period, the medical status of subjects was reviewed in preparation for their entry into the chamber.

Phase IIa Test Activities

Crew Health

Test subjects complained of eye and mucous membrane irritation shortly after the test began. Investigation revealed significantly elevated formaldehyde levels (up to 0.21 mg/m^3 [ppm]). Newly installed insulation/sound-proofing material was suspected to be offgassing; therefore the material was removed. The formaldehyde levels gradually declined.

One test subject developed biochemical evidence of hypothyroidism two months following test completion. The subject was evaluated and followed prospectively until the thyroid function tests returned to baseline five months later. The test subject appeared to be clinically normal. Consultation with a thyroidologist was conducted. It was concluded that the thyroid changes were likely due to ingestion of excess iodine.

Phase III Test Activities

A change in the composition of the prime crew was made before the Phase III chamber test when a prime crew test subject developed a disqualifying medical condition prior to start of the test. One of the back-up test subjects moved into the prime crew position.

Crew Health

One test subject fell on the stairs, sustaining a leg laceration. The injury was examined by the crew medical officer. Under audio and video guidance from the medical officer, the chamber crew provided treatment for the wound. The wound healed satisfactorily without complications.

One test subject sustained an overuse injury of the knee associated with the cycle ergometer. The test subject refrained from lower-extremity exercise for a period of three weeks, until the condition had resolved.

One month after the test began, one test subject was noted to have decreased hemoglobin and red blood cell counts. This anomaly persisted for the remainder of the test, but resolved one month after conclusion of the test. Two other test subjects were also noted to have slightly decreased hemoglobin and red blood cell counts at the conclusion of the test, with resolution of the laboratory abnormalities occurring in these individuals one month after conclusion of the test. After consultation with a hematologist, it was concluded that the hematology changes were likely due to adaptive changes of exercise in two test subjects and to iron deficiency anemia in one test subject (see Chapter 5.1).

Water Quality

The crew began by drinking iodinated water, 5 mg/L, as was customary for ground-based and space flight crews. Because one test subject from the Phase IIa test had developed subclinical hypothyroidism following test completion, the thyroid function tests of this crew were monitored closely. When the thyroid function tests were evaluated 30 days after the chamber test began, the thyroid-stimulating hormone (TSH) level was 2 to 4 times higher than baseline and the thyroxine

levels had fallen slightly. The 24-hour urine iodine samples showed excretion of 7 to 16 mg iodine. On the 35th day of the test, the iodine was removed from the drinking water following installation of an anion-exchange resin. A 0.2-micron filter was installed at the use port distal to the deiodinator. Subsequently, water samples showed < 0.05 mg/L iodine from the galley sink. Forty-eight hour microbial counts from the deiodinated galley use port were 3 cfu/100 ml or less (with most counts < 1 cfu/100 ml). Thyroid function tests and urine iodine levels were monitored for the remainder of the test. The thyroid function tests returned close to the baseline levels by the completion of the test. Urine iodine levels persisted at ~1 mg/L through the end of the test, although urine iodine samples three days after completion of the test showed values of 0.2 to 0.3 mg/24 hours, which is within the normal range. The cause for the persistently elevated urine iodine levels in the chamber for the last six weeks of the test, at levels greater than that which was expected from the food alone, is uncertain.

Air Quality

Formaldehyde levels slightly above spacecraft maximum air concentration (SMAC) levels (0.06 to 0.07 mg/m^3) were identified early in the test and during the last month. No signs or symptoms of skin or mucosal irritation due to the formaldehyde were identified in the crew. The crew was exposed to low levels of diethylamine (~1.58 mg/m^3) leaking from one component of the air revitalization system, but no symptoms of mucosal irritation were reported.

Posttest Activities

Three of four test subjects had a 6- to 9-pound weight loss during the Phase III test. The hematology values in the Phase III test subjects returned to baseline levels after cessation of the exercise protocol.

The thyroid function tests in the Phase IIa test subject, as mentioned previously, returned to baseline seven months after study completion. One test subject in the Phase III test developed biochemical evidence of hyperthyroidism five months after study completion (seven months after discontinuing iodinated water consumption). Consultation with a thyroidologist was obtained. It was concluded that the thyroid test changes were likely due to excess iodine ingestion even though the iodine had been discontinued some months earlier. Thyroid function tests returned to baseline 10 months after study completion (12 months after discontinuing iodinated water consumption). The test subject appeared to be clinically normal. There were no other significant changes in the crew health, which could be attributed to the chamber stay.

Recommendations for Future Chamber Studies

Medical monitoring of the test subjects was hampered by inadequate documentation of medical history and physical examination and a lack of essential laboratory tests and ancillary studies obtained prior to the start of the project. Therefore, after completion of the LMLSTP study, test subject medical selection requirements were written and approved by the Aerospace Medicine Board (see Appendix). These include appropriate medical history, physical examination, laboratory, and ancillary testing, with comprehensive documentation essential to the medical evaluation of an individual's health status.

Two injuries occurred over the course of the project. A laceration from contact with the ladder was effectively managed by the in-chamber crew acting under direction of the physician medical officer using telemedicine audio/video. In selected cases of minor illness or injury, appropriately trained chamber crew can provide limited medical care under the direction of the medical officer who has conducted telemedicine evaluation of the test subject. Such treatment must be carefully monitored by the physician medical officer. Medical supplies for the chamber should be expanded from the current minimal configuration to include appropriate supplies, medication, and equipment that could be used in these cases.

The second injury, an overuse injury during an exercise protocol, resolved after the test subject refrained from lower-extremity exercise for a period of three weeks. Increases in workload during exercise protocols should be carried out with appropriate consultation and communication between the subject, the principal investigator, and the medical officer. This may help to decrease the probability of the occurrence of training injuries resulting from increases in workload.

Three test subjects exhibited decreased hemoglobin levels. Two of these cases were found to be due to increased plasma volume as the result of exercise, while the third case was determined to be due to iron deficiency. Although exercise-induced "anemia" is likely to occur in this setting, the medical officer must rule out other possible causes of anemia including occult bleeding, hemolysis, marrow failure, anemia associated with illness, nutritional deficiency, or other causes.

Iodine will continue to be used for the disinfection of the water. Although iodine will be removed from the drinking water, iodinated water will still be present in the shower water and wash water. Ongoing monitoring of the water recycling system will be necessary to ensure proper functioning of the filters and resins and maintenance of microbial control.

The Phase IIa crew experienced skin and mucous membrane irritation from formaldehyde offgassing from newly installed insulation/sound-proofing material. Formaldehyde was specifically monitored from various locations during the

Phase III test by analysis of badge samples to ensure levels remained within the acceptable range. Particular attention to crew symptoms must continue when new or untested materials are introduced into a sealed environment.

Appendix

Subject: Policies and Procedures for Selection Medical Examinations and Medical Certification of Closed Chamber Study Test Subjects

Responsible Individual(s): physicians conducting selection medical examinations and medical certification of closed chamber studies test subjects.

1. PURPOSE

The purpose of this document is to provide standard policies and procedures for selection medical examinations and medical certification of closed chamber studies test subjects.

2. SCOPE

These policies and procedures apply to physicians who conduct medical evaluations to determine the medical qualification of individuals undergoing selection for positions as human test subjects in closed chamber studies in which Institutional Review Board (IRB) review and approval is required.

3. REFERENCES

a. Air Force Instruction 48-123, "Medical Examination and Standards."
b. NASA Management Instruction (NMI) 7100.8, "Protection of Human Research Subjects."
c. NASA IOHERD, "Human Experimental and Research Data Records," Privacy Act of 1974, Systems of Records.
d. NASA 10 HIMS, "Health Information Management," Systems of Records.
e. JSC Management Instruction (JMI) 1382.8, Privacy Act of 1974.11
f. JSC-20483, "Revision B II JSC Institutional Review Board – Guidelines for Investigators Proposing Human Research for Space Flight and Related Investigations."

4. PROCEDURE

Medical evaluation of individuals for the purpose of medical selection and medical certification for participation in closed chamber studies human research tests shall be conducted in accord with the above referenced policy directives. In addition to the published directives, the following procedures will apply.

a. Medical examinations and evaluations will be conducted by physicians who are familiar with the medical issues of long duration, closed chamber tests.
b. The minimum medical standards for selection of closed chamber studies test subjects will be those standards that are required to be met for Air Force Class III medical certification. The document defining those standards is Air Force Instruction 48-123, "Medical Examination and Standards."
c. Additional requirements for certification may include mission or test specific medical requirements as established by the responsible test physician.
d. Completion of SF form 93 and the NASA Medical Survey (JSC form 1639) is to be done by the subject prior to the physical examination. The physician will review these forms, interview the subject with attention to positive disclosures on the forms and make appropriate comment on the medical record.
e. A comprehensive history and physical examination shall be conducted for each subject and will be documented in typewritten or computer-printed standard narrative format including:
   1) purpose of the examination
   2) history of any present illness
   3) past medical history including an appropriate discussion of illnesses, surgery, injuries, transfusions and allergies
   4) family medical history
   5) social history
   6) health habits including alcohol, drug and cigarette use
   7) occupational history including exposure to toxins, radiation or pathogens.
   8) Physical examination of all systems will be comprehensive in nature, however a female subject may provide medical records of pelvic exam nation and Pap smear within the preceding year by her private physician, as an alternative to examination by the NASA physician.
f. Appropriate documentation of the history and physical examination will include the specific negative as well as the specific positive findings for each system that is examined. The history and physical examination will be documented in standard narrative format that will be computer-printed or typewritten. In addition to the narrative, an SF form 88 will be completed by the physician.
g. The minimum laboratory tests that will be obtained will include the following:

1) CBC with differential count and reticulocyte count
2) urinalysis
3) chemistry panel
4) lipid profile
5) thyroid panel, free T4, thyroid autoantibodies
6) serum iron, iron binding capacity, percent iron saturation, ferritin
7) hepatitis A Ab, hepatitis BsAg, hepatitis C Ab
8) HIV antibody
9) stool hemocult if 40 years of age or older
10) urine pregnancy test, if female

h. Other tests/examinations will include:
1) height, weight and vital signs
2) visual acuity and intraocular pressure
3) audiometry
4) tuberculin skin test, unless previously positive or if the individual has received BCG in the past
5) chest x-ray within 5 years
6) pulmonary function test
7) treadmill exercise ECG (Bruce or Cunningham)

i. For medical screening purposes, a psychiatric evaluation will be conducted by a psychiatrist and a psychologist with expertise in the area of crew selection for unusual or extreme environments. This evaluation will be comparable to the 'select-out' evaluation administered to astronaut applicants and will consist of psychological testing and a structured clinical interview. Selection criteria will be the same as the selection criteria and procedures currently in use for astronaut selection. However, modifications will be made depending upon the length of the mission:
1) *Missions < 30 days.* A subset of the current psychological battery will be given; specifically, the Family History Questionnaire, MMPI-2 and NEO-PI-R will be used. A full version of the current structured clinical interview will be administered.
2) *Missions 30 or more days.* All tests which are a part of the current psychological battery will be included. At present, these are FHQ, MMPI-2, NEO-PI-R, and FSSCT. A full version of the current structured clinical interview will be administered and any further diagnostic tests applicable to astronaut selection at that time.
3) The medical screening process will share psychological test and interview data, as well as interview time, with the suitability ('select-in') process.

Psychiatric certification of the test subject by the examining psychologist and psychiatrist as "qualified for closed chamber test crew" will be required for subject participation as a crewmember.

4) A separate, non-medical 'select-in' evaluation will also be conducted on each applicant by a psychologist with expertise in assessing psychological suitability of candidates for extreme or confined environments.
However, the results of this evaluation do not affect determination of a subject's Class III medical certification, and recommendations derived from this 'select-in' examination are forwarded to the closed chamber test selection committee for consideration in their crew selection decisions.

j. Dental examination must be conducted within one year prior to the selection physical examination. The subject shall provide certification of dental health documenting the absence of current dental pathology. All required dental care should be completed by the time of selection examination or soon thereafter.

k. Medical consultation will be obtained at the discretion of the examining physician.

l. Additional laboratory or ancillary tests may be ordered at the discretion of the examining or consulting physicians.

m. The examining physician will determine whether the subject meets the requirements for Air Force Class III medical certification.

n. The Aerospace Medical Board may consider waiver for conditions that are disqualifying for Air Force Class III medical certification, if in the opinion of the Board, such disqualifying condition would not constitute a threat to the health and safety of the subject or other persons, or to the successful completion of the test.

o. Any medical condition or defect that develops in a test subject who is certified must be reported to the test physician. Any condition that in the opinion of the physician presents a hazard to the individual's health or to mission completion is cause for withholding certification for initial participation or disqualification for continued participation. To be considered waiverable, any disqualifying condition should meet the criteria as outlined in the above directives.

Effective date: February 4, 1998
Revision: May 21, 1998

3.1

Architecture

Janis H. Connolly, M. Arch., Assoc. AIA

SUMMARY

For the Lunar-Mars Life Support Test Project (LMLSTP), the retrofit of an existing 6-meter (20-foot) vacuum chamber, renamed the Life Support Systems Integration Facility (LSSIF), was challenged to provide for a human's basic needs, in addition to those that would be required given the unique nature of the environment and test objectives. Located within a building at the Johnson Space Center, only a limited volume was available within the geometry of the chamber, which was already divided into three levels. Each floor provided approximately 29.1 square meters (314 square feet gross area) and 226.5 cubic meters (approximately 8000 cubic feet gross volume) for crew functions and equipment. Required was an overall plan to divide each of the levels into functional spaces, several serving as dual- or triple-use areas. The lower level was dedicated to most of the crew's public and group activities, the second level housed systems equipment, stowage, and occasional exercise equipment, and the upper level provided for crew privacy.

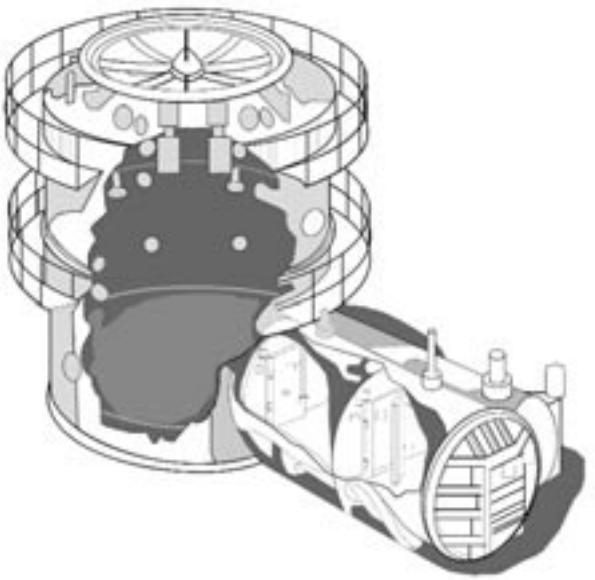


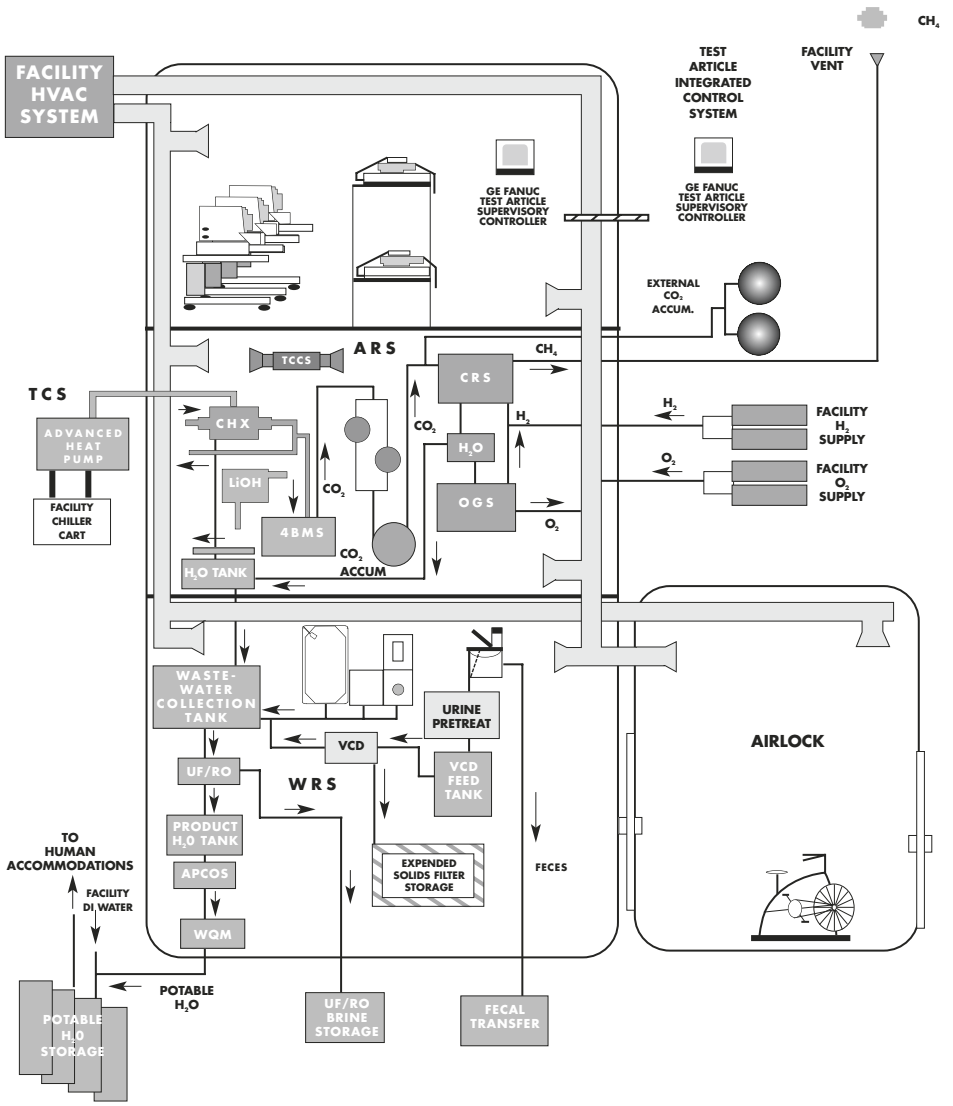
Figure 3.1-1 *Exterior rendering and cutaway of LSSIF complex with airlock*

Of importance was that the interior configuration and outfitting would need to address the safety of the crew. The design addressed potential mechanical and electrical hazards and endeavored to eliminate them. Fire safety and warning systems were employed. A lift was required on the upper level to allow for the safe exit of a crewmember in the event of an emergency. A considerable challenge confronting the team charged with the interior configuration was the materials from which the vacuum chamber had been constructed. Metal was the construction material of choice – an acoustics challenge to be sure. The selection of all interior surfaces and materials had several critical criteria that had to be met: they should be nonflammable, should produce minimal outgassing (within acceptable limits to the life support system), should be easy to maintain and clean using cleansers compatible with the recycling systems, depending upon use could not be porous, and would support acoustics abatement when possible.

The "20'-Chamber" design for the LMLSTP was to provide for basic needs and to allow the outfitting of a number of crew functions: external/internal communications, general meeting capability, personal hygiene, health care, food preparation and associated stowage, dining, exercise, sleep, crew privacy, general stowage, cleaning of clothing, recreation, trash management, and installation of equipment required to support the tests.

LMLSTP Phase II

The LSSIF (or commonly referred to as the "20 foot chamber") was retrofit for a series of three tests. Entitled Phase II, Phase IIa, and Phase III, each test had its own objectives yet needed to provide for the same crew functions. The schematic section below illustrates the divisions of the chamber by floor. The exterior image shows the mid and upper levels with support structure surrounding the chamber.



KEY

APCOS = aqueous phase catalytic oxidation subsystem
ARS = air revitalization subsystem
4BMS = four-bed molecular sieve
CHX = condensing heat exchanger
CRS = carbon dioxide reduction subsystem
DI = deionized
GE FANUC = programmable logic controller used for the LMLSTP
HVAC = heating, ventilation, and air conditioning
OGS = oxygen generation subsystem
TCCS = trace contamination control subsystem
TCS = thermal control subsystem
UF/RO = ultrafiltration/reverse osmosis
VCD = vapor compression distillation
WQM = water quality monitor
WRS = water recovery subsystem

Figure 3.1-2 *Architectural section of LSSIF during Phase II test*

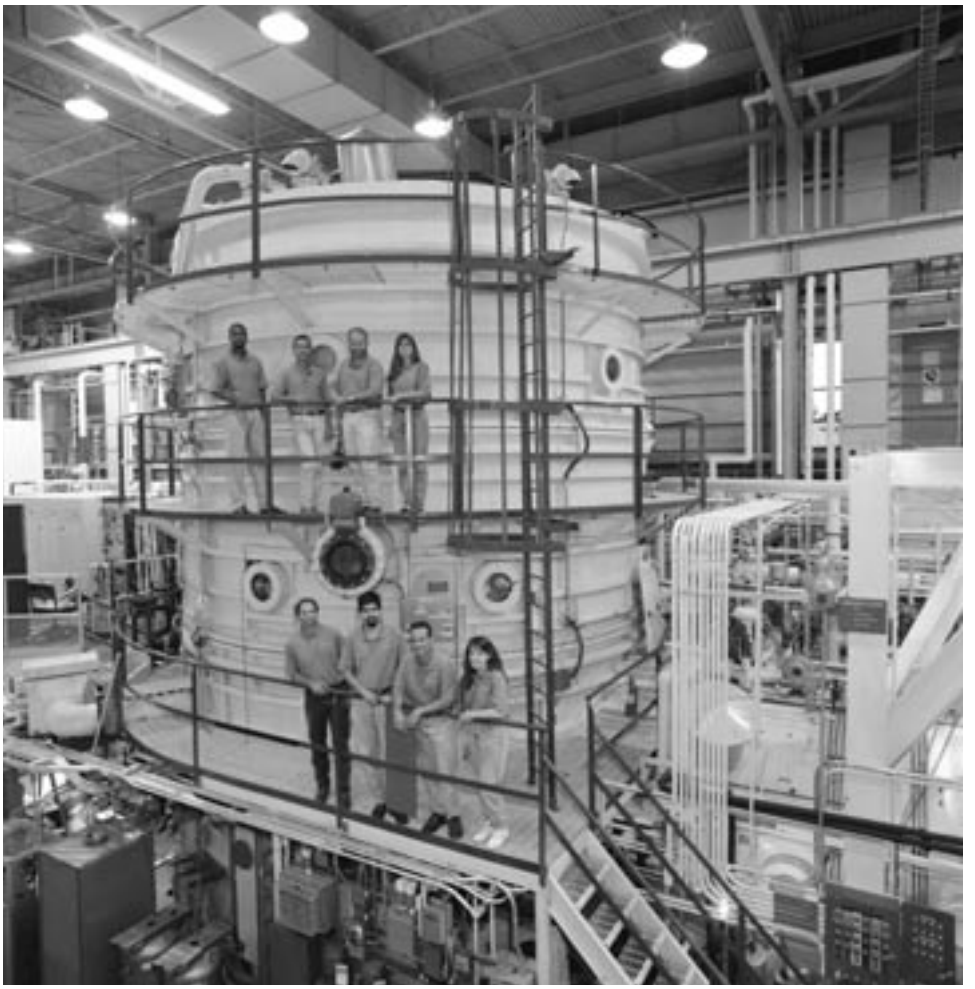


Figure 3.1-3 *Exterior of LSSIF for Phase II with prime and back-up crewmembers*

The entrance to the LSSIF is an airlock that is attached external to the cylindrical shell at the first level. Within the airlock, a volume separate from the living quarters was dedicated to crew exercise. The airlock housed the treadmill and exercise bicycle for Phase II. Other than carpet on the floor, no other material was attached to the airlock shell. General illumination was attached to the airlock wall. Electrical and audio outlets provided power for hardware and personal audio equipment used while exercising.

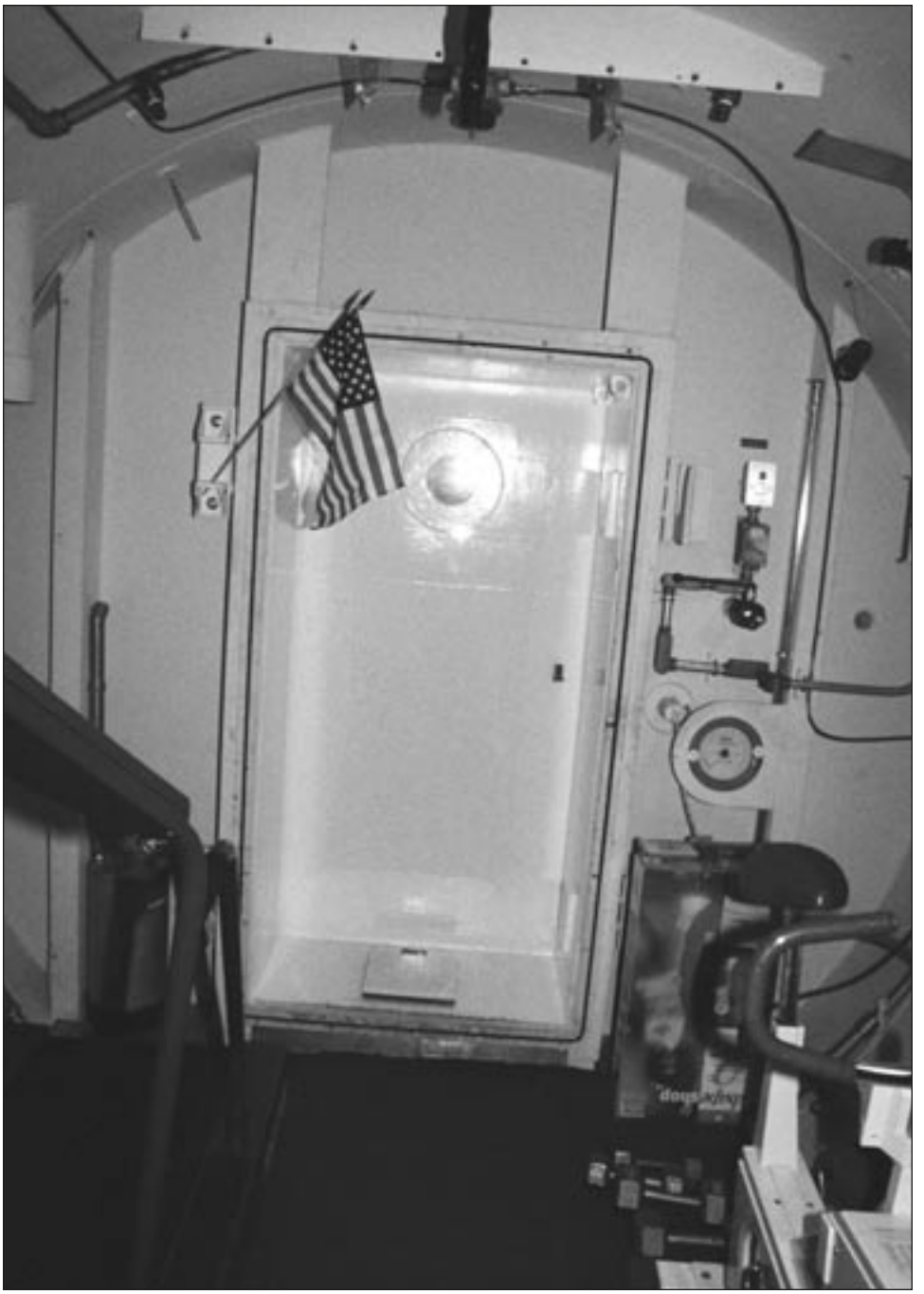


Figure 3.1-4 *View of airlock toward chamber exit, treadmill on left, exercise bicycle on right*

Figure 3.1-5 *View of Phase II crewmember Pat O'Rear during exercise on treadmill*

The lower level provided accommodations for food preparation and stowage in the galley. Also included on the lower level was the hygiene facility (for hand washing, showers, and fecal waste and urine collection), the wardroom (a table located centrally in one half of the level), the laundry facility, communications, and

translation staircase to the upper levels. In addition, a transfer lock was provided to allow for equipment, personal items, food, supplies, and samples to be imported into and exported from the chamber. In general, those functions that supported group or public types of activities were allocated space on the lower floor. Gathering for meals, holding group meetings, communications between the crew and the control room staff and guests, and videoconferences with a variety of remote sites were all held in the common space. This public versus private separation of space was created to allow for the greatest amount of privacy for individual crew functions on the upper level.

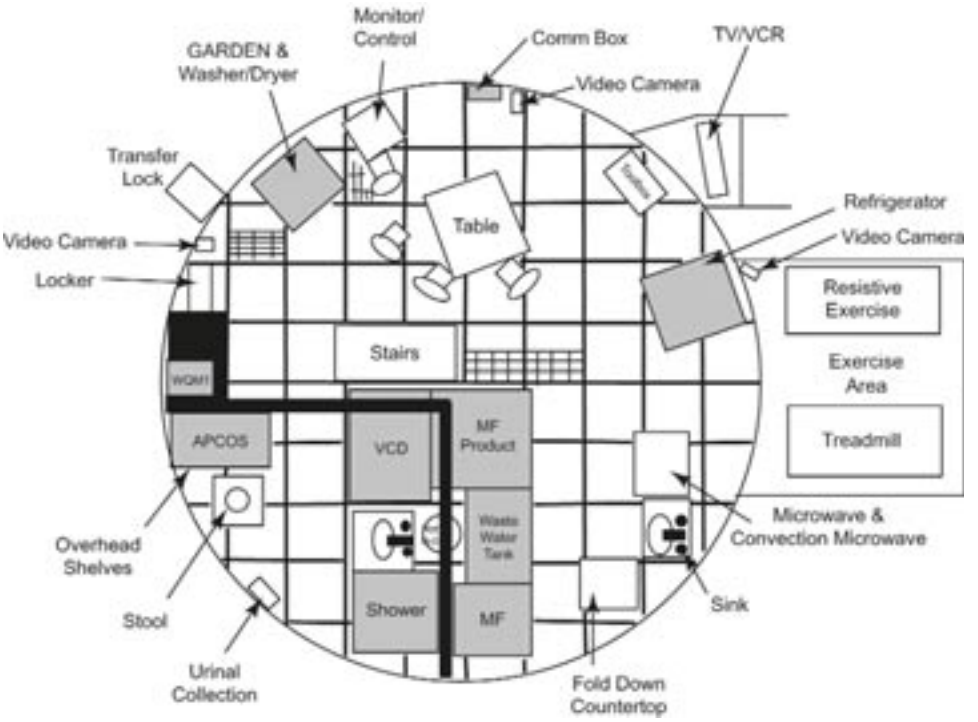


Figure 3.1-6 *LLSIF lower level floor plan*

Figure 3.1-7 *Lower-level galley*

Within the chamber, the metallic walls were covered with an insulation material to provide general acoustic abatement. General illumination was provided by fluorescent fixtures which were diffused to assist in light distribution. Carpeting was installed over the floor plates in such a fashion to allow for access to the volume directly beneath the floor level. The color of the materials was limited to commercial availability and only to materials with the properties necessary for the closed-loop life support environment. Special attention was paid to the use of adhesives that also possessed the properties compatible with the closed system to attach the materials.

The galley was equipped with a sink and stowage cabinetry of stainless steel. Two microwave ovens allowed for preparation of the food. Food preparation surfaces with nonporous characteristics provided a surface that was easy to maintain, minimizing the potential for microbial growth.

Adjacent to the galley was the wardroom area that served multiple functions. The most prominent use was for crew dining, although the table served as a workstation and location for the crew to gather for group communication, either for recreation or for more formal presentations or interviews, as mentioned above. Group messaging and logging of food consumption, daily activities and crew exercise, as well as water usage, were accomplished by manually recording these activities onto clipboards attached to the front face of the refrigerator.

Figure 3.1-8 *Crewmembers Vickie Kloeris, Laura Supra, and John Lewis at wardroom table; communications center visible behind John Lewis*

Figure 3.1-9 *Phase II crew, Katy Hurlbert and Doug Ming, preparing meal at wardroom table*

The compact washer/dryer unit was placed close to the table. Every fourth day, the crew was allowed to wash their clothing. The table could then serve as a place to fold the laundry prior to stowing it within the crew quarters. A computer workstation provided a means of monitoring various functions and systems within the chamber at a location on the lower level where cameras provided direct communication with the control room.

Figure 3.1-10 *Compact washer/dryer unit located near the wardroom table*

Figure 3.1-11 *Phase II crew, Patrick O'Rear, John Lewis, and Katy Hurlbert, gathered at wardroom table. Note locations of washer/dryer and workstation*

Various items were transferred several times per day through the small equipment lock. The items transferred included personal items for the crew, samples to be tested by investigators outside of the chamber, equipment supporting experiments, tools, and biological samples. Located adjacent to the washer/dryer, the transfers were accomplished by loading the transfer cart on either side of the equipment lock. To transfer, one hatch was opened, the transfer cart was placed into the equipment lock, and the hatch then closed. Communication was given to the crew, or to the control room, that the hatch on the opposite side could be opened. By securing the hatches on either side in the appropriate order, the integrity of the internal environment of the chamber was maintained.

Figure 3.1-12 *Phase II crewmember John Lewis performs transfer duties*

Figure 3.1-13 *Phase II materials being placed in the transfer cart by Katy Hurlbert and readied for placement within the transfer lock*

Personal hygiene was accommodated on both the lower and upper levels. On the lower level, full-body cleansing could be performed. This level included the shower stall, a one-piece premolded unit, hanging space for towels, stowage for personal belongings, a curtain for privacy, and a flow meter control to monitor water usage. One shower per day was allotted to each crewmember, occurring most often immediately after the conclusion of the exercise session.

Figure 3.1-14 *Solid fecal waste was collected in the toilet portion of the hygiene area. It was bagged and exported from the chamber. A curtain allowed for personal privacy during use*

Figure 3.1-15 *The urinal collection area provided for crew hygiene plus the hanging of towels and a posting area for checklists and logs*

Figure 3.1-16 *The premolded shower unit defined the majority of the hygiene area*

During the Phase II test, the second level of the chamber was utilized only for equipment and additional stowage. The crew did translate through the second level by staircase that led to the private quarters on the third floor. The stairs were quite steep, and translation had to be taken with care, especially if transferring equipment or supplies from one level to another.

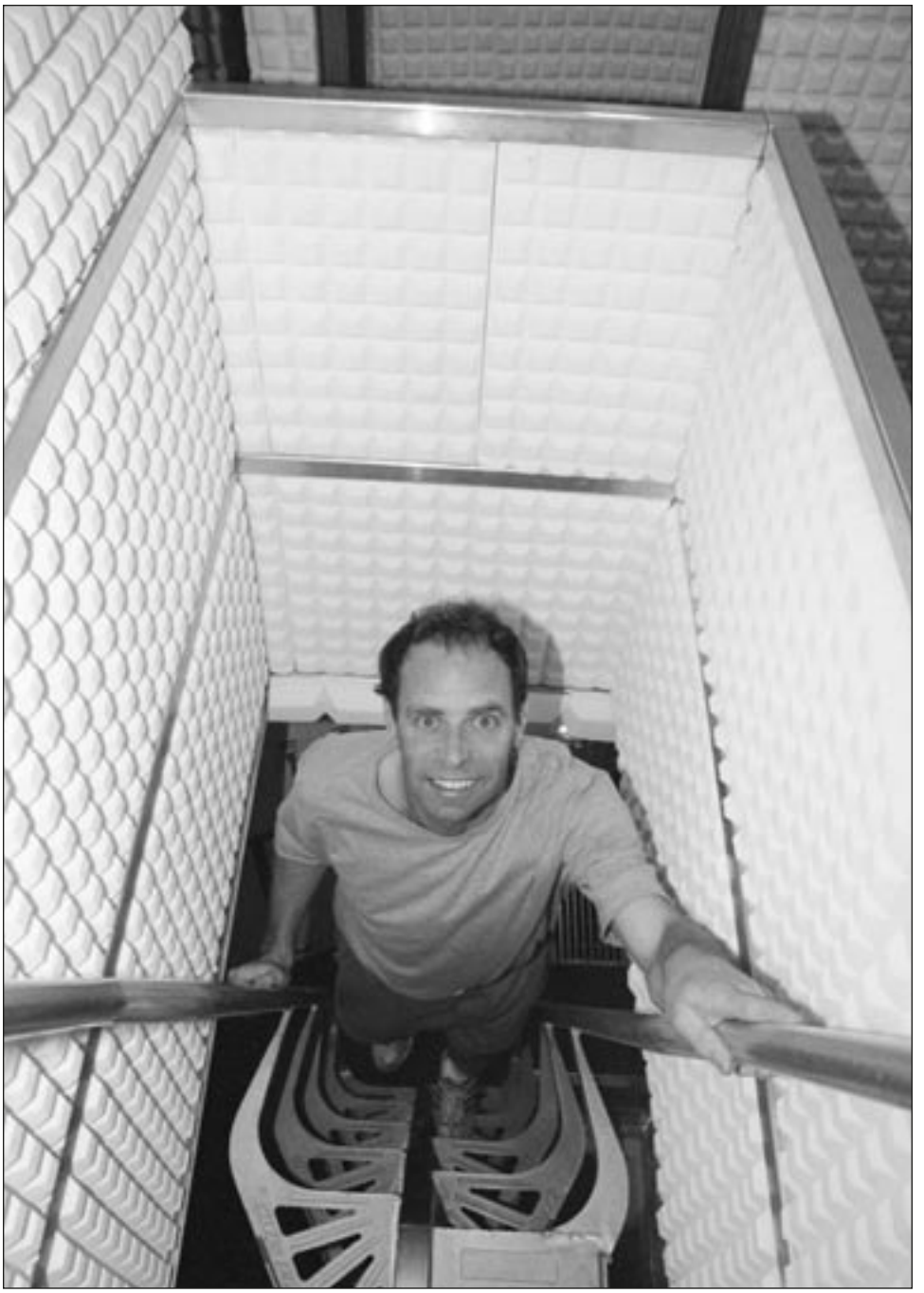


Figure 3.1-17 *Phase II crewmember Doug Ming climbs the stairs from the second to third level. Note the acoustic material surrounding the stairwell. Equipment on the second level emitted noise that the material helped abate*

Above the equipment level, the entire space was dedicated to the crew and their individual, private quarters. The layout of the floor was divided in half with two quarters on either side of a central hallway. The stairs terminated in the central portion.

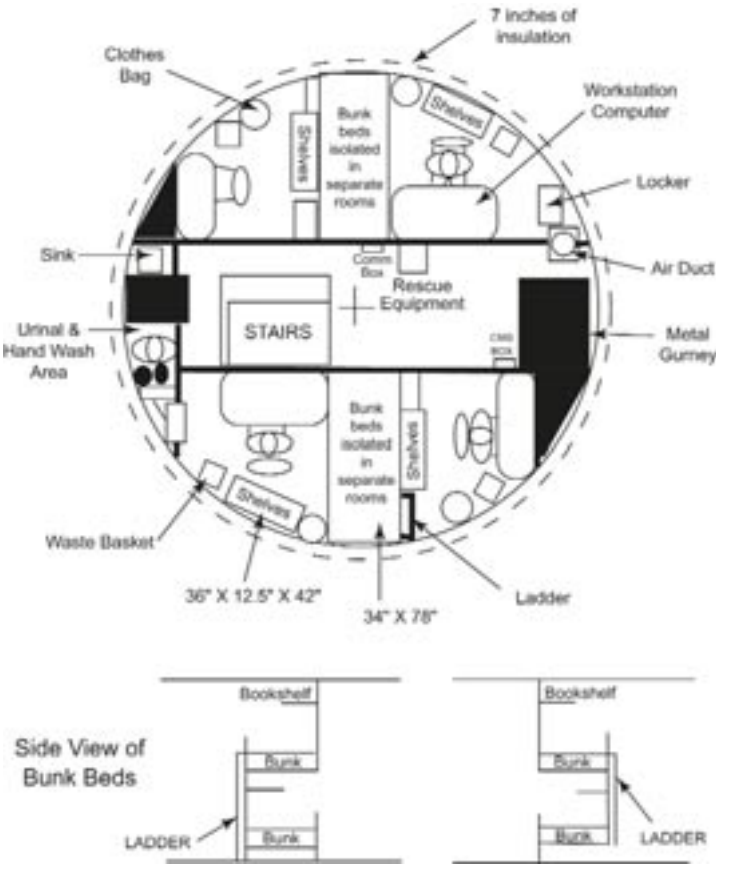


Figure 3.1-18 *Floor plan for third level of chamber. Detail of bunk area illustrates the utilization of the bed from each crew quarter*

For the sake of economy, each of the quarters was provided a twin-sized bed. By placing them in bunk fashion, one over the other, the crew quarters were provided additional floor space. In one quarter, the crew had a lower bunk, and in the adjacent quarter, the other crew was given an upper bunk. Acoustic material was installed to provide as much privacy as possible within the allowable limits imposed by the life support system. Each quarter was equipped with controllable lighting and ventilation, a computer workstation, stowage, and private communications capability. The individual quarters were also an area for the crew to display personal items.

Figure 3.1-19 *Crewmember Laura Supra at workstation within her personal quarters*

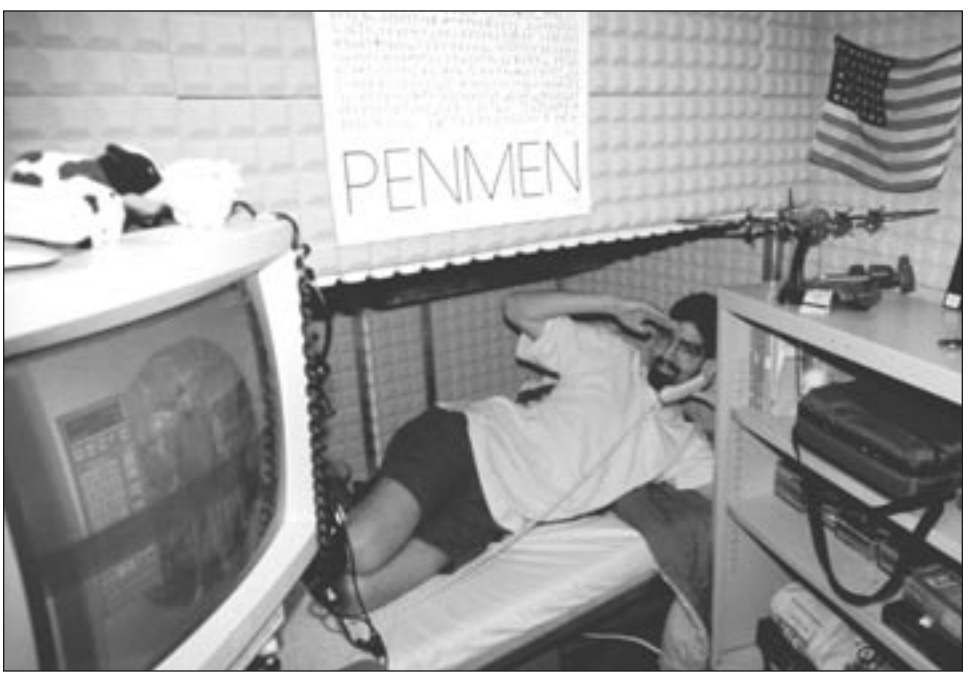


Figure 3.1-20 *Phase II crewmember John Lewis relaxes in lower bunk. Note acoustic lining of the bunk area to provide additional privacy*

A partial hygiene facility was installed on the upper level of the chamber, providing hand washing capability and urine collection. This volume was quite compact as it was located in a compartment at the top of the stair. A sliding door provided privacy.

LMLSTP Phase IIa

The second test conducted within the LSSIF provided accommodations for the crew of four similar to those provided during Phase II. In addition to those accommodations already constructed, the second level provided a laboratory workbench amidst the systems equipment. The work area was lined with the acoustic insulation, and the work surface was again of a stainless steel material that would allow for ease of maintainability and cleaning. Simple shelving on the lower level adjacent to the wardroom table area provided a location to store additional food and supplies for housekeeping of the chamber interior.

Figure 3.1-21 *First-floor pantry provides additional stowage for food and supplies*

In addition to the treadmill and bicycle ergometer, the airlock housed a resistive exercise machine for this particular test. Again the crew spent a portion of each day performing a series of exercises to maintain their health.

Figure 3.1-22 *Phase IIa crewmember Terry Tri works out on the resistive exercise equipment*

Phase IIa saw the inclusion of various cooperative research objectives to evaluate the habitability of the chamber, food system, sleep, training, and environmental assessments, to name but a few. The chamber proved an appropriate venue to study these issues, and the findings can be found elsewhere in this body of work.

LMLSTP Phase III

The final phase of the tests to be held within the LSSIF saw minimal changes in the interior configuration of the three levels. One of the more prominent changes, however, was the replacement of the acoustic insulation that lined the walls of the chamber. A concern had arisen over formaldehyde offgassing exposure from the

maroon-colored insulation used during Phase II and Phase IIa. In keeping with the priority of crew safety, the maroon-colored insulation was removed and replaced with an available nonhazardous golden-color material that met the life support system requirements.

The airlock remained the location for the majority of the exercise devices. Both the treadmill and the resistive exercise equipment dominated the volume attached to the chamber.

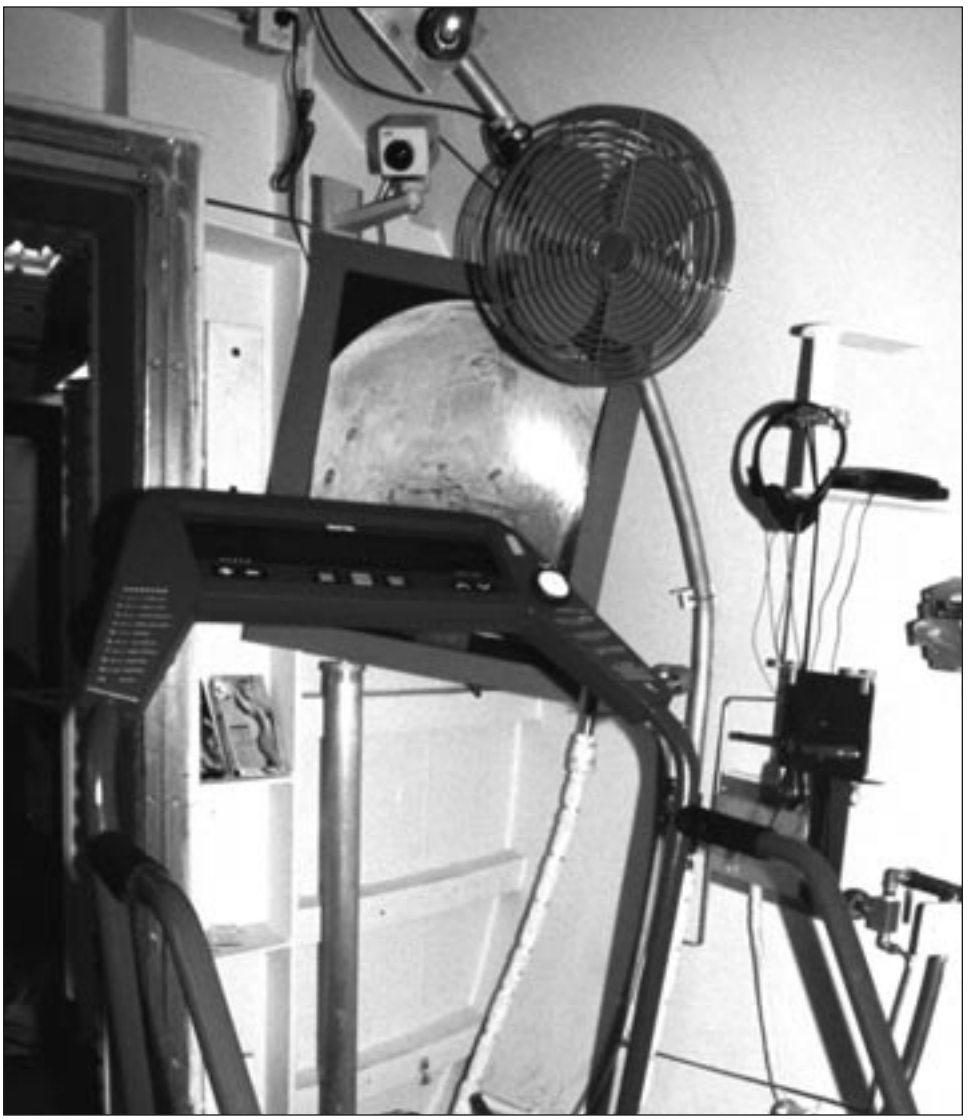


Figure 3.1-23 *Treadmill located within the airlock*

The same hardware for the wardroom and galley remained. Each area still provided a multipurpose work surface, communications capability, food preparation and serving capability, and provisions for record keeping, trash management, and cleaning (see Figure 3.1-7). General illumination using fluorescent lighting remained installed.

The lower level contained the personal hygiene area comprised of the urinal, fecal collection device, shower, and hand wash (see Figure 3.1-16). Each area provided stowage of hygiene supplies, test and monitoring equipment, and personal belongings.

Another change in equipment for the lower level was the washer and dryer unit. A unit was installed for Phase III that allowed the clothing to be laundered and dried within the same component. This allowed for a saving in volume that was then allotted to the "GARDEN"- Growth Apparatus for the Regenerative Development of Edible Nourishment. Not only did the GARDEN provide fresh produce, it also provided a change in the color scheme of the lower level. The prominent "purple" glow emitted from the "growth lights" was evident from all locations on that level.

Figure 3.1-24 *Lettuce grown in the GARDEN unit provided fresh produce during Phase III*

During Phase III, the second floor remained dominated by the systems equipment necessary to support chamber functions. In addition, a piece of exercise equipment, the bicycle, was relocated to that level, the maintenance/laboratory workbench was again used, and stowage volume was available for additional provisions.

The upper level of the LSSIF supported the crew of four by providing their personal quarters (including locker stowage, bookshelf, bunk bed, and computer workstation), a partial hygiene facility, and the lift necessary to allow the safe exit of a crewmember in the event of an emergency.

Figure 3.1-25 *View of personal quarters stowage and bunk*

Figure 3.1-26 *A bank of stowage lockers gives the crew additional volume for personal belongings*

Cameras were installed in various locations throughout the chamber, except for the private areas, providing views of chamber life to the community. Intercoms on each level gave the crew the ability to communicate with each other and the control room. A camera within the control room gave the chamber crew the ability to see what events were occurring within that area.

As experienced by previous crewmembers, the staircase, which allowed for movement between levels, challenged individuals with or without carrying supplies or equipment. The staircase was quite steep and very narrow and provided just enough tread to place only one foot, step by step.

Figure 3.1-27 *Phase III crewmembers tightly gathered on the staircase for a photo opportunity*

SUMMARY OF CHAMBER ARCHITECTURE

The retrofit of an existing vacuum chamber on site at the Johnson Space Center served to house humans involved in the development and test of life support systems equipment. The interior volume was preselected, the "shell" geometry defined and unchangeable, and movement between levels supported by a steep staircase. Given these initial conditions, a team of engineers, coupled with limited consultation from architectural designers, began the task of preparing the interior to support a crew of four humans each for three successive tests. There were assumptions made regarding the interior configuration, the most challenging being the use of existing equipment to outfit the chamber. Little custom design of crew accommodations was provided. This meant that the equipment and accommodations would have to be fit as best as reasonable within the given geometry.

The chamber was contained within a building and provided no direct viewing to the natural exterior environment. Camera views outside could be placed on the monitors if desired. No sunlight was available. Window viewing was limited to the airlock "porthole" where, on occasion, visitors could view the chamber crewmembers and communicate on a limited, infrequent basis.

Materials were chosen with properties that would not prove toxic to the humans in this closed environmental system. Unfortunately, one material, the maroon acoustic insulation used in Phase II and Phase IIa, outgassed formaldehyde and had to be replaced. Available colors for this type of material were limited, forcing a selection of a color that was considered least objectionable. A wall color was selected, and the ceilings of all three levels were covered with white insulation. Materials and color selections for any future advanced life support facility should be made based upon a scheme that incorporates the entirety of the chamber complex, considers material availability and appropriate use, and allows the crew a measure of control over the appearance of the interior by color changes.

Other materials were chosen based upon the need for ease of maintainability and clean-up – surfaces that were not porous, thereby limiting potential microbial growth – and allowing acoustic abatement whenever possible. Carpeting covered the metal grating floor plates but allowed for access to the volume below the plates. Materials needed to be compatible with all the life support systems within the chamber. The risk of outgassing had to be minimized, and the outgassing that did occur would need to be tolerated by the crew and the life support systems. These criteria will need to be applied once again when the construction of the new test facility is undertaken. One recommendation is that the limited "palette" of materials and colors be investigated more thoroughly and that additional tests be conducted on more materials to determine their viability for this type of use. This will allow for more flexibility on the selection of surface application, as well as for future selections of color and texture.

Lighting was limited to fluorescent fixtures located as required within the airlock and on all three levels of the chamber. Additional task lighting was made available in the private quarters. Future lighting will again need to address not only general illumination, but also both general and task lighting that the crew can control. This will allow for a full range of lighting levels to suit the functions supported within the test chambers.

Movement between levels was made possible with the use of a steep "ship's" staircase. Nearly vertical in nature, this provided a great challenge for the crew, with or without carrying equipment or supplies, yet was economical in volume utilized. Future trades would need to be conducted as to what type of translation can be provided and how each candidate solution would impact the interior configuration. In the LSSIF, the stair was at the central portion of the chamber. All interior outfitting had to address the location of that stair on all three levels. Economy is a significant consideration, but location will be a significant design driver to allow for maximization of the volume.

The lower level supported a number of functions in relatively close proximity. Given the defined shell and the equipment necessary to support test and chamber activity, those functions deemed group and public were assigned to the lower level.

This included the galley (food preparation, clean-up, and stowage) and wardroom functions (dining and general meeting), laundry, and overall chamber communications (audio and visual). The location of private functions, the shower and toilet, was placed in the lower floor due to the commercial shower unit dimensioning. The toilet function was close to the equipment lock that allowed the easy exporting of biological waste. This placement, while out of the direct line of sight, was still quite close to the most public of utilized spaces. In future test facility designs, the placement of a full-body hygiene facility and toilet needs to be away from the dining and public gathering locations.

The second level provided for few crew accommodations. By design, it was the equipment level. During a portion of the chamber tests, additional exercise equipment was located on this level. In addition, a maintenance/laboratory workstation was installed, providing a necessary surface for repairs. All future workstation environments must be evaluated for proper lighting, ventilation, surface area, and ability for the crew to access the equipment that requires maintenance or change-out of components.

The upper level provided sleep quarters and for the privacy of each individual crewmember. Separate quarters were outfitted with bunk beds (each secluded from the adjacent crewmember), stowage for supplies and personal belongings, a workstation, and private communication capability. All quarters were lined with acoustic insulation material to further isolate the sound of the equipment on the level below the quarters and to keep sound from traveling between the quarters. External to the quarters, a small hygiene facility provided for hand washing and urination. To further provide for the safety of the crew, a lift was installed on the upper level over hatches on each level that, in the event of an emergency, would allow for the incapacitated crewmember to be taken to the lower level and out of the chamber. In future crew quarters designs, the types of activities that the crew will conduct need to be traded with the amount of space required to support those functions. In addition, the crew, if so desired, should be able to reconfigure their personal spaces within the limitations of the exterior geometry.

Chamber studies on all habitability issues affecting the well-being and performance of the crew must continue to be conducted. Any future facility that will test advanced life support systems will provide an environment to study a wide variety of issues from lighting, color, configuration, and function, to communication, training, maintenance, and repair. The internal configurations of the test facilities and their evaluations should be developed and designed in tandem with those disciplines addressing human performance. By utilizing a multidisciplinary approach, coupled with advances in technology and materials application, the lessons learned will have direct applicability to humans leaving the familiar habitat of Earth and being sustained by an environment designed for maximum performance.

3.2

Habitability: an Evaluation

Frances E. Mount, Ph.D., CHFP

SUMMARY

Habitability is one aspect of long-duration missions that becomes more important as the mission length increases. The impact of a poorly designed switch or lack of stowage area is different for a mission of six months compared to a mission of one week. With habitability and human factors studies which took place during the early phases of the Lunar-Mars Life Support Test Project (LMLSTP), each subsequent phase built on the previous, and the final designs were improved based on what was learned.

Information concerning habitability issues was solicited from crewmembers during LMLSTP Phases IIa and III. One format used to obtain information was the Space Operations Issues Reporting Tool, or SOIRT. A second was a "habitability issues" questionnaire containing 59 questions used to rate the acceptability of different habitability categories on a Likert scale, from 1 to 7. Shortly before each crew completed its mission, a debrief was held with each member individually. The completed questionnaire was used to address subject areas where a crew member had given a low rating for habitability.

Introduction

For both LMLSTP Phase IIa and Phase III, there were two distinct project objectives. The first objective was to gather information regarding habitability and human factors/crew interface issues. Any issues identified and noted during Phase IIa could lead to an improvement or change before the next chamber mission, Phase III. In addition, any habitability and human factors information collected can also be factored into the design and development of future test bed architecture and mission design. Ultimately, the knowledge gained through the Phase IIa and Phase III studies could lead to greater expertise for development of the actual long-term manned vehicle to Mars or elsewhere.

The second objective was a usability study on the use of the SOIRT, (see Appendix A for a copy of the SOIRT). Feedback and comments from the use of this

product can lead to improvement for future use during flight missions as well as use as a tool for simulated long-term missions that are held on the ground. This tool is designed to assist the responder in organizing thoughts about an issue and its possible solution.

Background of the Project

Habitability is one aspect of a long-duration mission that can become more important the longer the mission lasts. A poorly designed switch or lack of stowage area impacts differently a mission of six months versus a mission of one week. Habitability, or the quality of daily living, is a nebulous concept and is presumed to comprise the following elements: environment, architecture, mobility aids and restraints, food and drink, garments, personal hygiene, housekeeping, communication, and off-duty requirements (1). The habitability experienced during a space flight mission is greatly influenced by the presence and design of vehicle systems that interface with and support the crew. The living and working spaces within which the crew operates must provide both the essentials of life as well as the support necessary for the crew to be productive in accomplishing the mission (2). With habitability and human factors studies taking place during the early phases of LMLSTP, each phase can build on the previous, and the final designs can be improved based on what has been learned.

The purpose of the SOIRT is to provide a process for identifying human factors and habitability issues that may impact space crew operations and mission success. During extended-and long-duration missions, with which NASA has had limited experience, habitability is an important issue. Incidents or issues which would seem innocuous at home may interfere with performance in an isolated environment.

The SOIRT was developed a few years ago with the hope of flying it as a standard item on Shuttle missions. The project was approved in a peer review process but not funded. Now with very limited funding, the SOIRT prototype has been completed, has been tested by two Shuttle crews for usability, and has received positive feedback. However, this tool was used for the first time during the Phase IIa LMLSTP study.

Issues and/or concerns, including crew interaction with hardware and habitability, can be identified through the use of the SOIRT. Debriefings with the crew near the end of their mission also give an additional set of information on habitability issues. The added value of these tools is that any interface and habitability issues identified can be documented, which can then be used in the design of future crewed habitats developed for research into long-mission habitability.

The LMLSTP provided an opportunity to evaluate SOIRT in the real setting of extended-duration missions in order to identify the need for potential modifications to the tool. Appendix A includes screen views of the SOIRT.

Methods and Operations

Subjects

All crewmembers were encouraged to participate in this study in order to obtain a comprehensive list of issues. All crewmembers did participate – four for each mission.

Hardware

The only hardware used for support of the project was the standard computer system supplied to each crewmember. An electronic version of the SOIRT form was added to the standard computer software. Additionally, hard copies of the SOIRT were provided. Each crewmember was encouraged to use the evaluation tool as often as he or she felt a need.

SOIRT

The SOIRT is a means for describing human factors issues during space operations and long-duration ground missions. The SOIRT contains a brief introduction to the purpose of the SOIRT, and it is then divided into three sections. The first section, entitled "General Information," contains spaces for personnel, location, time, date, and other information pertinent to the issue. The second section of the SOIRT is the "Description of Issue," which includes a free-form area to describe the issue and a checklist of items. The checklist was divided into three general categories: "Environment," "Human," and "Equipment/Systems." Personnel may check any of the items under each category that apply to the issue (many issues relate to more than one of the different categories). On-line definitions and examples are provided for each item. The third section of the SOIRT is "Causes & Possible Solutions." There are three categories included in this section to indicate the severity of the issue and to gather data on ways to preclude occurrence or recurrence of the issue. The SOIRT user checks the appropriate category for this issue. There is also a space provided for the user to identify the cause of the issue and provide suggestions for preventing the issue in the future.

The SOIRT was provided in an electronic format on each crewmember's personal computer and as hard copy. All crewmembers in Phase III chose to use the electronic format. In Phase IIa, five hard copies were filled out by the crew, four of which were filled out as a group effort. The output from the electronic SOIRT is in the form of a text file used by the investigator. Participants were encouraged to fill in a SOIRT form whenever the need arose.

Habitability Issues Questionnaire

The habitability issues questionnaire (see Appendix B) was developed to compile questions for the individual crewmember debriefs in any areas of habitability in which issues were found during their mission.

There were 59 questions that the crew used to rate the acceptability of their mission. The categories were human performance capabilities, the environment, communication, crew safety, health management, workstations, quarters and systems, hardware and equipment, clearances for operations, and scheduled activities. These categories were rated on a scale from 1 to 7, with 1 being "Completely Unacceptable" and 7 being "Completely Acceptable." There was also a choice of N/A for "Not Applicable."

An additional five open-ended questions addressed caution and warning, SOIRT, unplanned hardware modifications, noise, recreation, privacy, training, research, tasks and equipment, and other areas of concern not evaluated in the questionnaire.

Debrief

An hour-long debriefing was held with each crewmember while he or she was in the chamber. All crewmembers were asked the same set of questions (see Appendix C); however, some areas were covered more thoroughly depending on each crewmember's responses. Individualized questions were also addressed covering any subject area where a crewmember had given a low rating on the habitability questionnaire.

Procedure

Before the Phase IIa and the Phase III studies started, a half-hour overview briefing was given to the crew on the goals and objectives of the SOIRT, with the request that each crewmember sign the consent form. In addition, approximately a half-hour was taken per participating crewmember for instructions on filling in the SOIRT and to answer any questions or concerns the crewmember may have.

Approximately one hour for each crewmember was required during the last week of the chamber stay to answer the habitability issues questionnaire, which was used as a guide during the debrief. Additionally, one hour of time per crewmember was taken for the habitability debrief during the last week. The amount of time used to complete the SOIRT was not recorded. Thirteen SOIRT forms were filled in, and it is assumed not a great deal of time was spent on the activity.

No further data were gathered from crewmembers after they exited the chamber, with one exception. One debrief took place in two parts due to an interruption by an alarm sounding during the debrief. The second part took place after the end of the chamber stay.

There were no risks to the participants, since the only crew interface required was a standard computer. There were no constraints on the participants. All names were removed when the comments were combined by topic, and individual responses were not used.

Each crewmember completed the entire questionnaire during the chamber stay and participated in a debrief session the last week of the chamber stay, with the exception mentioned above.

There were five hard copy SOIRTs filled in with information pertaining to issues which the crew felt needed to be addressed; of these five, four had been filled in as a group effort in which all of the crew were involved in the development and documentation of the issues. Two SOIRTs related to issues of the private/sleep areas, covering comfort and privacy design. Another SOIRT addressed the design of the control button for the transfer lock. The fourth SOIRT covered the shower. The fifth SOIRT hard copy discussed the insufficient space for frozen foods.

All of the information discussed in the crew debriefing was taped and transcribed, resulting in more than 80 pages of typed material. This information has been integrated with the responses to the questionnaires and the filled-in SOIRTs. Overall, there are 20 topic areas covered, some with subtopics. This information (data) has been condensed, integrated, and combined to form a cohesive discussion about each topic area, and the following presents edited highlights of the data. Where suggested by crewmembers, requirements or recommendations have been included.

Table 1 summarizes the human factors and habitability topic areas and some of the specific issues within each topic area that are addressed in this section. The table lists the areas in the order in which they are discussed.

Table 3.2-1. Summary of issues addressed in each topic area

Topic Area	Issues
Environment	Temperature
	Ventilation
	Noise
	Odors**
Stowage	Work and sleep areas
	Food pantry
	Medicine cabinet*
Clothing/Personal Belongings	Clothing supplies and laundry*
	Constraints*
Tools/Maintenance	Laboratory bench*
	Hardware consumables*
	Portable workbench concept*
Housekeeping	Peroxide cleaner
	Task assignments*
	Carpet and soap dispenser problems
	Disposal item usage*
	Vacuum**
Duties/Assignments	Assignment of roles*
	Definition of roles*
Communications	Comm boxes
	Personal communications
Exercise Equipment	Training*
Hazards	Stairway
	Sharp edges
	Electrical issues*
	Cramped spaces*
Meals and Food	Cycles*
	Group meals*
	Galley space**
	Food selection**
	Water**
Trash	Wet vs. dry - verbal procedures*
	Fecal trash
Shower/Toilet	Shower water temperature and pressure
	Shower head design*
	Shower curtain*
	Height of commode above floor*
Labeling and Coding	Labeling of items transferred in and out*
	Establishment of a dedicated labeling system*
Transfer Lock	Mechanical/electrical difficulties
	Transfer procedures
	Problems with varying diameter size
Caution and Warning/Emergency Systems	Facility emergency system*
	Alarms for specific emergencies*
	Lack of audible alarms*
	Alarm system**
	Alarm noise levels**
Procedures	Written vs. verbal procedures*
	Lack of procedures for some tasks*
Lighting	Issues with first and second levels
	Lack of bed and bathroom lights
Performance	Increase in physical strength*
Furnishings and Outfitting	Walls** Sleep areas*
	Floor grate** Chairs*
	Dining table*
	Power outlets*
	Workstations*
Workstations	Lack of writing space
	Sharp edges*
	Hardware changeout*
	First-floor workstation**

**Mentioned only in Phase IIa **Mentioned only in Phase III*
No asterisk indicates mentioned in both phases

Findings and Discussion

Note: The following information contained under each topic area is the subjective input from crewmembers (shown in italics). In some instances, all eight crewmembers gave inputs on the same topic; in other instances only one crewmember commented on a topic. All inputs were used. The recommendations that follow the crew comments are primarily based on those inputs. For a few topics, recommendations were made based on the author's knowledge about the topic.

ENVIRONMENT

Ventilation/Temperature

The environment – ventilation and temperature – was fine almost all of the time. Once or twice, it felt a little bit drier than normal or a little bit warmer than normal but not to the point where it was uncomfortable. In the airlock it was warm when people were exercising – even with the fans going.

The temperature was uncomfortable at night sometimes while sleeping. On each side of the hallway, one of the rooms gets the air first, before it goes on to the second room. If the crewmember closest to the ventilation system is cold, and/or if that ventilation system is closed down, air flow to the second room stops. The lack of ability to control the temperature and the airflow through each individual sleep area was a problem at times. Comments were made that without a fan it does tend to get a little stuffy in the sleeping areas.

The air composition, pressure, and ventilation were terrific. All the oxygen levels were within specification. The air was clean. There were no odors (for Phase IIa). The Phase III crew found that the odor from lifting the lid of the fecal collection container was overwhelming. In the bathrooms there was a vent and a charcoal filter on the commode to keep the odors down.

Recommendation: Procedures for reducing trash odors should be investigated. Similar to the fecal material transfers done twice a day, wet trash should be transferred twice daily.

Low Humidity

It was too dry. One crewmember's skin reacted to the dryness. Due to the dryness, it was easier to get scratches and/or cuts.

Noise

First Level

On the first level, there are a lot of pumps going on and off, and it is irritating. A real problem is the sink pumps under the bathroom sink. Those pumps come on and off for five minutes every half-hour. They basically don't have enough vibration insulation on them. They vibrate the whole cabinet. Then the cabinet leans up against the

shower, and the shower vibrates. It is all stainless steel and aluminum, so it makes quite a bit of noise. The Urine Crystallization Unit was found to be one of the units that was very loud and bothersome. The crew insulated a few places to reduce the noise level. One of the crewmembers adjusted the Urine Crystallization Unit so that it ran at night while everyone was on the third level and could not hear it.

The sump pump is a problem and can be heard when it is on. Closing the bathroom door helped.

On the first floor the noise of the systems, combined with the poor placement of the television speakers on the television, made it difficult to hear the TV.

If someone is having a heavy workout in the exercise area and wants to play loud music, it can be a problem. The music carries into the Level 1 common area and sometimes makes it difficult to communicate or watch TV.

Otherwise, on the first level, there are no high frequencies and no irritating frequencies. It is a low-level calm.

Second level

The second level is very noisy because of the compressors and the blower out back. There is considerable noise because of the hardware. Long periods of concentration and thinking would be a problem on this level. It was hard to communicate on this level because of the noise. Crew did not want to spend time on the second level due to noise.

Third level

In the sleep areas, any sort of noise going on at a workstation or a telephone communication would be an irritant to the person adjacent to you. It would be easy to hear any sort of noise. If one crewmember was trying to sleep and was not a heavy sleeper, he/she would be awakened by someone's alarm clock, by phone calls and even by the other person rolling around in bed, as well as other normal sleeping noises. The transmission of noise through the walls was a problem. There should be some type of sound barrier between one sleep area and the next one.

There wasn't enough perceived privacy to have a personal conversation in the sleep area. Voices, music, and other noises can be heard very clearly from one sleep area to another.

Recommendation: Provide temperature control in each sleeping area so that each crewmember can adjust his/her sleeping area to the proper comfort zone. Provide more acoustic insulation between sleeping areas, and provide crewmembers with ear plugs or protection for future chamber tests. Earplugs should also be worn when working on the second level (the loudest level) for long periods of time. Loud equipment should be run at night and away from the sleep quarters. Equipment should be tested prior to a mission in an integrated operational setting, and predetermined noise levels should be identified as acceptable.

STOWAGE

Stowage was a problem brought up by all of the crewmembers. They had differing opinions about what the problem was, but there was a consensus that stowage was a problem.

Lacking was a stowage area to go along with the work areas. There were no places to store tools, filters, and other consumables. Additionally, file space was lacking. Cardboard boxes were used as substitute file space in some cases.

Just before the mission started, it was requested that some system be added to hang clothes. In the private sleep areas, hooks and some sort of small poles to hang clothes were added and were a great help because the lockers were full. Additionally, there was a strong need for more shelving in the crew quarters.

There was a formal plan for the stowage of food in a food pantry. The shelves in the pantry were extremely effective. For a 90-day test there would be a definite need for more freezer space for food. If there are to be perishables on future studies, more refrigerator space would be needed. The pantry stowage area behind the television/monitor was very difficult to access. The step was difficult to traverse while ingressing or egressing due to the limited height of the stowage area.

A bigger medicine cabinet was seen as necessary, both on the third-level urinal and the first-level urinal.

Recommendation: Provide stowage at work areas (e.g., bins, shelves, etc.). Provide more shelves in sleeping areas. Increase medicine cabinet volume. Pantry area-type stowage should be easy to access. Provide perhaps a table in which the top lifts up for stowage. Consider providing stowage beneath the tabletop accessible by lifting the top.

TOOLS AND MAINTENANCE

A laboratory bench was found to be a necessity that was not in the original plan. One was built on the second level before the start of Phase IIa and enhanced the working environment. The laboratory bench was not used for maintenance.

Hardware diagnostics were done, and there was a need for more voltmeters, specialized tools, different types of fittings, screws and bolts, and all the basic hardware consumables. Using the transfer lock, needed tools and supplies were sent in – even those not planned for in advance.

There were a few unexpected maintenance procedures. The microwave malfunctioned, and there were some problems on the air and water side that were phenomenal. A log was kept on the maintenance problems, but a report is not being written nor is it planned to be written.

There is a perceived strong need for some kind of portable maintenance workbench and/or a dedicated work area for maintenance. There was no problem

with using the dining table as a workbench at some times, but it did interfere occasionally.

Recommendation: A general workbench is required: provide a folding, movable/portable worktable. A maintenance log should be required.

HOUSEKEEPING

The only cleaner or disinfectant allowed was a spray bottle with peroxide. Housekeeping was a shared activity. When things got dirty, the person who cleaned them was the person whom it bothered the most. The system worked fairly well. The tasks that were neglected were the vacuuming and washing out the sinks in the first-level urinal area. Peroxide was not an effective cleaning agent and would be a problem for long-duration missions.

Housekeeping problems occurred because the carpet was fraying. Another problem occurred when the soap dispenser got slightly clogged and tended to spray a lot. A lot of soap sprayed all over the inside of the sink areas and on the walls and mirrors. The vacuum cleaner was difficult to carry up and down stairs.

Disposable items were used for eating and other meal activities, with the exception of some bowls and other utensils used for the microwave. The microwave was difficult to clean with just the peroxide in a spray bottle.

Requirement: A cleaning agent is required in addition to the peroxide disinfectant.

DUTIES AND ASSIGNMENTS

Roles had been assigned, but some of the duties were a little undecided within those roles. But for the most part, it was very clear to the crew what one's main areas of responsibility were. Assignment of major roles and responsibilities pre-test was found to be essential. Each crewmember understood his/her area of responsibility.

Recommendation: Role definition is necessary before start of mission.

COMMUNICATIONS

Personal

The time allocated for family conversations was sufficient. There were not any real restrictions, unless the family happened to visit or call during a press conference or similar activity. The telephone was preferred for a very personal conversation.

In some cases there was not enough perceived privacy to have a truly personal conversation. Crewmembers commented that it would have been nice to have a telephone in a different area where one can go for private conversations, like the "Cone of Silence" or something.

Most of the time, family communications were fine. "Viewpoint Pro" (video-conferencing software) instructions caused a problem sometimes. Very simple instructions were then provided to help out the family member or visitor.

The time spent with personal communication was sufficient.

With only one video-conferencing room, it could have created problems if family members for different crewmembers came in at the same time. On occasion, one crewmember completed a visit and found that another crewmember's family was coming. But the area was evidently private enough.

Telephones

Sometimes one could hear other crewmembers on the telephones, especially in the adjacent sleep area. But it depended on the level and how loud they were talking. This perceived lack of privacy for telephone conversations bothered some crewmembers.

Control Room Communication

It was available at all times, and we had good communication with them.

The control room communication was readily available. Either the squawk boxes or the telephones were used for communication between the control room and the crew.

Some crewmembers felt the instruments for communication with the support crew were a problem. It may have been a training issue. If a person did not know where to place the microphone with respect to his/her mouth, the sound "broke up" and then understanding was limited to only parts of the sentence. For certain crewmembers, it never improved over the test duration.

EXERCISE EQUIPMENT

A great deal of time and effort was given to training on the resistive exercise device, itself, with respect to the computer because it is complicated. Not enough training was received on how to do the exercises properly. It took time and work to learn how to use the equipment properly.

Recommendation: Fully train on exercise equipment use as well as equipment control procedures prior to start of chamber stay.

HAZARDS

Stairway

1) *There is an oxygen generator system that is right at the top of the steps. When the system's drawers are pulled out, they are exactly over the steps. If someone came up the steps when the drawers were out, it created a dangerous situation. The situation actually resulted in a wound that drew blood.*
2) *Sharp and/or rough edges on the stairway created problems for the crewmembers.*

Sharp Edges

1) *There is a hose clamp connector on the urine collection hose. During operation, it was necessary to put on and take off the funnel of the urine collection system. In turning the funnel into the connection, there is a relatively sharp edge on the connector hose that sliced fingers.*
2) *There was a hastily clipped-together addition to the resistive exercise device that provided a step for performing the heel raising exercises. It was thrown together at the last minute, has sharp edges, and has caused bloody ankles with the crew.*
3) *Crewmembers have had a lot of cuts from the rough edges of corners and cabinets.*

Crewmembers were shocked from contact with circuits that originally had covers on them but were taken off for one reason or another.

Crewmembers have hit their heads on the stairs and scraped their shins on the stairway. The design of the stairway was poor and hazardous.

Power left on while working on something was a problem at times.

Clip lights in the crew quarters became extremely hot when left on.

Food items had to be maneuvered in the convection oven while cooking. This caused crewmembers to burn their fingers. The oven should be sized for crew size and food system.

The cross structure of the seat frame on the aerial exercise equipment pinched fingers. A warning label should have been provided.

The storage area behind the TV is a tight space. Crewmembers scraped their backs on ceiling-hung hooks in the pantry storage area. The area had a low ceiling that did not allow crewmembers to stand up completely. Latch hooks were located at the point where one began to stand up when leaving the pantry area.

STRONG Recommendation: Wherever possible, hazards should be removed before human testing starts. For instance, provisions should be made such as providing an oven mitten.

MEALS AND FOOD

The 20-day cycle worked out well. There were assumptions that certain foods were going to taste a certain way, but they did not. With the cycle came the ability to change something that was liked or disliked. Tasting more potential foods before a study would be helpful. One crewmember would have liked a greater variety.

Some problems with the meals included burning a few items. Different items required different cooking times and temperatures, so cooking them at the same time was a challenge. With only one microwave oven and one convection oven,

baking temperatures were being compromised. Either the temperature was balanced (incorrect for each item) or baked at different times (then complete meal not ready at once). It was difficult to cook an entire meal at one time.

Only dinner was eaten together as a group. Breakfast and lunch were not. Since different crewmembers woke at different times, breakfast was eaten alone. At lunch time crewmembers were busy with other things, and it was difficult to eat together.

Recommendation: The total food system – including types of food, number of crewmembers, quantity of food, number and quality of ovens, and cooking times – has to be considered collectively.

It was difficult to clean dishes using the small amount of water allotted.

Recommendation: A low-volume sink which recycles water should be developed.

SHOWER AND TOILET

Shower Water

It was found that, to some extent, the temperature was unacceptable. It was too hot in a lot of cases and did not quickly readjust to a medium temperature. The shower knob was not acceptable. It was hard to get the correct temperature. It is a problem because of the tight water restriction. Such a long adjustment time resulted in wasting a lot of water to get the right temperature. The amount of water (six pounds) was sufficient and was not an issue WHEN the shower temperature was good.

Recommendation: Turn the temperature down on the hot water tank to where the maximum, if it is running hot, will not be scalding or near scalding, or redesign how the water is delivered. Scalding water is also a safety issue.

Shower Design

The shower head was too short, and the user had to stand right up against the wall of the shower.

The water pressure was low.

The shower curtain was replaced midway through the test. It was basically falling apart. It did not have adequate grommets to withstand heavy use.

Recommendation: The shower head should be extended and/or adjustable to accommodate people of different heights.

Toilet

The urine collection system is separate from the fecal collection system. The first floor facility included the urine and the fecal collection systems, while the third floor had only the urine collection system.

The commode, itself, was a little too high, with its legs a little too long. The toilet was difficult to use and it took time to adjust to it. The toilet was designed to be

high so that the space underneath could be used for storage; this space was used to store a general-use bucket. It took some time to adjust, because the user is sitting up relatively high while going to the bathroom. What the crew ended up doing was using the bucket as a footstool in order to sit in a more natural position.

The privacy in toilet area was acceptable to the crew.

Recommendation: Toilet redesign should be done with an emphasis on ergonomics and anthropometrics.

TRASH

There were two types of trash: wet trash and dry trash. Fecal trash was separate. And all of it was passed out of the chamber. There were no trash or odor problems inside the chamber after the realization in the beginning of the test that the wet trash should be passed out once a day.

Initially, the fecal trash didn't have to go out everyday, but the carbon filter was expended in IIa as in III and resulted in strong odors. After that problem, the fecal trash went out every day also.

There is a lack of clarity on the definition of dry trash versus wet trash. The definition was no clearer at the end of the test than at the start.

Recommendation: Need clear definition of wet and dry trash. Require that each crewmember understand the difference.

The trashcan in the galley had a regular lid. The lid had to be touched in order to throw trash away during meal preparation. This resulted in an unsanitary condition during meal preparation.

Recommendation: The galley should be supplied with a trash can that has a foot control for its lid. A lid which pivots open easily would also be acceptable.

LABELING AND CODING

Only occasionally, items passed through the transfer lock were labeled sufficiently, such as which water samples were to be taken at which time.

There was no labeling or coding system in place, although one was developed by the crew throughout the test period. There were no labels. One thing that was defined very clearly was the mailbox. It was used very successfully for all incoming mail.

It is strongly suggested that a set of procedures be established, advising the crew early in the mission that it should define locations where things will always be placed. This is especially important when there are two or three different crewmembers expecting supplies or a large variety of items coming in for them. Supplies and hardware items should have planned locations established before the start of the mission. Another reason that there were difficulties is that there is not adequate storage for the variety of items that were needed. There is a tool space, but it is small and inefficient. There are not enough lockers. The crew

talked about each crewmember having his/her own locker or cabinet on the first level, because there is a variety of items lying around for each crewmember and not enough space to keep it. There was a need for more dedicated storage for certain things. This should be based on what the crew would like to have in different areas.

There was a continual resupply, and the space left when something was used was immediately filled up with the replacement. Most of the things that came in were used and then passed back out. They were therefore usually left on the common area or left directly next to the transfer lock door. That was actually very successful. Things that needed to be done right away were left on the table, easily noticed, and it did not require much effort tracking people down to make sure they knew about it. Things that were left right by the transfer door were also written down on the transfer list. So if a transfer came around, it was ready to go; that was very effective.

Since things constantly go in and out, a dedicated labeling system for lockers probably would not have helped much, because the labeling would require many changes. The kinds of things that come in and out quickly would not require labeling. It would be helpful to have a dedicated labeling system for the kinds of things that were not needed very often and that were resupplied through a transfer. Every crew should decide where it wants to store items and then go ahead and label those areas.

Some things were labeled well. There were a couple of systems that were missing a few labels that were needed for identification purposes – e.g., the water systems.

When items were passed through the transfer lock into the chamber (e.g., equipment for an experiment), everything was labeled adequately. It was normally labeled with a name and could be directed to the correct person.

Recommendation: Before the mission, allow each crew to thoroughly plan where to stow supplies and hardware that are not constantly passed in and out. Have crew establish dedicated labeling system for these items.

TRANSFER LOCK

Mechanically and electrically, there were difficulties transferring things in or out. The electrical signal that allows the outer door to open malfunctioned once, and the button to open the outer door also malfunctioned.

Other problems with the transfer had more to do with procedures. In the beginning of the test, there were no clear transfer guidelines. Transfer operations were learned real-time while they were performed. There was trouble knowing what was acceptable to transfer out. One would have to check with all other three crewmembers for each transfer. It took two weeks to determine a procedure to transfers items out.

Things did not always have labels. However, most of the items transferred had labels on them. There was a communication system set up to read what was coming in or going out of the control room. And then the crew would agree to, or acknowledge, what was coming in.

Occasionally, a few last-minute additions were transferred, and if the right person was not there to handle it, then it is possible that the item was set aside.

The biggest problem with transfers was when the incoming tray was overstuffed. The pass-through diameter on the inside was a little bit smaller than the diameter on the outside. That made a few transfers out of the chamber extremely difficult. The crew sometimes needed to reach in and reposition items so that everything would make it through the smaller diameter.

Recommendation: Specify a location to store "transferred in" items until all crewmembers can retrieve them. Redesign the transfer lock so the diameter is the same throughout.

CAUTION AND WARNING OR EMERGENCY SYSTEMS

There are two distinct parts to the warning or emergency systems. The facility emergency system sounds an alarm when one of the sensors goes off, such as combustible gases, fire, or smoke. The second system is the alarms on the computer screens for specific systems. If a system became off-nominal, there would be a notification on the computer screen. There was not a problem with the system alarms, but an additional, audible alarm for those on the computer would be good to have.

The alarms for the facility were a different matter. If a facility emergency system alarm was activated, crewmembers would not know why. Only the control room would have knowledge of the anomaly. There was no way to know if there was a trace contaminant in the air or a spark that set off the UV detector. The crew had no way of knowing what it was dealing with and had no control. The crewmembers thought there should be some type of indication to the crew in the chamber so they would have knowledge of the situation. A panel that displays the problem and its source is needed so that the seriousness of the problem can be ascertained by the crew.

Additionally, there is a need for some audible alarms to notify a crewmember if something is wrong with some equipment. That could help eliminate the necessity of some support staff.

Recommendation: There is a need for a panel that displays the problem and its source so the cause and seriousness of the problem can be ascertained. A read-out about a problem in each crewmember's room (and on all levels) would be useful. Additionally, there is a need for some audible alarms to notify a crewmember if something is wrong with a piece of equipment. These things could help eliminate the necessity of some of the people being on the outside. Alarm notifications on computer screens should include audible alarms.

It is difficult for a crewmember who is exercising in the airlock to hear the communications with the external support crew from the main chamber's first-level squawk box. The noise of the exercise machines doesn't allow this.

Recommendation: There should be consideration of a squawk box in the airlock.

PROCEDURES

There were few written procedures for operations throughout the chamber stays. There were some verbal procedures with unplanned tasks. Some tasks were worked from previous experience.

Some procedures given were hard to follow.

Recommendation: Standardize procedure formats, and familiarize crew with this format prior to the start of the chamber stay.

LIGHTING

Lighting needs to be improved on the first and second levels. For Office of Public Affairs (PAO) events, there was not enough lighting. Extra lights had to be brought down to the first level for the events.

There are no independent lights in the upstairs bathroom. In order to see, it was necessary to turn on the hall light which sends a lot of light into the other people's rooms at night. Additionally, since all urination is recorded, a light in the toilet area is a necessity.

A bed light would have been helpful. Getting in and out of the top bunks was difficult. It was found to be necessary to bring in an incandescent light for the personal workstation since the general room lighting is not as optimal as a task light while reading, writing, etc.

The lighting was poor on the second level because the lights are set up differently, due to the hardware on that level. Work lights were necessary on the second level. With their use, illumination was not a problem.

Flashlights and work lights were necessary and useful.

Recommendation: Add an independent light in the upstairs bathroom, even if it is a night-light or a low-intensity light.

Include a bed light. With the top bunks, getting in and out is difficult.

Include incandescent task lighting for the workstations and more lighting for PAO events.

A few incandescent lights could simulate evening or morning sunlight on levels I and III.

PERFORMANCE

Physical

Physical performance capabilities went up considerably – most likely due to the regular exercise program. There was a quantifiable change in physical strength from week to week. Strength changes were quantified, since performance on the resistance machine was tracked.

Cognitive Skills

No changes were noted at all for Phase IIa. One crewmember in Phase III felt that cognitive skills were improved because there was a greater awareness of his/her surroundings. The Phase III crew suggested tasks were done more efficiently in the chamber than outside of the chamber.

FURNISHINGS AND OUTFITTING

Walls and Ceilings

The sound insulating foam frequently fell off the walls and ceilings. Many times, the Velcro holding the foam in place failed. Other times, the foam was held in place by friction only and fell out when bumped or jostled.

Chairs

The chairs in the lounge (first level) and the chairs in the sleep area were acceptable. But they do not move much, and they do not lean back.

The chairs in the dining area should be more adjustable so one can lounge in them. They are pretty rigid and should be flexible. The chairs are acceptable for meals but not for watching a movie or trying to relax. They are not very comfortable over a long period of time. Chairs frequently rolled into a grate in the floor.

The furniture could all be improved. The crew spent a lot of time in the area of the table and chairs. A couch where one could stretch out a little bit more would have been a welcome addition. More comfortable chairs should be used for future tests.

With the limited amount of space in the chamber, it is not going to be possible to have another set of chairs, unless they are of the fold-away, plastic type. And those normally are not very comfortable anyway.

The four chairs upstairs in the sleep quarters have improper back support, and the seat, itself, was too deep. The chairs on the first level should probably be on wheels and be much lighter. The crew spent a lot of time relaxing in the evening and sometimes experienced sore backs from the chairs on the first level.

Dining Table

The dining table on the first level was used for meals and work. It was also used as an area for public affairs activities with the cameras. For the camera arrangement the crew sat around that table. It was also used as a workbench for mechanical repair tasks. The dining table was also used for filling out forms, writing in journals, and other paperwork tasks. The table was used often for a variety of tasks, such as working on the micro-water samples. Everyone was careful to try not to take up the whole table if others were going to need it.

It would be nice to have a workbench that could fold up or pop out, one that could handle some weight. One that would be big enough to be worthwhile to get stuff off the dining table.

Bed

The beds were, for the most part, comfortable. At first it took some adjustment; they were not queen-sized, premium-quality mattresses. They felt relatively hard at first, but after two or three days one adjusted and was fine after that. It was a little difficult getting in and out of the bed.

Sleep Areas

Tight quarters. The space needs to be utilized more efficiently for storage. Shelving needs to be added. The drawers for stowage of the clothes have hinges; they open up, and they swing down. If not careful, they come down and smash on your head. They also make a lot of noise and disturb others who are sleeping. The cabinet to hold clothes can be improved; it is really noisy.

Some fold-away stools are needed in the rooms upstairs. If somebody visits in your room, he/she has to stand. And if two crewmembers are looking over some documents or looking at something on the computer, for a period of a half-hour they are standing or kneeling.

One cannot sit on a top bunk, and on the bottom bunks there is a headroom problem. Therefore, both options are uncomfortable. If there was a fold-away stool, it could be used in the sleep area: unfold it when wanted and stow it when not wanted. There would be room to stow a stool.

One crewmember did have a back injury associated with the exercise device. That crewmember perceived the recovery to be a little bit longer than normal and attributed that to the bed and, to some extent, the chair at the workstation. The crewmember compensated by using some towels for additional lumbar support, but that did not seem to help much. Over time, the crewmember's back improved.

One crewmember brought a bed recliner frame with webbing straps that one can put a pillow on to recline in bed. The crew all remarked that it would be nice to have some sort of lounging recliner capability downstairs while watching a two-hour movie or socializing – maybe an adjustable futon bed.

Power Outlets

There was an insufficient supply of utility outlets/plugs, and some had to be brought into the chamber during the stay. They were needed for the telephone, the cameras, and other pieces of equipment that require power. Power strips were used, and a lot of electrical things ended up getting plugged into one outlet, which caused some logistical problems.

Computer Workstations

The workstations were "terrific." Everything was considered in the design. A little more work space would have helped, however. Keeping the keyboard underneath the desktop helped. When working on tasks such as scheduling and budgets, with papers spread out, there was a definite shortage of work area.

On the first level, there is a need for a better workstation. There was some trouble with the sliding keyboard. Crewmembers would bang their knees on that. There were sharp edge issues. What is needed is a physically bigger workstation and a more efficient work space.

A dedicated storage area would have been a nice addition for holding the two-way camera equipment. A storage area for floppies, electronic storage devices, batteries, and such would also be helpful.

The crew would have liked:

- *A place to relax and be comfortable, to watch a movie without sitting in a hard chair.*
- *Colored pictures on the walls as well as carpeting on the third level. The insulation is completely white.*
- *Brighter pictures on the first level to cover up the purple insulation.*
- *Resolution of wire and cable management issues.*
- *More electrical outlets.*
- *More switch guards. There are a lot of switches, electrical and nonelectrical, that are not protected.*

SIGNIFICANCE

Phase III experienced an occurrence of the same or similar problems experienced during Phase IIa. Complaint about the transfer lock, while a nuisance, is not an engineering problem for a Mars mission, nor is it life threatening. Safety and health issues (i.e., sharp edges, noise levels, etc.) should be addressed before another chamber test is considered. Common safety practices and standard design goals should always be followed when a chamber test is planned.

The topics of poor communication, emergency alarms, size of ovens, inability to clean a surface, sharp edges, no stowage space, noise, odors, poor lighting, etc., are issues that should be carefully and systematically studied. These technologies/topics are those that will be of serious consequence and import during an actual long-term mission. While for different reasons, interrupted and/or poor communication will be the reality on a mission to Mars. For example, the inability to find a solution to clean a surface could lead to health problems. Noise levels that are unacceptable could lead to permanent problems. Constantly finding the oven too small or inadequate and lacking privacy could result in short tempers and low morale during an actual long-term mission.

The LMLSTP had as its primary goal the testing of the regenerative life support systems. The LMLSTP was a long project and went through many iterations. These two habitability studies, for Phase IIa and Phase III, were only two of many such studies to help define requirements and resolve human factors issues in long manned missions. The subjective inputs from the crew on the issues of human factors and habitability should increase our awareness of habitability concerns, which will lead to better design for each new long-term crewed mission.

The LMLSTP gave us opportunities to study the behavior and performance of the different crews. Subjective input from the crews gave us added knowledge for future vehicle designs with the goal to enhance productivity. The human component in a system for a long-term mission must be planned in and integrated fully into the design process.

Building on what has been learned from these studies and information from ensuing studies will yield the knowledge to design a facility that can serve as an analog for a Mars mission. Of course, if used as an analog, care must be taken to change operations to reflect the constraints of a real space mission. Tasks, such as transferring items in and out twice a day, would have to be eliminated to reflect the realities of life on a long-term mission. The higher level of autonomy representative of potential Mars mission operations, with all of the inherent resupply, logistics, and communications restraints, would have to be considered. Additionally, mission designers would have to ensure that all the necessary tools, diagnostic equipment, and other support supplies are factored into the planning of the mission.

Acknowledgments

Thanks must go to the wonderful Lockheed Martin support during this project. Dr. Jurine Adolf accomplished the task of putting the SOIRT on the crewmembers' computers. Ms. Jacquie Minton gave invaluable support during the information-gathering part of the project.

References

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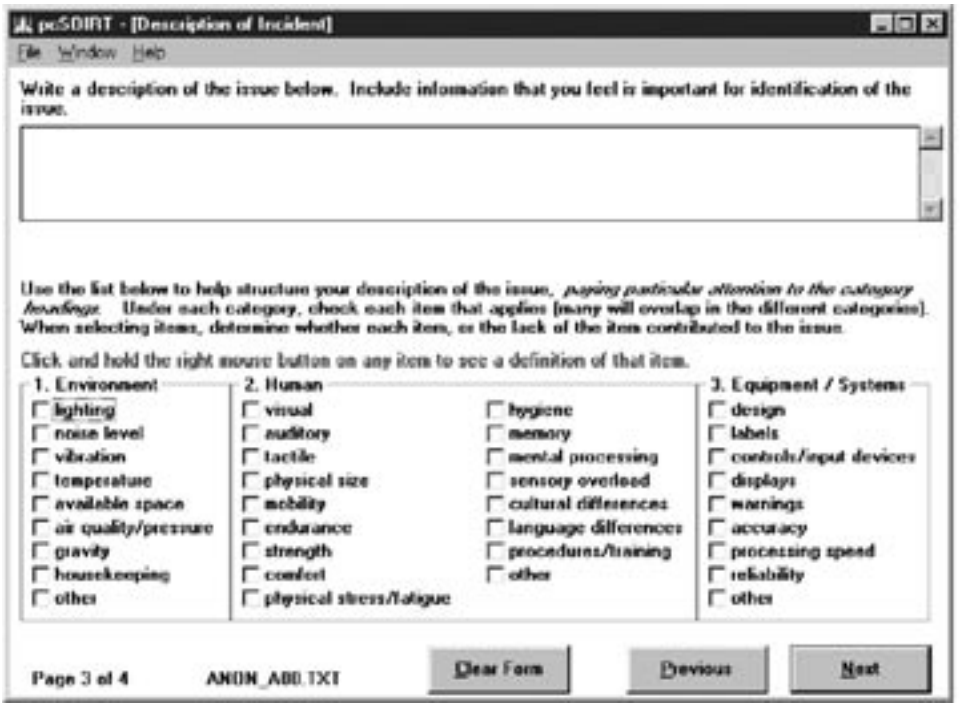
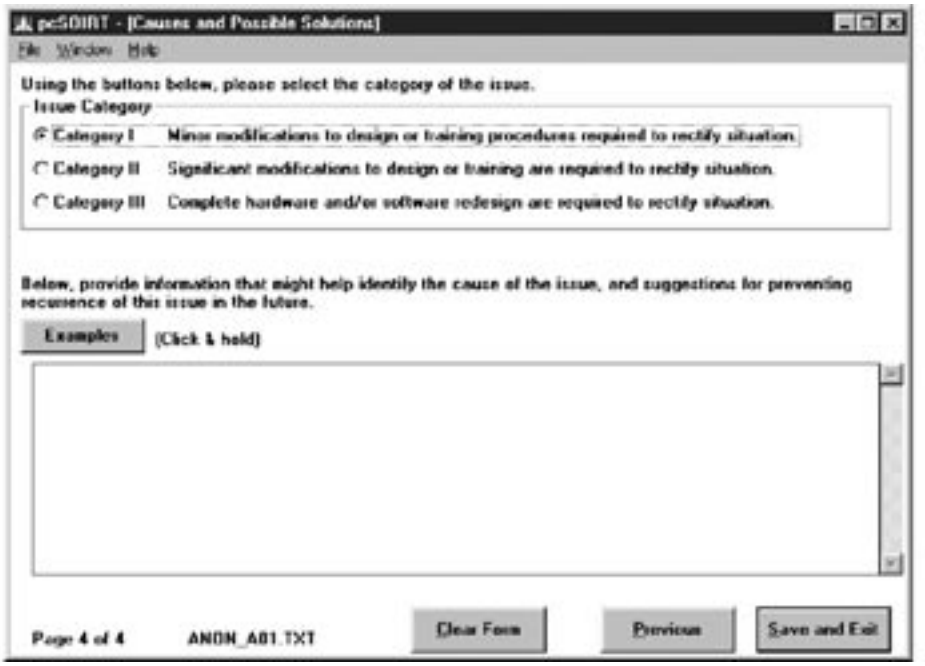
2. Bond R, Campbell P. 1995. Operational monitoring of Mir habitability (PIPS Database #810530). Internal NASA Document.

Appendix 3.2-A

Information presented in the SOIRT

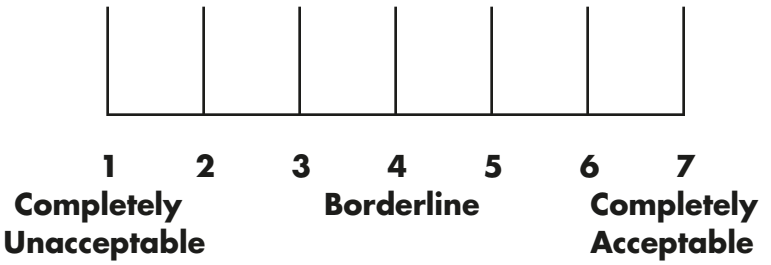


Appendix 3.2-A continued



Appendix 3.2-B

Questionnaire - Lunar-Mars Life Support Test Project (LMLSTP) Habitability Issues



Use the above scale to rate the acceptability of the environment in terms of its compliance with the following design considerations.

Appendix 3.2-B continued

Rate the overall acceptability of any changes you experienced with the following human performance capabilities (enter N/A if no change was experienced):

1. _____Vision
2. _____Olfaction
3. _____Taste
4. _____Hearing
5. _____Reaction time
6. _____Motor skills
7. _____Strength
8. _____Cognitive skills

Rate the overall acceptability of the environment based on the following:

9. _____Noise
10. _____Lighting
11. _____Odor
12. _____Ability to control temperature
13. _____Humidity
14. _____Temperature
15. _____Contaminants
16. _____and/or humidity

Rate the acceptability of the following:

17. _____Crew communication
18. _____Communication with Control Room
19. _____Personal communication (family)

Rate the acceptability of the following systems with respect to crew safety:

20. _____Mechanical
21. _____Electrical
22. _____Fire detection/protection
23. _____Emergency equipment

Rate the acceptability of the following health management methods:

24. _____Nutrition
25. _____Water
26. _____Sleep
27. _____Exercise
28. _____Preventive medical care
29. _____Diagnostic medical care
30. _____Medical treatment

Rate the acceptability of the following:

31. _____Personal
32. _____Labeling and coding
33. _____Displays and controls
34. _____Control station (first floor)

Appendix 3.2-B continued

Rate the acceptability of the following quarters and systems:

35. _____Personal hygiene
36. _____Body waste management (first floor)
37. _____Body waste management (third floor)
38. _____Crew quarters
39. _____Galley and wardroom
40. _____Exercise area
41. _____Staircase
42. _____Hallways
43. _____Passthrough
44._____Recreation
45. _____Trash
46. _____Stowage
47. _____Preventive maintenance
48. _____Diagnostic maintenance

Rate the acceptability of the following hardware and equipment:

49. _____Tools
50. _____Clothing

Rate the acceptability of clearances for operations performed within the following:

51. _____Staircase
52. _____Hallways
53. _____Passthrough

Rate the acceptability of workload in terms of scheduled activities in the following areas:

54. _____Procedures for experiments
55. _____Maintenance
56. _____Recreational time (other)

57. Provide any comments on the operation of the caution and warning system. For example: sound and number of tones, lights, text on caution and warning (C&W) panel, and frequency of alarm annunciation.

58. Please provide any comments regarding the SOIRT.

Appendix 3.2-B continued

59. Describe any significant unplanned hardware modifications the crew made.

60. Describe specific sources of significant noise with: related events, locations, durations, noise characteristics.

61. Describe significant positive and negative aspects of the following during your mission:

• Recreation

• Privacy

• Training

• Research tasks and equipment

Appendix 3.2-C

Phase III Chamber Crew - Habitability Debrief Questions

PROCEDURES

- What specific difficulties occurred during any crew procedures? (ex. communication between the crew)
- In what ways could this be improved?

AIRLOCK TRANSFERS

- Did you experience any difficulty with the translation of items to and from the chamber via the airlock?
- Did you experience any difficulty locating items inside the chamber?

ANTHROPOMETRICS

- Were there any issues in the chamber related to hardware, chairs, beds, etc. not fitting your body size/shape?

HUMAN PERFORMANCE CAPABILITIES

- Did you experience:
  - Changes in your strength?
  - Changes in your motor skills? Fine vs. gross?
  - Changes in your cognitive skills?

NATURAL AND INDUCED ENVIRONMENTS

Atmosphere Composition and Pressure

- Was the chamber ventilation acceptable? Was the chamber temperature comfortable? Would you like to have the capability of adjusting the chamber temperature yourself?
- Were there ever any unpleasant odors in the chamber atmosphere?
- Do you have any other comments on the atmosphere inside the chamber?

Acoustics

- Did you use any noise-suppressing devices (e.g., ear covers, ear plugs) during your sleep and during non-sleep periods? Did they affect the quality of your sleep, either positively or negatively?
- Did the effect of noise increase or decrease with your time spent in the chamber?
- Did noise interfere with your concentration? How often? Ability to monitor Control Room-Chamber communications? How often?

Personal Communication

- Did you experience any problems with the communication system during family conferences?

Appendix 3.2-C continued

- Were you provided sufficient privacy during family conferences?
- How often were you provided the opportunity for family conferences? How long did each family conference last? Was this sufficient?

Other Communication

- Was Control Room communication readily available at all times?

CREW SAFETY

- Talk about the Caution and Warning system a little bit. Where does the alarm sound (e.g., chamber, control room, or both?), and what is the procedure once it sounds?
- Were any false alarms activated during your chamber run? If so, how often? If so, what was the procedure for discovering the cause of the alarms? Do you feel that this procedure could be improved?
- Did you find any sharp edges in the chamber? (Mentioned bedposts) Did they cause any hazardous conditions? If so, did you consciously have to avoid them?
- Do you feel that the emergency equipment was sufficient? (Mentioned that there was no first aid kit? and no fire extinguisher) Do you have any suggestions for other emergency equipment that you feel is important?
- Were there any electrical hazards in the chamber that you identified? If so, did you or another crewmember repair those hazards?
- Were there any other hazards (e.g., mechanical, thermal) you noted?

HEALTH MANAGEMENT

Food

- Was your food selection adequate? Were there enough choices? Was there always enough food?
- Did the food inventory system help you keep track of the food?
- Did you experience any problems heating food in the microwaves? If so, do you have any suggestions for eliminating this problem?
- Were meals typically eaten together at specific times and if so, did this cause any problems for the crew in preparing and heating food for all four at one time?

Water

- Was the taste and temperature of the drinking water acceptable?
- Was the shower water acceptable in terms of temperature and hardness or softness?

ARCHITECTURE

Lighting

- Was the lighting sufficient for all tasks and if not what alternatives did you use? (portable lighting, flashlights?)

Appendix 3.2-C continued

- Was portable lighting easily utilized wherever needed (i.e., power and restraints available to install the portable lighting)?
- Did lighting levels change on each level of the chamber? (If yes, did your eyes need to adjust to the change?)

WORKSTATIONS

- Did you encounter any problems at any of the various workstations such as:
  - Illumination
  - Ventilation
  - Control/display placement and integration
  - Configuration
  - Communication
  - Access to power or other utilities

LABELING AND CODING

- Did you find sufficient labels, decals, and placards on items such as experimental equipment, food, personal items and so on to easily determine the item and how it should be used, and if not, how could this be improved?
- When items were passed through the airlock, was everything well labeled so you knew what it was and what you should do with it?

ACTIVITY CENTERS

Shower

- Did you feel that the shower system was sufficient, or is some additional means of full-body cleansing desirable? Waste Collection System (WCS)
- Did you experience any difficulties using the chamber toilet?

Crew Quarters

- Privacy
  - Did you feel a need for more privacy than what was available in the chamber?
  - How did you achieve your desired level of privacy?
- Sleep Accommodations
  - Did you experience difficulties sleeping in your quarters? If so, why?

Wardroom

- Was the dining table on the first floor used a lot? Was it used exclusively for meals or for work as well?

Exercise Equipment

- Did you prefer using the resistive device or the ergometer? Would you have preferred different exercise equipment? If so, why?
- Did you feel that you had sufficient time to exercise on the days you were scheduled to exercise?

Appendix 3.2-C continued

Trash Management Facility

- Was there a need for trash to be stored in the chamber, or did it all get passed through the airlock as it accumulated?
- Did trash cause any odor problems inside the chamber?

Stowage Facility

- Was there a sufficient number of stowage facilities?
- Was there a formal stowage plan for each type of item being stowed?
- Did you have a stowage area for personal hygiene and other personal items? Was it sufficient?
- Describe a system for stowage that you would want on longer chamber runs.

HARDWARE AND EQUIPMENT

Tools

- What were the most commonly used tools? What diagnostic equipment was used?
- Was the complement of tools available sufficient for required tasks? If not, what additional items would be needed?

Crew Personal Equipment/Clothing

- Did you feel that you lacked any personal equipment that may have been too large to pass through to you after the chamber was locked?

DESIGN FOR MAINTAINABILITY

- What unexpected maintenance procedures did you have to perform?
- Were you trained for them ahead of time, or did real-time training occur?
- Were there any crew safety issues during maintenance procedures?

HOUSEKEEPING

- Do you have any comments on the housekeeping procedures?

CULTURAL/GENDER DIFFERENCES

- Did any gender issues arise?

GENERAL

- What other advice would you have for us on how to improve habitability within the chamber?

3.3

Acoustic Noise During the Phase III Chamber Test

Tico Foley, Ph.D.

SUMMARY

In the Lunar-Mars Life Support Test Project (LMLSTP) chamber, crewmembers collected various sound level measures starting with the entry day ceremonies and ending with the welcome home celebrations. Crewmembers recorded sample A-weighted overall sound pressure levels in the different chamber areas. These dB(A) levels were in the 80s and 70s in the mechanical area; 70s and 60s in the common living and work areas; and 50s and 40s in the individual crew quarters. Medical personnel evaluated crewmembers for hearing threshold shifts comparing audiometric readings before and after the chamber experience. Given knowledge and awareness of noise levels during the chamber experiences, crewmembers altered their activities and environment to reduce exposure to noise. Crewmember hearing threshold data did not show a significant difference between measures taken pre-test when compared to those taken at egress. However, hearing was improved when these measurements were compared with audiometric measures taken 30 days post-test, suggesting temporary hearing loss for crewmembers during the preparation and execution of the chamber test. The discussion relates the chamber findings to the operational requirements for space stations and planetary habitation, as well as for long-duration exposures on Earth.

INTRODUCTION

The electrical power suddenly went off in every building at NASA Johnson Space Center in Houston, Texas. Four crewmembers participating in the Lunar-Mars Life Support Test Project were living and working in a sealed 20-foot chamber. They had been in the chamber for several weeks as part of a 91-day evaluation of a bioregenerative/physicochemical life support system. Instantly, it was totally black and absolutely quiet. Soon flashlights, then emergency generators kicked in; and lights, and computer fans, and air conditioners, and circulating pumps cycled on. Even though the crewmembers had been measuring the noise levels inside the chamber

before the power outage, they had said it wasn't very noisy. The contrast of silence, compared with the once imperceptible machinery hum, finally made a point to the crewmembers about their noise environment.
The first time a crewed mission goes to Mars in recorded terrestrial history, the crewmembers will ask more than once, "Are we there yet?" The journey will take about six months or longer each way and the stay will be for more than a year. Machinery will constantly reprocess or manufacture food, water, and air. Life support and thermal control systems will push air and water around for breathing and cooling. Noises will come from the pumps and fans; from the movements of parts and fluids; and from crewmembers – their work, entertainment, and communication equipment. Each little noise will add to the next, each will be enclosed and reverberated within the confines of the spacecraft and habitation areas - all day and all night. A return to the moon or living in orbit on a space station will put people in similar environments - locked up in a metal can for a long time.

Habitable areas within the 20-foot chamber

At NASA Johnson Space Center in Houston, Texas, scientists and engineers are preparing for these journeys by building prototypes and analogues of the equipment and procedures. One such container is the so-called "20-foot chamber" which, from the outside, looks like a huge coffee can standing on one end. Inside the chamber, the volume is divided into three floors. On the first floor there is an appended habitable entryway that extends horizontally beyond the diameter of the main structure.

For the LMLSTP the round part of the first floor contained three major areas. A general living quarters occupied half this space. This community area included a dining/work table, crew work areas, communications equipment, test equipment, and video equipment for entertainment. A combination clothes washer and dryer was surmounted by a salad growing machine. The remaining half was divided into a kitchen and a bathroom. The kitchen included food storage and preparation facilities. The bathroom included a toilet, shower, and hand wash sink for hygiene purposes. Forming the walls for these areas were cabinets containing the water recycling equipment. On the first floor there was also an appended airlock for entry and egress that was used by the crewmembers for some exercise equipment, and hidden behind the television was a tunnel recess that was used for storage.

The main purpose for the second floor was to contain the mechanical equipment used for air revitalization. Due to the arrangement of the stairs, the crewmembers had to pass through the second floor on their way between the first and third levels. Crewmember activities on the second floor were limited to maintenance around the equipment, repairs at a lab work bench, access to stored items, and use of the stationary bicycle for exercise.

Third floor accommodations included primary crew quarters: four individual private areas with bedding, controllable lighting and ventilation, private communications, personal storage, and personal work area. A partial body cleansing facility, urinal, and entertainment equipment were also on level three. Two bedrooms were on each side of a hallway. The stairway ladder was centrally located. The urinal/lavatory was at one end of the hallway and an opening for escape and ventilation was at the other end. Some storage cabinets completed the hallway. The individual bedroom areas were so small that a visitor would usually stand in the doorway.

More information and description of the 20-foot chamber and the Lunar-Mars Life Support Test Project Phase III can found at the Internet site: http://advlifesupport.jsc.nasa.gov/ by following the link to "LMLSTP" and then to "Phase III." Details of the 20-foot chamber facility and equipment layout may be found in Chapter 3.1 Architecture.

*Acoustic noise and hearing: measuring and perceivin*g

Undesirable sounds in the air in the hearing range of about 20 Hz to 20,000 Hz are what we commonly call noise. Noise loudness is measured with sound level meters. The total noise exposure over a period of time is measured with a noise dosimeter. Loud noise can interfere with communication, cause stress and annoyance, reduce useful work, or even cause hearing loss. In order to identify hearing loss, repeated measures are taken at several frequencies of the softest sound that can be heard, before and after an exposure to noise. Usually this screening is just performed at frequencies associated with speech intelligibility.

An accepted (4) rough guide for evaluating perceived changes in sound level suggests the following guidelines as judged by an average listener: a 3 dB change is just barely perceptible, 5 dB is a clearly noticeable change in loudness, 10 dB doubles the apparent loudness. But these secondary sources do not state how far above the threshold these judgments were obtained – clearly at the threshold there would be no basis for judging a sound to be twice as loud as one not heard. These perceived loudness comparisons should not be confused with the changes of sound pressure or sound energy. Doubling sound pressure is equivalent to a 6 dB change; doubling the sound energy results in a 3 dB change.

There is a difference between the effects of noise usually encountered in an eight-hour workday, and the same noise level endured for 24 hours a day for days on end. Government regulations (2) set standards for the maximum noise level to which an individual may be exposed during an eight-hour day at 90 dB, with the expectation that the individual will then be able to recuperate from temporary hearing losses during the remaining 16 hours of the day. Individuals exposed to any noise levels over 85 dB must be monitored for hearing loss and be included in hearing conservation programs. The U.S. Environmental Protection Agency (1981) suggests that an eight-hour average noise level over 75 dB presents a reasonable risk for hearing loss. The requirements for the International Space Station (5)

state that (a) each payload rack or item of continuously operating equipment must emit less than noise criterion curve (NC) 40 and (b) the total ambient noise in a habitable area must be less than NC 50, which are roughly equivalent to 49 dB(A) and 58 dB(A) respectively. Sleep areas are required to be between NC 25 and NC 40. These Space Station requirements are designed to assure communications, comfort, performance, and hearing protection. No intermittent noise levels are permitted to equal or exceed 80 dB on the International Space Station.

Just to get an appreciation for what these decibel levels mean, the author measured the noise at home and at work. With the air conditioner and refrigerator compressors off, the center of the living room sound levels were measured at about 45 dB(A). At the office the sound level meter was placed 60 cm from the computer screen and processor unit. With the computer turned on, the noise level measured between 47 dB(A) and 55 dB(A), depending on whether there were conversations occurring in the background at other desks. The sound measured at approximately 2 meters from a small gasoline driven tractor mowing grass reached levels slightly over 80 dB(A). These measures are similar to those reported in the literature (9).

Goals of this study

The conditions and duration of the mission in the 20-foot chamber for Phase III of the Lunar-Mars Life Support Test Project are more similar to those expected for Space Station than they are for a typical ground-based industrial work setting. This study was designed to monitor and describe the acoustic noise environment of the 20-foot chamber during its extended 91-day operation. In order to do this, measures were taken of the noise levels at multiple locations within the chamber; also crewmembers had audiograms (hearing threshold tests) before and after the chamber experience.

SOUND LEVEL METER

We wanted to monitor the acoustic noise in the chamber so that recommendations for hearing protection could be provided to crewmembers if the conditions required this. In addition, we wanted to describe the noise environment as a baseline measure for other Earth-bound analog test facilities.

The crewmembers collected 283 sample measurements of acoustic noise from the chamber during their 91-day stay. The crewmembers used either a Brüel & Kjær Sound Level Meter (Model 2231) or an Ametek Audio Dosimeter (Model MK-3) to obtain an A-weighted overall sound pressure level measure at each of the locations. These locations were determined using one of two schemes.

In the first scheme, each crewmember was asked to subjectively identify the noise sources and noisier locations in the chamber. Each of these locations was discussed and the crew arrived at a consensus of 25 locations they believed should be measured (they selected some quiet locations as controls). The plan was for the

sound level meter to be placed 60 cm. from each noise source, but in many cases other machinery or walls prevented this, so the sound level meter was placed closer to these noise sources or noisy locations.

The second scheme used predetermined locations geographically spaced around the chamber on each floor, at four elevations above the floor. The four approximate heights were based on locations where a hypothetical fiftieth-percentile person's ear might spend some time: 10 cm above the surface (lying down), 75 cm above surface (sitting on the surface), 120 cm above the surface (sitting on a chair), and 150 cm above the surface (standing). The actual locations were specified at each 30 degree increment within the outer wall about 60 cm from the wall (equipment permitting), at the center axis of each floor, at locations where crewmembers were likely to spend more time (e.g., at computer workstation, lying in bed, in front of stove, at dining room table, at an exercise machine, etc.), and then a measurement location was placed in the middle of any large area not already represented within 60 to 100 cm. Since this was a screening measure, most data were rounded off to the nearest unit.

On the first floor (see Table 3.3-1) the acoustic noise from 105 measurements averaged 63.2 dB(A). The noisiest general area was the airlock at 63.7 dB(A), with major noise contributors including exercise machines and ventilation fans blowing air through ducts. The only measurements that equaled or exceeded 70 dB(A) were from intermittent noise sources. The television was the loudest measured equipment noise source (average 75.2 dB(A)). Other first floor noise sources that were measured at greater than 70 dB(A) included the waste management toilet fan (average 70.6 dB(A)) and the treadmill that was noisier when used at a running pace than when used at a walking pace (running pace 74.3 dB(A) vs. walking pace 63.5 dB(A)).

The second floor (see Table 3.3-2) was much noisier; crewmembers reported not wanting to spend a lot of time lingering there. The average for 94 measurements

Table 3.3-1 *First Floor Overall Sound Pressure Level Measurements*

	1st Floor (Total)	LR/DR	BATH	KITCHEN	AIRLOCK
n =	105	48	14	18	25
AVE =	63.2	63.2	62.8	62.8	63.7
SD =	3.0	3.0	4.0	1.4	3.4
range =	18.3	18.3	15.6	6.0	13.3
max =	78.3	78.3	75.6	67.0	74.3
min =	60.0	60.0	60.0	61.0	61.0

Note: Floor measurements include rooms; units are in decibels (A-weighted).
(LR/DR = Living room and dining room area)

was 74.6 dB(A). Only two of these 94 samples were measured at less than 70 dB(A); at other times these same two locations (behind some equipment cabinets) were measured in the 70s.

On the second floor there were two general locations associated with equipment used to revitalize the air that accounted for 12 measurements in excess of 80 dB(A). This equipment was constantly operating except for maintenance times and for a planned 10-day event when alternate equipment was tested. One location was near the Trace Contaminant Control System (TCCS) (noise measurements from 72 to 81 dB(A)) and the other area was in the vicinity of equipment used to remove the carbon dioxide from the air, the Four-Bed Molecular Sieve (4-BMS), the 4-BMS Accumulator, and the lithium hydroxide (LiOH) back up (noise measured predominantly in the 80s, from 77.2 to 88.0 dB(A)). The high noise levels of this equipment were associated with the compressors, blower fans, and the air flowing across the grids.

The third level (see Table 3.3-3) was quieter than the other floors and the noise levels were similar to those measured by the author in a single-family residence.

Table 3.3-2 *Second Floor Sound Level Measurements*

	2nd Floor Total)
n =	94
AVE =	74.6
SD =	3.9
range =	19.9
max =	88.0
min =	68.1

Note: Units are in decibels (A-weighted).

The overall average of 84 measurements was 51.5 dB(A). Measurements taken in the immediate proximity of the beds averaged 42.4 dB(A) with a range of 40.3 to 44.9 dB(A). The noisiest area on the third level was in the hallway, averaging 59.8 dB(A), especially near the ladder that led to the noisy second floor where a couple of measurements were in the low 70s.

Table 3.3-3 *Third Floor Sound Level Measurements*

	3rd Floor (Total)	ROOMS	BEDS	DOORWAY	WC	HALL
n =	84	33	9	16	8	18
AVE =	51.5	47.8	42.4	54.3	53.2	59.8
SD =	6.3	2.8	1.7	1.5	2.5	4.7
range =	31.3	13.7	4.6	5.3	7.2	16.9
max =	71.6	56.0	44.9	57.3	59.2	71.6
min =	40.3	42.3	40.3	52.0	52.0	54.7

Note: Floor measurements include rooms; units are in decibels (A-weighted).
(WC = Water Closet – urinal and lavatory room)

GENERAL NOISE LEVELS - DOSIMETER

We wanted to see how the acoustic noise levels varied throughout a typical 24-hour day – for each crewmember, for special events, and on each chamber level.

For these purposes the crewmembers used the Ametek Audio Dosimeter (Model MK-3) in the dosimeter mode. This dosimeter allowed an equivalent A-weighted average to be collected each minute as loudness equivalent (Leq 1 min) of a 24-hour period (1440 samples each day). After downloading to a desktop computer, the resulting data could then be graphed as noise level versus time (see for example Figure 3.3-1) or as a histogram of number of minutes for each selected noise level interval (see for example Figure 3.3-2). Each crewmember wore the dosimeter for two different 24-hour periods. In addition, one crewmember volunteered to wear the dosimeter for about an hour before and an hour after entering the chamber on the first day, as well as an hour before and after exiting the chamber on the last day (see Figure 3.3-3). On each of three other days, the dosimeter was placed in a central location in a bedroom, among the equipment on the second level, and attached to the ceiling in the middle of the first floor. The data from these three days measurements at fixed locations are not presented, but are consistent with the sound level meter data reported above.

The 24-hour data from the dosimeter attached to a moving crewmember was also consistent with that obtained by the sound level meter. Knowing the sound levels from Tables 3.3-1 through 3.3-3, one can almost visualize the crewmember moving from floor to floor based on the noise level known to exist at that floor. The first floor background noise was measured in the mid 60s with an occasional spike that could be attributed to voices either from one of the crewmembers or from the

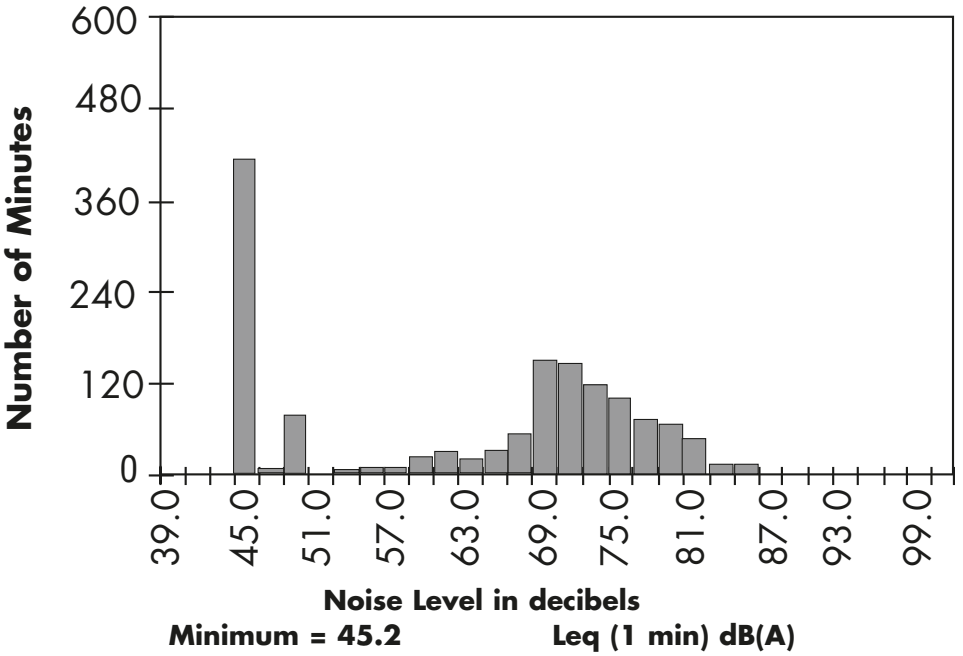


Figure 3.3-1 *Typical noise levels encountered by a crewmember during a 24-hour period.*

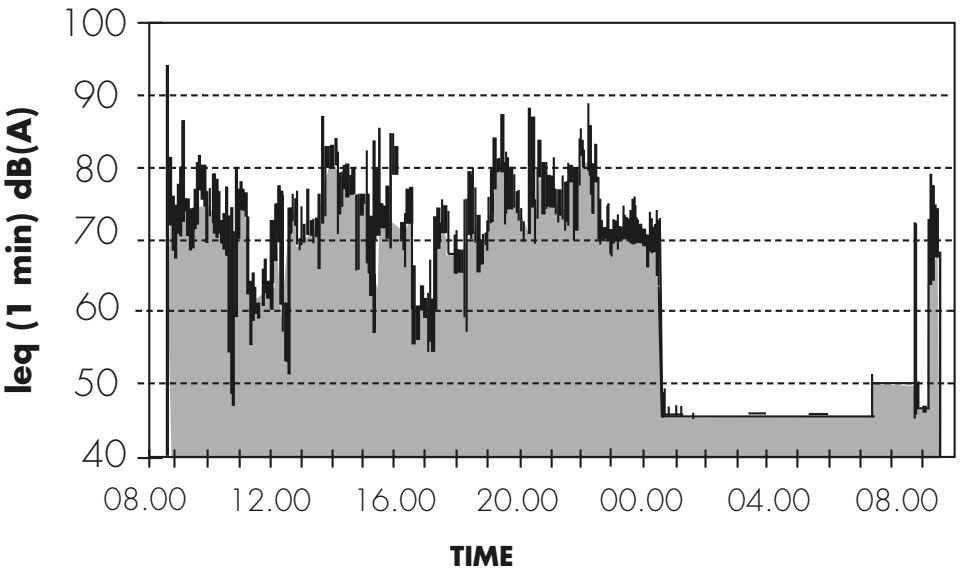


Figure 3.3-2 *Typical distribution accumulated duration of noise at each loudness category as encountered by a crewmember (from raw data in Figure 3.3-1 above).*

amplified communication loudspeaker. Periods of time with noise measured above 70 dB(A) represent transitions through or short visits to the loud second floor. Rest or quiet work on the third floor can be seen during the periods of time below 60 dB(A).

An interesting contrast between the noise inside the chamber and that, which greeted the crewmembers upon egress, is illustrated in Figure 3.3-3. It was clearly a tumultuous welcome back with noise levels often above 90 dB(A) during the first 10 minutes, and above 80 dB(A) for most of the hour.

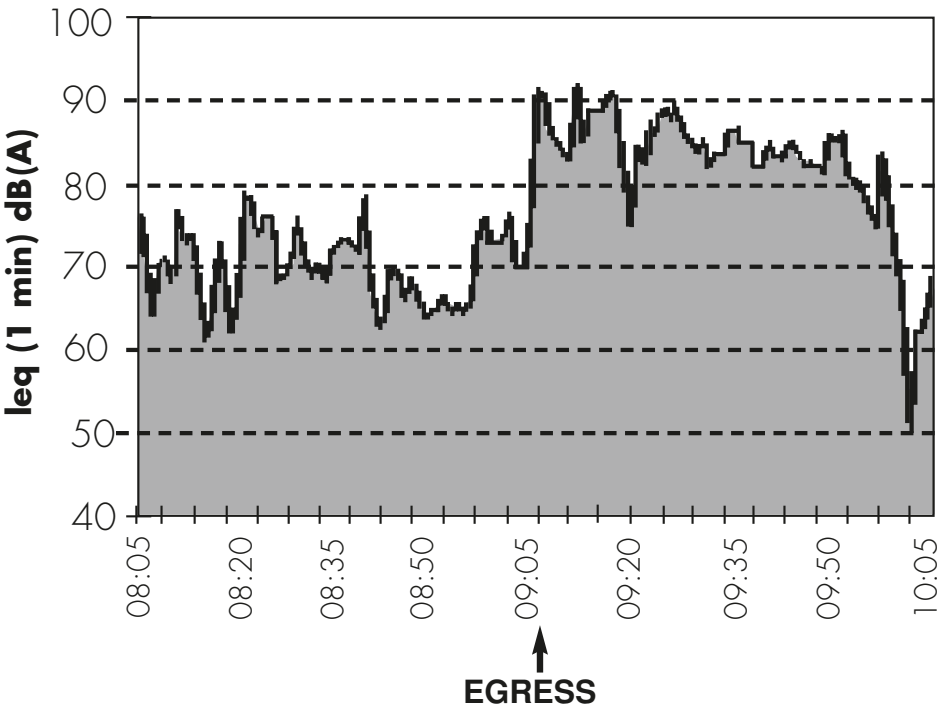


Figure 3.3-3 *Noise levels encountered by a crewmember one hour before and one hour after exiting the chamber after a 91-day stay in the chamber.*

HEARING TESTS - AUDIOGRAMS

We wanted to know whether or not the noise levels inside the chamber would affect the threshold hearing levels of the crewmembers, as a result of their 91-day experience.

The hearing tests were conducted under standard conditions at the health clinic at NASA Johnson Space Center. At the clinic there is a special sound-insulated closet, about the size of a large telephone booth with a sound-insulated door. The

person to be evaluated enters the closet and puts on a headset through which sounds are transmitted. When the person hears a sound he or she is supposed to push a button, and the monitoring computer records this response. The computer presents sounds at predetermined frequencies and at incremental amplitudes, getting softer until there is no response and then louder to double-check the person's threshold of hearing. The procedure tests each ear independently starting with sound frequencies of 1000 cycles per second, and then proceeding with 500, 1000 (again), 2000, 3000, 4000, 6000, and 8000 Hz. The first measure at 1000 Hz is considered a training run, so each ear receives seven data collection measures, for a total of 42 audiogram measurements per crewmember.

The four chamber crewmembers each had a series of three audiograms (or hearing tests). The first audiogram was performed with the intention of establishing a baseline measure. This test was conducted at the time the crewmember had the medical examination to meet the eligibility criteria for being a test subject. Three crewmembers had this "pre-test" audiogram about seven months before entering the chamber; the fourth crewmember had the test a little less than a month before entering the chamber. The second audiogram was performed for all crewmembers within two hours after the crewmembers left the chamber. The purpose of the "egress-day" test was to determine whether or not there had been any shift in the crewmember's hearing threshold. A slight temporary hearing threshold shift was expected. The third audiogram was obtained between 6 and 14 weeks after chamber egress. The purpose of this "post-test" audiogram was to determine whether or not the hearing threshold had returned to baseline. It was expected that there would be no permanent hearing threshold shift.

As reported here, an audiogram measure of 0 (zero) indicates that the threshold of hearing is the same as that for an average person without hearing loss. Larger numbers indicate hearing loss, or in other words, it takes a louder noise before the individual being tested pushes the button indicating he or she has heard the noise. Medical doctors appear to disagree on what level of threshold shift should be cause for concern. Of course bigger shifts should cause more concern. Shifts of 5 dB should not be taken seriously on such a screening device, as they are in the range of expected test errors. The author's personal experience in the audiometry test chamber suggests that there could easily be an error of judgment between when a person actually hears a sound and when the person believes he or she heard the sound. In fact, it was common for the crewmembers to have measures differing by 5 dB between the two measures on the same ear at 1000 Hz at the same administration.

Audiogram measures on the pre-test ranged from 0 to 50 dB; on the egress-day from 0 to 50 dB; and on the post-test from 0 to 45 dB. For two crewmembers, all measures were at or below 20 dB; for another crewmember, all measures were at or below 25 dB; and for a final crewmember, one ear had all measures at or below 10 dB while the other ear had evidence of hearing threshold loss in the 45 to 50 dB range for all three test administrations.

A visual inspection of graphs of the audiometry data reveals many crossing lines and no clear distinction among pre-test, egress day, and post-test audiometry measures. As a typical example of this phenomenon Figure 3.3-4 displays the group averages of the audiometry data. For each crewmember, there were frequencies at which the hearing threshold for the pre-test was higher than that obtained on egress day. Similarly, for each crewmember, there were frequencies at which the hearing threshold on the post-test exceeded those obtained on egress day. There were also frequencies for each crewmember at which the egress day measures exceeded both of the other measures. Because of concern for privacy rights, individual crewmember data are not presented here.

A modified sign test was performed on the data as an indication of the overall tendency of the measured hearing threshold. The arithmetic differences between the egress day and pre-test measures were obtained for each data pair (see Table 3.3-4). A normal approximation to the binomial probability distribution testing degrada-

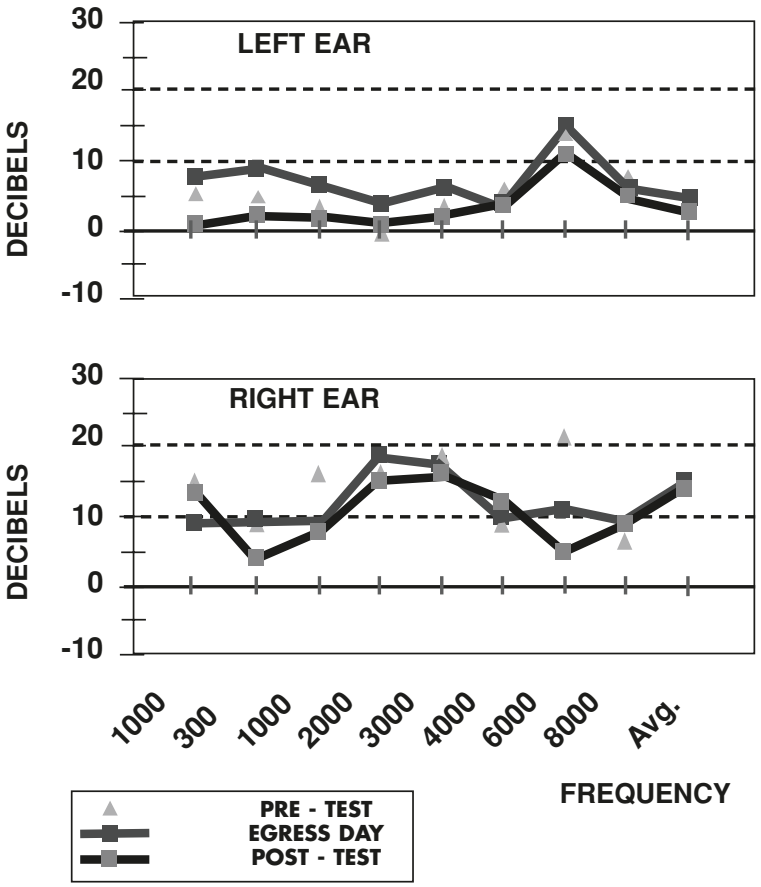


Figure 3.3-4 *Group averaged audiometry hearing test data for four crewmembers*

tions versus improvements showed no significant difference (p=0.19). However, a similar comparison showed that the crewmembers' hearing as measured at the post-test was better than either the pre-test or the egress day measures (p=0.0045 and p=0.0004, respectively).

Some features of the data deserve mention. Of the 56 possible comparisons among the pre-test, egress day, and post-test, there were five for which the egress day measure suggested a temporary hearing threshold loss and the post-test measure suggested that this loss had not returned to the pre-test baseline level. For only

Table 3.3-4 *Audiometry Comparisons*

hearing threshold change across time	pre-test vs. egress day	pre-test vs. post-test	egress day vs. post-test
Loss	19	9	9
Same	23	23	17
Better	14	24	30
loss vs. better probability=	0.1922	0.0045	0.0004

Note: Units for loss, same, and better are number of comparisons.

one crewmember did this difference equal or exceed 10 dB (one ear at 6000 Hz and the other ear at 4000 Hz). This was the same crewmember who had the 30 dB difference between the training measure and the data collection measure at 1000 Hz and the only crewmember who showed any hearing loss across time that exceeded 10 dB. Further inquiry into this variability is necessary.

Another post hoc analysis was performed on the audiometry data. All the data measured for each frequency were arithmetically added (see Table 3.3-5). A visual analysis suggests some attention should be paid to 6000 Hz, especially during the pre-test and egress daytime intervals. This is another indication that there is essentially no difference between the audiometry measures at pre-test and on egress day, while hearing thresholds appear to be lower when measured at post-test.

How can we explain the appearance that the chamber stay may have improved the hearing of the crewmembers? After seeing the raw data, some reviewers commented that it appeared the chamber experience itself may have been related to improved hearing threshold measures. In fact, as mentioned earlier, the noise of the cheering crowds that greeted the crewmembers as they emerged from the chamber was greater than that found in the living areas of the chamber. This loud celebration noise may have confounded the results of the egress day measures which were taken within two hours after egress. Another confounding factor may have been the crewmembers' environment during the pre-test baseline measures. During the pre-test time frame,

Table 3.3-5 *Arithmetic Sums of Threshold Hearing Measures*

	Frequency (Hz)								
	500	**1000**	**2000**	**3000**	**4000**	**6000**	**8000**	**sum**	**range**
pre-test	55	80	65	90	60	140	55	545	85
egress day	75	60	90	95	55	105	60	540	50
post-test	25	40	65	75	65	65	55	390	50
sum	155	180	220	260	180	310	170		
range	30	40	25	20	10	75	5		

Note: Column Headings are in Hertz; cell units are arithmetic sums of decibels.

the crewmembers were heavily involved in the development, assembly, and testing of the mechanical equipment for the chamber. All pre-test hearing measures were taken in the afternoon, after at least a half-day of work. The crewmembers were also involved in the operation and maintenance of this equipment during the 91 days of the chamber test. After egress day and a week or so of intensive debriefings, the crewmembers took holidays and vacations and the equipment was all turned off. So the post-test measures were obtained after the crewmembers had not only been in a different noise environment, but also under different stress conditions.

An alternative hearing measure that could be taken on site would help resolve some of the confounding aspects. Such a measure would also be useful in space vehicles for on-orbit and Mars transit hearing tests. Human perception during space flight has not been fully investigated. Repeated measures, preferably on several mornings, would more reliably establish a baseline.

DISCUSSION

Living in a moderately noisy work area 24 hours a day is different from facing the same noise level for an eight-hour work shift and then returning home to relative quiet. The rules for permissible noise levels have changed. Mir astronauts (1) and International Space Station astronauts face extended exposure to loud acoustic noise levels that are within the safety standards for factory workers. These extended exposure times could result in communication difficulties and cause hearing damage; they could also be annoying or stressful and cause degradation in work performance.

After the first data were analyzed and reported to the crewmembers in the chamber, they took steps to reduce the noise to which they were being exposed. Noise insulation barriers were constructed, redundant equipment was turned off, and schedules were changed so that the noisiest equipment was not operated when the crewmembers were in the same area. Follow-up acoustic data to measure the effects

of these changes was not collected because such data collection had not been scheduled to systematically measure these differences.

The results of this study will be used to provide motivation to reduce the noise levels of the air and water revitalization equipment. A continuing benefit for this and subsequent habitation study crews is an increased awareness of the noise levels in a closed environment. Plans are being made to repeat the noise monitoring activities on the next use of the 20-foot chamber and then in other Earth-bound analogs of space vehicles.

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3.4

Assessment Of Sleep Dynamics In A Simulated Space Station Environment

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SUMMARY

Based on prior experience, it is believed that the unique environmental conditions and work-rest schedules aboard orbital spacecraft (i.e., the International Space Station (ISS)) will result in sleep decrements and fatigue in astronauts. This report details methods for estimating sleep variables and circadian rhythms in a simulated work-rest environment that mimics the schedule of ISS crew activities. Eight healthy subjects in two separate studies stayed for 60 days (Phase IIa) and 91 days (Phase III) in a closed life support test facility at Johnson Space Center. Subjects wore an activity and ambient light monitor (Actillume™), completed sleep logs twice daily, and collected timed saliva and void-by-void urine samples for 48 hours. This protocol was repeated four times during the 60-day chamber study and six times during the 91-day study; results were compared with samples collected before and after each chamber stay. Sleep variables (latency, duration and efficiency) were estimated from the Actillume™ data (objective) and from the sleep logs (subjective); acrophases for salivary melatonin and urinary melatonin sulfate were determined from concentration versus time profiles. Objective assessment of sleep efficiency, sleep duration and sleep latency were lower than the corresponding subjective assessments. In addition, the number of awakenings recorded by actigraphy was higher than those from the subjective sleep log scores. There were no significant differences in sleep variables between baseline and chamber stay periods. Changes in sleep variables were independent of chamber stay duration. Self-assessment of sleep quality scores did not reflect any sleep decrements. Wake period light intensity in the chamber was lower (50-100 lux) compared to baseline readings (1000-1500 lux). Salivary melatonin acrophase was delayed during the chamber stay by 2.7 hours and compared well with the urinary melatonin sulfate acrophase, which was delayed 3.0 hours. The chamber light conditions were similar to those of ISS and may be responsible for the melatonin acrophase delays noted during the chamber study. These results indicate that the methods tested here will be sufficiently sensitive to detect sleep decrements and contributing circadian rhythm changes in astronauts aboard ISS. Salivary melatonin levels could serve as a sensitive marker of determining circadian rhythmicity.

INTRODUCTION

Potential disturbances of circadian rhythmicity in the space flight environment and consequent decrements in performance efficiency and in the well-being of astronauts are major concerns of NASA. In addition to changes in environmental factors, such as the absence of a gravity vector and ultra-shortened light-dark cycles, other factors that contribute to the development of sleep disturbances and fatigue during space flights include the abnormal length of working periods (high work load effect), continuous deviation of the sleep-wake cycle duration from 24 hours ('migrating day') effect, and cyclic noise disturbances.

With respect to sleep during space flight, a continuous reduction of sleep time and an increase in sleep latency were reported from earlier missions (6) and more pronounced sleep disturbances were reported with dual-shift crews (5, 12). Results of a simulation study reflecting the schedule of work-rest periods indicate a distinct increase in awake time as well as a decline of the sleep efficiency index and a descynchrony of circadian rhythms (7, 18). In a more recent study (16) that analyzed crew sleep patterns on Shuttle missions, decreased sleep duration and increased use of sleep medications during dual-shift missions compared to those used on single-shift flights was reported. In an even more recent investigation (14), in-flight use of medications from astronaut debriefings after 79 U.S. Space Shuttle missions was evaluated. From the 219 records obtained, 45% reported usage of medications for sleep disturbances. Furthermore, sleep medications were less efficacious and were therefore administered for longer periods of time (4, 14). In addition to these physiological and sleep disturbances, in order to meet operational demands, crewmembers have been assigned shift-work schedules during certain dual-shift missions.

It is well documented that sleep deficits, biological asynchrony with work-rest activities, and sleep-promoting medications will impact alertness and induce fatigue (2). This presents a very high risk for shuttle and ground-operations of the space program and, particularly to crew health and safety. Current strategies for minimizing sleep decrements due to shift-work during flights are based on the theory that exposure to bright light aids shift workers by altering or re-orienting their circadian rhythms (17). To better prepare the subjective night-shift crew and to support launch and landing time activities, crewmembers are entrained to match their work schedules to their sleep-wake activities using artificial light and simultaneous sleep shift schedules. Limited data have been collected from these astronauts before flight, during the light assisted sleep-shifting period in the days just before flight, and immediately after flight (19). In this study salivary melatonin and cortisol rhythms were examined to determine the effectiveness of this entrainment protocol in accomplishing the desired shifting of the endogenous rhythms to match in-flight work-rest activities. Results of this investigation indicated that

targeted shifts were achieved for both cortisol and melatonin rhythms before flight and were restored immediately after return to Earth. However, ambient light levels on the Shuttle were low and may have been insufficient for circadian entrainment.

In order to augment sleep quality, pharmacological agents are often prescribed during flight, in addition to pre-flight entrainment. However, a systematic evaluation of the effectiveness of light treatment on the maintenance of in-flight work-rest demands is missing due to a lack of methods and technologies that are both sufficiently sensitive and flight-suitable. To fill this gap, the present study was conducted to evaluate objective and subjective data collection methods for sleep quality and contributing variables in a ground-based analog environment in human subjects confined to a closed chamber during as part of Phase IIa and Phase III Lunar Mars Life Support Test Project (LMLSTP). Information gained from this study will be useful in the identification and validation of sensitive, non-obtrusive techniques for evaluating sleep and circadian rhythms during space flight.

METHODS AND MATERIALS

Experimental Design

All procedures involving human subjects for this study were reviewed and approved by the Johnson Space Center Institutional Review Board. The test group consisted of eight subjects, three females and five males, from two separate phases of chamber confinement (Phase IIa and Phase III). Each phase consisted of one pre-chamber, four (Phase IIa) or six (Phase III) in-chamber and one post-chamber data collection session. Each session was 48 hours long during which the following activities were performed by the crewmembers:

An Actillume™ was worn on the wrist of the non-dominant arm of each crew member for 48 hours. The activity data recorded by the Actillume™ were autoscored for sleep, while the illumination data were analyzed for patterns of light exposure.

An electronic sleep/wake questionnaire was completed upon wake up and before bedtime using the Ames Interactive Reporting Log (AIRLOG). AIRLOG is a tool developed exclusively for research in aviation and ground transportation environments; the instrument was developed by NASA Ames Research Center and includes separate components that relate to the events of the day preceding the sleep period, the quality of sleep period, and the ensuing wake time. These data were analyzed to estimate subjective changes in sleep duration, latency, efficiency and quality during chamber stay.

Saliva samples were collected every two hours while subjects were awake using salivettes (Sarstedt, Inc., Newton, NC). Void-by-void urine samples were also collected during the 48-hour period. All saliva and urine samples were processed and stored at -40˚C until analysis. Samples were analyzed using commercial RIA kits to determine levels of melatonin and melatonin sulfate.

Data Analysis

Illumination data from the Actillume™ were analyzed for patterns and intensity of light exposure using vendor provided Action-3 software. Activity data were analyzed using Action-3 software using both the manual and autoscore options in the software to estimate objective sleep variables.

Data from the AIRLOG were analyzed to estimate subjective sleep quality, efficiency and latency. Salivary melatonin concentrations were determined using commercially available direct radioimmunoassay kit (ALPCO). Urine aliquots were assayed to determine 6-hydroxymelatonin sulfate levels by the method of Aldhous and Arendt (1).

Cosinor and cross-correlation methods were used to analyze salivary melatonin and urinary melatonin sulfate measurement data with respect to time (11). Cosinor analysis was based on least-squares fit of the cosine function to a series of observations. This technique allowed characterization of the mesor (the 48-hour time-series mean), acrophase (peak time, referenced to local midnight) and amplitude (half of the peak-to-trough variability). Phase shifts were calculated from the entire 48-hour session by subtracting the baseline acrophase from the in-chamber acrophase.

RESULTS

Objective measurements of sleep variables by Actillume™ showed no statistically significant differences between baseline (pre- and post-chamber) and in-chamber periods. These data suggest that crewmembers adjusted with the Space Station analog work-rest activities (Table 3.4-1). Light intensity during waking periods in the chamber was lower compared to baseline readings (Figure 3.4-1). Similar readings of light intensity have been observed on two earlier space flight missions as well (15).

Self assessment of sleep variables (sleep latency, number of awakenings, sleep duration and sleep efficiency) by AIRLOG showed no changes between chamber stay and baseline (Table 3.4-1). In addition, sleep quality scores did not reflect any sleep decrements during chamber stays.

A comparison of the sleep variables data from the objective and subjective scores indicate that subjective assessment scores of sleep by the crewmembers were higher than the respective objective measures derived from actigraphy. This observation confirms the general notion among sleep researchers that perception of sleep decrements is always less than actual deficits. Sleep diaries have been used extensively in clinical and research environments to evaluate subjective sleep quality (10). Subjective sleep scores are also useful in linking circadian parameter estimates (e.g. acrophase, mesor) with aspects of sleep quality and personality. It is necessary to assess sleep deficits using both subjective and objective data sets in order to identify any significant changes in sleep hygiene that may adversely affect alertness and performance during space flight. Subjective estimates of sleep latency, duration and efficiency are often inadequate by the very nature of their being subjective, therefore, an objective estima-

tion of these variables, such as actigraphy data, in conjunction with the subjective sleep logs may provide a more comprehensive assessment of sleep hygiene in space. Results from this study indicate that the methods tested here are suitable for in-flight assessment of sleep during long-duration flights. Non-obtrusive wrist-actigraphy appears to be a valuable diagnostic method for the assessment of sleep decrements in astronauts.

It is well known that rectal temperature and urine melatonin sulfate are good indices for determining circadian rhythmicity (3,13). Due to the inconvenience caused by rectal probes during space flight, this is not a preferred means of data collection for astronauts. Although urine sample collection is non-invasive, it places increased demands on spacecraft stowage. Earlier reports indicated that there is good correlation between salivary melatonin and serum melatonin levels suggesting that salivary melatonin rhythm is an accurate predictor of circadian rhythmicity (8). Cosinor analysis of salivary melatonin and urinary melatonin sulfate excretion rates from the present study yielded valuable information on the applicability of salivary data for the assessment of circadian rhythms. When circadian variables derived from both markers are in agreement, acrophase estimates calculated from time profiles of both markers and an accepted measure of circadian shifts, are also in agreement (Figure 3.4-2). Regression analysis of these data indicated that good correlation exists between estimates from the two sets of data (Figure 3.4-3; $r = 0.79$). However, the correlation between delayed salivary melatonin rhythm and sleep duration, although weak ($r = 0.42$) suggests that the desynchronized melatonin rhythm and sleep period may have affected the sleep quality in the chamber crewmembers as depicted by reduced sleep duration (Figure 3.4-4). These results suggest that salivary melatonin rhythms may be successfully employed for estimating circadian rhythms and related sleep decrements in astronauts during space missions. Further analysis of these data is in progress to evaluate the correlation between temperature and salivary melatonin rhythms; results from these analyses may confirm that salivary melatonin can be utilized as a reliable chronotherapeutic marker in place of temperature.

ACKNOWLEDGMENTS

We express our sincere appreciation to the staff of the LMLSTP Project Office for their support of this study. The authors also wish to thank the crewmembers for their compliance and enthusiastic participation in the study. We also thank Ms. Ladonna Miller from the Space and Life Sciences office for coordinating the study. We are grateful to Dr. Mark Rosekind for making AIRLOG, the hand held sleep diary developed at the Ames Research Center, available for the study.

Table 3.4-1 *Sleep variables in chamber crewmembers**

	Objective Measurements		Subjective Measurements	
	Baseline	**Chamber**	**Baseline**	**Chamber**
Duration(h)	6.62 ± 0.31	6.00 ± 0.24	6.78 ± 0.27	6.21 ± 0.21
% Efficiency	88.50 ± 1.44	88.10 ± 1.73	96.40 ± 1.16	95.66 ± 1.03
Latency (h)	0.27 ± 0.06	0.20 ± 0.05	0.20 ± 0.05	0.24 ± 0.05
WASO**	0.90 ± 0.12	0.86 ± 0.15	N/A	N/A
Quality	N/A	N/A	1.31 ± 0.11	1.08 ± 0.21
Number of Awakenings	4.65 ± 1.43	4.22 ± 0.59	7.11 ± 0.31	7.33 ± 0.30

*Values are Mean ± SEM of 8 subjects
**Wake after sleep onset

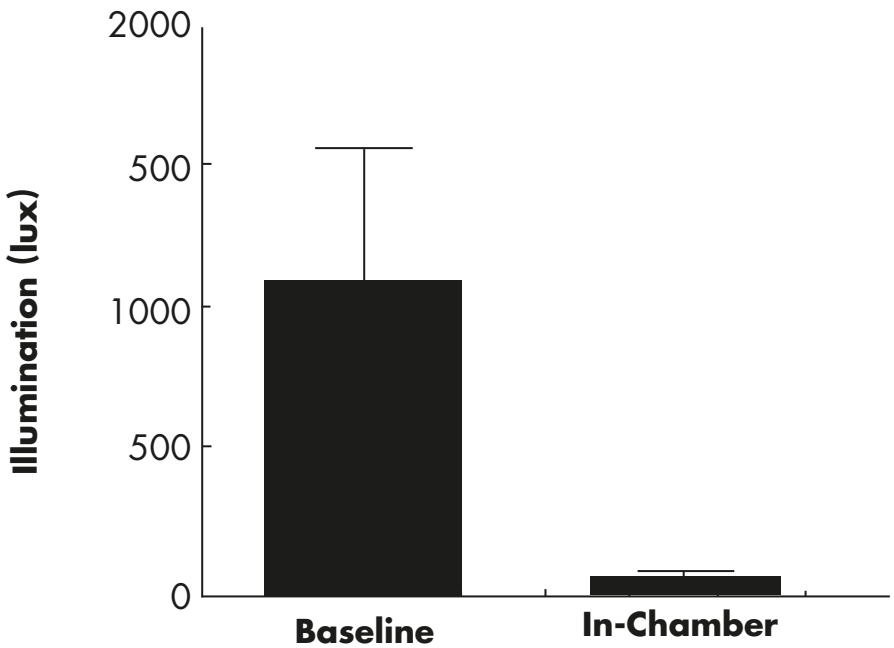

Figure 3.4-1 *Light Exposure During Wake Period*

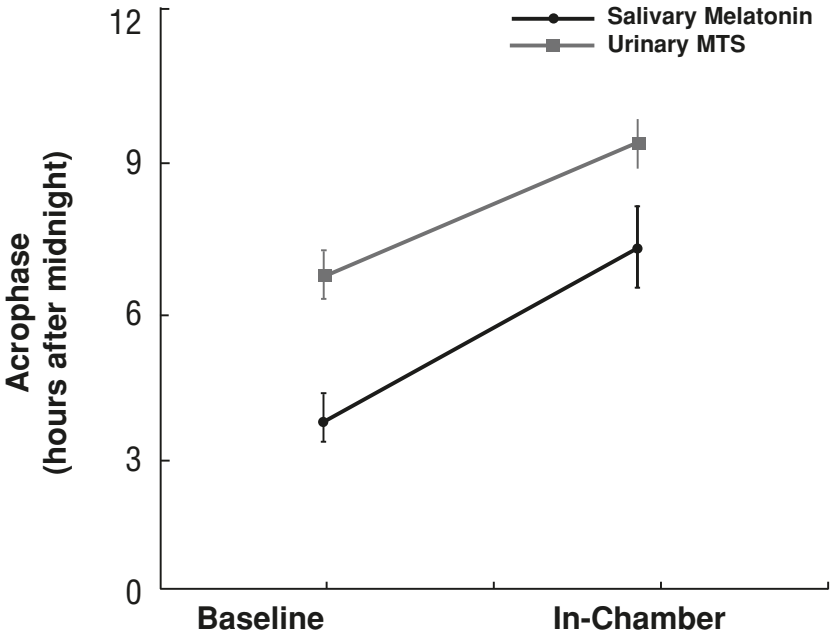

Figure 3.4-2 *Comparative Estimates of Circadian Rhythm Changes*

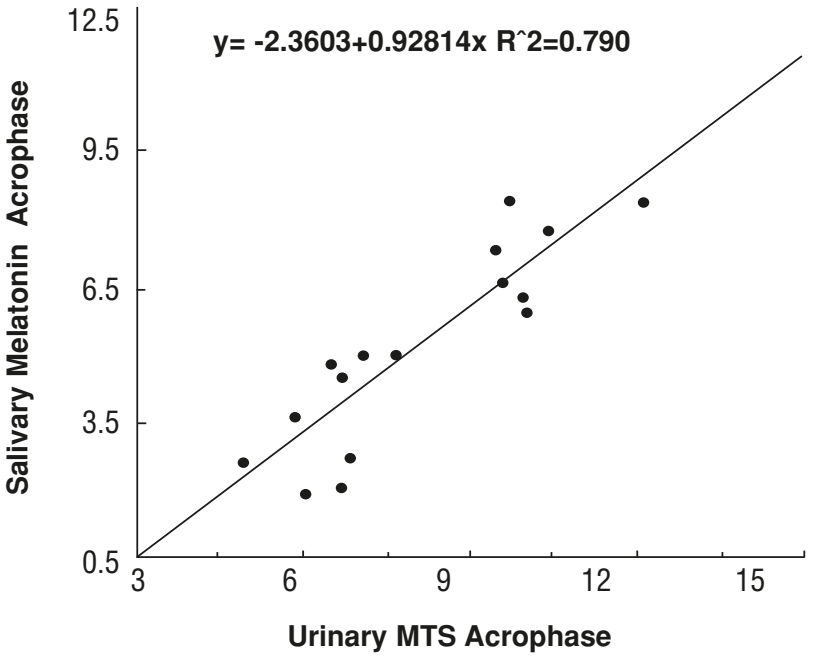

Figure 3.4-3 *Correlation between Urinary MTS and Salivary Melatonin Acrophases*

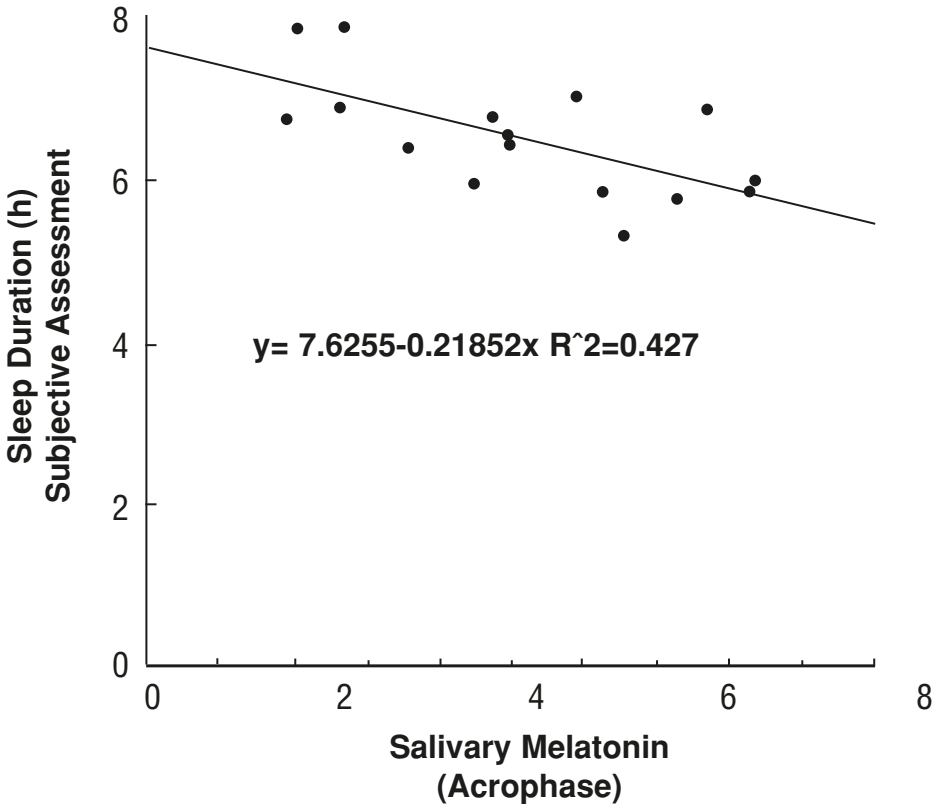

Figure 3.4-4 *Correlation of Rhythm Markers (Salivary Melatonin Acrophase) with Sleep Duration*

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3.5

Operational Psychology Countermeasures During the Lunar-Mars Life Support Test Project

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SUMMARY

The Crew and Thermal Systems Division at the NASA Johnson Space Center conducted a series of human-rated tests designed to advance technology in closed life support systems. As the duration of these tests lengthened, the psychological factors associated with placing humans in these environments became increasingly salient to successful mission completion. A number of psychological activities were conducted to ensure successful operations and protect crewmember well-being, including individual crewmember selection, crew composition, training and preparation, family inclusion, educational briefings, in-mission tracking, operational interventions, and postmission repatriation. This article describes these activities, the rationale behind their design, the similarities and differences to techniques utilized for space flight, and considerations related to designing psychological countermeasures for confined environments. In addition to testing physical and engineering systems, the chambers studies series functioned as an effective test bed for developing operational concepts and countermeasures for extended space missions.

Introduction

Between 1995 and 1998, the Crew and Thermal Systems Division at the NASA Johnson Space Center conducted a series of ground-based tests designed to advance technology in closed life support systems. The regenerative technology was tested with human crews in four tests, or phases, whose objective was to ultimately produce equipment and processes that could be incorporated into a variety of lunar, Martian, and low-Earth orbit stations and vehicles. The four tests were termed Phase I (15 days, one person), Phase II (30 days, four persons), Phase IIa (60 days, four persons), and Phase III (91 days, four persons). The crewmembers performed a number of technical and research tasks in the areas of engineering, station maintenance, medicine, and life science. In addition, they had exercise, housekeeping,

media events, educational outreach, and other duties related to the conduct of high-profile confinement missions. There are many sources for detailed descriptions of the physical living environment, its phases, physical layout, engineering, accommodations, and schedules, including Barta and Dominick (1), Laws and Foerg (2), Ming et al. (3), and Meyers et al (4).

When considering any mission within an extreme or confined environment, there are a number of psychological issues that the planner must address. Obviously, as the severity of the environmental, work, or personnel factors increases, or as the importance of goal attainment increases, it becomes more critical that the psychological issues involving the crewmembers are dealt with in a proactive manner. Over time, the duration and complexity of the LMLSTP phases increased, and the psychological aspects associated with maintaining crew health, well-being, productivity, and team functioning became increasingly salient.

A number of psychological activities and countermeasures were conducted to ensure successful completion of the phases and to protect the crewmembers' psychological health and well-being. These operational activities, including crew selection, training and preparation, family inclusion, control room team management, in-mission tracking, management consultation, in-mission interventions, postmission debriefings, and so forth, differed from the psychological research conducted during some of the phases. The psychological countermeasures were implemented specifically and solely to assure that the objectives of the tests, including psychological health and readiness objectives, would be achieved. The team that designed and provided the psychological countermeasures had previous experience doing so for missions in other ground-based, underwater, and space environments.

The purpose of this chapter is to give an overview of the psychological countermeasures designed for the closed-loop living environment, and some of the operational considerations that steered their implementation. Although all of the issues that arose during a particular phase are not included here, a sampling of issues is discussed. A more detailed discussion of the driving factors behind the design of psychological countermeasures can be found elsewhere (5).

Early Assumptions

Like all human missions, the advanced human life support enclosed system study final report had its own distinctive configuration and set of constraints that shaped the conduct of its missions. These included:

1. The division and project management that conducted the studies had never before conducted or participated in a human-rated confinement project.

2. The project would consist of multiple tests of increasing complexity and duration.

3. It would be necessary to place system experts, who were inexperienced in confined operations, inside the chambers in order to repair and maintain the systems.

4. There was interest within the life science research community in using the series to perform non-engineering studies pertaining to extended confinement.

5. Everyone within the organization was highly motivated to have a successful test series, and management was aware of the importance of psychological factors.

Phase I

During the very early planning stages of the series, the project management requested general psychological requirements. Those that were submitted covered a wide range of individual, team, and environmental topics, such as the necessity for meaningful work versus "make work," reasonable work-rest schedules, exercise capability, several types of communication capabilities, a systematic procedure for psychologically selecting and preparing participants, sleep protection, and basic habitability (e.g., privacy, leisure resources). These basic guidelines were incorporated into the overall study design.

The 15-day, one-person test was the first to be conducted. The simplicity of this phase did not require a large number of countermeasures from the psychological team. Due to its relatively short duration, this test was not expected to be as psychologically challenging as the longer, multi-person tests. Its duration was similar to that of a Space Shuttle mission, although it only involved one person. This latter fact was the only potential source of concern. However, it was offset by several beneficial factors:

1. Basic psychological requirements had been applied to accommodate fundamental psychological needs inside the chamber.

2. Tasks were straightforward and meaningful.

3. The participant was a Crew and Thermal Systems Division engineer who had been highly involved in the development of the regenerative life support systems being tested.

4. The outside control team was small and was composed of friends and colleagues of the participant inside the chamber.

5. The duration of the test was short.

6. The participant possessed the necessary personality characteristics and motivational set needed to cope with the solo conditions of the test.

Table 3.5-1 *Psychological Countermeasures*

	Phase I (15 Days)	**Phase II** (30 Days)	**Phase IIA** (60 Days)	**Phase III** (91 Days)
Basic Operating Requirements	X	X	X	X
Selection (select out)				
Psychological testing	X	X	X	X
Structured interview	X	X	X	X
Selection (select in)				
Psychological testing		X	X	X
Structured interview		X	X	X
Reference interviews				X
Crew Assembly				
Psychological testing		X	X	X
Situational assessments				X
Peer evaluations				X
Staffing w/committee		X	X	X
Training and Preparation				
1st crew briefing with crew-psyc. basic factors of long-duration confinement		X	X	X
Team building			X	X
Lessons Learned briefing		X	X	X
Confined team operations/Leadership				X
Individual Psyc. Planning		X	X	X
Advanced Psyc. Factors/ Lessons Learned Briefing			X	X
Effective communication training for control room (CR) personnel			X	X

Table 3.5-1 continued *Psychological Countermeasures*

	Phase I (15 Days)	**Phase II** (30 Days)	**Phase IIA** (60 Days)	**Phase III** (90 Days)
Control team/crew resource management training			X	
1st family meeting			X	X
2nd family meeting			X	X
3rd family meeting				X
4th family meeting				X
Lessons Learned/Psyc. Factors briefing with project management				X
Individual crewmember prep meeting				X
Monitoring				
Individual and group psyc conferences w/crew	X	X	X	X
Individual and group psyc conferences w/control room team			X	X
Individual and group psyc conferences w/families		X	X	X
Posttest				
1st debrief (~3 days post test)		X	X	X
2nd debrief (~14 days post test)		X	X	X

As shown in Table 3.5-1, the only measures taken for this phase by the psychological team were basic requirements, selection, and monitoring. In Phase I, selection only consisted of a review of the participant's ability to fulfill the mission, and monitoring was accomplished through informal visits to the chamber/control team to see that things were proceeding well. With the exception of providing the basic psychological requirements, this phase could have been accomplished successfully without any involvement of psychology. The human accommodations incorporated into this first test were rudimentary but sufficient for the duration. Later missions benefited through input from professionals in the physical design and accommodations area.

It is important to note that the motivational set of the Phase I participant was particularly high in part because he had been involved in developing many of the life support systems that were being tested in the closed facility. In confinement situations, it is very important for the participants to have meaningful work to perform; situations in which "make work," too little work, or meaningless tasks are scheduled will have a demoralizing effect on the participant. Previous U.S. experience on board the Mir station, as well as in other confined, ground-based settings, has highlighted this fact. Conversely, when someone is very interested and heavily invested in a task, motivation is high to endure any difficulties to see it through to the end. This latter case was the situation with all of the crewmembers in the enclosed system study series.

Phase II

This test was a four-person, 30-day test. Although still relatively brief from a duration perspective, the project was moving toward greater complexity by using four participants, moving to a larger chamber, adding additional hardware maintenance tasks to the internal workload, and gradually extending the duration. From a psychologist's perspective, this cautious extension was a wise decision, because the organization was new to crewed test beds and had a number of management and logistical lessons yet to learn. There was not only the matter of learning to structure and manage the activities of the confined participants, but also the job of learning how to staff and structure the activities of the outside control room (CR) and their relationship to the inside team.

In an early planning meeting between project management and the psychology team, several decisions were made that would affect not only the course of Phase II but of subsequent phases as well. These decisions addressed programmatic design issues of a psychological nature that, in the past, had direct effects on the success of other confinement tests and analogous missions. Among the key decisions were:

1. To promote a shift from a "test mentality" to a "mission mentality." This involved the organization as a whole adopting a somewhat different view of what they had been doing for years. Instead of simply providing a metabolic load for the system, the essential, multifaceted role of the human inside the closed environment was recognized. The addition of a few medical and life science objectives resulted in a more diverse set of test objectives to be carried out. Education outreach objectives were increased over Phase I, and methods similar to those used in space missions were utilized. Hence, instead of "subjects," the participants would be called "crewmembers" and comprise a cohesive "crew." Distinct job roles for each of the crewmembers would be identified and a crew "commander" would be formally designated. Together, the crew, the control room (CR) personnel, and project management were tasked with carrying out the ground-based "mission."

The establishment of a crew identity and mission mentality was especially important to the psychology team. Crew psychological health, well-being, and productivity are greatly facilitated by the motivation and focus that is derived from a mission context. This is particularly helpful when mission durations lengthen, and a greater burden is placed on individual and team coping strategies. In addition, the mission model offers a template from which the organization can draw a number of solutions to issues such as control room staffing schedules, management of human research data, and so forth.

2. To use the early, relatively brief phases to create a cadre of experienced crew members for later, more difficult phases and follow-on programs. It was anticipated that the later phases would be considerably longer and that programs which followed LMLSTP might be far more complex and psychologically challenging. It was recommended that the organization pursue a "farm club" approach with respect to crew selection; the objective being to expose as many engineers as possible to the briefer confinements before tackling the tougher missions. This would result in a local group that was operationally and psychologically experienced from which to draw crew members. The farm club approach requires that the early phases not be overly difficult and that they be an explicit part of building a potential crew member pool for later projects.

3. To compose crews that are diverse in gender and experience but which represent all of the essential technical skills needed inside the chamber. There are no magic numbers that comprise the "best" crew size or the "best" gender mix. The principal driver for these issues is ensuring that you have the necessary technical skills and minimum number of people inside the chamber to most effectively achieve the mission objectives. However, duration plays a role here as well. Considering the limited scope of this article, suffice it to say that it was recommended that mixed-gender crews be used.

As Table 3.5-1 indicates, the psychological countermeasures for Phase II were increased over those for Phase I, but were still at a relatively low level due to the brief duration. As mentioned above, the development of the mission context was the single most effective and far-reaching psychological act. After that, psychological selection activities had the greatest impact.

In all types of missions (e.g., space, military, polar, ground studies, etc.), many diverse factors influence who is actually assigned to a mission, and psychological information is only one part of the overall selection process. However, within that part, individuals are sought who are psychologically suited for the target mission and who work well together as a team. In general, the determination of individual suitability and team compatibility for long-duration missions is a systematic, multi-

stage process that involves psychological testing, structured screening interviews, structured reference interviews, skill-based training and selection exercises, sociometric ratings, formal briefings, individual strategy sessions, and other techniques. The specific techniques are chosen and adapted according to the characteristics of the target mission. Similarly, the psychological training and preparation of the individual crewmembers, the crew, the CR personnel, and the crew families varies according to the demands that the target mission is expected to place on them. In-mission monitoring and support procedures, as well as postmission repatriation and tracking activities, also must be tailored to the individual mission.

For Phase II, selection and compatibility were determined by an abbreviated version of psychological testing and through a structured interview, which was a combination of two screening ("select-out") and suitability ("select-in") interviews used for selecting astronauts for extended missions. The select-out testing and interview process addressed the clinical psychological fitness of potential candidates, and the select-in testing and interview process addressed the psychological suitability of each candidate for the target mission. The criteria were scaled to a level appropriate for a ground-based test bed of 30 days' duration. Training and preparation consisted of two crew briefings and an individual planning session with each crewmember, in which lessons learned from previous similar missions were passed along, potential issues were identified, and personal and team strategies were created and reviewed. As in preparation for space missions, the lessons, issues, and strategies that were covered were divided into three categories, specifically those that applied to: 1) the individual crewmember, including family issues; 2) the crew as a whole, including leadership; and 3) the wider organization, including management and control room relations.

This cautious foray into extended, human-rated test beds was successful from a psychological perspective as well as from a mission perspective. As anticipated, the team functioned very well as a unit, and the individual crewmembers felt that they could easily stay in the chamber much longer. As usual, the project analyzed the mission for lessons that could be applied to the next mission. One of these was the need to further clarify the roles and responsibilities of key individuals within the wider organization and the manner in which these roles interfaced with that of the crew, especially that of the crew commander.

Phase IIa

The next mission extended the duration for a four-person crew to 60 days. The increased psychological demand inherent in this phase necessitated a slightly greater set of countermeasures. A somewhat more stringent version of the selection testing and interviewing process was applied to the individual candidates, and significantly more effort was expended on assembling a compatible crew.

Additionally, activities were added in the areas of preparation of CR personnel and family members. The issues facing CR personnel were significant, because the organization had little experience maintaining a rested external team over an extended period of time. The psychological team passed along CR lessons learned from other environments and promoted three general themes: 1) further inclusion of the CR group as an integral part of the team and acknowledgment of their contribution, 2) mutual understanding of the daily issues facing the crew and the CR groups and strategies for managing that interface, and 3) a reasonable work-rest schedule for individuals in the CR. The organization as a whole was very eager to pursue these themes, because there was a vital, preexisting team spirit and a high tendency toward inclusion in general. During the mission, both of the groups made it clear that they valued the other's continuing efforts.

Similar to the Phase II experience, one of the principal lessons learned from Phase IIa was that the definition of the work roles and responsibilities of key people inside and outside of the chamber must be made clear and specific to an extraordinary degree. This somewhat mitigates the misunderstandings and erroneous assumptions that naturally arise between two interdependent groups that are physically and visually separated. Disconnects and miscommunications of intention between crews and their control rooms can lead to a "we-they" phenomenon that is a classical occurrence in a variety of venues (polar, space, military, etc.). This did not occur in Phase IIa, because both groups consciously worked to lessen the issue.

Beginning with Phase IIa and continuing into Phase III, the crewmembers' families or significant others were brought together before the mission and during the mission for psychological information about the mission, and to create an environment of mutual support between them. In Phase IIa, these informal gatherings occurred over lunch. Inclusion of family members is a powerful crew support method for many reasons, in addition to being a source of strength for the crewmembers' families.

Phase III

With the advent of Phase III, the project had at last reached a mission duration that approached some of the briefer Mir space flights. In addition to increased length, the project included a large number of science experiments or technology demonstration projects from the life sciences. Scientists were interested in various effects of extended confinement, the suitability of specific exercise and nutrition protocols, and a number of habitability, training, and medical issues. From a psychological viewpoint, this increased workload was welcome; with the scientific, hardware maintenance, and education outreach tasks, there would be plenty of opportunity for meaningful work. However, the duration and complexity of the test bed were not trivial, and that warranted the application of more extensive psychological countermeasures.

Selection was more stringent; the criteria were in line with the increased demand on the individual to adapt effectively and to function well as part of a confined team. Individual selection activities were extended to include structured interviews of character references and more extensive reviews of each candidate's history in past work teams. It was essential that the applicant possess the skills to maintain a cohesive crew while still enabling diversity of opinion. The initial process of testing, interviewing, and history review resulted in eight candidates remaining, from which four would be assigned as prime crewmembers and four as back up crewmembers.

At this point, activities were devised that served both psychological training and selection needs. Within the group of eight individuals, some had worked together in the Crew and Thermal Systems Division for years and knew each other very well; some came from outside organizations and were unknown to most of the group. Some had been crewmembers in previous chamber missions, and some had no experience with either confinement or operational environments. The objectives at this stage were to: 1) identify a set of four people who would work well together as a prime crew, 2) prepare all eight individuals to work together in the event that back up crewmembers were rotated in during the mission, 3) provide a means for the eight to get to know each other in problem-solving situations and under conditions of mild stress, and 4) begin to integrate the new people into the Crew and Thermal Systems group that was fielding the mission.

An outdoor challenge course was used to accomplish these selection and team building objectives. An initial series of low-level, physical problem-solving situations was presented to the eight as a group, and the team worked these out as they saw fit. In a subsequent series of high-level traverses and climbs, the group split into pairs and triads to resolve the challenges. Membership in the pairs was rotated, so that people would have a chance to work with each other in a smaller unit. Each situation was debriefed by the group and the psychological team for lessons learned that might apply to the actual Phase III mission, and general information regarding teamwork styles for extended missions was passed along. The most significant benefit of this training, however, was the familiarization and integration of the eight team members with one another.

Following this preparation, the eight team members completed a sociometric form in which each of them gave their input regarding who they would select to be with them on the upcoming mission. This was an opportunity for the crewmembers to express their social preferences and to have their input factored into the crew assembly process. The psychological team combined this input with their own to develop recommendations for crew composition. Ultimately, of the four prime crewmembers that were assigned, two were Crew and Thermal Systems engineers who were crewmembers on previous enclosed system study missions, and two others were scientists with no previous confinement or mission experience. The early integration and sociometric activities would later prove to be a good investment when a prime crewmember had to be swapped out for a back up just prior to mission start.

Once the prime and back up crewmembers were assigned, the psychology team turned its attention to providing sufficient preparation of the crewmembers, their families, the control room, and project management. In some cases, we only provided encouragement and assistance to key people to prepare themselves for the mission, as was the case with meetings to further define job roles and responsibilities and a training workshop for CR personnel. In other cases, we provided direct training. This included more lessons learned briefings for the prime and back up crewmembers, individual planning sessions to discuss potential situations that might arise and to review strategies for dealing with them, information and integration meetings with the families, a training briefing for project management, and a five-day experiential training course on confined team operations for the prime crew.

The Confined Team Operations training was designed to prepare the prime crew in precisely the following areas: 1) confined living and working, 2) integration and organization as a team, and 3) actual mission operations experience. A working underwater station with a topside control and logistics facility was the setting for the week-long training. The crew was provided with a schedule, scientific and station maintenance goals, and crew organization goals that were comparable to those expected on the three-month mission. In addition, the design incorporated a condensation of a number of scenarios and conditions that had arisen on previous enclosed system study missions, or on analogous missions of three months' duration, which had the possibility of arising during their upcoming mission. The prime crew was provided with a blend of didactic, discussion, and hands-on experience regarding living and working in confined settings, leadership and followership, personal adaptation, safety, scheduling and rescheduling, time management, team norms, managing a relationship with the control center, contingency planning, conducting interviews with the media, analogue lessons learned, and other topics related to the upcoming mission. In addition, the designated commander had an opportunity to establish the leadership style that he would use, the entire crew had an opportunity to sort out their roles, and the experienced crewmembers brought the others up to speed on all aspects of the organization, procedures, and points-of-contact among other things.

Approximately one month prior to the start of the Phase III mission, the psychological team held individual meetings with the prime and back up crewmembers to review their readiness for the mission, and to jointly identify any issues that needed to be handled. For the back up crewmembers, the main issue was certainly one of psychological readiness, because it is very typical for back up crewmembers, once prime crew assignments are made, to shift their attention to the demands of their daily jobs rather than the demands of the impending mission. It is not a fault; it is simply a matter of probability and expectation. They reasonably conclude that there is a higher probability that they will be on the outside of the chamber during the mission than on the inside, and it is not easy for a back up crewmember to maintain a state of mental preparedness for something he or she is not likely to do.

Consequently, the individual meetings were, in part, designed to relight the process of mental preparation in the back up crewmember. Approximately two weeks prior to the mission start, the prime crew commander had to be removed from the crew for medical reasons. A back up crewmember was rotated in, and a commander was assigned from the remaining prime crew. This was a major disruption, but it came with some excellent lessons attached.

The tracking of the crew's psychological health and well-being was accomplished through regular visits to the chamber at various times during the work week and on weekends. Of particular interest were aspects of the ongoing mission such as individual workload, technical concerns including failed equipment, the development of threats to the mission completion, or to crew health and well-being, the amount and quality of sleep, significant news from home or work, mood and humor, any significant mission events, and crew cohesion. Contacts were made with the control room team, managers, principal investigators, family members, and crewmembers. Contact with the crew was through two-way video meetings, telephone calls, and electronic mail. Family lunches also were held periodically during the mission. The psychological team served as a sounding board and information resource for all of these groups.

CONCLUSION

In conclusion, psychological countermeasures are a key aspect of any long-duration, human-rated test bed. They are most effective when they 1) are specifically designed for the demands of the target mission, and 2) address not only the crewmembers, but their families, key external personnel, and the wider organizational system that is fielding the test. Countermeasures act to increase the probability that the goals of the test will be met, and they do so by preventing difficulties and promoting performance readiness.

There is a direct, inverse relationship between the level of effort invested in the human aspects and the number of difficulties that arise during a confinement mission. Any reports from other such missions of difficulties with individual participants, within teams, between cultures, between external control personnel and internal participants, between participants and project managers, etc. can only be understood in light of the type, extent and quality of the interventions applied to prevent such problems. More often than not, when such problems occur, they can be traced back to the organization(s) that are fielding the mission and are responsible for implementing operational psychology countermeasures. How much effort should be invested? The answer to this depends upon how much difficulty and risk, and what kinds of difficulty and risk the organization is willing to accept.

The Advanced Human Life Support Enclosed System Study series was successful from both a technical and psychological point of view. The mission goals were attained, the crewmembers look back on their experience with a sense of satisfac-

tion and accomplishment, no significant mission management difficulties were encountered, and a step or two was taken forward on the road to the Moon and Mars. Early on, the project management recognized the importance of the human factor in the overall success of the series and was willing to invest in the pre-mission psychological activity necessary to ensure positive results.

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3.6

Spaceflight Cognitive Assessment Tool for the Lunar-Mars Life Support Test Project Phase III Test

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SUMMARY

Main Objective: To test a computer-based objective cognitive assessment tool in an analogue environment comparable to a space station.

A cognitive test was developed and tested. The Lunar-Mars Life Support Test Project (LMLSTP) Phase III test crew and back-up crew were briefed on the test, took the test four times for baseline data, and then took the test three times during a 91-day chamber test. The test was evaluated in terms of adequacy, training, scheduling, administration, and problems.

PSYCHOLOGY (COGNITIVE) SELF-EVALUATION

Introduction

Specific Aims and Objectives

For the purposes of the LMLSTP Phase III Test, the Behavioral Health and Performance Group (BHPG) assessed a cognitive assessment tool to assist in monitoring crew health during the 91-day stay in a sealed chamber. The Spaceflight Cognitive Assessment Tool (S-CAT) was designed as part of the behavioral medicine monitoring efforts to be used on the International Space Station (ISS). This tool will be incorporated into the routine medical monitoring regimen being conducted by NASA Johnson Space Center Medical Operations. The S-CAT was used to provide the medical support personnel with a valid measure that would help them make decisions regarding the cognitive well being and capabilities of the crew. In addition to routine health monitoring, this test could be used in case of accident, injury, or exposure to off-nominal environmental conditions.

There were two specific aims for the Phase III test:

- Evaluate the sensitivity and implementation of the S-CAT during a space-analogue mission; and
- Provide objective measures of cognitive functions that could be used in caring for the crew during the test.

The original intended objective of the Behavioral Health and Performance Group's LMLSTP Phase III effort was, in addition to evaluating the S-CAT, to assess a Behavioral Medicine Crew Assessment Battery. This battery was to include stress and mood assessment tools. Due to time and funding constraints, only the S-CAT was employed for this test.

Background

As of the end of 1996, there was no objective measure of cognitive functions available to space crews, although anecdotal reports from space crews suggested the space environment might adversely affect crew cognitive performance. crewmembers from short- and long-duration missions reported mild degradations in their ability to remember tasks and to recall information. Off-nominal conditions including accidents, injuries, and exposures to toxins can certainly affect an individual's ability to function. Operations aboard Space Station Mir clearly indicated the need for some form of objective cognitive and performance assessments.

Description of the S-CAT

A small team of extramural experts was assembled to assist the NASA Medical Operations BHPG develop a tool that could be used in the evaluation of the cognitive functions of space crewmembers within the time and environmental constraints of space missions. Expertise on this team included three clinical neuropsychologists and two experts in the construction and use of automated psychological tests.

Operational constraints significantly affected the development team's efforts in identifying the appropriate cognitive assessment tool. The constraints included: available computing equipment onboard ISS; the limited time available for completing the tool; results having to be immediately fed back to the crewmembers; and given the critical need and the short timeframe for development, the tool by necessity would be created from existing, validated tests and methods.

The S-CAT construction was based on a battery of tests derived from the Automated Neuropsychological Assessment Metrics (ANAM) developed for the U.S. Department of Defense (3, 4). The ANAM consists of validated tests that have been used in clinical settings to evaluate personnel with suspected brain injury (3). The ANAM tests used in the S-CAT were selected to meet the time and diagnostic requirements of the space environment. The following is the list of the S-CAT subtests and a brief description of what each measures:

- Code Substitution–memory
- Running Memory–sustained concentration
- Math-verbal working memory
- Match-2-Sample–visual working memory
- Code Substitution (Recall)–recall

The development team developed both a short and long version of the S-CAT. The short version, which was developed, required approximately 15 minutes to complete, and allowed routine monitoring of crew cognitive functions and provided initial diagnostic information in the event of an injury or toxin exposure. The long version was intended to provide an enhanced diagnostic capability (using additional tests) of the crewmember's condition, if necessary, and could be completed in 35 minutes.

Methods

Protocol

To implement the S-CAT, crewmembers had to learn and become proficient at each of the tests that comprise this tool. Crewmembers were first given a short familiarization briefing and documentation about the purpose and use of the S-CAT and then they completed four sessions taking the short version and two sessions of the long version. To reduce scheduling problems, the crewmembers attended training and baseline data collection sessions in groups of four. The team of S-CAT developers estimated that at least four sessions would be needed to overcome learning effects and to produce meaningful baseline data for each crewmember.

During the test, the same protocol planned for the Space Station Mir and the ISS was followed. Each crewmember was scheduled to take at least the short version of S-CAT once per month, coinciding with the monthly physical examination, for a total of three scheduled sessions during the test. Although the crew would be reminded when to take the S-CAT, they were responsible for actually scheduling and doing the S-CAT. The long version was scheduled at approximately test days 45 and 75 for a total of two times during the Phase III test. Following completion of the Phase III test, crewmembers would be debriefed and, if possible, take the short version of S-CAT one more time.

Since a back-up crewmember could be a replacement into the test chamber at any time, the back-up crew was trained and performed baseline S-CAT testing. Thus, all potential crewmembers were proficient and had recent baseline data.

Repeatedly taking the S-CAT ensured two objectives: there would be good data available from pretest and nominal operations over time with which to compare test results following a mishap; and crewmembers would maintain proficiency on the tests so that learning (or relearning) effects would not confound the data and lead to a misdiagnosis of the crewmember's condition. Table 3.6-1 is the schedule of S-CAT sessions for the Phase III test.

Table 3.6-1 *Scheduled S-CAT sessions for prime and backup crewmembers*

Pretest	In-test	Post-test
1 Familiarization @ 1 hour	Short @ 15 minutes on test days 30, 60, and 88 and as needed	1 Debrief @ 1 hour
4 Training_short @ 30 min	Long @ 35 minutes on test days 45 and 75 and as needed	1 Short @ 15 minutes < 30 days post-test
2 Training_long @ 35 min (combined with last two short sessions)		

Hardware

The hardware used during this project consisted of DOS-based notebook and desktop computers. The make and model of the computers used are unimportant except to note that they used a DOS-based (versus Apple) operating system that is required to run the S-CAT. The familiarization and initial exposure to the S-CAT was conducted at a training classroom with desktop computers in Building 12 at Johnson Space Center. Training sessions and the baseline data collection were completed at the Krug Life Sciences (now Wyle Laboratories) Parsec II building using notebook computers. During the Phase III test, the prime crew used their personal desktop computers in the test chamber and the back-up crewmembers used a notebook computer stored in the test control room.

Additional Issues

In addition to providing a tool to assist in evaluating crew capabilities during the Phase III test, an objective of this project was to answer three important questions regarding the implementation of the S-CAT.

S-CAT Practice and Baseline Sessions

First, what amount of training and practice is required to alleviate task learning effects? Based on experience using the ANAM tests, the S-CAT development team predicted that 5 to 10 sessions would be necessary to ensure that the subject learned the tasks well enough to work at his/her best performance level. However, the Phase III crewmembers had considerably less time than that available as they prepared for the test. The result was that crewmembers had just the one familiarization meeting and the four sessions for training and baseline data collection.

Control of S-CAT Data

The second question is what is the best way to control the S-CAT data? Significant results of poor performance on a test like the S-CAT, indicating a neuro-cognitive problem, is likely to be perceived by flight (and test) crewmembers as very serious and potentially career jeopardizing. This bias is believed even if the data would not be used to reach conclusions independent of other data. Hence, there is a reluctance on the part of the crewmembers to take such tests or to share the data with the flight surgeon, medical monitor, or flight managers.

Since the S-CAT cannot help the crew or medical personnel if it is not used, then the top priority was to encourage crewmembers to use the tool. The approach applied during the Phase III test was to allow the crewmember to control use of the data. In other words, if the crewmember did not want to share the data, then they did not. With this approach, the data collected was for the crewmember alone to assess his or her own capabilities without the fear of detrimental judgment from management or the flight surgeon/medical monitor. The counter argument to this approach was that crewmembers might not recognize impairment given the limited training on interpreting the performance scores. And, if they did, they might not report it to the flight surgeon/medical monitor for fear of some reprisal either immediately or upon completion of the mission. Ultimately, it was decided that the best option was to trust the crewmember to report any anomalies, although the crew surgeon could request that the crewmember take and report S-CAT results if test events warranted.

S-CAT Schedule

Similar to the question about data control was how often should the S-CAT be scheduled? The S-CAT would be used following an event that might have resulted in cognitive impairment of crewmembers. To determine whether or not a crewmember's cognitive abilities are different from normal, the post-event S-CAT test scores would be compared to S-CAT scores produced both before the mission and during the mission up to that point. However, the crewmember must be proficient at taking the tests in order for the scores to be truly meaningful. If the crewmember fails to maintain proficiency on the S-CAT, then performance degradations due to loss of skill over time might be interpreted incorrectly as a neuro-cognitive problem.

When should the crewmember take the S-CAT to maintain proficiency? The S-CAT development team was not certain of the maximum time interval between S-CAT administrations that could occur before problems were encountered. For space crews, one approach to the problem is to couple the S-CAT with other medical requirements. Since the S-CAT is to be a medical requirement, it is logical to incorporate it with the physical examinations that occur monthly. Based on the experience of the S-CAT development team with the ANAM tests, taking the test every thirty days or so was thought to be sufficient to maintain S-CAT proficiency.

Another approach to the S-CAT schedule question was to inform the crew of the importance of taking the S-CAT periodically and letting them determine the

specific schedule. On long-duration missions, especially when communications are disrupted or have lengthy delays, crews will have to act fairly independently from their Earth-based support group. In light of this more autonomous mission, perhaps the crew should set and follow a schedule of self-examination, including the S-CAT, which fits their respective work schedules. This is the approach taken for the Phase III test. While the crewmembers were briefed on the purpose of the S-CAT and the need to maintain proficiency, they were given a suggested schedule instead of hard dates on which to take the S-CAT. They were requested to complete the S-CAT around test days 30, 60, and 90 (officially, around day 88 so to avoid the exit day flurry of activities) and they were reminded when those dates approached. It is important to note that history suggests that if a task is not hard-scheduled into the extremely busy schedule of an astronaut, then it is not likely to be accomplished.

Results

Anomalies

There were some imperfections in the S-CAT software that caused the long version to be troublesome throughout the test. The training sessions were completed and several of the crewmembers attempted the long version during the test, but no meaningful baseline or in-test data were collected. The imperfections in the S-CAT software that caused the long version problems and other software-related issues have resulted in significant improvements being made to the S-CAT, including reduced conflict with a variety of computer platforms, easier installation, and fixing a previously unidentified data scoring error.

The group training that occurred for the Phase III test was not optimal. Each crewmember (or test subject) should be trained in a private area that is relatively free from distraction. Though the group setting did not seem to affect the last two pretest data collection sessions, the first two were probably not representative of what would occur in a more private setting. It is clear from direct observations and the numerous comments from the crewmembers that a requirement of using the S-CAT needs to be a fairly quiet location free of interruptions. The group sessions proved to be too distracting. For example, as one individual asked a question or commented, the others naturally stopped to listen or elaborate instead of continuing their session uninterrupted. Additionally, it was all but impossible for the back-up crewmembers to take the S-CAT in the control room due to the constant distractions of on-going communications, problem solving, and visitors.

Objective 1: Evaluate S-CAT Implementation

Training Time

For each of the prime and back-up crewmembers, performance asymptote on the S-CAT short version was reached after the familiarization session and two data collection/training sessions. If or when performance leveled off for the long version

of the S-CAT is inconclusive due to the software problems mentioned above. It seems as though the number of sessions required to achieve proficiency on the S-CAT might not be as much as the S-CAT developers originally thought. Training time of future subjects will be noted to compare with the results of the Phase III test. Training for the S-CAT for future use will be one familiarization and five baseline sessions.

Efficiency

The S-CAT appeared to be a reliable measure of cognition based on preliminary examination of the data. The S-CAT development team has recommended specific criteria for go/no-go decisions. Further assessment of the effectiveness of the tool is warranted with validation studies.

Data Control

There were no untoward events (head trauma, exposure to toxins) during the mission requiring cognitive ability evaluation so evaluation of the control of data and sharing of it with the medical monitor was not tested.

Schedule

Neither the short nor the long versions of the S-CAT were taken following the recommended schedule and some crewmembers did not complete either version the recommended number of times. Those who did complete the S-CAT did not follow any schedule. When they were reminded that it was time, they attempted to complete an S-CAT test, but did not necessarily accomplish fitting it into their schedule. One or two of the back-up crewmembers attempted to take the short version once or twice, but there was no meaningful use of, or data collected from, the S-CAT by the back-up crewmembers.

Based on the nearly unanimous recommendation from the prime and back-up crewmembers and on the history of manned space operations, the S-CAT sessions must be a requirement in the astronaut's flight timeline schedule, not just a recommendation. Otherwise, the probability that the S-CAT will be taken and, therefore, be a meaningful measure when needed is low. To paraphrase the gist of what the Phase III crewmembers reported, 'There will always be something more pressing to take care of or something more desirable to do than to spend the time doing a test that will not likely be used anyway.' The importance of this type of data to crewmembers and medical personnel is critical in the case of a cognitive event.

Objective 2: Provide Objective Measures of Cognitive Functions

There were no untoward events (head injury, exposure to toxins) that warranted assessment of any crewmember's cognitive functions.

Discussion

The S-CAT will meet the on-orbit cognitive assessment needs for future space missions, but it must be a hard-scheduled medical requirement rather than a suggested tool with a recommended schedule. Further, the S-CAT should be scheduled during the normal work day to reduce time and energy conflicts in order to ensure the crewmember is "working" at full, nominal levels rather than tired and rushed at the end of the day.

The issues over control of the S-CAT data were untested in the Phase III test. Control over the data is to remain with the crewmember unless an untoward event occurs. Depending on the event, the data may be used by the crew surgeon for medically-based diagnostic and management recommendations.

The long version was not perfected for the Phase III test, so no meaningful data were collected. Even so, the lessons learned regarding the training, baseline data collection, and implementation of the long version were quite valuable. These lessons were used in preparation for the NASA-7 mission and ISS implementation.

Further development of the S-CAT continues with improvements to data presentation displays, multiple language capability, installation improvements, diagnostic criteria, selection of different tests (as deemed necessary), and compatibility with the Microsoft Incorporated Windows95® operating system.

SIGNIFICANCE

A computer-based objective cognitive assessment tool was successfully developed and tested in a 91-day chamber study to be used on Space Station Mir and the ISS. The lessons learned from this chamber study included giving a thorough briefing on the importance of this tool, hard-scheduling training/baseline and operational use of this tool, and having the crewmembers maintain control over their own data unless there is a medical event.

Future research directions include validation studies with both normal and clinical populations. Possible operational applications may include the military, physicians, underwater divers, or other high-risk occupations.

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3.7

Sociokinetic Analysis as a Tool for Optimization of Environmental Design

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SUMMARY

For centuries, architects and planners have pursued the design of human environments with the understanding that a relationship exists between social behavior and the particulars of the built environment. Examples of designs abound which were intended to encourage (or to discourage) specific modes of interaction, from Baron Haussmann's construction of the grand boulevards of Paris to the communally minded dormitories of the Fourierist and the Shaker utopias.

Despite the amount of theory which has been applied to the precise relationship between social and human behavior and the environment – which we may think of as the arena in which these interactions take place – only one study has been conducted which sought by quantitative means to identify the elements of a given environment that would render it ideal for its intended use. In his study *The Social Life of Small Public Spaces*, sociologist William Whyte innovated a method for critiquing the design of public parks that was unique in that it sought to objectify the critical criteria. The sole criterion in Whyte's study was whether people used the parks he studied, and whether they engaged in social interaction while doing so (1). As a result of his work, radical changes were made to the design of public plazas throughout New York and other metropolitan areas; despite this fact, this important effort has yet to be duplicated or expanded upon.

One of the experiments developed for the Lunar-Mars Life Support Test Project (LMLSTP) Phase III test was designed to develop this idea for application to the design of enclosed environments, such as those for long-duration space missions.

Introduction

What is social interaction? In terms of the built environment, social interaction is a dynamic constant that expresses itself as a kind of *kinesis*, or a choreographic pattern, by which the members of a group occupy a given place. The impetus and

effectors to any given interaction certainly lie partly in the realm of personal and group psychology, and behavioral psychology shows us many ways of viewing the inherent relationships within any set of group transactions. However, personal or subjective studies alone are insufficient to explain the full set of behaviors that we describe as social interaction, in no small part because the environment in which these interactions happen contains formal elements which (whether by accident or by design) tend to stimulate or to suppress specific behaviors.

In general, these cues seem almost impossibly complex to identify, isolate, or characterize in terms of their behavioral impact. The design of hermetic habitats for long-duration human support in extreme environments (e.g., Arctic/Antarctic research, space exploration, or lunar/Mars bases), however, renders the need to do so as a matter of the highest importance. Under such circumstances, the habitat itself takes on a uniquely influential role as the primary or sole environment and is thus critical in either supporting or undermining the mental health, productivity, and interactions of its inhabitants. Therefore, the conscious control of environmental cues such as programming, acoustics, and orientation becomes fundamental to the facility's design and, by extension, to the success of the mission.

In order to enable the architect to exercise such control with any kind of precision, tools must be developed that are capable of generating hard requirements based on objective data. One such tool is **sociokinetic analysis** – that is, the study of the patterns in which a group of individuals within a given environment make use of that environment. This method involves a) the capture of hard data on the use of volumes within a hermetic habitat and b) the application of statistical analysis to their use by a resident group. Strict documentation of the habitat is then weighed against the results in order to force certain environmental design cues to the forefront.

The sociokinetic analytical method was pioneered at NASA's Johnson Space Center (JSC) in 1997. Its first run involved an objective study of the use patterns of JSC's 20-foot chamber during Phase III of the LMLSTP over the 91-day time span from September through December 1997.

Test Conditions

Camera locations included two cameras mounted on Level One, one mounted in Level Two, and one in the corridor area of Level Three. The following floor plans show the levels and the camera locations:

Level One — Camera 1: Common Room and Camera 2: Airlock

As the floor plans suggest, Level One was a multifunctional area consisting of galley and wardroom, science workstations for advanced life support studies, the principal personal hygiene and waste compartments (shower and toilet), and a dedicated exercise area in the airlock. The airlock was smaller than other rooms and was loud when any one crew person was using it for exercise. In addition, the airlock was the only location in the chamber from which the crew could be observed by anyone walking through the Building 7 highbay.

The common room housed the entertainment center (TV and VCR) as well as the only table large enough for communal activities or large work tasks. Despite its cramped and interstitial location, it housed all of the equipment most desired for group functions.

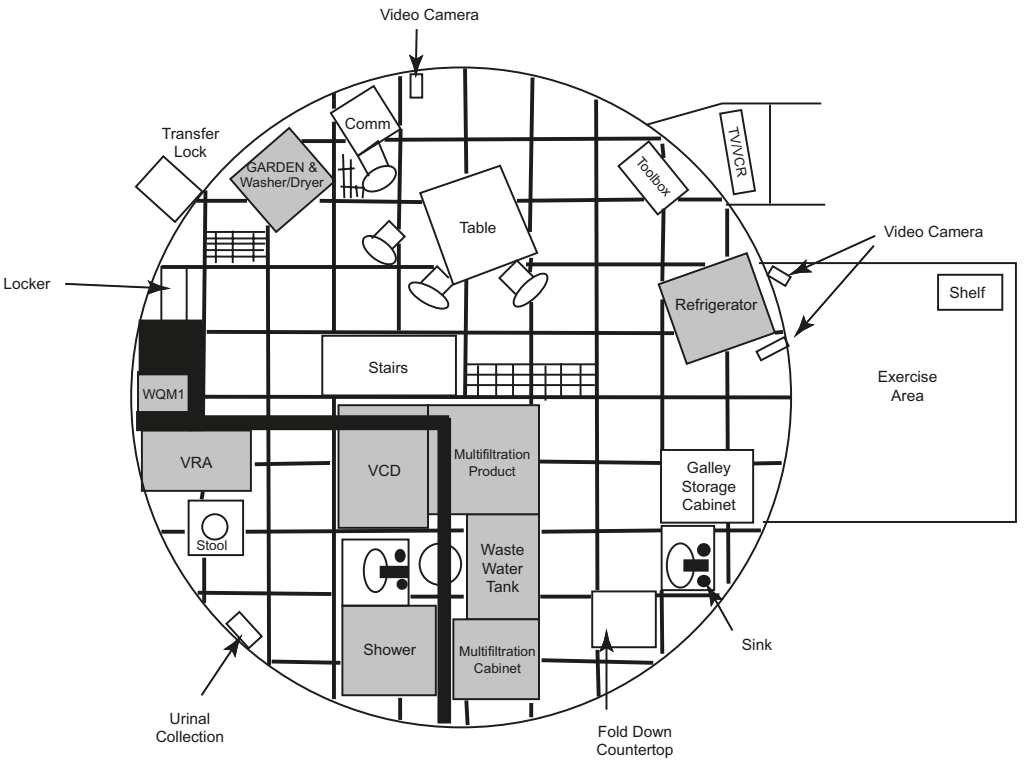

Figure 3.7-1 *Level One camera placement*

Level Two – Camera 3: Maintenance/Workstation

Level Two housed all principal equipment to support the basic functions of the chamber that were internal to it, including bioreactors and gauges. The average noise level in Level Two was 70 dB (3), and the lighting was provided by fluorescent fixtures arrayed vertically along the walls so that the occupants experienced a combination of glare and reflection at all locations on that level. A generous workstation table was provided on this floor, the same size as the wardroom table on Level One but without the crowding of the latter area.

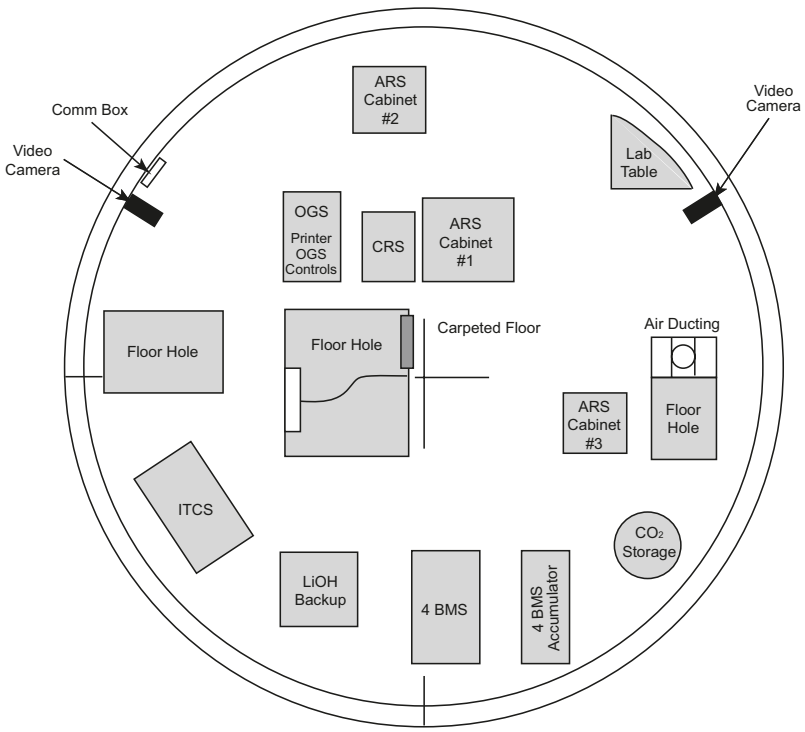

Figure 3.7-2 *Level Two camera placement*

Level Three – Camera 4: Crew Quarters

Level Three was the uppermost and most private level of the chamber, housing four identical crew quarters and a toilet all opening from a central landing at the head of the stairs.

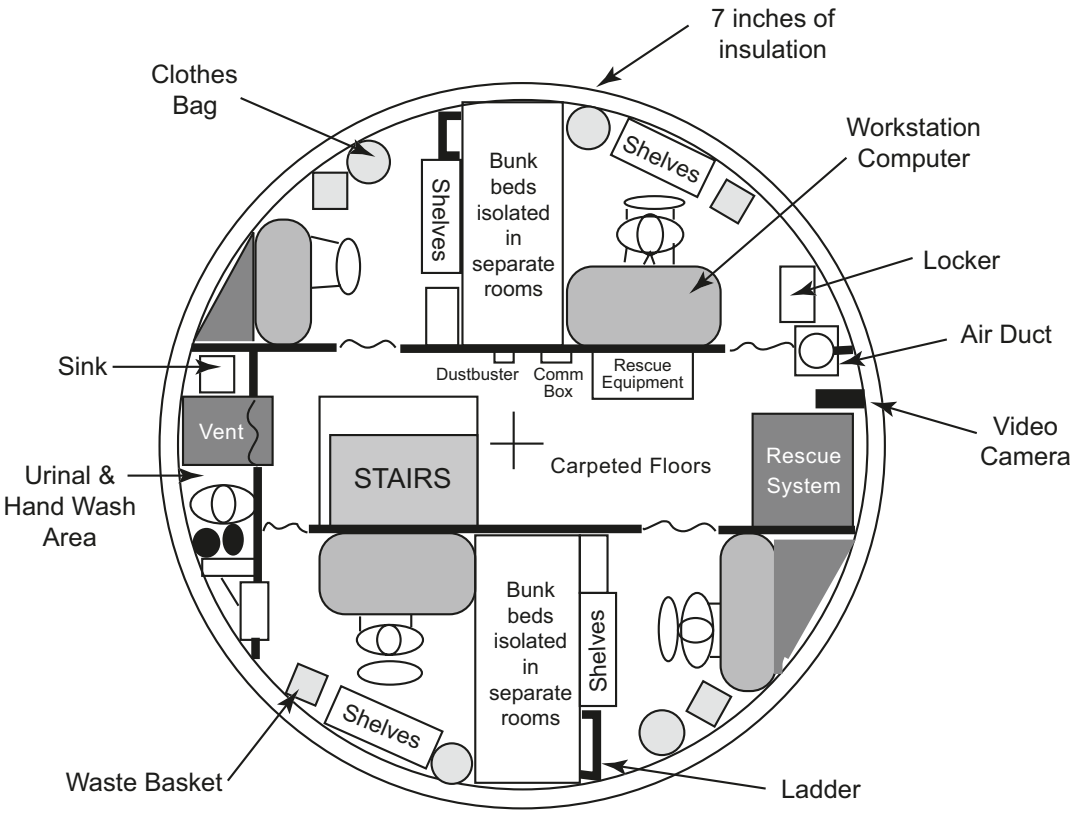

Figure 3.7-3 *Level Three camera placement*

Methods

In order to establish the habitable desirability of the various segments of the 20-foot chamber, it was essential first to find a quantitative, nonintrusive method of studying the patterns of use by which the four-person crew occupied the facility over three months. Because intrusiveness of any measurement system would inherently affect the data collected, the approach was winnowed down to one that used the four cameras, already present in the chamber, by which the Control Room maintained contact with the crew. The feed from these cameras was then recorded

24 hours a day, seven days a week, over three week-long spans of time. Since the question of adaptation or change was also a consideration, it was determined that data should be taken in the early, middle, and late stages of the test, specifically, weeks 3, 7, and 11.

Because the camera in Level Three records the public segment of that floor, off of which all four crew quarters are located, it was possible to note when the crewmembers were enjoying the privacy of their personal quarters without invading that privacy. Thus, the advantage was gained of having complete reasonable access to the crew's activities in a manner that was not intrusive. For instance, the Control Room protocol required that the crew be alerted to the fact that taping would commence at 00:00 that night on the evening prior to the onset of each week under scrutiny. Despite this alert, however, by week 3 – the first week studied – this was of negligible impact to the data because by this time the crew had become accustomed to the constant vigilance of the Control Room staff and had begun to ignore the presence of the cameras, or to accept them as a simple fact of daily life. An on-screen video time stamp was used which permitted the researchers to verify the time of the actual recording against the time marked on the cassette.

At the completion of the test, a total of 512 hours of video was then tracked using statistical analysis software known as SPSS 7.5, and the period of each crewmember's tenure on each floor was quantified in units of seconds. These units were then tracked against time, total duration, and the simultaneous activity of other crew.

The principal questions under consideration were:

- Did the crew's preference for group versus private areas or other use patterns change over the duration of their confinement?
- All things being equal (i.e., specific site-related activities aside), did the crew prefer more private locations or more public/shared locations?
- Were there any marked social patterns or behaviors that were anomalous to nonconfined groups?
- Were there any marked behaviors that reflect in an unambiguous fashion on known conditions of the crew's environment?

Findings

First, an analysis was made of the percentage of time the crew spent on each floor during each week of the test. Although there was less variation from week to week than had been anticipated, a slight but steady trend was seen toward less use of the airlock and Level One (the group areas) in favor of Level Three (private zones), as shown in Table 3.7-1.

Although this difference is not considered statistically significant, a significant trend was detected in the comparison of individual room usage within each week. Furthermore, this trend held true across all three weeks of testing. This trend was identified via post hoc Tukey's analysis as shown in Table 3.7-2.

Table 3.7-1 *Percentage of time spent on the floors*

	Week 3 (%)	Week 7 (%)	Week 11 (%)
3rd Floor	51.6	54.9	54.8
2nd Floor	4.0	2.7	3.0
1st Floor	41.4	40.0	39.9
Airlock	3.0	2.4	2.3

Table 3.7-2 *Post hoc: room usage differences within each week*

	3rd Floor	2nd Floor	1st Floor	Airlock
3rd Floor	—			
2nd Floor	S	—		
1st Floor	S	S	—	
Airlock	S	NS	S	—

S = Significant
NS = Not significant

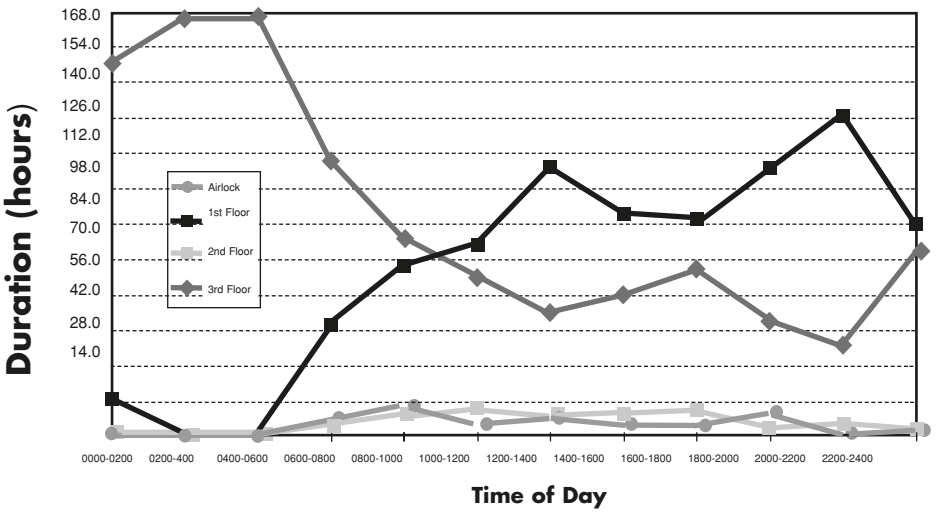

Figure 3.7-4 *Duration of floor use and time of day*

Of greatest interest for future refinements of this test is the analysis of the use patterns against time of day over the study period. Figure 3.7-4 (above) shows a temporal analysis which averages the usage over all three weeks. It is important to note that while the rates of use for the airlock and Level Two appear to be similar,

the incidence of that use is utterly different. Because of the exercise function in the airlock, its use by only a single crewmember at a time was extended throughout the waking day with small peaks between 08:00 and 10:00 and between 18:00 and 20:00 as personnel used it for exercise. Level Two usage, however, was almost exclusively in 20-second increments steadily throughout the day – in other words, the amount of time it took for a person to traverse the Level Two landing on the stair while in transit between Levels One and Three. Occasionally, crewmembers would spend slightly larger blocks of time in Level Two in order to check or maintain equipment, but the greatest percentage of use stems from the transit function which was more or less constant throughout the day.

Another important (although less marked) data point was the set of locales for socialization. While Level One group interactions included any number of crewmembers up to four, the group interactions which took place on Level Three were noted to be interactions of never more than three and predominantly of only two persons at one time. Moreover, crewmembers were never seen entering one another's private quarters. The group appears from very early on to have established an unofficial protocol whereby people talking would stand just outside or in the doorway of another person's room, thus establishing a territorial boundary between the semi-private realm of the corridor/landing and the private realm of the crew quarters. Mutual-boundary interactions also took place as crewmembers stood in their own doorways and conversed with one another across the landing.

Use of Level One was almost perfectly mirrored by the use of Level Three; in other words, when personnel were not on one, the chances were very high that they were on the other (rather than in the airlock or Level Two). In many instances this use at specific times of day is unsurprising. Level Three, for example, was the area of choice between 24:00 and 08:00, while the crew was sleeping; and Level One was most popular around 12:00 and 20:00, at lunch and dinnertime.

However, this mirroring is also unaccompanied by anything but a static, constant baseline in the airlock and Level Two areas, suggesting that the use or disuse of Levels One and Three had bearing on one another but no bearing on the occupancy of Level Two and the airlock. Thus we note that two of the four areas of the habitat (some 35-40% of its total available area for habitable use) went virtually unused except by necessity, while the other two areas became in essence the whole inhabited volume of the test chamber.

IMPLICATIONS

By separate use of these terms we are establishing a distinction between "habitable" volume and "inhabited" volume. The difference between the former and the latter is that the former – "habitable" volume – **can** be occupied by humans, whereas the latter – "inhabited" volume – **will** be occupied and used by humans.

This is tremendously useful data because it tells us which environments the crew found acceptable, and which they did not. There is nearly overwhelming evidence here that the crew preferred Levels One and Three of the 20-foot chamber over the Airlock and Level Two. This is true to such a degree that these areas almost constitute wasted volume in that, despite the expressed needs of the crewmembers for greater privacy and flexibility within the habitat, they largely rejected the use of two semiprivate areas which could have been utilized as offline workstations and/or relaxation areas. In addition, the semiprivate landing area of Level Three could have become less of a public site for mutual-boundary interactions had it been possible for the crew to interact "offline" in some nonprivate room other than the Level One common area.

Thanks to this pattern of nonuse we are able to identify environmental factors which people clearly find unacceptable to the point of rejecting their use. The airlock is a small, cylindrical area that was not comfortably outfitted but rather housed only the exercise and other mechanical equipment. Furthermore, it was the only part of the habitat exposed to the exterior, so that something of a "goldfish bowl" sensibility may have held sway. Other than exercise, there was no other activity associated with the room and it had nothing to offer by way of welcome.

Level Two, on the other hand, had a pleasant level of illumination, carpeting, and a cozy corner or two to offer. Only two factors were less than optimal in its outfitting, yet these appear to have had a decisive effect on the usability of the room: the very loud acoustic environment, and the direct-glare lighting. While the room appeared calm, it was extremely difficult to make oneself heard for the noise generated by the equipment. Also, although the lighting levels were acceptable for tasks, the angle of lighting was extremely unpleasant.

Thus we have managed to derive a few important rules for design of inhabitable built environments:

1. Finishings, dimensions, and privacy affect the usability of the area,
2. High-glare illumination can render an area unusable to the resident population, and
3. An unacceptably loud acoustical environment can render an area unusable to the resident population.

The private/semiprivate/public boundary issues raised by interpersonal communications on Level Three also suggest some useful rules of programming (i.e., functional allocation of volume) for future hermetic habitats:

4. In a restricted habitat, private rooms are considered inviolable territory and will not be invaded unless by explicit invitation, and
5. The territory immediately adjacent to private rooms may/will be annexed as a semiprivate social center (unless other areas specifically intended for offline socializing are provided).

In any event, it is clear that environmental conditions do affect the efficiency and usability of the facility.

Forward Work/Conclusion

The next step from this point is naturally to repeat this test using specific factors as control and as test items in the habitat's environmental design. Because of this, the Hab element of JSC's BIO-Plex test facility was designed to allow investigators to use this method in testing specific questions concerning programming and volumetric allocation during future tests with human subjects. Specifically, the Hab is designed to accommodate the following tests for narrowing the field of questions:

1. Reconfigure the chamber between extended habitation tests in order to vary the balance of common, semiprivate, and private areas
2. Reconfigure the chamber between tests in order to vary the location of circulation and semiprivate areas and their relationship to common and/or private rooms
3. Reconfigure the chamber between tests in order to vary the relationship between common areas and workstations (i.e., galley, maintenance bench, office vs. wardroom/sitting room)
4. Control acoustic environment throughout the chamber
5. Configure and reconfigure lighting to test preferences for indirect, direct, and chromatically adjusted illumination, and
6. Change color and finishes to balance preference for "hot" vs. "cool" environments.

Sociokinesis – or, the movement patterns of a group – is a new but potentially highly valuable field of study in that it combines the fields of behavioral studies with environmental design. In its maiden run, this method already has established that there is a quantifiable relationship between environmental factors and human behavior. Taken to greater levels of detail and pursued in a diligent and scientific fashion, this study stands to offer a truly innovative set of data to guide designers in enhancing productivity and well-being through more usable environments. With proper follow-up this work will contribute significantly to the process of mitigating human-system risk for long-duration and exploration missions, as well as to the productivity and efficiency of many types of terrestrial structures and dwellings, such as submarines, Arctic stations, and other hermetic enclaves.

Acknowledgments

Many thanks to Dr. Frances Mount for her support of this project and of the implementation of this test at the last minute before Phase III. Also this work is much indebted to the diligent assistance of Michele Segal, who spent her summer viewing hundreds of hours of videotape and generating statistical models for analysis.

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4.1

Environmental Monitoring Air Quality

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SUMMARY

Air pollutants were quantified during the Phase II (30 day), Phase IIa (60 day), and Phase III (90 day) tests. Measurements from the Phase II test demonstrated a generally stable and safe atmosphere; however, measurements of ammonia and formaldehyde were incomplete. Near day 10 a large amount of methane entered the atmosphere and Freon® 113 was unusually high most of the time. There were periodic "bursts" of ethanol and isopropanol imposed on a steady state level of methanol. The Phase IIa test, which was the first opportunity to measure formaldehyde, was plagued with excess formaldehyde offgassing from various materials in the test chamber. This led to mucosal irritation in one crewmember. Methanol was unusually high, and at one point carbon monoxide had accumulated nearly to its long-term spacecraft maximum allowable concentration (SMAC). In contrast to the Phase II test where an accidental release of methane occurred, methane accumulated steadily throughout the Phase IIa test. Ammonia levels in the Phase IIa test quickly reached a low, steady-state concentration. Except for formaldehyde, all contaminants met standards for acceptable air quality. The Phase III test demonstrated much improved control of formaldehyde even though it exceeded its long-term SMAC late in the test. Ammonia accumulated steadily during the 90 days, reaching approximately 1/8 of its long-term SMAC. During the final days of the test, the air was characterized by rather rapid rises in irritant compounds and methylcyclosiloxanes. The trace contaminant control system (TCCS) suffered degraded performance during this time, and this is the likely cause of the increases in concentrations. Even though air quality standards were exceeded for irritants late in the test, there were no reports from the crew that the air was causing symptoms.

Introduction

The pollutants present in the atmosphere of a sealed environment represent the summation of many interacting dynamic processes. Those processes can be roughly separated into pollutant sources and pollutant sinks. This simple division, however, masks the complexity inherent in the behavior of each of the sources and sinks. Some examples will illustrate this point.

Air revitalization systems are necessarily thought of as sinks for air pollutants; however, there are examples where such systems have been the source of serious spacecraft pollution, or have converted relatively non-toxic pollutants to hazardous pollutants. Humans are generally regarded as pollutant sources; however, inhaled air is "scrubbed" of many pollutants by the human respiratory system before being exhaled into the vehicle atmosphere. Materials can be the source of offgassing of trace contaminants released from their molecular structures; on-the-other-hand, materials can provide surfaces for the condensation and absorption of less volatile air pollutants. The task of understanding and controlling the sources and utilizing the sinks to produce a healthy, respirable atmosphere in a sealed environment is not a simple one. A summary of circumstances that have lead to potentially unhealthy levels of air pollution during ground-based or on-orbit operations are given in the introductory subsections below with a perspective on how they relate to the Advanced Human Life Support and Enclosed System Study.

Materials Offgassing as a Source of Pollution

All polymeric materials release volatile substances that have been trapped in the polymeric matrix or can be formed as a result of slow decomposition of the material. All non-metallic materials are screened for offgassing rates before being accepted for use inside space vehicles and modules. In addition, the aggregate of offgassing produced in a module is estimated from the sum of offgassing from all components (in Spacelabs) or is tested after the module has been configured for flight (Spacehab and ISS modules). If uncured materials are present, this can produce a dramatic effect on the rate of offgassing into the module's atmosphere. For example, an initial test of the Node 1 module for the ISS gave an offgassing rate of 0.3 T units/day; however, a subsequent test conducted after further curing of adhesives used in the module gave a rate of only 0.02 T units/day. The major components contributing to Node 1 offgassing were methanol and propenal. As we will show later in this chapter, careful attention to materials offgassing can preclude serious problems with air pollution, even in ground-based tests such as the Advanced Human Life Support and Enclosed System Study.

Systems Leaks as a Source of Air Pollution

Chemicals are an integral part of many systems that comprise sealed environments, especially in heat-exchange loops. Perhaps the most notorious system leak occurred during the NASA/Mir Program when the Mir heat-exchange loops repeatedly leaked an aqueous solution of ethylene glycol. At times the magnitude of the leak was sufficient to elicit symptoms of respiratory irritation in crewmembers. Ethylene glycol condenses on cool surfaces and does not readily evaporate, hence, its spread throughout the station took place on a time-scale of weeks to months. Leaks of Freon® from refrigerator coolant loops have also been observed during

space operations aboard Mir; however, most Freon® is very low in toxicity and has relatively high exposure limits. The Closed Environment Chamber used chilled water from facilities supplies for thermal control; therefore, the risk of systems leaks involving potentially toxic compounds was much less than was experienced on the ageing Mir space station.

Experiment and Payload Leaks Cause Pollution

Experiments and payloads generally contain smaller volumes of chemicals than systems; however, some of the chemicals are highly toxic. Certain experiments use strong bases, which can cause permanent eye damage if they were to escape containment, and others use strong fixatives, which can cause severe eye and upper airway irritation. For example, paraformaldehyde fixative used in the Fundamental Biology Investigation-1 leaked past several containment barriers during the Mir-18 flight, but caused no apparent effect on crew health. The cause of the leak was failure to adequately control the heat-sealing process used for the containment bags. Other, less serious leaks have been observed from Shuttle payload experiments. The experiments conducted during the Closed Environment Living Study generally did not involve toxic chemicals that could escape into the atmosphere; however, an "experiment" conducted near the end of the 90-day test did contribute substantially to air pollution. Addition of food processing activity and waste disposal processes will add new risks to air quality.

Accumulation of Human Metabolites

Carbon dioxide is the major anthropogenic pollutant present in sealed environments. A major subsystem of the air revitalization system of space vehicles is dedicated to removal of this single compound. Failure to control this pollutant can quickly lead to physiological effects on the crew. To improve resource utilization, regenerable carbon dioxide removal systems have been developed; however, the sophistication of these systems can leave them more vulnerable to failure than the traditional, non-regenerable lithium hydroxide-based filtering systems. Periodically, levels of carbon dioxide spike up on the Shuttle if the lithium hydroxide filters are not changed on schedule. At times on Mir the level of carbon dioxide slightly exceeded the U.S. standard of 5.3 mmHg. There were no known effects on crew health. Since different types of carbon dioxide removal systems and different modes of operation of the systems were to be used in the Closed Environment Living Experiment, we expected that there might be some excursions in carbon dioxide concentrations.

Utility Chemicals Causing Air Pollution

Utility chemicals include such diverse items as hardware cleaners, degreasers, glues, personal hygiene materials, medications, and anti-fogging solutions. Problems with such chemicals in the air are rare; however, water-soluble compounds such as alcohols will be removed from the air through the humidity condensate and can end up polluting the water if the humidity condensate is being recovered for purification. For this reason, the use of alcohol-based hand cleaners and alcohol-containing hygiene wipes are strictly controlled on the ISS, but do not need strict control on the Shuttle where humidity condensate is not recovered. Volatile components of utility chemical formulations tend to appear periodically in air samples over a broad range of concentrations. Several major pollutants (e.g. 2-propanol) in the Closed Environment Living Chamber atmosphere exhibited this characteristic.

Propellant Entry as a Source of Air Pollution

Perhaps the most toxic air pollution event in human space flight experience occurred as a result of propellant entering the habitable volume of the vehicle. At the conclusion of the Apollo-Soyuz Program in July 1975, the descending Apollo capsule was equilibrating its low internal pressure with the increasing, outside, atmospheric pressure at the same time thrusters were firing. This resulted in nitrogen tetroxide being pulled into the capsule causing illness and even unconsciousness in the crew. Modern vehicles are designed so that this cannot happen; however, there is a small risk that propellants could lodge on a crewmember's extra vehicular activity (EVA) suit and be brought into the habitable volume through the airlock. Propellant entry will, of course, not be an issue for the ground-based Closed Environment Living Experiment.

Combustion as a Source of Air Pollution

The highest environmental health risk in modern space vehicles results from the possibility that a fire could occur inside the cabin. Aboard the Shuttle there have been experiences involving wiring shorts, pyrolysis of electronic components, and motor burn-out that have resulted in concern about toxic combustion products in the atmosphere. Perhaps the worst was the production of formaldehyde from Delrin® polymer that burned as a result of a seriously overheated motor in the refrigerator-freezer on STS-40. Aboard Mir there were at least two major pollution events resulting from fire or pyrolysis of materials. The solid fuel oxygen generator caused a spectacular fire that nearly resulted in abandonment of the Mir space station, and the "BMP" trace contaminant removal system produced large amounts of carbon monoxide when it overheated, apparently due to improper operation. As expected,

wiring fires and other combustion events proved to be a very small risk during the study; however, incineration of waste material during the Phase III test demonstrated that high-temperature operations pose significant air quality risks.

Microbiological Metabolites as Air Pollutants

Microbes pose a threat to crew health not only from their ability to cause infectious disease but also because they can produce noxious air pollutants. The best example of this occurred during STS-55 when urine and other waste materials were being put in the contingency waste container and disposed of by squeezing the container contents into space. The crew reported that the odors generated by doing this were unbearable. Air samples and subsequent ground-based testing revealed that microbes had metabolized the contents into methyl sulfides, which penetrated the walls of the bag and created a noxious odor. Waste management is a major concern for air quality management in long-term space flight and in simulations such as the LMLSTP.

MATERIALS AND METHODS

Volatile Organic Compounds

Air samples were acquired periodically in 500 ml, passivated canisters that had been evacuated, proofed for cleanliness, and spiked with 3 surrogate standards (C13-acetone, fluorobenzene-D5, and chlorobenzene-D5). The samples were analyzed by gas chromatography (GC) and GC/mass spectrometry (MS) according to work instructions (WI) 003 and 004, respectively, in the Johnson Space Center (JSC) Toxicology Laboratory. The Toxicology Laboratory is ISO 9000 certified.

During the Phase II test, formaldehyde was monitored using Fourier Transform Infrared (FTIR) spectroscopy of grab sample canister (GSC) contents. The major limitation of this method is that its detection limit is near 2 mg/m3, which is well above the long-term exposure limit for exposure to this irritant. For tests IIa and III, formaldehyde badge samples were obtained periodically, most often from chamber level 1, with nominal sampling durations of 24 hours. This improved the formaldehyde detection limit by approximately 100-fold. The diffusion-controlled, badge samples were analyzed by the chromotrophic acid colorimetric method according to WI-006 in the JSC Toxicology Laboratory.

During parts of the Phase IIa test, when formaldehyde became a crew health issue, the badge measurements were confirmed with two active sampling methods. In the first method, formaldehyde was trapped in impingers containing a 1% sodium bisulfite solution, and the solution was subsequently analyzed by a chromotrophic acid colorimetric method. In the second method (EPA TO-11), formaldehyde was reacted with dinitrophenylhydrazine, which was coated onto silica gel beads in a tube. The tubes were extracted with acetonitrile, and the solution was analyzed by high-pressure liquid chromatography.

Ammonia was monitored during Phases IIa and III with an Interscan Model 2900, which used an electrochemical cell to detect ammonia. The instrument was calibrated with a gas permeation source at 4 mg/m^3.

Toxicological Assessment of Mixtures of Pollutants

The mixture of pollutants present in the atmosphere was assessed according to methods applied to spacecraft atmospheres. The average toxicity index for each toxicological group (Tgrp) was calculated for groups of "n" toxicants found at their respective concentrations (Cn) for 30 to 90 days and causing similar toxic effects or targeting the same organ system (e.g. respiratory system irritants, cardiotoxicants, carcinogens, etc). The equation below was used with 180-day spacecraft maximum allowable concentrations (SMACs):

$$Tgrp = C_1/SMAC_1 + C_2/SMAC_2 + ... + C_n/SMAC_n$$

The atmosphere was considered acceptable if each Tgrp value was <1.0. Certain SMACs have been set lower because of the effects of space flight (e.g. immune effects, hematological effects, etc.), hence, for the Earth-based application in this study, a few of the SMACs may be lower than necessary to fully protect crew health.

Findings

Phase II 30-Day Test

Even though the atmosphere throughout the test was acceptable for human respiration based on the T-value calculations from nine GSC samples, several

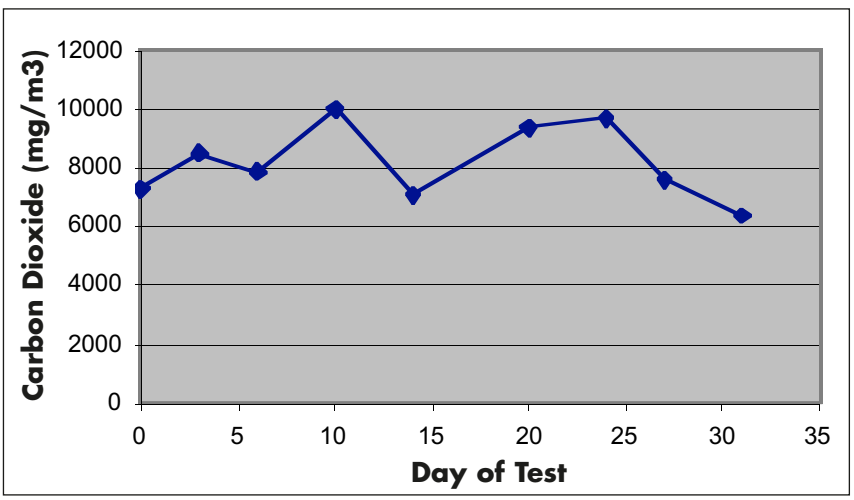

Figure 4.1-1b *Carbon Dioxide in the 30-day test*

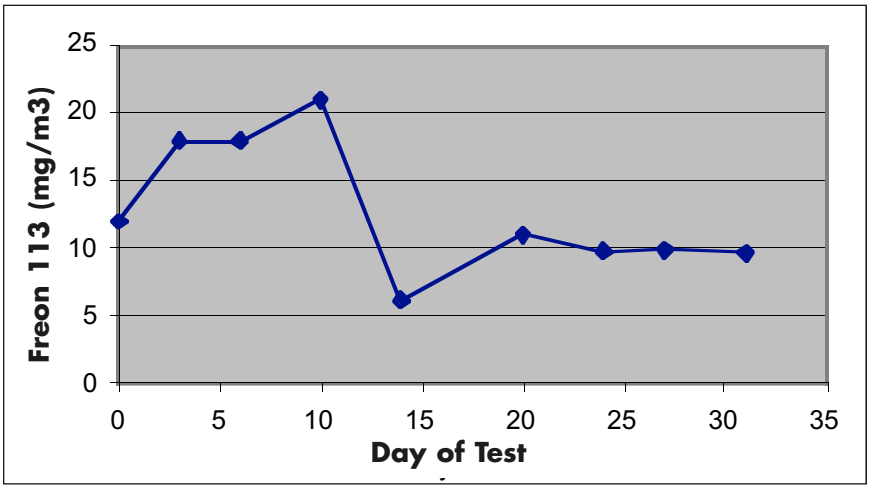

Figure 4.1-2 *Freon® 113 in the 30-day test*

atmospheric anomalies occurred during the test. On test day, six the carbon dioxide reduction system failed due to flooding of the methane/water separator. After replacement of the faulty, low-level water sensor, a methane leak was detected from one of the separator fittings and this was replaced. This occurred over a period of approximately three days and caused unusually high levels of methane and, to a

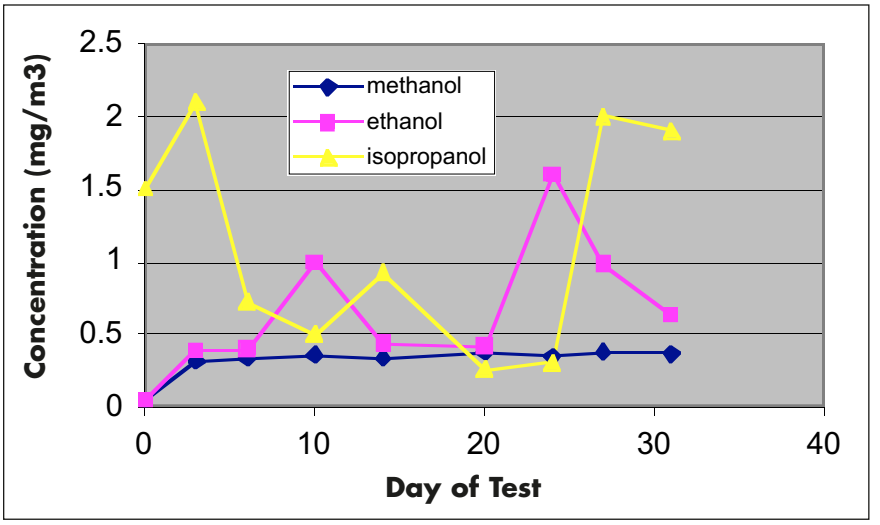

Figure 4.1-3 *Alcohols in 30-Day test*

lesser extent, carbon dioxide in the day 10 sample. The methane concentration slowly decayed throughout the remaining 20 days of the test.

The concentration of carbon monoxide was somewhat higher than that typically

observed in space vehicles and the level of Freon® 113 was much higher than typically observed in space vehicles. The carbon monoxide concentrations increased from trace to approximately 4 mg/m3 by day 10 and stayed near that level until the end of the test. The Freon® 113 concentrations were relatively high before the test began (12 mg/m^3) and increased through day 10 to about 20 mg/m^3, after which they stabilized at about 10 mg/m^3. This compound probably originated from the pre-test cleaning of electronic components of hardware.

Some of the low-molecular-weight alcohols exhibited interesting behavior. Ethanol and isopropanol concentrations varied from about 0.3 to 2 mg/m^3 during the test. The variation was undoubtedly due to the use of these alcohols in the hand wipes and sterilizing pads. This is in contrast to methanol, which maintained a steady state concentration of about 0.35 mg/m^3 throughout the test. Methanol originates primarily from hardware offgassing and one would expect the continuous rate of production and the rate of removal to result in a nearly uniform concentration.

As noted in the methods section, formaldehyde was measured during the 30-day test using FTIR spectroscopy on aliquots taken from the GSCs. This resulted in a method that was relatively insensitive to formaldehyde and led to concentrations that were consistently reported as less than the method detection limit. The method was replaced by a much more sensitive badge-sampling method, and this change proved to be a fortuitous improvement, as the 60-day test demonstrated.

Phase IIa 60-Day Test

The dynamics of air pollutants during the Phase IIa test were much different than during the Phase II testing. From an air-quality perspective the 60-day test can be summarized as a learning experience about the importance of controlling materials offgassing.

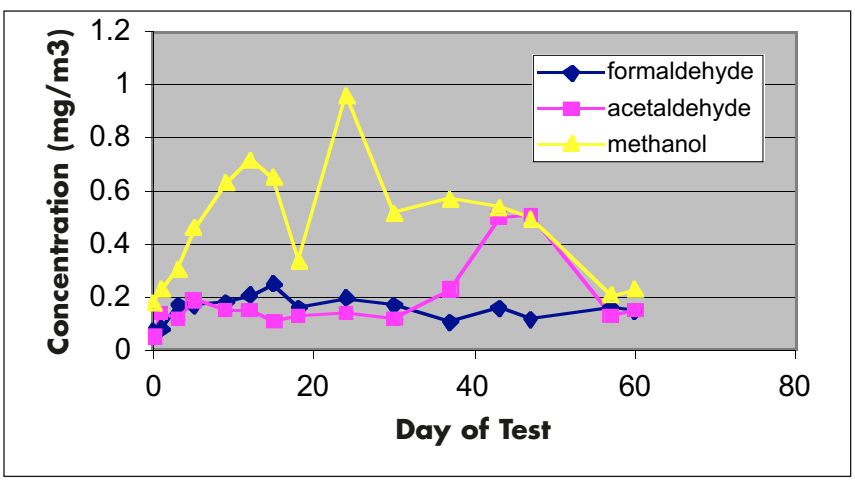

Figure 4.1-4 *Selected Airborne Pollutants During the 60-Day test*

Steady-state concentrations were not achieved for methanol, acetaldehyde, and formaldehyde until the last few days of the study. Formaldehyde was of particular concern because the measured values increased to 0.25 mg/m^3 by day 15, whereas the long-term SMAC is only 0.05 mg/m^3 (8).

The accuracy of the badge method was confirmed by comparing it to an impinger method and an U. S. Environmental Protection Agency (EPA) method. The day 27 badge result from level 1 was 0.17 mg/m^3, the coincident impinger sample was 0.17 mg/m^3, and the average of four EPA-type samples was 0.18 mg/m^3. A number of materials inside the chamber quickly underwent offgas testing to determine their rate of formaldehyde production. Most materials did not offgas detectable levels of formaldehyde; however, the poster murals were found to release measurable amounts of formaldehyde and were removed from the chamber on day 17. The airborne formaldehyde dropped from its high of 0.25 mg/m^3 on day 15 to 0.16 mg/m^3 on day 18.

Three compounds, coming primarily from anthropogenic sources, showed very different concentration profiles. During this test the primary methane source was

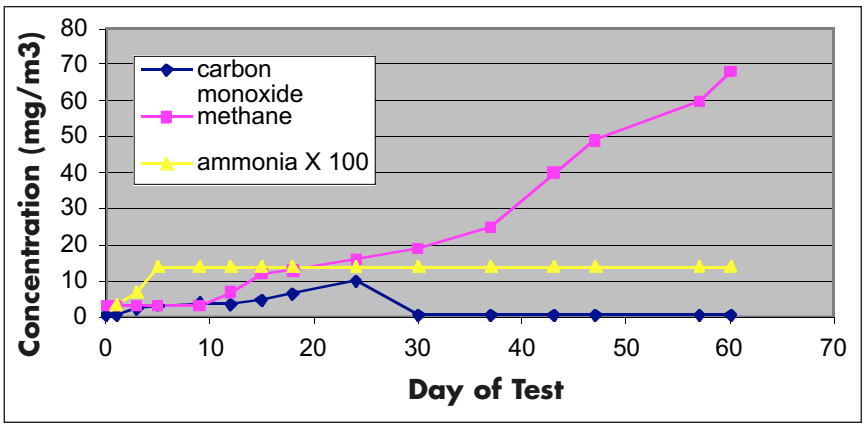

Figure 4.1-5 *Anthropogenic Pollutants in the 60-Day test.*

the human occupants; there was no evidence of a system leak such as that seen during the Phase II test. Methane concentrations increased steadily with time as the test progressed. Carbon monoxide also exhibited this behavior until day 30 when an abrupt drop in the concentration occurred. After this time, carbon monoxide was never found above a trace amount (about 0.5 mg/m^3). Ammonia concentrations reached a steady state level of 0.14 mg/m^3 by day 5 of the test and did not change from this level in the remaining 55 days.

Phase III 90-Day Test

Air pollutants were better controlled during most of this test than during the Phase IIa test; however, there was evidence that a new source of air contamination

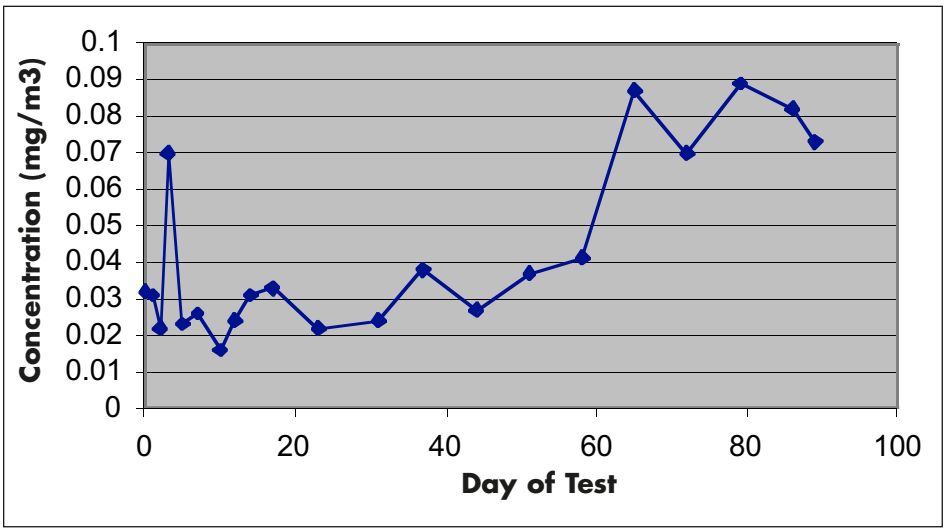

Figure 4.1-6 *Formaldehyde in 90-Day test*

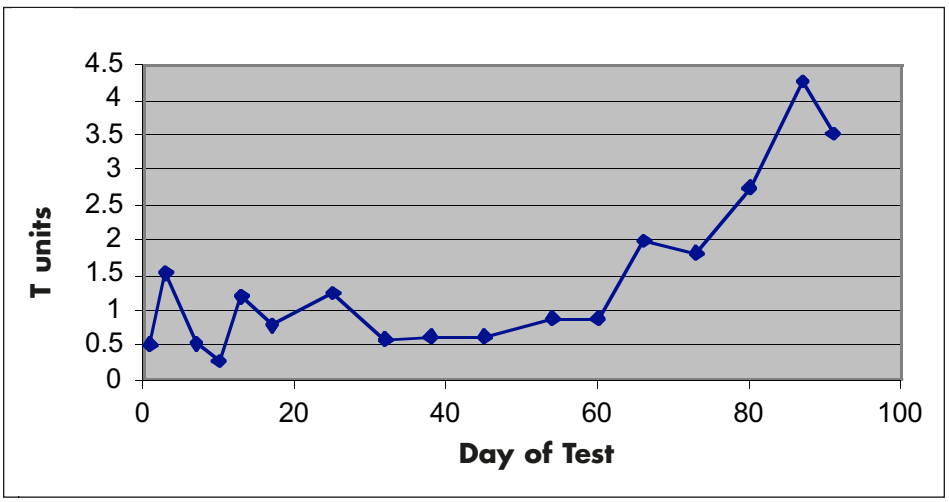

Figure 4.1-7 *T Value for Irritants in the 90-Day test*

was introduced late in the test and this caused a large increase in the concentration of respiratory irritants. Separate from this was a slight increase in formaldehyde toward the end of the test, but this was apparently due to an anomaly in a catalyst bed rather than excessive offgassing of materials as found in the 60-day test. The formaldehyde profiles are shown in Figure 4.1-6 and the Tgrp for the irritants is shown in Figure 4.1-7. Another distinct difference between the 60-day test and the 90-day test was the accumulation of ammonia during the latter test. The abrupt increase of common pollutants near the end of the test is shown in Figure 4.1-9.

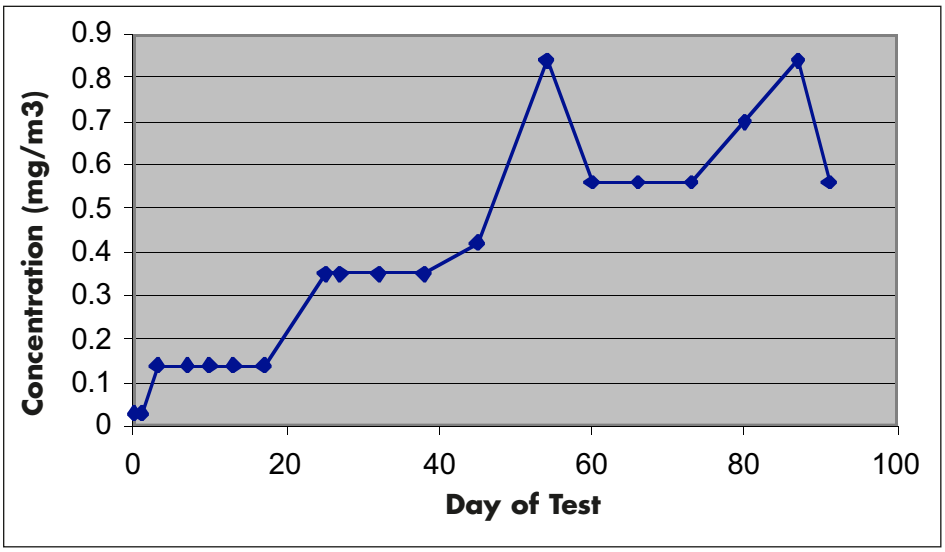

Figure 4.1-8 *Ammonia in the 90-Day test*

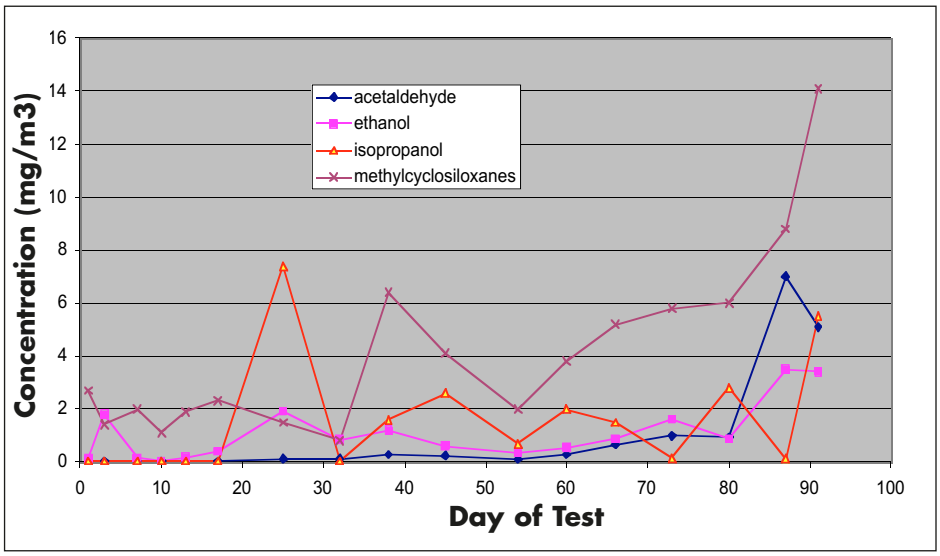

Figure 4.1-9 *Major Pollutants in the 90-Day test*

Discussion

Phase II 30-Day Test

Even though the air quality seemed to be acceptable during this test, there were important limitations to the methods used to measure pollutants. Specifically, the FTIR method of quantifying formaldehyde from aliquots of the GSC samples

proved to be too insensitive to provide useful information. Formaldehyde concentrations during this test may have been comparable to those measured during the Phase IIa test because the materials used in both tests were similar. Had we recognized the importance of measuring low concentrations of formaldehyde, we would have been better prepared to conduct the Phase IIa test in an uneventful manner.

The total T-values, with the contribution from carbon dioxide removed because it acts independently of other pollutants, and formaldehyde and ammonia not quantified, ranged from 0.32 to 0.58 during the 30-day test. This suggests that the trace pollutants were collectively quite stable during the test and that the atmosphere was easily within acceptable limits for human respiration. Given these low T-values, there was no need to separate the compounds according to toxicological groups.

Phase IIa 60-Day Test

Pollutant levels during this test were significantly higher than those typically encountered in space flight or during the Phase II test. In part this was due to excess offgassing from polymeric materials that had not received adequate testing for their offgassing properties. This led to concentrations of formaldehyde well above accepted limits and resulted in symptoms being reported in one crewmember. A concerted effort was mounted to identify the source(s) of the formaldehyde, with limited success during the test. Removal of murals on day 17 reduced the formaldehyde concentrations somewhat, but these items apparently were only one of the sources of formaldehyde.

The search for other sources of formaldehyde included evaluations after the study and a "bake out" study after the crew left the chamber. This bake out study demonstrated that the equilibrium between formaldehyde sources and removal processes was shifted to produce higher airborne concentrations as the chamber temperature increased. Post-test analyses by the Crew and Thermal Systems Division also indicated that the melamine foam acoustic tiles and carpeting were important sources of formaldehyde. A 40 g sample of the foam reached an equilibrium concentration of 0.5 mg/m^3 inside a 10 L bell jar. These tiles were removed from the test chamber and replaced with solamide tiles for the Phase III test.

One crewmember reported eye and upper-airway irritation as the formaldehyde concentrations climbed to their peak of 0.25 mg/m^3 on day 15 of the test. These symptoms should be expected at this level of formaldehyde, but not in every crewmember. There is a population of persons who are much more sensitive to the irritant properties of formaldehyde than the general population. The SMAC of 0.05 mg/m^3 was set to protect even sensitive individuals (8). In contrast, the Threshold Limit Valve (TLV®) of 0.3 ppm (0.4 mg/m^3) was set to protect the majority of workers, with the understanding that "the recommended formaldehyde 0.3 ppm ceiling TLV® will not protect that portion of the workforce reported to be responsive to low ambient concentrations of this chemical."

There were a number of adjustments in the trace contaminant control devices throughout the 60-day test. Normally, methanol is generated at a fairly constant rate from materials offgassing. The large changes in methanol concentration suggest that changes in the trace contaminant control devices caused most of these concentration changes. On the other hand, the drop on day 18, as depicted in Figure 4.1-4, may be from removal of materials on day 17 in an attempt to reduce offgassing of formaldehyde.

Carbon monoxide increased steadily during the first 24 days of the test because there was no removal mechanism as shown in Figure 4.1-5. The measurement on day 24 was 10 mg/m^3, which is just below the long-term SMAC of 11 mg/m^3 (10 ppm) for this compound. On day 25 the high temperature catalytic bed was started and this caused a dramatic drop in concentration. This action seemed to have no measurable effect on the steadily rising methane concentrations; however, methane is known to be more difficult to oxidize than carbon monoxide.

The total T-values for all measured pollutants except carbon dioxide and formaldehyde ranged from 0.15 (pretest) to 1.84 (day 12). The T-values reached much higher numbers than during the Phase II test. Four of the T-values (days 5, 12, 24, and 37) were significantly above 1, and these were broken down into toxicity groups to determine if any single group exceeded a value of 1. The following groups were identified and ranges found: irritants without formaldehyde (0.38-0.46), neurotoxicants (0.11 to 1.05), respiratory system injury (0.26 to 0.55), hepatotoxicants (0 to 0.71), gonad toxicants (0.11 to 0.55), immunotoxicants (0 to 0.12), carcinogens (0 to 0.23), and cardiotoxicants (0.05 to 0.95). The only unacceptable value was for neurotoxicants, which was due to the one relatively high value of carbon monoxide found on day 24. Since long-term SMACs were used to calculate the T values, and the exposure was no more than a few days, there was an extremely low risk of any neurotoxicity.

Phase III 90-Day Test

Until day 80 the total T-values, without carbon dioxide and formaldehyde, ranged from 0.06 to 1.89, which was comparable to the Phase IIa result. The remarkable increase in acetaldehyde and ethanol late in the test can be attributed in part to fermentation processes such as the baking of bread. These processes are known to produce large amounts of ethanol and metabolic products such as acetaldehyde. The cause of the increase in concentration of the methylcyclosiloxanes is unknown.

The slight increase in formaldehyde concentrations after day 60 has been attributed to incomplete oxidation of methanol in a catalytic bed (12). This cause was determined after the 90-day test by evaluating the performance of the catalyst bed. Under test conditions of 200 deg C, approximately half the input methanol was reacted, but 2/3 of the reacted methanol was converted to formaldehyde rather than water and carbon dioxide. Further investigation suggested that the

catalyst had been poisoned by organic sulfur compounds (12). The highest formaldehyde levels reached (0.09 mg/m^3) were still well below those expected to elicit symptoms in most individuals.

The cause of the increase in ammonia during the test (see Figure 4.1-8) was due to venting of the bioreactor head gas and headspace above the waste-water tanks directly into the TCCS beginning on day 21 (10). Apparently, the ammonia-conversion catalyst in the TCCS was not fully capable of converting the additional load of ammonia. Hence, the ammonia concentration began to increase at this time and had not reached a steady-state concentration by the end of the 90-day test.

SIGNIFICANCE

The findings reported here underscore the need for comprehensive air quality analyses to determine whether preventative measures to limit pollution have been effective, to ascertain if the ARS is capable of dealing with the pollutant load on a sustained basis, to detect any new sources of air pollution, and to judge whether the air has been acceptable for crew health. These goals can be achieved only in a test chamber or space vehicle due to the complex interactions between the sources and sinks. Such interactions will only be made more complex as food preparation and waste processing systems are integrated into habitats.

During the LMLSTP the analyses were retrospective, yet they still provided valuable insight into the dynamic changes that were occurring in the chamber. NASA is on the threshold of being able to analyze spacecraft air for trace pollutants on a near-real time basis, and this will further enhance the value of air quality assessments. Future research should focus on understanding the risks that specific air pollutants pose to crew health, and then developing analyzers capable of addressing those risks using a minimum of resources. That research must be conducted in realistic, ground-based environments before analytical hardware is flown in space vehicles, which one can only hope will be headed to Mars in the not-too-distant future.

Acronyms

ARS	Air Revitalization System
EPA	U.S. Environmental Protection Agency
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GSC	Grab Sample Canister
ISS	International Space Station
JSC	Johnson Space Center
LMLST	Lunar Mars Life Support Test
MS	Mass Spectrometer
SMAC	Spacecraft Maximum Allowable Concentration
TCCS	Trace Contaminate Control System
TLV®	Threshold Limit Value
Tgrp	Toxicological Group
WI	Work Instruction

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4.2

Water Chemistry Monitoring

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SUMMARY

The Lunar-Mars Life Support Test Project (LMLSTP), within the Advanced Life Support Program, is the first time NASA has attempted the direct recycle of water for human consumption since the late 1960's. The direct recycle of potable water from urine, wash water, and humidity condensate, as planned for International Space Station and future planetary missions, is not practiced on Earth. This is partially due to concern over the health impact of incomplete removal of potential contaminants in the recovered water. Since direct recycle is not an established practice, the United States Environmental Protection Agency has not developed water quality standards for recycled water. Thus, NASA has established its own stringent requirements for recycled water.

The Medical Operations Water and Food Analytical Laboratory (WAFAL) of the Medical Sciences Division of NASA Johnson Space Center (JSC) was responsible for ensuring that the quality of water generated during the LMLSTP chamber studies was medically acceptable for human consumption. As a result, water quality monitoring and technical support for the development of water recycling systems were provided from the inception of Phase I (1994) through the Phase III test (1997). A comprehensive water sampling and analysis protocol was accomplished to verify that the NASA requirements were met. Salient indicator quality parameters such as total organic carbon (TOC), pH, conductivity, total microbial content, color, iodine, turbidity, and trace metals were verified to ensure requirements were met before the water was consumed. Comprehensive organic analyses for volatile organic compounds, semivolatile organic compounds, alcohols, amines, carboxylates, formaldehyde, urea, glycols, anions, and cations were also performed. If the requirements were not met, the water was reprocessed until they were met. In addition to the water analyses, the test subjects were monitored to ensure that no health changes occurred. Analyses were also provided for nonpotable water sources, such as in-process samples, atmospheric moisture (humidity condensate), and plant condensate, to support engineering evaluations.

During Phase I, potable water was provided from the facility's public water supply system and was not recycled. For Phases II and IIa, potable water was generated from the recycle of wastewater using physicochemical methods, while Phase III water recycling systems were based on a combination of physicochemical and biological recovery systems. For each chamber study, trained crewmembers collected samples for chemical analysis using WAFAL-provided sampling equipment. After collection, samples were then transferred from the test facility to WAFAL for chemical analysis and to the microbiology laboratory for microbial analysis. Chemical results are reported in this chapter, while microbial results are reported in Chapter 4.3. With the exception of minor exceedances, the test provided water for consumption that met the established NASA potability requirements for recycled water. On numerous occasions, however, this required that the water be reprocessed in order to meet these requirements. Most of the reprocessing excursions were required due to high total organic carbon (TOC) and microbial content. On several occasions reprocessing was required due to high nickel and lead.

Introduction

A major goal of the Advanced Life Support Program is to develop and validate technologies for regenerative life support systems for long-duration space missions (lunar, Mars, and orbital). One of the regenerative systems needed to achieve this goal is the water recovery system to produce potable water from various wastewaters. During the course of the chambers project, four separate and distinct tests with human test subjects were conducted, each progressively more complex in terms of the water recovery system.

Within the Medical Sciences Division, the Medical Operations' Water and Food Analytical Laboratory participated in the design, development, and testing of the water recovery systems for the LMLSTP. Specifically, WAFAL was responsible for: 1) providing assistance with the design of the water recovery systems, 2) providing analytical support for the testing of hardware components and the integrated systems, 3) developing water quality standards and monitoring requirements, and 4) supporting technology development through the analysis of humidity condensate and other liquids.

Water quality standards and monitoring requirements for the chamber studies were based on U.S. Environmental Protection Agency (EPA) standards and NASA Man-Systems Integration Standards (MSIS) (NASA-STD-3000), a set of standards specifically developed by NASA for recycled water (1). These standards were developed to document relevant human engineering requirements applicable to the space environment and are listed in Table 4.2-1. U.S. EPA standards are legally enforceable regulations levied by the U.S. government on water supplied by public water systems (2). In addition, the EPA provides health advisories and MCL goals which are non-enforceable guidelines for drinking water. Health advisories are estimates of acceptable drinking water levels for a chemical substance based on health

effects information; a health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials (2). NASA uses these advisories and MCL goals as required and to the extent possible to determine the acceptability of recycled water.

Table 4.2-1 *NASA Man System Integration Standards, Rev B, Volume III*

Parameter	Units	Potable Maximum Contaminant Level	Hygiene Maximum Contaminant Level	U.S. EPA Maximum Contaminant Level
Physical Parameters				
Total solids	mg/L	100	500	
Color True	Pt-Co	15	15	
Taste	TTN	3	–	
Odor	TON	3	–	
Particulates (maximum size)	micron	40	40	
pH		6.0-8.5	5.0-8.5	
Turbidity	NTU	1.0	1.0	
Iodine	mg/L	0.5-4.0	0.5-6.0	
Total I	mg/L	15	15	
Trace Metals				
Arsenic	µg/L	10	10	50
Barium	µg/L	1000	1000	2000
Cadmium	µg/L	5	5	5
Chromium	µg/L	50	50	100
Copper	µg/L	1000	1000	1300
Iron	µg/L	300	300	
Mercury	µg/L	2	2	2
Manganese	µg/L	50	50	
Nickel	µg/L	50	50	100 (HA)
Lead	µg/L	50	50	15
Selenium	µg/L	10	10	50
Silver	µg/L	50	50	100 (HA)
Zinc	µg/L	5000	5000	2000 (HA)

HA = Health Advisory

EPA Drinking Water Standards and Health Advisories, October 1996, EPA 822-B-96-002

Table 4.2-1 continued *NASA Man System Integration Standards, Rev B, Volume III*

Parameter	Units	Potable Maximum Contaminant Level	Hygiene Maximum Contaminant Level	U.S. EPA Maximum Contaminant Level
Physical Parameter				
Anions				
Chloride	mg/L	200	200	
Nitrate (NO3 as Nitrogen)	mg/L	10	10	10
Sulfate	mg/L	250	250	500
Sulfide	mg/L	0.05	0.05	
Cations				
Ammonium (as N)	mg/L	0.5	0.5	30 (HA)
Magnesium	mg/L	50	50	
Calcium	mg/L	30	30	
Potassium	mg/L	340	340	
Total Acids	µg/L	500	500	
Cyanide	µg/L	200	200	200 (HA)
Halogenated				
Hydrocarbons	µg/L	10	10	
Total Phenols	µg/L	1	1	
Total Alcohols	µg/L	500	500	
Total Organic Carbon (TOC)	mg/L	0.5	10	
Uncharacterized TOC	µg/L	100	1000	

HA = Health Advisory
EPA Drinking Water Standards and Health Advisories, October 1996, EPA 822-B-96-002

Methods

For each chamber study, potable water samples were collected and analyzed during pretest, test, and post test operations. Samples were collected using WAFAL-provided sampling equipment consisting of benzalkonium chloride disinfectant wipes (PDI, Orangeburg, NY), cleaned Teflon® sample bottles, and labels. To collect a sample, the sample port was disinfected using a wipe. Next, approximately 250 ml of fluid was purged from the sample port and discarded. Then, 1000 ml of fluid was collected into a prelabeled sample bottle. The sample bottle

was transferred as soon as possible (usually within six hours) from the sample location to the laboratory for analysis. Upon receipt at the WAFAL, the sample was allocated and preserved according to the WAFAL Water Sample Receiving, Allocation, Preservation, and Storage Procedure (3).

Samples of recycled water collected from the potable water storage tanks during the chamber studies were analyzed for pH, turbidity, iodine, color, conductivity, anions, cations, trace metals, total organic carbon (TOC), volatile organics, semivolatile organics, alcohols, formaldehyde, amines, carboxylates, organic acids, diols (glycols), and urea. These samples were analyzed in an attempt to characterize at least 80% of the organic components of the recycled water. Other samples, such as the shower, galley, handwash sink, wastewater feed to the Water Recovery System (WRS), and effluent samples from many of the WRS subsystem components were also collected at various stages in the water treatment process. These samples were analyzed for a number of inorganic and organic parameters to verify system performance at various stages of water recovery and to collect data for future reference.

Analytical methods used for the analysis of water samples during the chamber studies were based on instrument manufacturer instructions, procedures outlined in Standard Methods for the Examination of Water and Wastewater (4), published methods from EPA, and other methods developed in the laboratory. Conductivity, pH, turbidity, and total organic carbon (TOC) were measured following standard methods and manufacturer instructions. Two instruments were used to measure TOC, a Sievers Model 800 TOC analyzer or an O.I. Analytical Model 1010 TOC analyzer.

Semivolatile organic compounds were assessed by the EPA 625 solvent/solvent extraction GC/MS method with a Hewlett Packard (HP) 5890 Series II GC coupled directly to a HP 5971 MSD. Three 15 ml methylene chloride extractions of a 500 ml sample were made at a pH of 11 or higher to obtain the base/neutral fraction. For the acid fraction, the pH of the sample was lowered to 2 or less and the sample was again extracted with methylene chloride. The extracts were dried with anhydrous sodium sulfate and concentrated to 0.5 ml with a Zymark TurboVap II automatic concentrator. An internal standard was added and the concentrated samples were analyzed. Extraction recoveries were assumed to be 100% and when a compound was extracted into both the base/neutral and acid fractions, each fraction was added together to get the total concentration for that compound.

Volatile organics analyses were determined by a headspace GC/MS method using a target list consisting of the EPA Method 624 compounds along with 24 additional compounds (4, 7). The system consisted of an HP 7694 headspace sampler attached to a HP 5890 Series II gas chromatograph coupled directly to an HP 5972 mass selective detector. The 5890 GC was equipped with an electronic pressure controlled inlet that was set to constant flow mode, with vacuum compensation on. Before analysis, each sample was equilibrated by agitating and heating the sample at 85°C for 15 minutes. Then a 3 ml volume of the headspace from each sample vial

was transferred to the instrument for analysis. Targeted compounds were confirmed and quantified using a five level calibration curve.

Iodine, iodide, triiodie, and hypoiodous acid were measured with a Shimadzu UV-265 UV-visible spectrophotometer, according to a method described by Schultz et al (5). In cases where contaminants in the sample interfered with the analysis, a leuco crystal violet method was used (4). Color was assessed using a Shimadzu spectrophotomer at an absorbance of 455 nm (6). Color levels in the samples were quantified using calibration curves generated with dilutions of a platimum-cobalt color standard.

Trace metals were determined by graphite furnace atomic absorption (GFAA) analysis with a Thermo Jarrell Ash Smith/Hiefje 400 atomic absorption spectrophotometer, according to standard methods (4). Formaldehyde was determined by direct aqueous phase o-2,3,4,5,6-pentafluoro-benzyl hydroxylamine (PFBHA) liquid-liquid extraction with a HP 5890 Series II GC coupled directly to an HP 5971 MSD, (8, 9). Five milliliters of sample were reacted with PFBHA, extracted with 0.5 ml of hexane, and chromatographed.

Cations, anions, diols, urea, amines, carboxylates, and alcohols were assessed using methods developed in-house. Inorganic anions and cations were assessed by ion chromatography using a Dionex 4000I ion chromatograph (IC). Direct acqueos injection GC/MS with a HP 5890 Series II GC coupled directly with an HP 5971 MSD was used to measure six C1-C4 alcohols. A 0.5 ml aliquot of sample and calibration curve standards were analyzed and quantified in the selected ion mode. A Waters quanta 4000 capillary electrophoresis unit (CE), was used to measure methyl, ethyl, and n-propyl amine and 5 C1-C4 carboxylic acids. Samples were injected onto a capillary and analyzed using 2.5 mM potassium hydrogen phthalate and 0.25 mM tetradecyl-trimethylammonium bromide electrolytes. Analytes were detected by indirect UV absorbance at 214 nm. Calibration and peak determination were performed by spiking with standard solutions of the targeted compounds.

Findings

Phase I

The main objective of this test was to verify the ability of a wheat crop to provide air revitalization to a crewmember for 15 days (10). Water recycling was limited to condensation of humidity from the air (human respired air, evaporation, plant transpiration). This recovered water, known as humidity condensate, was used to rehumidify the Variable Pressure Growth Chamber (VPGC) plant growth area and airlock, or to provide water for replenishing the plant nutrient solutions. No recovered water was used for human consumption during this test.

In preparation for Phase I, a system checkout pretest was conducted in April 1995 using a human metabolic simulator (HMS) in place of the human test subject. During this pretest, the HMS was sealed inside the airlock compartment of the VPGC while a crop of wheat was grown in the plant chamber. Two samples of

plant atmospheric moisture (condensate) were collected from the condensate collection tanks located on the outside of the VPGC, one from each side of the chamber (sides A and B).

For the actual Phase I test, a human test subject lived in the airlock compartment for 15 days while a crop of wheat grew in the plant chamber. Potable water was not recycled during Phase I. Drinking water for the crewmember consisted of water from the JSC public water supply that was deionized, filtered using a 0.2 μm microbial filter, and iodinated using a microbial check valve (MCV) to simulate spacecraft water supplies with iodine as the disinfectant (11). Two potable water samples were taken from the faucet at the sink in the airlock compartment of the VPGC: one before the start of the test and one at the end of the test. Four additional samples were collected in response to crewmember comments concerning a distinct iodine taste and odor. One of these samples was taken at the inlet of the MCV and another at the outlet of the MCV. Two more were taken at the outlet at the sink. A summary of the potable water analytical results obtained during Phase I is listed in Table 4.2-2.

Table 4.2-2 *Phase I Potable Water Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
pH		4.38	4.80	4.59	2
Conductivity	μS/cm	3.52	11.57	7.54	2
Turbidity	NTU	0.003	0.057	0.03	2
Total Solids	mg/L	5.5	56	30.75	2
Iodine					
I2	mg/L	0.03	5.42	3.16	6
I3-	mg/L	0.00	0.05	0.03	6
I-	mg/L	0.00	2.96	1.11	6
HOI	mg/L	0.00	6.50	3.48	5
Total I	mg/L	3.30	13.00	8.62	5
Trace Metals					
Arsenic	μg/L	ND	3.8	2.8	6
Chromium	μg/L	ND	1.8	0.4	5
Copper	μg/L	ND	3.7	0.7	5
Iron	μg/L	ND	9.6	4.7	5
Manganese	μg/L	ND	1.6	0.5	5
Molybdenum	μg/L	ND	1.3	0.3	5

Table 4.2-2 continued *Phase I Potable Water Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Lead	µg/L	ND	0.6	0.1	5
Selenium	µg/L	ND	3.2	2.5	5
Zinc	µg/L	0.3	13.2	3.3	5
Anions (IC)					
Chloride	mg/L	ND	0.130	0.065	2
Cations (IC)					
Ammonium					
(as Nitrogen)	mg/L	ND	0.0008	0.0004	2
Total Organic Carbon					
Total Inorganic					
Carbon	mg/L	0.255	0.371	0.313	2
Purgeable Organic					
Carbon	mg/L	<0.028	0.005	0.025	2
Nonpurgeable					
Organic Carbon	mg/L	0.244	0.427	0.336	2
Total Organic Carbon	mg/L	0.244	0.432	0.338	2
Volatile Organics					
Acetone	µg/L	1.50	7.75	4.63	2
2-Butanone	µg/L	12.47	39.79	26.13	2
Iodomethane	µg/L	ND	1.31	0.66	2
Tetrahydrofuran	µg/L	19.52	24.07	21.80	2
Extractable Organics					
Benzothiazole	µg/L	ND	2.3	1.2	2
2-(2-Butoxyethoxy) ethanol acetate	µg/L	ND	2.0	1.0	2
Butylated hydro-xyanisole (BHA)	µg/L	ND	1.3	0.7	2
3-t-Butylphenol	µg/L	ND	1.6	0.8	2
4-Chloro-3,5 dimethylphenol	µg/L	ND	0.5	0.3	2
Cyclohexanone	µg/L	1.0	2.5	1.8	2
Decamethylcyclo-pentasiloxane	µg/L	ND	0.9	0.5	2
Di-n-butylamine	µg/L	ND	25.1	12.6	2
n,n-Dibutylformamide	µg/L	ND	0.90	0.45	2
2,6-Di-t-butyl-1, 4-benzoquinone	µg/L	ND	2.7	1.4	2

Table 4.2-2 continued *Phase I Potable Water Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
3,5 Di-t-butyl-4 -hydroxybenzaldehyde	µg/L	0.2	0.4	0.3	2
2,4-Di-t-butylphenol	µg/L	ND	0.1	0.1	2
Dibutyl phthalate	µg/L	ND	0.5	0.3	2
Diethyl phthalate	µg/L	ND	0.6	0.3	2
Diiodomethane	µg/L	ND	1.4	0.7	2
Dimethyl phthalate	µg/L	ND	0.2	0.1	2
Dioctyl phthalate	µg/L	ND	5.8	2.9	2
Dipropyplene glycol methyl ether	µg/L	ND	76.3	38.2	2
2-Ethyl-1-hexanol	µg/L	ND	1.5	0.8	2
bis-2-ethylhexyl ester adipic acid	µg/L	ND	0.2	0.1	2
2-Hexanol	µg/L	ND	3.3	1.7	2
Iodoform	µg/L	ND	8.8	4.4	2
Methyl sulfone	µg/L	ND	4.6	2.3	2
4-t-Octylphenol	µg/L	ND	1.0	0.5	2
Octamethyl-cyclotetrasiloxane	µg/L	ND	0.1	0.1	2
Phenol	µg/L	ND	5.1	2.6	2
2-Phenylphenol	µg/L	ND	2.2	1.1	2
2-Phenyl-2-propanol	µg/L	ND	0.4	0.2	2
Toluene	µg/L	1.6	3.9	2.8	2
Aldehydes					
Formaldehyde	µg/L	9.5	12.6	11.1	2
Carboxylates					
Formate	µg/L	ND	560	280	2
Acetate	µg/L	ND	140	70	2
Organic Carbon Recovery	percent	13.03	132.76	72.90	2

The potable water analysis results show that all chemical parameters met the U.S. EPA water quality specifications. Although the Phase I water source was the JSC public water supply, the results were also compared to the NASA Man-System Integration Standards for recycled water. The potable water samples did not meet MSIS specifications for pH, iodine, and total phenols. The pH of samples collected before and after the test were 4.80 and 4.38, respectively, as compared to the MSIS requirement range of 6.0 to 8.5. This low pH was due to the addition of

iodine to the potable water. Iodine added to water hydrolyzes to hypoiodous acid and iodide, forming a slightly acidic solution. Iodine also imparts a yellowish-brown color to the water as well. Iodine results ranged from 0.33 to 5.42 mg/L. The MSIS specification for this parameter is 0.5 to 4.0 mg/L, which is considered the desirable range for taste considerations and microbial control. The sample collected at the end of the test had 10.4 μg/L of total phenols, which exceeded the MSIS limit of 1 μg/L for total phenols. This amount included 5.1 μg/L phenol, 1.6 μg/L 3-t-butylphenol, 0.5 μg/L 4-chloro-3,5-dimethylphenol, 1.0 μg/L 4-t-octyphenol, and 2.2 μg/L 2-phenylphenol. None of these were a health concern at the levels detected. The source of these phenol compounds is unknown, and the MSIS phenol specification is presently under review.

To determine the amount of organic material that could be accounted for through the analysis of individual organic compounds present, the percent of organic carbon recovered was calculated. This was accomplished by adding the organic carbon content of each individual organic compound detected. This value was then divided by the measured TOC, thus giving the percent of organics recovered. Total organic carbon levels for the six samples analyzed ranged from 244 to 432 μg/L, while the organic carbon recovery for the sample collected before the start and after the end of Phase I was 13% and 133%, respectively.

Phase II

The main objective of this test was to verify the performance of integrated physicochemical air revitalization, water recovery, and thermal control systems for a four-person crew for 30 days (12). Humidity condensate and wastewater from the shower, handwash, galley, laundry, and urinal were collected and recycled for potable use by the Phase II water recovery system (WRS). This water system consisted of a vapor compression and distillation subsystem (VCD), an ultrafiltration/reverse osmosis subsystem (UF/RO), and a post-treatment subsystem, as shown in Figure 4.2-1. The VCD is a rotating still that distills urine and produces a urine condensate that is mixed with washwater and humidity condensate for further processing. The UF/RO is a two-stage membrane filtration system designed to remove organic molecules and dissolved salts from the wastewater. The post-treatment subsystem provides final polishing of the recovered water. Processed water was stored in one of four potable water storage tanks that had a 0.2 μm microbial filter and a MCV positioned at the inlet of each of the tanks for microbial control and for adding iodine to the product water. Each tank also had the capability of being heated to disinfect the tank, if required.

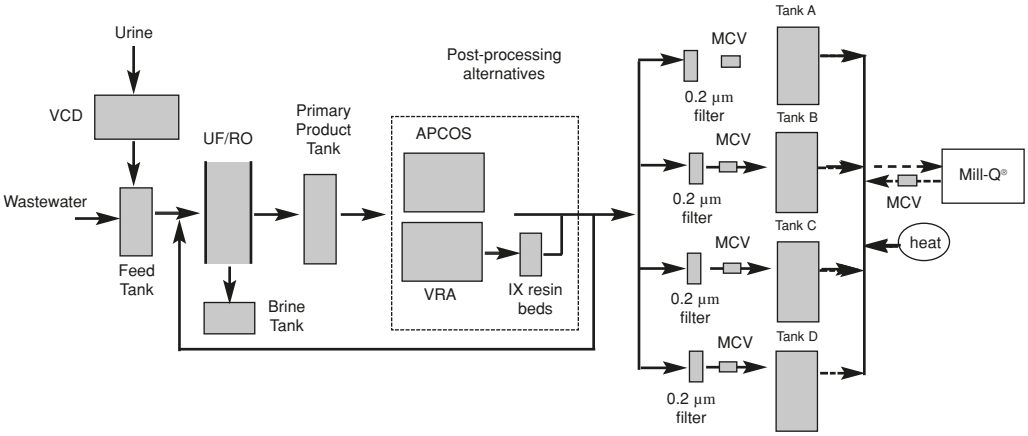

Figure 4.2-1 *Phase II Water Recovery System Schematic*

A series of system verification tests were conducted before the start of Phase II as outlined by Verostko et al. (13). A viral challenge test was also conducted to verify the capability of the water recovery system to remove viruses and provide potable water that met the NASA MSIS. No water produced from these tests was consumed. Once samples were collected, the product water was discarded. During the viral challenge test, several samples of processed water were collected for microbial analysis and one sample was collected for chemical analysis. Microbial and viral results of the challenge test are discussed in Bouma et al. (14). Results from chemical analyses indicated that all parameters met MSIS requirements except pH and color. The pH of the sample was 4.79 as compared to the MSIS required range of 6.0 to 8.5. Again, this low pH was due to the added iodine and did not represent a health hazard. The color of the sample was 37.6 platinum-cobalt (Pt-Co) units. Although this level was above the MSIS limit of 15 Pt-Co units, the color was due to the iodine and did not represent a health hazard. All parameters met U.S. EPA standards.

Three WRS donor mode tests and an integrated air revitalization system/water recovery system test were performed to validate the ability of the WRS to produce potable water from wastewater. During these tests, actual wastewater from human donors was processed. Although the final product water was not consumed by the donors, it was sampled and analyzed. The first donor mode test was initiated in March 1996 with a water recovery system consisting of a VCD, a UF/RO, and an Aqueous Phase Catalytic Oxidation Subsystem (APCOS) for post treatment. Chemical results of recovered water samples collected on days 3 and 6 of this test exceeded the MSIS limits for TOC, color, iodine, and turbidity. The measured TOC levels were 792 and 5170 µg/L, color levels were 44.4 and 48.2 Pt-Co units, iodine levels were 4.07 and 4.38 mg/L, and turbidity levels were 0.601 and 2.94 NTU,

respectively. To assist in troubleshooting, six additional APCOS recovered water samples were collected and analyzed for TOC. Levels ranged from 42 to 780 μg/L. Next, the APCOS was isolated and flushed with deionized water several times and the fluid water sampled. These flush water samples had TOC levels from 137 to 8900 μg/L. Based on this information, the test was halted and updates to the APCOS were performed to improve its capability to process the water. Another donor mode test (#2) was conducted in April 1996 and again, samples of processed water exceeded the MSIS limit for TOC. The high TOC levels were attributed to the breakdown of the carbon-based catalyst in the APCOS subsystem. As a result, the APCOS subsystem was abandoned as the post treatment subsystem in the Phase II WRS.

The APCOS was replaced with another catalytic oxidation system, called the Volatile Removal Assembly (VRA). A 2-phase gas separator and an ion-exchange resin bed were installed along with the VRA for a third donor mode test which was conducted in May 1996. During donor mode test #3, two recovered water samples were collected, and the TOC results for these samples were 268 μg/L and 433 μg/L. Results also showed that the samples exceeded MSIS specifications for pH and color, because of the iodine added to the water. These results indicated that the water recovery system consisting of a VCD, UF/RO, VRA, 2-phase gas separator, and an ion-exchange resin bed was capable of producing potable water that met the MSIS TOC standard. However, the reliability of the WRS with the VRA in place of the APCOS was unclear for a 30-day test duration because this new WRS configuration had not been extensively tested. Hence, a back up WRS post-processing subsystem consisting of a commercial Millipore Milli-Q® water purification system was provided for the Phase II test.

On June 12, 1996, the Phase II test began with three of four potable water storage tanks filled with about 211 kg (465 lbs) of water from the JSC public water supply that had been deionized, filtered with a 0.2 μm microbial filter, and iodinated with a MCV. Samples were collected from the storage tanks once they were filled completely with recycled water. This normally occurred every two days. During the test, one of the four tanks would be “in use,” one tank would be “on hold” awaiting completion of analytical tests, one tank would be a “spare,” and one tank would be “filling” with processed water. The tanks were configured such that they were sequentially cycled from the fill, hold, spare, and use modes.

Processing of wastewater for reuse began on day 1. The crew initiated use of one of the water tanks containing deionized water, while wastewater from hygiene activities was collected in one of two wastewater tanks. Crewmembers continued to consume deionized water during this period. The first sample of recovered water collected on June 16, 1996 did not meet MSIS specifications for TOC and color. The TOC level was 2290 μg/L, and the color measurement was 43.3 Pt-Co units. This sample contained 2160 μg/L of acetic acid, 1670 μg/L of propionic acid, 376 μg/L of formaldehyde, 127 μg/L of lactic acid, 5.4 μg/L of acetone, and about 12 μg/L

of several semivolatile organic compounds. The organic carbon recovery for this sample was 86.1%. It appears the ion-exchange bed located after the VRA failed early on day 2 of the test (11). This water was not consumed but was reprocessed by the backup Milli-Q® postprocessing system. After reprocessing, the water met potability requirements and was eventually consumed.

During the 30-day test, nine potable water tank samples did not meet the potable water specifications for TOC and had to be reprocessed by the Milli-Q® system The TOC of these samples ranged from 2010 to 2530 μg/L, with acetic and propionic acid levels ranging from 2160 to 3510 μg/L and 720 to 1670 μg/L, respectively. The organic carbon balances indicated 71.6 to 89.5% accountability for these samples. Potable water tanks reprocessed by the Milli-Q® system did meet MSIS TOC specifications, ranging from 105 to 243 μg/L, and were subsequently used for consumption. A summary of results from the potable water tanks consumed during Phase II, is presented in Table 4.2-3. The prevalent organics identified in these samples were acetic acid (60 to 165 μg/L) and acetone (9.6 to 32.0 μg/L). Bis-2-ethylhexyl phthalate was also found at levels ranging from 0.13 to 233. 9 μg/L. However, this compound probably originated from laboratory contamination. The organic carbon balances indicated 11 to 51% accountability for the post-Milli-Q® samples.

Potable water tank samples collected also did not meet the MSIS limits for pH

Table 4.2-3 *Phase II Consumed Potable Water Tank Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Conductivity	μS/cm	1.96	6.19	3.66	7
pH	pH units	4.65	6.01	5.36	7
Turbidity	NTU	0.003	0.07	0.02	7
Iodine (UV/VIS)					
I2	mg/L	2.82	3.89	3.47	7
I-	mg/L	0.05	0.79	0.50	7
IOH	mg/L	0.05	0.18	0.09	7
Total Iodine	mg/L	3.54	4.46	4.05	7
Color	Pt/Co	31.10	42.2	37.83	7
Cations (CE/IC)					
Sodium	mg/L	0.11	0.995	0.27	7
Potassium	mg/L	0.14	0.244	0.08	7
Ammonium (NH4-N)	mg/L	0.26	0.505	0.11	7
Calcium	mg/L	ND	0.209	0.03	7

Table 4.2-3 continued *Phase II Consumed Potable Water Tank Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Metals					
Silver	µg/L	ND	0.6	0.09	7
Chromium	µg/L	0.4	2.4	0.61	7
Iron	µg/L	0.7	3.1	0.93	7
Manganese	µg/L	0.3	0.7	0.14	7
Nickel	µg/L	1.0	1.8	0.59	7
Zinc	µg/L	0.1	0.6	0.26	7
Total Organic Carbon					
TIC (Sievers 800)	mg/L	0.092	0.565	0.227	7
TOC (Sievers 800)	mg/L	0.105	0.243	0.166	7
Volatile Organics					
Acetone	µg/L	9.60	32.03	19.90	7
Naphthalene	µg/L	ND	2.98	0.43	7
Tetrahydrofuran	µg/L	ND	1.88	0.27	7
Extractable Organics					
Benzothiazole	µg/L	ND	0.1	0.01	7
Benzyl alcohol	µg/L	ND	0.3	0.04	7
n-Butylbenzene-sulfonamide	µg/L	ND	0.4	0.06	7
4,4'-Butylidenebis					
(6-tert-butyl-m-cresol)	µg/L	ND	1.3	0.54	7
Di-n-butyl phthlate	µg/L	ND	0.3	0.13	7
2,6-Di-t-butyl					
-4-methylphenol	µg/L	ND	2.6	1.44	7
Diethyl phthalate	µg/L	ND	0.1	0.01	7
Diiodomethane	µg/L	ND	0.5	0.19	7
2-Ethyl-1-hexanol	µg/L	ND	0.4	0.11	7
bis-2-Ethylhexyl adipate	µg/L	ND	1.4	0.33	7
bis-2-Ethylhexyl phthalate	µg/L	ND	28.1	4.06	7
1-Hexadecanol	µg/L	ND	1.5	0.21	7
Iodoform	µg/L	ND	1.9	0.53	7
Methyl sulfone	µg/L	ND	1.5	0.93	7
Pentacosane	µg/L	ND	0.3	0.04	7
1-Tetradecanol	µg/L	ND	0.9	0.23	7

Table 4.2-3 continued *Consumed Potable Water Tank Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Toluene	µg/L	ND	1.7	0.56	7
Triethylamine	µg/L	ND	0.6	0.09	7
2,2,4-Trimethyl-1, 3-pentanediol diisobutyrate	µg/L	ND	0.2	0.06	7
Aldehydes (GC/MS)					
Formaldehyde	µg/L	9.0	17.2	12.60	7
Carboxylates (CE)					
Acetate	mg/L	0.06	0.165	0.08	7
Organic Carbon Recovery	percent	11.07	50.88	32.77	7

and color. Color levels exceeded MSIS limits in both the pre- and post-Milli-Q® samples and ranged from 31.1 to 43.3 Pt-Co units. pH levels also exceeded MSIS limits and ranged from 4.20 to 7.2 in the pre-Milli-Q® samples and from 4.65 to 6.01 pH units in the post-Milli-Q® samples. As before, the pH and color exceedances were attributable to the iodine in the potable water. Comprehensive results for these samples and other samples collected during Phase II are discussed in Homan et al. (15) and Koenig et al. (16). Further discussion on the performance of the Phase II WRS can be found in Verostko et al. (13).

Phase IIa

The Phase IIa test was conducted to demonstrate the specific life support systems developed for use on the International Space Station (ISS). This test incorporated integrated air revitalization, water recovery, and thermal control systems similar to those planned for ISS use in order to provide a liveable habitat for 60 days for dour crewmembers. The water recovery system for Phase IIa included a VCD, a multifiltration subsystem, and a volatile removal assembly with an ion-exchange resin bed as shown in Figure 4.2-2. The VCD is a rotating distillation unit that distills urine and produces the urine condensate which is mixed with washwater and humidity condensate for further processing. The multifiltration subsystem provided mixed-bed deionization and activated-carbon absorption. The VRA provides for the wet oxidation of organic (primarily nonpolar) that escaped the multifiltration unit. The VRA effluent was then treated by an anion-exchange resin to remove the oxidized organics (organic acids). Further details on these subsystems may be found at http://advlifesupport.jsc.nasa.gov/.

The VCD, VRA, and ion-exchange resin beds had previously been tested in

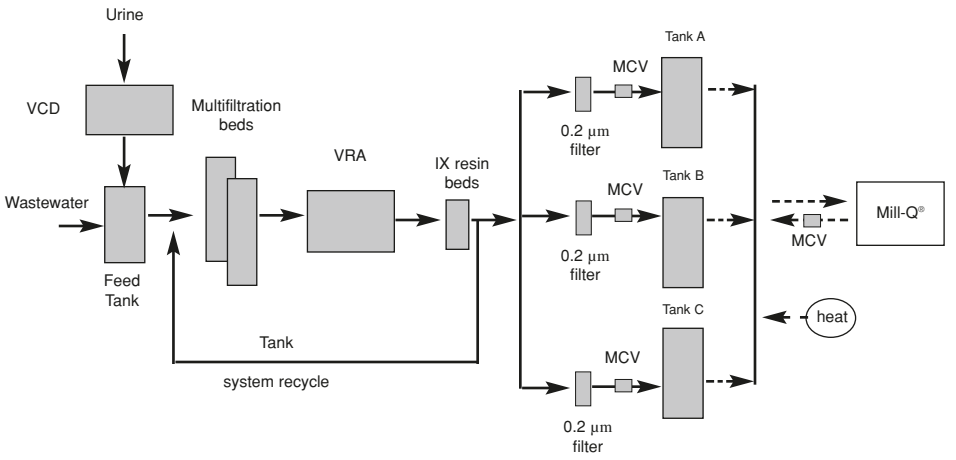

Figure 4.2-2 *Phase IIa Recovery System Schematic*

Phase II. As in Phase II, the Phase IIa system included a modified commercial Milli-Q® system to provide for reprocessing of the potable water if it did not meet potability requirements. The Phase IIa system was designed to accept wastewaters from the urinal, shower, handwash, and air revitalization system condensing heat exchangers (humidity condensate), as was the Phase II WRS. In addition, the Phase IIa WRS was required to process simulated wastewaters expected on the ISS, such as condensate from animal experiments, wastewater from the Crew Health Care System (CHeCS) water quality monitors used for offline water quality monitoring, and condensate from the off-gassing of equipment and new materials introduced into the ISS environment.

In order to test the ISS systems for Phase IIa, Phase II subsystems were configured as closely as possible to subsystems used in the Marshall Space Flight Center Stage 10 Water Recovery System tests (17). The Phase II UF/RO subsystem was replaced with a multifiltration subsystem. The laundry wastewater was removed as an input to the wastewater feed stream since a clothes washer is not planned for ISS.

The amount of water to be processed was reduced from 211 kg (465 lbs) to 52 kg (115 lbs) to reflect the water usage rates expected. For process control, an in-line process control water quality monitor (PCWQM) was added for continuous monitoring of the processed water's conductivity, TOC, and iodine (I_2) levels. If any of the three parameters were out of specification, the product water was rejected and returned to the inlet of the system for reprocessing. After processing, the water was stored in one of three potable water storage tanks containing a 0.2 μm microbial filter and a MCV at the inlet of each of the tanks.

As with previous tests, several pretest verification tests were performed prior to the 60-day test including a subsystem check, an integrated wet functional test, and a WRS Demonstration Test. During subsystem checks, each subsystem was operated individually using deionized water. Next, the subsystems were plumbed

together for the integrated wet functional test and deionized water was processed through the entire integrated system. Then, actual wastewater from human donors was processed by the integrated system during the WRS Demonstration Test. The processed water was not consumed by the donors, but instead was sampled and discarded. Six potable water samples were collected during the Phase IIa WRS Demonstration Test. Table 4.2-4 shows a summary of the results. Other samples of shower, wastewater feed, multifiltration effluent, and VRA effluent were also collected and analyzed for engineering evaluation.

The Phase IIa test began in January 1997 with water from the JSC public water supply that was deionized, filtered with a 0.2 μm filter, iodinated using a MCV, and

Table 4.2-4 *Phase IIa WRS Demonstration Test Potable Water Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Conductivity	μS/cm	1.97	4.76	3.05	6
pH	pH units	4.65	5.76	5.11	6
Turbidity	NTU	0.03	0.11	0.06	4
Iodine (UV/VIS)					
I2	mg/L	2.21	3.22	2.83	6
I-	mg/L	0.24	0.73	0.50	5
IOH	mg/L	<0.05	0.17	0.03	5
Color	Pt/Co	31.40	34.80	33.58	4
Anions					
Chloride	mg/L	0.026	0.077	0.042	4
Phosphate	mg/L	<0.01	0.076	0.025	4
Sulfate	mg/L	<0.01	0.083	0.021	4
Cations					
Sodium	mg/L	0.002	0.017	0.008	4
Potassium	mg/L	0.005	0.014	0.008	4
Ammonium (NH4-N)	mg/L	<0.001	0.005	0.002	4
Calcium	mg/L	<0.005	0.008	0.002	4
Metals					
Aluminum	μg/L	1.90	34.00	11.15	4
Barium	μg/L	<1	1.20	0.30	4
Copper	μg/L	1.1	4.40	3.00	4
Iron	μg/L	<2	10.70	4.08	4
Manganese	μg/L	<1	2.10	1.35	4
Nickel	μg/L	1.60	5.10	3.88	4
Zinc	μg/L	<1	4.10	1.55	4

Table 4.2-4 continued *Phase IIa WRS Demonstration Test Potable Water Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Total Organic Carbon					
TIC (OI 1010)	mg/L	0.196	0.258	0.217	3
NPOC (OI 1010)	mg/L	0.187	0.621	0.353	3
TIC (Sievers 800)	mg/L	0.164	1.220	0.398	6
TOC (Sievers 800)	mg/L	0.139	0.555	0.273	6
Volatile Organics					
Toluene	µg/L	1.70	4.88	3.07	4
Extractable Organics					
Acetophenone	µg/L	ND	1.60	0.53	4
Anethole	µg/L	ND	1.00	0.25	4
Benzaldehyde	µg/L	ND	0.50	0.13	4
Benzyl alcohol	µg/L	ND	1.60	0.40	4
Decamethylcyclo-pentasiloxane	µg/L	ND	0.90	0.23	4
Di-n-butyl phthalate	µg/L	0.80	1.30	1.00	4
Diethyl phthalate	µg/L	ND	0.20	0.05	4
Diiodomethane	µg/L	ND	0.70	0.18	4
Dodecamethylcyclo-hexasiloxane	µg/L	ND	5.00	1.25	4
2-Ethyl-1-hexanol	µg/L	2.00	5.70	3.53	4
bis-2-Ethylhexyl phthalate	µg/L	ND	0.50	0.23	4
Iodoform	µg/L	ND	2.30	0.58	4
3'-Methylaceto-phenone	µg/L	ND	4.50	1.68	4
Methyl 4 -hydroxybenzoate	µg/L	ND	1.50	0.38	4
1-Methyl-2 -pyrrolidinone	µg/L	ND	1.30	0.33	4
Phenylethyl alcohol	µg/L	ND	1.80	0.63	4

Table 4.2-4 continued *Phase IIa WRS Demonstration Test Potable Water Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Alcohols **(DAI/GC/MS)**					
Methanol	µg/L	ND	274	68.50	4
2-Propanol	µg/L	ND	175	43.75	4
Aldehydes (GC/MS)					
Formaldehyde	µg/L	6.60	14.20	10.63	4
Carboxylates (CE)					
Oxalate	mg/L	<0.2	0.53	0.13	4
Organic Carbon Recovery	percent	7.24	78.19	38.12	4

filled in two of the three potable water storage tanks. The WRS processed wastewater nominally for the first 29 days. On day 29, problems with the VCD, the VRA, and the ion-exchange resin bed occurred. Several VCD components, the resin in the ion-exhange resin bed, and the phase separator in the VRA were replaced. It was noted that the ineffective phase separator of the VRA caused gas to accumulate in the ion-exchange resin bed, rendering the bed ineffective (18). A sample of processed water collected during this period had a TOC of 840 µg/L which exceeded the MSIS limit of 500 µg/L. This water was not consumed but instead was reprocessed through the backup Milli-Q® post processor so that the recovered water would meet potability standards.

A total of 51 recovered water samples were collected during Phase IIa. A summary of results of the consumed recovered potable water tank samples is reported in Table 4.2-5. The potable water tank samples analyzed met MSIS limits except for TOC, pH, iodine, copper, and selenium. Typically, when the results exceeded specifications, the potable water tanks were reprocessed through the Milli-Q® system before human consumption. Potable water tanks were reprocessed through the Milli-Q® system 11 times for not meeting potable water chemical specifications. The tanks were also heat disinfected eight times for not meeting potable water microbial specifications. The TOC levels of the consumed recovered water ranged from 174 to 523 µg/L acetate (< 0.012 to 0.65 mg/L), lactate (< 0.012 to 1.10 mg/L), and oxalate (< 0.12 to 0.41 mg/L) were the organic acids detected. Acetone (not detected to 6.40 µg/L) and toluene (not detected to 9.53 µg/L) were the only volatile organic compounds identified. Methyl sulfone (not detected to 54.5 µg/L) was the only semivolatile organic found above 10 µg/L. Methanol (not detected to 233 µg/L) and 2-propanol (not detected to 154 µg/L) were the only alcohols detected. Only one of the 51 samples had detectable levels of urea (0.302 mg/L). Low concentrations of formaldehyde (< 2.0 to 13.8 µg/L) were also found. The

Table 4.2-5 *Phase IIa Consumed Potable Water Tank Results Summary*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Conductivity	µS/cm	1.40	12.20	3.20	51
pH	pH units	3.91	6.28	4.85	51
Turbidity	NTU	0.02	0.40	0.07	33
Iodine (UV/VIS)					
I2	mg/L	1.72	4.02	3.46	51
I3-	mg/L	<0.05	0.11	0.01	51
I-	mg/L	1.84	5.40	3.39	51
Total Iodine	mg/L	2.73	7.50	3.87	51
Color	Pt/Co	19.20	43.30	36.97	51
Anions (IC)					
Chloride	mg/L	<0.01	0.04	0.01	32
Nitrate (NO3-N)	mg/L	<0.01	0.25	0.02	32
Phosphate	mg/L	<0.01	0.17	0.01	32
Sulfate	mg/L	0.07	0.09	2.50	32
Cations (CE/IC)					
Sodium	mg/L	<0.001	0.007	0.001	32
Potassium	mg/L	<0.001	0.046	0.006	32
Ammonium (NH4-N)	mg/L	<0.001	0.009	0.001	32
Magnesium	mg/L	<0.001	0.001	0.001	32
Calcium	mg/L	<0.001	0.373	0.019	32
Metals					
Aluminum	µg/L	<1	6.8	2.16	32
Arsenic	µg/L	<1	1.2	0.04	32
Barium	µg/L	<1	1.9	0.13	32
Chromium	µg/L	1.2	9.7	0.74	32
Copper	µg/L	<1	1770	80.98	32
Iron	µg/L	<2	43.7	4.64	32
Manganese	µg/L	<1	24.7	2.10	32
Nickel	µg/L	<1	43.4	4.31	32
Selenium	µg/L	<1	12.20	0.60	32
Zinc	µg/L	<1	5.20	0.91	32
Total Organic Carbon					
TIC (Sievers 800)	mg/L	0.073	3.290	0.364	51
TOC (Sievers 800)	mg/L	0.174	0.523	0.286	51

Table 4.2-5 continued *Phase IIa Consumed Potable Water Tank Results Summary*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Volatile Organics					
Acetone	µg/L	ND	6.40	0.49	32
Toluene	µg/L	ND	9.53	3.21	32
Extractable Organics					
Acetophenone	µg/L	ND	0.3	0.01	32
Benzaldehyde	µg/L	ND	0.8	0.3	32
Benzothiazole	µg/L	ND	0.7	0.03	32
Benzyl alcohol	µg/L	ND	7.0	0.9	32
Benzylbutyl phthlate	µg/L	ND	3.6	0.1	32
2-Butoxyethanol	µg/L	ND	3.1	0.10	32
2-(2-Butoxyethoxy) ethanol	µg/L	ND	0.8	0.03	32
tris-2-Chloroethyl phosphate	µg/L	ND	0.9	0.03	32
Decamethylcyclo-pentasiloxane	µg/L	ND	0.2	0.02	32
1,4-Diacetylbenzene	µg/L	ND	0.3	0.01	32
Di-n-butyl phthlate	µg/L	ND	1.1	0.50	32
Diiodomethane	µg/L	ND	1.8	0.28	32
Diisopropyl adipate	µg/L	ND	0.9	0.30	32
N.N-Dimethyl-benzylamine	µg/L	ND	0.6	0.02	32
Dodecamethylcyclo-hexasiloxane	µg/L	ND	1.2	.29	32
2-Ethylhexanoic acid	µg/L	ND	1.7	0.05	32
2-Ethyl-1-hexanol	µg/L	ND	2.7	0.88	32
bis-2-Ethylhexyl phthalate	µg/L	ND	1.7	0.14	32
1-Formylpiperidine	µg/L	ND	0.6	0.07	32
4-Hydroxy-4 -methyl-2-pentanone	µg/L	ND	3.4	0.59	32
Iodoform	µg/L	ND	4.8	2.53	32
1-Methyl-2-pyrrolidinone	µg/L	ND	3.6	0.49	32
Methyl sulfone	µg/L	ND	54.5	19.36	32

Table 4.2-5 continued *Phase IIa Consumed Potable Water Tank Results Summary*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Monomethyl phthlate	µg/L	ND	4.8	0.15	32
Neomenthol	µg/L	ND	0.2	0.01	32
Octamethylcyclo-tetrasiloxane	µg/L	ND	0.6	0.03	32
Pentacosane	µg/L	ND	1.2	0.06	32
sec-Phenethyl alcohol	µg/L	ND	0.2	0.02	32
Phenol	µg/L	ND	1.00	0.08	32
2-Phenyl-2-propanol	µg/L	ND	0.5	0.02	32
Squalene	µg/L	ND	1.8	0.09	32
Tributyl phosphate	µg/L	ND	0.5	0.02	32
Alcohols					
(DAI/GC/MS)					
Methanol	µg/L	ND	233	25	32
2-Propanol	µg/L	ND	154	5	32
Aldehydes (GC/MS)					
Formaldehyde	µg/L	ND	13.8	4.70	32
Carboxylates (CE/HPLC)					
Oxalate	mg/L	<0.10	0.41	0.04	32
Acetate	mg/L	<0.12	0.65	0.05	32
Lactate	mg/L	<0.12	1.1	0.07	32
Non-volatiles (HPLC)					
Urea	mg/L	ND	302	9.44	32
Organic Carbon Recovery	percent	2.67	142.02	29.31	31

ND=None Detected

organic carbon recoveries of the potable water samples ranged from 2.67 to 142%.

Two of the 51 recovered water samples analyzed did not meet MSIS pH requirements. These samples measured 3.91 and 3.75. All 51 samples exceeded color requirements. This is attributable to the iodine present in the potable water. One sample slightly exceeded the iodine requirement (4.02 vs 4.0 mg/L limit). One sample exceeded the 1000 µg/L copper limit at 1770 µg/L and another sample exceeded the 10 µg/L selenium specification at 12.2 µg/L.

Several other in-process samples of condensate, handwash, galley, shower,

wastewater feed, multifiltration bed effluent, VRA effluent, and ion-exchange bed effluent were also collected and analyzed for engineering evaluation.

Phase III

Phase III was the first test to incorporate biologically-based wastewater processing. The overall objective of this test was to conduct a 90-day test of integrated physicochemical and biological life support systems for air revitalization, water recovery, thermal control, and solid waste management (19, 20). In terms of wastewater processing, biologically based systems were used to initially process the wastewaters and physicochemical systems were used for polishing and post-processing. The components of the Phase III WRS included an immobilized cell bioreactor (ICB), a trickling filter bioreactor (TFB), a reverse osmosis subsystem (RO), an air evaporation subsystem (AES), an ammonia removal subsystem (NH4RS), and the Milli-Q® polishing subsystem which was previously used in Phases II and IIa. In addition, an upgraded and refurbished APCOS was also available for use in this test. After processing, the recovered water was stored in one of four potable water tanks. A 0.2 μm microbial filter and a MCV were positioned at the inlet of each of the tanks for microbial control and for adding iodine to the product water. As in the previous tests, each tank and its contents had the capability of being heated to disinfect the tank if microbial water quality requirements were not met. A schematic of the Phase III WRS is depicted in Figure 4.2-3. The Phase III WRS was required to process laundry, shower, handwash, and oral hygiene wastewaters, along with urine, humidity condensate, and incinerator condensate from the processing of human solid wastes.

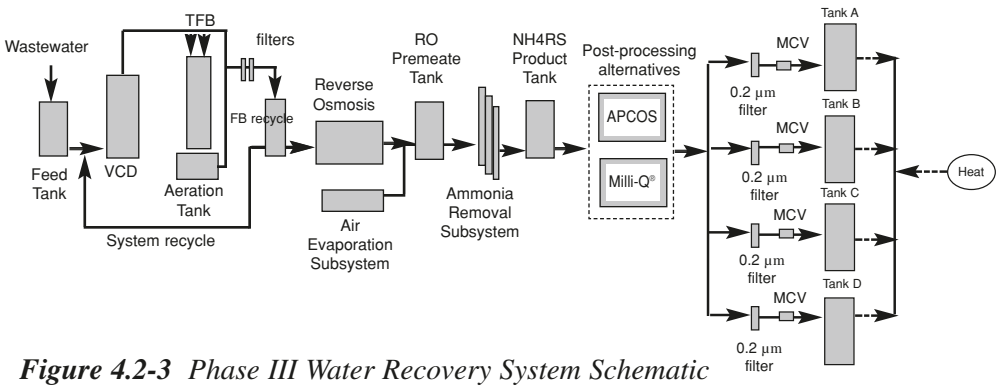

Figure 4.2-3 *Phase III Water Recovery System Schematic*

In preparation for Phase III, a demonstration test was performed from March through June 1997. This test processed urine and hygiene water generated by human donors but the water was not consumed. The recovered water was analyzed during the final two weeks of the test to evaluate the system's capability to produce potable water. Ten samples were collected and analyzed. The analytical results from these samples can be found in Table 4.2-6. All 10 samples exceeded the MSIS

specification for color. Color levels ranged from 41-64 Pt/Co units, which resulted from the iodine in the water. Five samples collected in the first week of the two-week collection period exceeded the MSIS specification of 10 mg/L for nitrate and ranged from 18 to 20 mg/L. Nitrate levels from samples collected in the second week of the collection period ranged from 0.88 to 1.08 mg/L. All other parameters routinely met MSIS and U.S. EPA standards.

The Phase III test began on September 19, 1997. The WRS processed water nomi-

Table 4.2-6 *Phase III WRS Demonstration Test Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Conductivity	µS/cm	18.30	333.0	170.0	10
pH	pH units	5.93	6.11	6.03	10
Turbidity	NTU	0.00	0.03	0.01	10
Iodine (UV/VIS)					
I2	mg/L	3.8	5.63	4.69	10
I3-	mg/L	0.054	0.296	0.154	10
I-	mg/L	1.35	6.28	3.411	10
IOH	mg/L	<0.05	0.13	0.60	10
Color	Pt/Co	41.0	64.4	51.8	10
Anions (IC)					
Chloride	mg/L	1.83	31.70	15.92	10
Nitrite (NO2-N)	mg/L				
Nitrate (NO3-N)	mg/L	0.88	20.00	10.21	10
Sulfate	mg/L	0.50	8.92	4.37	10
Cations (CE/IC)					
Sodium	mg/L	1.23	53.42	26.63	10
Potassium	mg/L	0.091	1.86	0.905	10
Magnesium	mg/L	<0.002	0.017	0.008	10
Calcium	mg/L	1.48	25.21	12.389	10
Metals					
Aluminum	µg/L	1.1	9.0	2.4	10
Barium	µg/L	<1	20.8	6.28	10
Chromium	µg/L	<5	58.0	16.2	10
Copper	µg/L	<1	3.2	1.04	10
Iron	µg/L	7.9	144.0	71.36	10
Nickel	µg/L	<1	3.3	1.11	10
Selenium	µg/L	<1	1.5	0.26	10
Zinc	µg/L	<1	8.5	4.47	10

Table 4.2-6 continued *Phase III WRS Demonstration Test Results*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Total Organic Carbon					
TIC (OI 1010)	mg/L	1.230	0.462	0.988	6
NPOC (OI 1010)	mg/L	0.106	0.210	0.171	6
TIC (Sievers 800)	mg/L	0.457	1.930	1.096	10
TOC (Sievers 800)	mg/L	0.097	0.246	0.161	10
Extractable Organics					
Cyclododecane	µg/L	ND	15.4	3.9	10
Diiodomethane	µg/L	ND	4.9	1.8	10
Dodecanol	µg/L	ND	16.3	3.0	10
Iodoform	µg/L	ND	6.2	2.6	10
1-Octanol	µg/L	ND	53.8	29.9	10
2,2,4-Trimethyl-1, 3-pentanediol diisobutyrate	µg/L	ND	21.6	2.16	10
Aldehydes (GC/MS)					
Formaldehyde	µg/L	<2	4.6	2.1	10
Carboxylates (CE/HPLC)					
Lactate	mg/L	<0.25	0.44	0.044	10
Organic Carbon Recovery	percent	2.02	46.5	41.5	9

nally throughout the test, except from days 47 to 56 when the APCOS was being tested. Water processed by the APCOS exceeded MSIS limits for lead and nickel and was only consumed after further processing by the Milli-Q®. During this period, crewmembers conserved water, until the Milli-Q® system could reprocess the APCOS effluent. Details concerning the performance of the APCOS subsystem can be found in the *Lunar-Mars Life Support Test Project: Phase III Final Report* (21).

A total of 52 recovered water samples and 15 in-process samples were collected for analysis during Phase III. The summary data for the consumed recovered potable water tank samples are listed in Table 4.2-7. Color and pH levels were consistently above MSIS specifications because of iodine addition to the recovered water. Recovered potable water samples consistently met U.S. EPA requirements. Two samples exceeded the NASA potable water requirement for TOC. One recovered water sample collected on October 16, 1997 had a TOC level of 615 µg/L, slightly above the 500 µg/L limit. The elevated TOC was attributed to the presence

of isopropanol that was used to disinfect the sample port for microbial sample collection. The probable cause of this was that the sample port, after being disinfected with isopropanol, was not sufficiently flushed prior to the collection of the chemical sample. The sample was recollected with an acceptable TOC level (0.055 mg/L). Another sample collected on November 11, 1997 also was above the limit, with a TOC level of 1.55 mg/L. This was also attributed to the presence of isopropanol. The tank was processed through the Milli-Q®, which lowered the TOC level (64 µg/L), but the total microbial count at 48 hours exceeded the 100 CFU/100 ml MSIS limits. After heat disinfection, the tank was certified on November 14, 1997 for crew consumption. The average TOC level of all the Phase III consumed recovered water samples was 146 µg/L and ranged from 55 to 291 µg/L.

Table 4.2-7 *Phase III Consumed Potable Water Tank Results Summary*

Iodine levels were elevated in three samples collected from tank A between

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Conductivity	µS/cm	2.6	51.5	15.0	45
pH	pH units	4.42	6.37	5.80	46
Turbidity	NTU	<0.01	0.16	0.03	33
Iodine (UV/VIS)					
I2	mg/L	0.69	4.69	2.61	46
I3-	mg/L	0.008	0.127	0.047	46
I-	mg/L	0.26	7.87	2.40	46
IOH	mg/L	<0.05	0.17	0.04	46
Total Iodine	mg/L	3.56	8.63	5.11	46
Iodine (LCV)					
Total I	mg/L	0.250	6.920	3.245	6
I2	mg/L	0.109	0.163	0.275	6
I-	mg/L	0.445	0.543	1.956	6
Color	Pt/Co	7.3	51.0	28.0	46
Anions (IC)					
Chloride	mg/L	0.31	3.97	0.86	36
Nitrite (NO2-N)	mg/L	<0.01	0.50	0.11	36
Nitrate (NO3-N)	mg/L	0.40	3.60	0.57	36
Sulfate	mg/L	<0.01	0.10	0.03	36
Cations (CE/IC)					
Sodium	mg/L	0.69	7.270	1.076	36
Potassium	mg/L	<0.02	0.514	0.066	36
Calcium	mg/L	0.344	1.910	0.917	36

Table 4.2-7 continued *Phase III Consumed Potable Water Tank Results Summary*

Parameter	Units	Minimum Concentration	Maximum Concentration	Average Concentration	n
Physical Parameter					
Metals					
Aluminum	µg/L	<2	17.9	0.8	40
Chromium	µg/L	<5	36.6	0.915	40
Copper	µg/L	1.5	331	80.1	40
Iron	µg/L	<5	14.2	3.1	40
Manganese	µg/L	<1	3.3	0.6	40
Molydenum	µg/L	<1	10.2	2.6	40
Nickel	µg/L	6.9	125.0	22.0	40
Lead	µg/L	2.3	38.2	11.1	40
Zinc	µg/L	3.7	123.0	12.3	40
Total Organic Carbon					
TIC (OI 1010)	mg/L	0.083	0.667	0.591	5
NPOC (OI 1010)	mg/L	0.127	0.300	0.221	5
TIC (Sievers 800)	mg/L	0.054	0.722	0.467	45
TOC (Sievers 800)	mg/L	0.055	0.291	0.146	45
Extractable Organics					
2-Ethyl-1-hexanol	µg/L	ND	8.9	0.3	40
4-Hydroxy-4-methy l-2-pentanone	µg/L	ND	47.4	4.4	40
Iodoform	µg/L	ND	5.6	0.1	40
2-Methyl-2,4 -pentanediol	µg/L	ND	34.1	3.6	40
Methyl sulfone	µg/L	ND	25.4	2.2	40
Aldehydes (GC/MS)					
Formaldehyde	µg/L	<21	2.2	1.31	36
Organic Carbon Recovery	percent	0.00	97.9	8.83	36

October 20 and 22, 1997, before and after heat disinfection for two hours, and overnight (4.74, 4.57, and 4.69 mg/L, respectively). On day 35 of the Phase III test, the test physician determined that it was necessary to remove the iodine from water consumed by test subjects based on crewmember physiological data. See chapter 5.5 for further discussion. To remove the iodine, a system of ion-exchange resins and activated carbon, called the iodine removal subsystem, was incorporated in the galley on October 23, 1997. Samples from the galley showed that total iodine levels

(iodine and iodide) were reduced to <10 μg/L. Iodine was not removed from the potable water tanks. The crew consumed water only from the galley, while water from the potable water tanks containing two to four mg/L of iodine was used for hygiene purposes only.

Nickel and lead levels in a few cases exceeded the MSIS limits. Six samples exceeded the nickel standard of 50 μg/L, ranging from 50.4 to 125 μg/L, and three samples exceeded the lead standard of 50 μg/L, ranging from 66 to 184.4 μg/L. A sample collected from tank B on November 3, 1997 had an elevated nickel level (64.9 μg/L), but the corresponding galley sink sample was within limits. Another sample collected on November 9, 1997 from tank C was not certified potable due to elevated levels of nickel (206.9 μg/L), aluminum (19.8 μg/L), lead (184.8 μg/L), and nitrate (19.9 mg/L) which all exceeded MSIS requirements. After reprocessing the water with the Milli-Q®, the parameters were within limits and the tank was certified on November 12, 1997. A tank B sample collected November 11, 1997 had elevated nickel (328 μg/L), aluminum (15.2 μg/L), and lead levels (106 μg/L) but was also processed through the Milli-Q® which lowered the nickel, aluminum, and lead levels to within acceptable levels.

Elevated nickel (302 μg/L) and lead (66.0 μg/L) levels in a sample collected on November 13, 1997, along with a too-numerous-to-count microbial result prevented another tank from being certified potable. After being reprocessed by the Milli-Q®, heat disinfected, and recirculated through a MCV, another sample collected one day later still had an elevated total microbial count. The tank was heat disinfected and the water recirculated through a MCV again before this tank was finally certified potable on November 17, 1997 with a note to investigate why the iodine level was in the lower range of acceptability.

The nickel level (125 μg/L) was elevated in the tank C sample collected on November 17, 1997, but the corresponding galley sink sample was within limits. Another sample collected on November 21, 1997 also had a slightly elevated nickel level (50.4 μg/L) but was certified without reprocessing with the qualifier to analyze the nickel on the corresponding galley sink sample. This was done, and the nickel level was found to be within limits.

Discussion

Physical Parameters

Conductivity, turbidity, color, iodine, and pH were routinely analyzed in samples collected from the potable water storage tanks during Phases I, II, IIa, and III. Of these, pH and color frequently exceeded the NASA MSIS limit because of the addition of iodine to the potable water. Phase I samples had a pH range from 4.38 to 4.80, Phase II samples ranged from 4.65 to 6.01, Phase IIa samples ranged from 4.65 to 5.76, and Phase III samples ranged from 4.42 to 6.37 pH units. Color levels measured were 31.1 to 42.2 Pt-Co units in Phase II, 31.4 to 34.8 Pt-Co units in Phase IIa, and 7.3 to 51 Pt-Co units in Phase III. This parameter was not analyzed

in Phase I. Iodine levels measured in Phase I ranged from 0.03 to 5.42 mg/L, from 2.82 to 3.99 mg/L in Phase II, from 2.21 to 3.22 mg/L in Phase IIa, and from 0.69 to 4.69 mg/L in Phase III in the tank samples. Conductivity levels seen in the potable water samples were 3.52 to 11.57 μS/cm in Phase I, 1.96 to 6.19 μS/cm in Phase II, 1.97 to 4.76 μS/cm in Phase IIa, and <2.6 to 51.5 μS/cm in Phase III. Turbidity levels were typically low and ranged from <0.01 to 0.40 for all samples tested from Phases I through III.

Inorganic Parameters

The inorganic parameters detected in the potable water samples were low and usually significantly less than the MSIS and EPA standards. No traces of fluoride, bromide, or magnesium were found in any of the potable water samples analyzed. However, other anions and cations such as sodium, potassium, calcium, chloride, nitrate, nitrite, phosphate, and sulfate were detected in some of the samples. During Phase I, chloride was detected in one sample at 0.13 mg/L. In Phase II, sodium (0.11 to 0.995 mg/L), potassium (0.14 to 0.244 mg/L), ammonium (0.26 to 0.50 mg/L as nitrogen), and calcium (not detected to 0.21 mg/L) were measured in the samples analyzed. For Phase IIa, chloride levels ranged from 0.03 to 0.42 mg/L, nitrate levels were <0.01 to 0.25 mg/L as nitrogen, phosphate levels ranged from <0.01 to 0.17 mg/L, and sulfate levels were 0.07 to 0.09 mg/L. Sodium, potassium, calcium, and ammonium levels were all less than 0.1 mg/L, while calcium levels reached a maximum of 0.373 mg/L. Higher levels of ionic compounds were detected in the Phase III samples, probably due to the biologically based water processing systems that were used in this test. Chloride (0.31 to 34.0 mg/L), nitrite (not detected to 0.50 mg/L), nitrate (0.40 to 3.60 mg/L), sulfate (not detected to 0.10 mg/L), sodium (0.69 to 7.27 mg/L), potassium (not detected to 0.514 mg/L), and calcium (0.344 to 1.91 mg/L) were detected in Phase III potable water samples.

Trace metals detected in potable water samples, but typically below potability requirements, included arsenic, barium, chromium, copper, iron, manganese, molybdenum, nickel, lead, selenium, and zinc. No mercury or cadmium was detected. Arsenic levels varied from nondetectable in all Phase II and Phase III samples to a maximum of 3.8 μg/L in a sample from Phase I. Barium and manganese levels did not exceed 4 μg/L in any of the samples analyzed. Chromium levels ranged from not detected to 1.8 μg/L in Phase I, from 0.4 to 2.4 μg/L in Phase II, from 1.2 to 9.7 μg/L in Phase IIa, and from nondetectable to 36.6 μg/L in Phase III samples. Copper was detected at levels exceeding the MSIS. One tank sample collected during Phase IIa had a copper level of 1770 μg/L that exceeded both the 1000 μg/L MSIS specification and the EPA action level of 1300 μg/L. EPA has found copper to potentially cause stomach and intestinal distress, liver and kidney damage, and anemia when people are exposed to it at levels above the action level for relatively short periods of time. The source of high copper in the Phase IIa sample was never isolated. Levels of copper in Phase I samples were nondetectable

to 3.7 μg/L and Phase III samples contained from 1.5 to 331 μg/L of copper. All samples from Phase II had nondetectable levels of copper. Iron was detected in many of the potable water samples. Iron was found in Phase I samples (not detected to 9.6 μg/L), in Phase II samples (0.7 to 3.1 μg/L), in Phase IIa samples (<2.0 to 43.7 μg/L), and in Phase III samples (not detected to 14.2 μg/L). Nickel was detected in 4 of 6 samples analyzed during Phase I at 1.0 to 2.6 μg/L, in 5 of 7 post-Milli-Q® samples during Phase II at 1.0 to 1.8 μg/L, in post-Milli-Q® samples during Phase IIa at 1.2 to 42.4 μg/L, and in tank samples during Phase III at 6.9 to 12.5 μg/L. Nickel levels in 3 samples from Phase III exceeded the NASA MSIS specification of 50 μg/L with one sample also exceeding the EPA Health Advisory of 100 μg/L. The high nickel levels observed in Phase III may have resulted from the APCOS water processing subsystem as discussed in the LMLSTP Phase III final report (12). This is also the case for lead. While lead levels in all Phase II tank samples were nondetectable, lead was found in Phase I samples from not detected to 3.2 μg/L, in the Phase IIa galley sink samples from <1 to 54.3 μg/L, and in Phase III from 2.3 to 38.2 μg/L. The NASA MSIS specification for lead is 10 μg/L and the EPA action level is 15 mg/L. Selenium was detected in Phase I (not detected to 3.2 μg/L) and Phase IIa (<1 to 12.2 μg/L) only; none was detected in Phases II and III. Zinc levels in the potable water samples measured from 0.3 to 13.2 μg/L in Phase I, from 0.1 to 0.6 μg/L in Phase II, from <1 to 5.2 μg/L in Phase IIa, and from 3.7 to 123 μg/L in Phase III. These zinc levels were very low compared to the MSIS limit of 5000 μg/L.

Organic Parameters

In general, the water recovery systems efficiently removed organic matter in the processed waters, so that few detectable organic compounds remained in the potable water consumed by test subjects. In summary, of the approximately 265 organic contaminants tested, about 35 compounds were detected in the Phase I samples, 25 compounds were detected in Phase II, 44 compounds were detected in Phase IIa, and 12 compounds were detected in Phase III. None of the compounds detected exceeded U.S. EPA maximum contaminant levels or health advisories that have been established. It must be noted, however, that EPA requirements are developed for normal terrestrial water supply systems and are not intended for water directly reused from spacecraft waste streams. As a result many of the compounds found in the recovered potable water do not have established limits.

TOC levels in the consumed recovered water samples averaged 338 μg/L during Phase I, 166 μg/L for post-Milli-Q® samples during Phase II, 286 μg/L during Phase IIa, and 180 μg/L during Phase III. Total carbon accountability is given in Table 4.2-8. The average TOC accountability ranged from 9 to 73%. Low accountabilities were normally observed in samples with low TOC as the analytical methods employed were unable to detect or identify the specific organic compounds at the low TOC values encountered. Higher levels of accountability were found for those sam-

ples with corresponding higher levels of TOC.

Low molecular weight compounds were detected in the potable water samples

Table 4.2-8 *TOC Accountability of Potable Water Samples*

Samples	TOC (μg/L)	Average TOC (μg/L)	Accountability Range (%)	Accountability Average (%)
Phase I JSC facility water	244-432	338	3-133	73
Phase II Pre-MilliQ recovered water	90-2530	1740	8-90	72
Phase II Post-MilliQ recovered water	105-243	166	11-51	33
Phase IIA consumed recovered water	174-523	286	3-142	29
Phase III consumed recovered water	55-291	146	0-98	9

consistently throughout the chamber studies. Acetone was detected in Phase I (1.5 to 7.8 μg/L), Phase II (9.6 to 32.0 μg/L), Phase IIa (not detected to 6.40 μg/L), and Phase III (not detected to 29.27) samples. Toluene was also detected at 1.6 to 3.9 μg/L during Phase I, 0.9 to 1.7 μg/L during Phase II, and not detected to 9.53 μg/L during Phase IIa. Formaldehyde levels during Phase I measured 9.5 to 12.6 μg/L, during Phase II these levels were 9.0 to 17.2 μg/L, during Phase IIa levels they ranged from not detectable to 13.8 μg/L, and during Phase III the levels were <2 to 12.2 μg/L. Acetate was found at levels ranging from nondetectable to 140 μg/L in Phase I, from 0.06 to 0.165 mg/L in Phase II and from <0.12 to 0.65 mg/L in Phase IIa. Other low molecular weight compounds detected less frequently included 2-butanone (12.5 to 39.8 μg/L) in Phase I samples, formate (not detected to 560 μg/L) in Phase I samples, 2-propanol (not detected to 154 μg/L) in Phase IIa samples, and methanol (101 to 233 μg/L) in Phase IIa samples.

Other organic compounds detected above 5 μg/L during the tests included dibutylamine (not detected to 25 μg/L in Phase I); dipropylene glycol methyl ether (not detected to 76.3 μg/L in Phase I); bis 2-ethylhexyl phthalate (0.3 to 28.1 μg/L in Phase II); benzyl alcohol (not detected to 7 μg/L in Phase IIa); 2-ethyl-1-hexanol not detected to 8.9 μg/L in Phase III); methyl sulfone (not detected to 54.5 μg/L in Phase IIa and not detected to 25.4 μg/L in Phase III); oxalate (<0.10 to 0.41 mg/L in Phase IIa); lactate (<0.12 to 1.10 mg/L in Phase IIa); urea (0.302 mg/L in Phase IIa; methylmethacrylate (not detected to 6.74 μg/L in Phase III); 4-hydroxy-4-methyl-2-pentanone (not detected to 47.4 μg/L in Phase III); and 2-methyl-2, 4-pentanediol (not detected to 34.1 μg/L in Phase III).

Phenolic compounds were detected in Phases I, II, IIa, and III at levels exceeding

the NASA MSIS of 1 μg/L for total phenols. Compounds detected during Phase I include 3-t-butylphenol (not detected to 1.6 μg/L); 4-chloro-3,5 dimethylphenol (not detected to 0.5 μg/L); 2,4-di-t-butylphenol (not detected to 0.1 μg/L); 4-t-octylphenol (not detected to 1.0 μ(g/L); phenol (not detected to 5.1 μg/L); and 2-phenylphenol (not detected to 2.2 μg/L). Iodine disinfection byproducts such as iodomethane (not detected to 1.3 μg/L); diiodomethane (not detected to 1.4 μg/L); and iodoform (not detected to 8.8 μg/L) were also detected. Only one phenol compound, 2,6-di-t-butyl-4-methylphenol (2.5 to 2.6 μg/L), was detected in Phase II samples, while iodinated compounds such as diiodomethane (0.4 to 0.5 μg/L) and iodoform (1.8 to 1.9 μg/L) were also found. During Phase IIa, phenol ranged from not detected to 1.0 μg/L and iodoform ranged from not detected to 4.8 μg/L. No phenolic compounds were found in Phase III samples, and iodoform was the only iodinated compound detected at levels up to 5.6 μg/L.

SIGNIFICANCE

The development of water recycling systems is of paramount importance for the success of long-duration missions. In turn, the monitoring of water quality provides concrete evidence of the capability of the water recycling systems to provide clean potable water and is required to verify that the water is potable and acceptable for human consumption. WAFAL analyzed about 160 water samples throughout the course of the Lunar-Mars Life Support Test Project. This project was the first time since the late 1960's that water recycling with human consumption was performed and the first time systems developed for the ISS were tested in this manner. Results from the analysis of samples show that the water recycling systems developed during Phases II, IIa, and III were capable of producing potable water which met NASA and U.S. EPA requirements after the water was treated using a commercial system. All recovered water samples analyzed met U.S. EPA standards. Generally, the majority of samples also met NASA potability standards.

On several occasions the organic and inorganic content of the water exceeded the NASA specifications and required the water to be reprocessed prior to consumption. Parameters of most importance where requirements were not met included total organic carbon, total bacteria, copper, lead, and nickel. During Phase II, the water was reprocessed seven times because total organic carbon requirements were not met. During Phase IIa, problems with the water recovery system required the recycled water to be reprocessed eleven times for TOC excursions and ten times for excessive microbial levels. While Phase III did not require reprocessing because a commercial Milli-Q® was used as a post processor, high microbial levels did require the potable water tanks to be heat sterilized three times. Thus, systems for polishing and disinfecting the potable water tanks appear necessary.

That exceedances were detected that required the water be reprocessed for potablity clearly demonstrates the need for onboard water analytical capabilities. Similarly, on several occasions microbial contamination in the potable water was

detected and required heat disinfection to assure microbial safety. This demonstrates the need for onboard microbial analysis capability and the ability to recover microbiological control.

Color and pH measurements consistently did not meet NASA standards because of the addition of iodine as a disinfectant in the potable water. With the help of these data, the MSIS specifications should be re-evaluated to determine more appropriate limits for pH and color in iodinated water. Another specification that should be evaluated is the MSIS total phenols specification. This level should be increased to agree with the EPA health advisory for phenol, which is 4 mg/L. Other standards outlined in the MSIS appear to be adequate. However, future work should concentrate on the development of short-term and long-term exposure requirements for the most critical water quality parameters.

The sampling and monitoring plan performed during these studies proved to be adequate. However, analytical methods for identifying organic constituents in recovered water at low levels should be improved. More work should be done to increase the organic carbon recovery of the potable water samples by either developing more sensitive methods of analysis and/or by testing a wider array of organic compounds, especially those of a biological nature such as proteins and biomolecules.

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ACRONYMS & ABBREVIATIONS

AES	Air Evaporation Subsystem
APCOS	Aqueous Phase Catalytic Oxidation System
ARS	Air Revitalization System
C	Conductivity
CHX	Condensing Heat Exchanger
CHeCS	Crew Health Care System
EPA	Environmental Protection Agency
F	Filter
GC/MS	Gas Chromatography/Mass Spectrometry
HA	Health Advisory
HMS	Human Metabolic Simulator
I2	Iodine
ICB	Immobilized Cell Bioreactor
ILSSTF	Integrated Life Support Systems Test Facility
ISS	International Space Station
IX	Ion Exchange
JSC	Johnson Space Center
MCL	Maximum Contaminant Level
MCV	Microbial Check Valve
MI	Matrix Interference
MSIS	Manned System Integration Standards
NA	Not Analyzed
ND	Not Detected
NH4RS	Ammonia Removal System
RO	Reverse Osmosis
STP	Standard Temperature and Pressure
TFB	Trickling Filter Bioreactor
TOC	Total Organic Carbon
UF/RO	Ultra Filtration/Reverse Osmosis
VCD	Vapor Compression & Distillation
VPGC	Variable Pressure Growth Chamber

VRA	Volatile Removal Assembly
WQM	Water Quality Monitor
WRS	Water Recovery System

Units of Measure

Pt-Co	Platinum-Cobalt units
μg/L	Micrograms per Liter
μS/cm	Microsiemens per Centimeter
mg/L	Milligrams per Liter
NTU	Nephelometric Turbitity Units
TON	Threshold Odor Number
TTN	Threshold Taste Number

Acknowledgements

We appreciate the contributions and support from members of the Advanced Life Support community, crewmembers of LMLSTP Phases I, II, IIa, and III, and especially members of the Medical Sciences Division, Water and Food Analytical Laboratory for their dedication to this project.

4.3

Microbiology

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Introduction

As NASA prepares for long-term missions aboard the International Space Station (ISS) and spacecraft destined for Mars, self-contained/closed chambers on Earth have become important test beds for microbiological evaluations. The insight gained from these studies directly benefits NASA as this knowledge is incorporated into the development of monitoring systems and countermeasures against microbiological contamination problems unique to long-duration space missions.

The microbiological study of these chambers and their crew also addresses many Earth-based concerns. Many office buildings are semiclosed systems, which can develop air and surface contamination (3, 5). Commonly referred to as "sick building syndrome," contamination in these facilities may affect up to 30% of new and remodeled buildings worldwide (6). The United States Navy has also investigated the problems associated with microbial growth in semiclosed systems to determine the potential health risks during long-term submarine missions (2, 4). While studies of office buildings and submarines give some insight into changes in the microbial levels and diversity created by an artificial ecosystem, all terrestrial models eventually have access to fresh air and water and can be thoroughly disinfected. The NASA closed-chamber studies provided the unique opportunity to evaluate undisturbed changes in microbial ecology and its relationship to the crew.

During the chamber studies, the primary objective of microbiological evaluations was to ensure crew health by monitoring microbial levels and changes in microbial ecology. In every phase of these studies, the scope of the analyses included the air, potable water, and surfaces that the crew directly contacted. The surfaces that were sampled included not only smooth surfaces, but also carpeting which builds high microbial levels and is difficult to disinfect. In addition, the ability of viral contaminants to survive the water treatment system was evaluated prior to the Phase II test using bacteriophages MS-2 and PDR-1 (1). Monitoring microbial concentrations and diversity provided a way to assess other problems that could affect life support and other systems, such as microbial degradation of materials and the potential fouling of process lines. The use of in-line coupons

provided a way to determine both the type of microorganism in the flow systems and the effect of these organisms on in-line materials.

Evaluations of the air, potable water, and surfaces were performed using standard culturing methodologies and biochemical identification. Physical techniques were applied to retrieve microbial samples from carpets, rugs, and biofilm evaluations of water lines from metal coupons that were then assayed for microbial concentration and identity. The viral challenge of the water reclamation system used standard culturing methodologies combined with plaque enumeration to determine phage survival.

The microbial concentration and diversification in the air and surface samples varied dramatically over time at any given sampling site. Evidence suggested that these changes were directly related to crew activities. These activities range from obvious activities such as cleaning to innocuous activities such as walking through the chamber. Microbial contamination of the air, especially fungal contamination, was maintained at minimal levels, possibly resulting from the complex air revitalization system. In general, the potable water system was successfully disinfected with iodine. The average bacterial concentration was generally kept below the NASA specification throughout the studies. After 60 days during Phase III, the unexpected emergence of various gram-positive *Bacillus* species as the dominant flora suggested potential long-term contamination problems.

Samples extracted from carpets and rugs indicated high levels of microorganisms, although the concentration would on occasion drop dramatically, possibly due to housekeeping patterns. The evaluation of metal coupons in a heat exchanger line indicated bacterial biofilm formation. The coupons were coated with a biocide that limited biofilm buildup initially, but inevitably did not prevent bacterial colonization. Lipid analysis of the coupons suggested viable bacteria were not detectable, but did indicate a bacterial presence.

In order to understand the risk of infectious disease among crewmembers during the chamber studies, microbial samples were collected from the throat, nose, urine, and feces of each crewmember upon entering and exiting. A second throat swab was collected from each crewmember for a viral culture. This data also allowed the identification of microorganisms that may not be considered normal human flora.

The information provided by these studies suggests that long-term space flight can be accomplished and that a unique environmental equilibrium between humans and microbial flora can be maintained. The living environment is in many ways healthier than the conditions found on Earth. However, the potential for long-term microbial contamination is also suggested by this data. The possibility of long-term contamination requires further study to ensure the health of the crew and the operational function of life support systems.

Methods

Microbial Control During Operations

During all phases of the chamber studies, potable water was disinfected with iodine targeted at 2 to 4 ppm. During scheduled and contingent housekeeping, surfaces were cleaned with benzalkonium chloride antiseptic towelettes (PDI, Orangeburg, NY). No HEPA (high-efficiency particulate arresting) or equivalent filter was attached to the air systems.

Sites for Water, Air, and Surface Samples

During Phase I, potable water was sampled directly from fully filled storage tanks. A single air sample site monitored air quality. Surface sample sites included the air return vent, air intake vent, bed rail, desk surface, urinal, cabinet door, sink edges, air conditioner, microwave, and rug. During Phase I, microbial analyses were performed on several sites in the Variable Pressure Growth Chamber (VPGC). Heterotrophic plate counts (HPC) were assessed from the distilled water source, nutrient sources (A and B), the water supply tanks (A and B), and two condensate sources. Two in-line coupons from the water system were analyzed for bacterial and fungal accumulation. Plant samples from the VPGC were evaluated for microbial concentration and identity. Surface samples at four separate air-return sites were evaluated. A single air sample source in the VPGC also was evaluated during the course of this study.

During Phases II, IIa, and III, potable water was supplied by separate water tanks (A, B, C, and D) which were periodically replenished and disinfected with iodine. HPC of potable water samples were taken initially and when the tanks were replenished. Air samples during these three phases were collected from the same locations on every floor of the Life Support Systems Integration Facility (LSSIF) (Figure 4.3-1). Surface samples were collected at similar locations during the final three phases. Minor adjustments were made depending on physical changes within the LSSIF.

Air Sample Preparation and Analysis

Air samples were collected inside the chambers with a Burkard air sampler (Figure 4.3-2), which impacted 84.9 liters of air onto either trypticase soy agar (Remel, Lenexa, KS) for bacteria or rose bengal agar (Difco, Ann Arbor, MI) for fungi. Trypticase soy agar plates were incubated at 35°C for 48 hours; rose bengal agar plates were incubated for five days at 30°C. Bacterial and fungal colonies were counted. Morphologically different bacterial colony types were streaked on blood agar plates for isolation and identification. Morphologically different fungal colony types were streaked on Sabouraud-dextrose agar (Difco, Ann Arbor, MI) for isolation and identification.

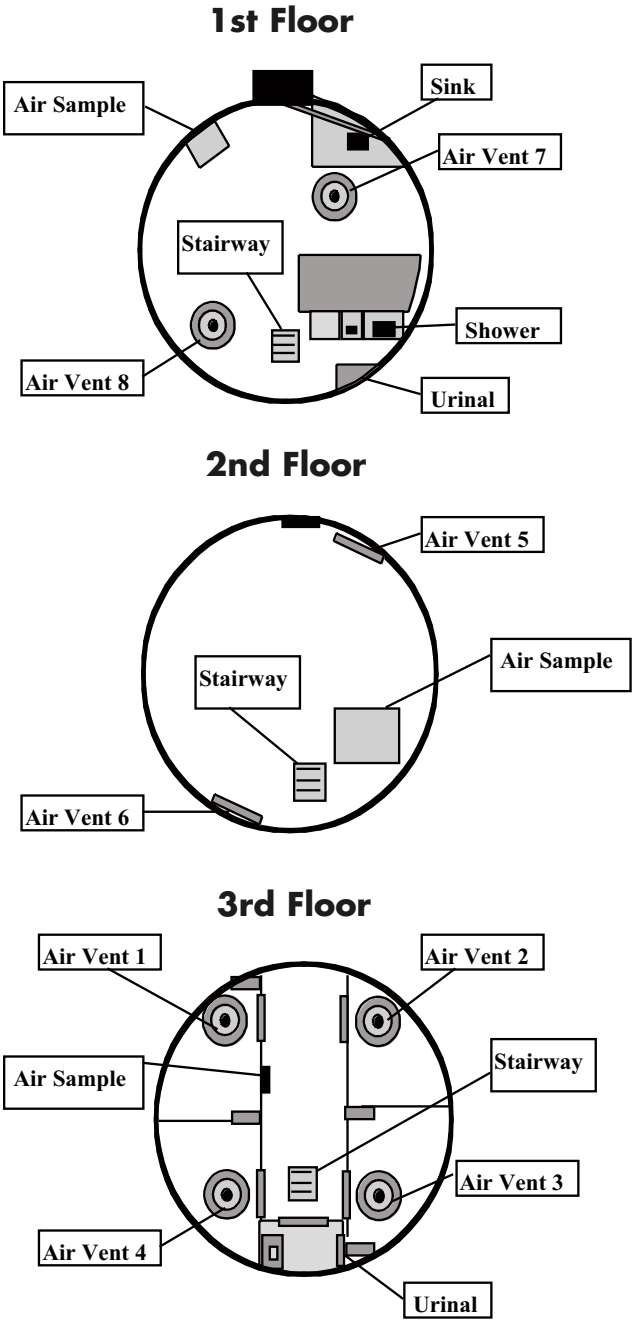

Figure 4.3-1 *Microbiology sampling sites in the Life Support Systems Integration Facility*

Figure 4.3-2 *Burkard air sampler being prepared for sample collection*

Surface Sample Preparation and Analysis

Surface samples were collected by swabbing a 25 cm^2 area with a moistened calcium alginate swab (Figure 4.3-3), which then was placed in 3 ml of trypticase soy broth (Remel, Lenexa, KS). The swabs in broth were vortexed, and the suspension was plated on trypticase soy agar for bacteria and rose bengal agar for fungi. Plates were incubated for enumeration and identification as described for air sampling.

Figure 4.3-3 *Collection of a surface sample from an air vent*

Potable Water Preparation and Analysis

Aliquots of 100 ml were passed through 0.22 µm membrane filters (Millipore, Bedford, MA). For HPC, the filter was placed on a pad saturated with TGE (trypticase/glucose/yeast extract, Difco, Ann Arbor, MI) or R2A broth (Remel, Lenexa, KS), and incubated at 35°C for 48 hours. Potable water specifications were based upon the current NASA specification at the time of the respective study. During Phases I and II, the specification was one colony forming unit (CFU) per 100 ml. During Phases IIa and III, the specification had been increased to 100 CFU per 100 ml.

Microbial Identification

All bacterial isolates were identified with either a Biolog Automated Identification System (Biolog, Hayward, CA) or a VITEK Identification System (bioMérieux, Hazelwood, MO). Fungi were identified microscopically by their morphological characteristics.

Coupon Analysis in the VPGC During Phase I

A manifold holding 30 stainless steel coupons was positioned in the flow stream to allow a representative flow of fluid across the coupon surface. The coupons were removed from the manifold and gently rinsed with deionized water before sonication in 90 ml of phosphate buffer (pH 7.0) to remove sessile bacteria.

Bacterial concentrations of this buffer were determined by HPC using serial dilutions.

Coupon Analysis for Phospholipid Fatty Acid During Phase II

Stainless steel coupons in the manifold were removed and gently rinsed with deionized water before sonication in 90 ml of phosphate buffer (pH 7.0) to remove sessile bacteria. Samples were shipped to Microbial Insights (Rockford, TN) for phospholipid fatty acid (PLFA) determination.

Coupon Analysis of the Condensate Heat Exchanger (CHX) During Phase IIa

Stainless steel coupons were place in the CHX system before the start of Phase IIa. Two coupons were removed for microbiological analysis at the start of the test (day 0), day 2, day 30, and day 60. The coupons were removed from the manifold and gently rinsed with deionized water before sonication in 90 ml of phosphate buffer (pH 7.0) to remove sessile bacteria. Bacterial concentrations of this buffer were determined by HPC analysis following serial dilutions.

Assays for Viral Challenge

Bacteriophage concentrates containing 1×10^9 plaque forming units (PFU) per 100 ml were used to inoculate the water recovery system (WRS) at the urine and wastewater collection tanks prior to the Phase II test (1). One-liter samples were collected at a variety of sources including the urine and wastewater collection tanks after disinfection with Oxone™ and sulfuric acid and downstream after vapor-compression distillation (VCD), after ultrafiltration/reverse osmosis (UF/RO), and after the aqueous phase catalytic oxidation system (APCOS). Samples were split to determine HPC and PFU. PFU were determined by first filtering 500 ml through a 0.45 μm cellulose acetate filter. The filtrate was plated in serial dilutions with phosphate-buffered saline. Host cells, *Escherichia coli* (MS-2) and *Salmonella typhimurium* (PRD-1), were grown in trypticase soy broth for 3 to 5 hours at 37°C, added to 1.5% agar, then inoculated with the viral samples. PFU were counted after 24 hours.

Assays for Crew Microbiology

Crew samples were obtained before and after the tests. These consisted of throat, nose, urine, and fecal samples. Throat samples were collected by swabbing the posterior pharyngeal vault of the crewmembers with the swab from the Culturette device (Becton-Dickinson). Nasal samples were collected by using the swab from the Culturette device moistened with phosphate buffer to swab both nares. Clean-catch midstream urine samples were collected in 4 oz. sterile specimen containers. Fecal specimens were collected in commode containers and transferred to bacterial transport medium for culture and to sodium acetate-acetic acid formalin fixative for ova and parasite examination. Quantification and isolation of organisms were performed by plating each sample onto selected media.

Bacterial cultures were incubated at 35°C and examined after 48 hours, and organisms were identified using the VITEK Identification System. Fungal cultures were incubated at 25°C and examined after five days, and organisms were identified by microscopic examination. Each culture was examined for the presence of medically significant organisms, and antibiotic susceptibilities were performed on the isolates. During Phase II, throat and nasal samples were collected by the crewmembers on day 7 and day 22. Samples were examined for bacteria and fungi, and the fecal samples were examined for ova and parasites.

Findings

Phase I

While the duration of Phase I was only 15 days, increases in surface colonization were apparent as nine of the 10 surface sites displayed over a four-log increase in bacterial concentration during the final week (Figure 4.3-4). Five of the 10 surface sites displayed a six-log increase. Of the bacteria identified, *Clavibacter* and *Curtobacterium* were the predominant genera. Fungal concentrations did not reflect the sharp increase seen with bacteria. A wide variety of fungal genera were identified including *Aspergillus* species, *Penicillium* species, *Acinetobacter* species, *Acremonium* species, *Microsporium* species, and Hyphomycetes.

No culturable bacteria were isolated from the initial samples from the Phase I potable water supply. However, on day 7, bacterial concentration increased to 230 CFU/ml. After 15 days, bacterial concentration decreased to nondetectable levels. Coliform bacteria were never detected during the study. *Burkholderia pickettii, Clavibacter michiganense,* and an unidentified *Clavibacter* species were the only bacteria isolated from potable water samples.

Measurement of biofilm formation on in-line coupons in the VPGC indicated both bacterial and fungal adhesion, although no pattern of progressive contamination was discernable (Table 4.3-1). This steady concentration of both bacterial and fungal levels was reflected in various liquid samples analyzed from the VPGC (Table 4.3-2) (Table 4.3-3). Microbial speciation of liquid samples indicated a wide variety of organisms, although none were medically significant (Table 4.3-4). Fungal levels from VPGC surface samples were relatively stable; however, bacterial concentrations increased dramatically, exceeding 10^8 CFU/cm^2 on certain air vents during crew egress. Identification of VPGC surface microorganisms indicated only common flora of no specific medical importance.

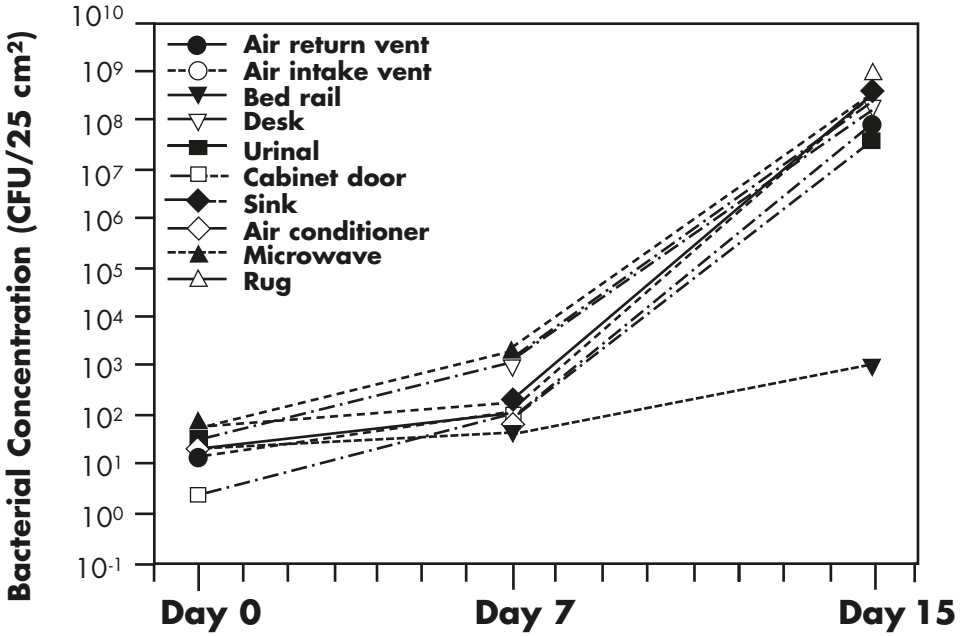

Figure 4.3-4 *Bacterial concentration (CFU/cm²) from Phase I surface samples*

Table 4.3-1 *Microbial adhesion (CFU/coupon) from in-line coupons from the VPGC before and after Phase I*

Date	Bacteria		Fungi	
	Coupon A	Coupon B	Coupon A	Coupon B
6/29/1995	1.0×10^7	1.1×10^8	3.4×10^2	2.0×10^2
6/29/1995	0	0	2.3×10^1	2.0×10^1
7/7/1995	7.7×10^6	1.0×10^6	1.1×10^2	6.0×10^1
7/10/1995	2.7×10^6	4.51×10^3	1.1×10^2	6.1×10^1
8/8/1995 (Phase I egress)	2.0×10^4	2.5×10^4	1.0×10^2	3.0×10^2
9/13/1995	2.9×10^5	1.5×10^6	3.6×10^2	3.6×10^2

Table 4.3-2 *Bacterial concentration (CFU/ml) of liquid samples from the VPGC before, during, and after Phase I*

Date	Deionized Water	Nutrient Source A	Nutrient Source B	Water Supply A	Water Supply B	Condensate Tank A	Condensate Tank B
6/29/95	1.1×10^2	5.6×10^5	1.3×10^6	NC	NC	NC	NC
6/29/95	1.4×10^2	1.0×10^5	9.7×10^2	NC	NC	NC	NC
7/7/95	2.4×10^2	9.5×10^4	6.0×10^5	NC	NC	NC	NC
7/10/95	1.4×10^2	3.3×10^5	5.5×10^5	5.0×10^2	5.6×10^2	2.8×10^6	4.0×10^3
7/18/95	NC	NC	NC	NC	NC	7.0×10^2	1.4×10^3
7/21/95	NC	NC	NC	NC	NC	4.7×10^2	4.5×10^2
7/24/95	1.6×10^2	2.8×10^4	3.0×10^4	3.4×10^4	6.0×10^4	3.1×10^4	2.0×10^5
7/31/95	1.1×10^2	1.7×10^4	5.3×10^4	7.2×10^4	4.0×10^4	1.1×10^5	1.0×10^5
8/8/95 (Phase I egress)	7.8×10^2	3.5×10^4	6.3×10^4	1.2×10^6	1.4×10^5	1.3×10^4	4.2×10^5
9/13/95	5.8×10^2	1.0×10^5	1.0×10^5	3.7×10^5	1.2×10^5	2.9×10^5	5.9×10^4

NC = Not collected

Table 4.3-3 *Fungal concentration (CFU/ml) of liquid samples from the VPGC before, during, and after Phase I*

Date	Deionized Water	Nutrient Source A	Nutrient Source B	Water SupplyA	Water Supply B	Condensate Tank A	Condensate Tank B
6/29/95	0	1.4×10^2	1.1×10^2	NC	NC	NC	NC
6/29/95	7.5×10^0	7.5×10^0	7.5×10^0	NC	NC	NC	NC
7/7/95	3.8×10^1	2.0×10^0	2.3×10^1	NC	NC	NC	NC
7/10/95	7.5×10^0	2.3×10^1	3.0×10^1	5.0×10^1	5.0×10^1	2.0×10^1	4.5×10^1
7/18/95	NC	NC	NC	NC	NC	0	0
7/21/95	NC	NC	NC	NC	NC	3.8×10^1	2.3×10^1
7/24/95	7.5×10^0	4.5×10^1	2.3×10^1	4.0×10^1	4.0×10^1	1.5×10^1	7.5×10^0
7/31/95	7.5×10^0	2.3×10^1	1.5×10^1	1.5×10^1	4.0×10^1	7.5×10^1	2.3×10^1
8/8/95	0	3.0×10^1	4.0×10^1	0	7.5×10^0	1.5×10^1	6.0×10^1
9/13/95	0	7.5×10^0	3.6×10^2	0	3.0×10^1	4.5×10^1	2.3×10^2

NC = Not collected

Table 4.3-4 *Microbial colonies isolated from the VPGC liquid samples during Phase I*

	Nutrient A	Nutrient B	Air Sample	Supply Tank A	Supply Tank B	Condensate A	Condensate B
Bacteria	*Acidovorax delafieldie, Bacillus* sp., *Clavibacter michiganense, Comomonas terrigena, Rhizobium loti, Rhizobium meliloti, Vibrio cyclosites*	*Acidovorax facilis, Curtobacterium flaccumfaciens, Variovorax paradoxus, Vibrio cyclosites*	*Bacillus* sp., *Cladosporium* sp., *Clavibacter michiganense*	*Burkholderia picketti, Comomonas acidovorans, Variovorax paradoxus*	*Cladosporium* sp., *Rhizobium loti, Salmonella* sp., *Variovorax paradoxus*	*Cladosporium* sp., CDC grp IVC-2, *Salmonella* sp.	*Burkholderia cepacia, Comomonas acidovorans, Rhizobium leguminosarum*
Fungi	*Acremonium* sp., *Fusarium* sp.	*Acremonium* sp., *Epicoccum* sp.	*Acremonium* sp., Hyphomycete, *Penicillum* sp.	*Acremonium* sp., Hyphomycete, *Fusarium* sp.	Hyphomycete, *Fusarium* sp.	*Acremonium* sp., Hyphomycete, *Fusarium* sp.	*Fusarium* sp., *Monilia* sp., *Cladosporium* sp.
	Distilled Water	**Air Intake A**	**Air Intake B**	**Coupon A**	**Coupon B**	**Rhizoplane A**	**Rhizoplane B**
Bacteria	*Burkholderia picketti, Rhizobium meliloti*			*Curtobacterium flaccumfacien, Clavibacter michiganense, Alcaligenes* sp.	*Rhizobium meliloti, Rhizobium loti, Aureobacterium saperdae, Rhodococcus aichiensis, Alcaligenes xylosoxydans*	*Pseudomonas putida, Pseudomonas* sp.	*Alcaligenes xylosoxydans, Xanthomonas maltophilia, Pseudomonas* sp., *Alcaligenes* sp.
Fungi	*Fusarium* sp.	*Fusarium* sp., *Penicillum* sp., *Aspergillus* sp.,	*Fusarium* sp., *Penicillum* sp., *Aspergillus* sp.,	*Acremonium* sp., *Fusarium* sp., *Rhodotorula glutinis, Trichoderma* sp.	*Fusarium* sp., *Acremonium* sp., *Trichoderma* sp.	*Fusarium* sp., *Trichoderma* sp., Hyphomycete, *Acremonium* sp.	*Acremonium* sp., Hyphomycete, *Penicillum* sp., *Trichoderma* sp., *Paecilomyces* sp.

Phase II

Patterns of bacterial and fungal colonization on surface samples during Phase II varied depending on the sampling site. The greatest numbers of bacteria were found at sample sites near wet areas such as the sink, urinal, and shower. These levels fluctuated more than two-log fold with no apparent pattern (Figure 4.3-5). The greatest numbers of fungi were found on the air vents and carpet samples. A subtle increasing trend in microbial concentration was observed during Phase II.

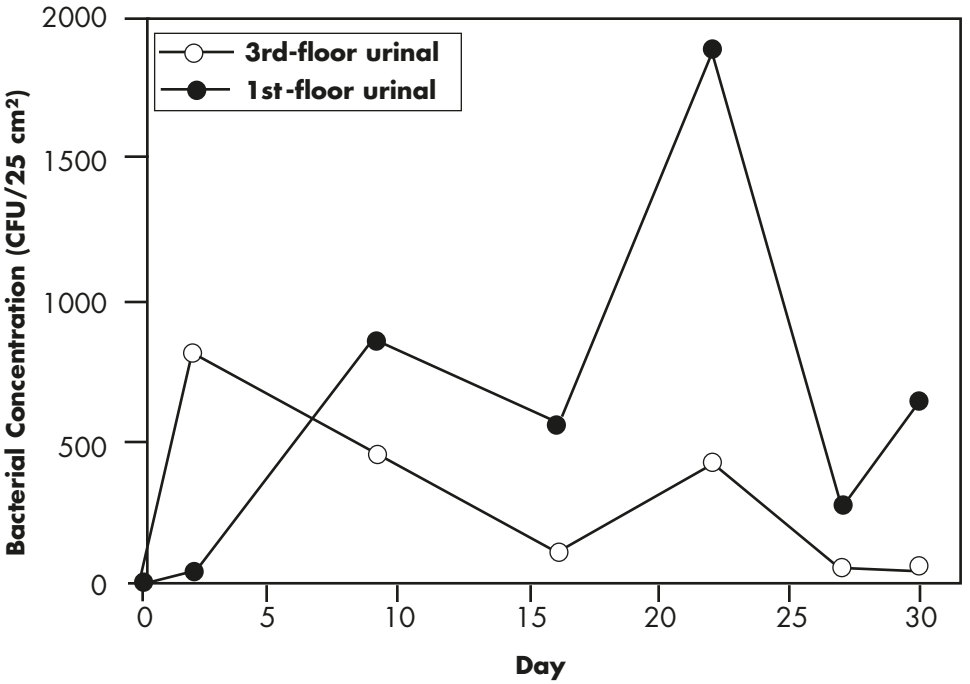

Figure 4.3-5 *First- and third-floor bacterial contamination (CFU/cm²) at urinals during Phase II*

Bacterial and fungal concentrations in air samples were consistently low, never exceeding 332 CFU/m³ for total bacteria and 339 CFU/m³ for total fungi. No trends were apparent based upon the day collected or location of the air sample.

During Phase II, no coliforms or anaerobic bacteria were isolated in the potable water system. Bacterial concentration generally remained at or below NASA specifications (Figure 4.3-6). Several bacterial species were isolated from the potable water tanks including *Sphingomonas paucimobilis, Burkholderia picketti, Stentrophomonas maltophilia,* and *Pseudomonas vesicularis*.

Bacterial viruses MS-2 and PRD-1 were injected into the WRS at the urine and wastewater collection tanks prior to Phase II to evaluate the ability of the WRS to remove or inactivate viral particles and prevent transmission to recovered potable water. Oxone/sulfuric acid and vapor compression distillation dramatically decreased the viral titer from 5.5×10^9 PFU/100 ml (MS-1) and 3.7×10^9 PFU/100 ml (PRD-1) to less than 1 PFU/100 ml. In comparison, HPC for these samples did not significantly decrease, remaining between 100 to 500 CFU/100 ml. The reverse osmosis unit also decreased viral density from 9.3×10^8 PFU/100 ml (MS-1) and 4.3×10^8 PFU/100 ml (PRD-1) to less than 1 PFU/100 ml. Bacterial concentration

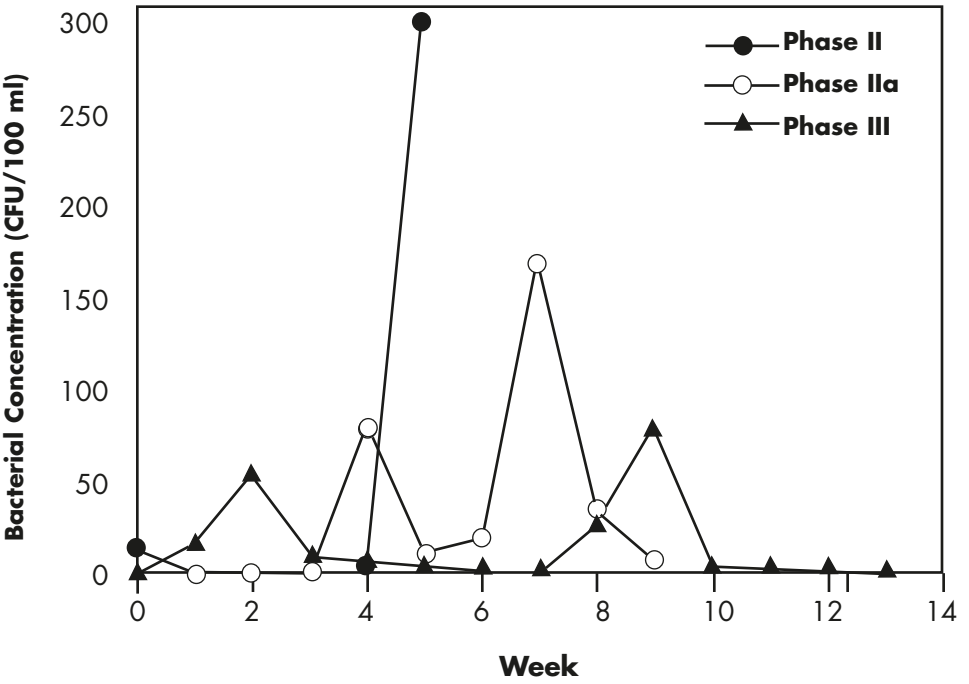

Figure 4.3-6 *Bacterial concentration (CFU/100 ml) in potable water during Phases II, IIa, and III*

decreased approximately one log, including a complete removal of all coliform bacteria. The retention of bacteria downstream of the reverse osmosis unit is possibly the result of contamination of the unit prior to installation. In combination, the units of the WRS removed all detectable MS-1 and PRD-1 viral particles.

Stainless steel coupons from Phase II were analyzed for PLFA content. The coupon that was in the final potable water system water line indicated viable bacteria, although it did have residue lipids that are indicative of gram-negative bacteria, gram-positive bacteria, and eukaryotes. Biomass, as measured by PLFA, was relatively low in the potable water line at 46 picomoles PLFA/coupon. Processing lines, such as the water from the UF/RO unit, contained diverse microbial communities with PLFA primarily from gram-negative bacteria. The total biomass per coupon from the UF/RO was 359 picomoles PLFA/coupon. Biomarkers indicative of gram-positive and sulfate-reducing bacteria were detected in the process line leading to the UF/RO unit that also contained very high levels of biomass at 3322 picomoles PLFA/coupon.

The crew microbiology results from Phase II were collected from eight subjects, and numbers 1, 2, 3, and 4 were chosen as crewmembers (Table 4.3-5). *Staphylococcus aureus* was recovered from the nose of crewmember 1 at entry, day 7, and day 22. *Klebsiella pneumoniae* was isolated from the nasal swab of crewmember 1 on day 30 (exit) and also from crewmember 2 on day 7 and day 30. *Streptococcus agalactiae* was isolated in low numbers from one urine sample on day 30. *Pseudomonas aeruginosa* was isolated from the throat swab of crewmember 4 on day 30. *Candida albicans* was isolated from crewmember 2's fecal sample at pre-entry and from the urine and fecal sample on day 30. It was also isolated from the throat and feces of crewmember 3 at pre-entry and from the feces on day 30. *Candida albicans* was isolated from the throat and urine of crewmember 4 at pre-entry and from the urine and feces on day 30.

Table 4.3-5 *Microorganisms isolated from Phase II crewmembers*

		SAMPLE PERIOD			
Crewmember	Sample	Pre-entry	Day 7/8	Day 22	Exit – Day 30
1	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, non-hemolytic No fungi isolated	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Staphylococcus* species, not *aureus* *Corynebacterium* species	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic *Neisseria* species No fungi isolated
	Nasal	*Staphylococcus aureus* *Staphylococcus* species, not *aureus* *Cladosporium* species	*Staphylococcus aureus*	*Staphylococcus aureus* *Corynebacterium* species	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Klebsiella pneumoniae* No fungi isolated
	Urine	No bacteria isolated No fungi isolated	No sample collected	No sample collected	*Streptococcus agalactiae* No fungi isolated
	Feces	Normal enteric flora *Aspergillus* species	No sample collected	No sample collected	Normal enteric flora *Trichosporon* species
2	Throat	*Streptococcus* species, alpha-hemolytic *Corynebacterium* species *Staphylococcus* species, not *aureus* *Neisseria* species *Cladosporium* species	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic *Neisseria* species	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic *Neisseria* species No fungi isolated

Table 4.3-5 continued *Microorganisms isolated from Phase II crewmembers*

		SAMPLE PERIOD			
Crewmember	Sample	Pre-entry	Day 7/8	Day 22	Exit – Day 30
2	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Cladosporium* species	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Klebsiella pneumoniae*	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Klebsiella pneumoniae*	*Staphylococcus aureus* *Corynebacterium* species No fungi isolated
	Urine	*Enterococcus faecalis* *Staphylococcus* species, not *aureus* No fungi isolated	No sample collected	No sample collected	*Staphylococcus* species, not *aureus* *Candida albicans*
	Feces	Normal enteric flora *Candida albicans* *Rhodotorula rubra* *Penicillium* species	No sample collected	No sample collected	Normal enteric flora *Candida albicans*
3	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Staphylococcus aureus* *Corynebacteria* species *Candida albicans*	*Streptococcus* species, alpha-hemolytic *Staphylococcus* species, not *aureus* *Neisseria* species	*Streptococcus* species, alpha-hemolytic *Micrococcus* species *Staphylococcus aureus*	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic *Neisseria* species *Haemophilus parainfluenzae* No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated	*Staphylococcus* species, not *aureus* *Corynebacterium* species	*Staphylococcus* species, not *aureus* *Corynebacterium* species	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Urine	*Staphylococcus* species, not *aureus* *Lactobacillus* species No fungi isolated	No sample collected	No sample collected	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans* *Rhodotorula rubra* *Penicillium* species	No sample collected	No sample collected	Normal enteric flora *Candida albicans*
4	Throat	*Streptococcus* species, alpha-hemolytic *Corynebacterium* species *Streptococcus* species, nonhemolytic *Neisseria* species *Candida albicans*	*Streptococcus* species, alpha-hemolytic *Neisseria* species, *Streptococcus* species, nonhemolytic	*Streptococcus* species, alpha-hemolytic *Pseudomonas aeruginosa*	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Bacillus* species No fungi isolated

Table 4.3-5 continued *Microorganisms isolated from Phase II crewmembers*

		SAMPLE PERIOD			
Crewmember	Sample	Pre-entry	Day 7/8	Day 22	Exit – Day 30
4	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated	*Staphylococcus* species, not *aureus* *Corynebacterium* species	*Corynebacterium* species *Staphylococcus* species, not *aureus*	*Micrococcus* species *Corynebacterium* species *Staphylococcus* species, not *aureus* *Aspergillus* species
	Urine	*Staphylococcus* species, not *aureus* *Enterococcus faecalis* *Candida albicans*	No sample collected	No sample collected	*Streptococcus* species, non-hemolytic *Staphylococcus* species *Lactobacillus* species *Candida albicans*
	Feces	Normal enteric flora No fungi isolated	No sample collected	No sample collected	Normal enteric flora *Candida albicans*

Crewmember (Backup)	Sample	Pre-entry
5	Throat	*Streptococcus* species, alpha-hemolytic *Corynebacterium* species *Haemophilus parainfluenzae* *Neisseria* species *Bacillus* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Urine	*Streptococcus* species, alpha-hemolytic *Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora *Candida albicans* No ova or parasites observed
6	Throat	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Micrococcus* species *Neisseria* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Epicoccum nigrum* *Acremonium* species
	Urine	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora No fungi isolated

Crewmember (Backup)	Sample	Pre-entry
7	Throat	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Candida albicans*
	Nasal	No bacteria isolated No fungi isolated
	Urine	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora *Candida albicans*
8	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Staphylococcus* species, not *aureus* *Haemophilus* species, not *influenzae* No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Bacillus* species *Staphylococcus aureus* *Corynebacterium* species *Cladosporium* species
	Urine	*Enterococcus faecalis* *Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora No fungi isolated

Phase IIa

The greatest numbers of bacteria during Phase IIa were found at sample sites near wet areas such as the sink, urinal, and shower, and the greatest numbers of fungi were found on the air vents and carpet samples. The numbers of microorganisms detected in surface and air samples remained relatively constant for Phase IIa. The second floor, which contained only air revitalization equipment, had the fewest bacteria and fungi during the Phase IIa study. The highest numbers of fungal species were found before and after crew entry and exit. The number of fungal genera isolated from all samples ranged from zero to five during habitation, with the most common genera being *Penicillium, Cladosporium,* and *Aspergillus.* The bacterial levels from carpet decreased during the Phase IIa study, while the fungal levels remained consistent.

While air samples collected on the first and third floors had similar bacterial concentrations, samples from the second floor had fewer bacteria. Levels of airborne fungi were greatest on the third floor. The genera *Penicillium, Cladosporium,* and *Aspergillis* were commonly collected.

During Phase IIa, no coliforms or anaerobic bacteria were isolated in the potable water system. Bacterial concentration generally remained below 100 CFU/100 ml (Figure 4.3-6). The primary isolate during Phase IIa was *Burkholderia cepacia*. Other species included *Burkholderia picketti, Acenitobacter calcaoceticus, Burkholderia pseudomallei,* and *Staphylococcus saprophyticus*.

During Phase IIa, an evaluation was performed of biofilm accumulation on metal coupons exposed to condensate in the air handling system. Coupons were either uncoated or coated with a biocidal coating. The coated coupons had less bacterial growth than uncoated coupons (Table 4.3-6). The numbers of attached bacteria increased with time on both types of coupons. The types of bacteria found on both coupon types were common water-associated species including *Bacillus brevis, Burkholderia picketti, Methylobacterium rhodinum,* and *Sphingomonas paucimobilis.* The diversity of bacterial flora on the coated coupons was less compared to the uncoated coupon. The biocide coating also reduced the loading on coupons by at least two-log fold after 60 days of exposure to the Phase IIa condensate, although the biocide coating did not eliminate biofilm formation. The bacterial numbers for the day 30 coupons had a predominance of *Bacillus* on both coupon types, which may result from a resistance of spores to the biocide. Fungal loading seemed to be less affected by the coupon coating than the bacteria, which like bacterial spores may be a result of reduced sensitivity of fungal spores to biocide action. The most predominant genera included *Aspergillis, Penicillium,* and *Trichosporon*. The initial increase in fungal concentration from day 2 to day 30 was followed by a subsequent decrease at day 60.

Table 4.3-6 *Microbial concentration on stainless steel coupons in the CHX condensate biofilms during Phase IIa*

	Bacterial Count (CFU/coupon)		**Fungal Count (CFU/coupon)**	
Day	Coated	Uncoated	Coated	Uncoated
0	NG	NG	NG	NG
2	NG	6.3×10^4	NG	NG
30	2.0×10^3	3.0×10^3	3.8×10^3	1.2×10^4
60	3.6×10^4	2.4×10^6	6.0×10^1	5.3×10^3

NG = No growth

Crew microbiology results from Phase IIa were collected from eight subjects, and numbers 1, 2, 3, and 4 were chosen as crewmembers (Table 4.3-7). *Staphylococcus aureus* was recovered from the throat swab of crewmembers 3 and 4 at pre-entry. It was also recovered from the nasal swab of crewmember 1 and from the throat swabs of crewmembers 2 and 3 on day 60 (exit). *Candida albicans* was recovered at pre-entry from the feces of crewmember 2 and from the throat swab and feces of crewmembers 3 and 4. It was recovered on day 60 from the throat swabs of crewmembers 1, 2, and 3 and from the nasal swab of crewmember 4.

Table 4.3-7 *Microorganisms isolated from Phase IIa crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit – Day 60
1	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Staphylococcus* species, not *aureus* No fungi isolated	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Candida albicans*
	Nasal	*Corynebacterium* species *Staphylococcus* species, not *aureus* No fungi isolated	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Staphylococcus aureus* No fungi isolated
	Urine	*Corynebacterium* species No fungi isolated	No bacteria isolated No fungi isolated
	Feces	Normal enteric flora *Rhodotorula* species *Trichosporon* species	No sample collected

Table 4.3-7 continued *Microorganisms isolated from Phase IIa crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit – Day 60
2	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic No fungi isolated	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Corynebacterium* species *Staphylococcus aureus* *Candida albicans*
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Micrococcus* species No fungi isolated	*Corynebacterium* species *Staphylococcus* species, not *aureus* No fungi isolated
	Urine	*Lactobacillus* species *Corynebacterium* species *Staphylococcus* species No fungi isolated	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected
3	Throat	*Corynebacterium* species *Staphylococcus aureus* *Candida albicans*	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Staphylococcus aureus* *Candida albicans*
	Nasal	*Staphylococcus* species, not *aureus* No fungi isolated	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Urine	*Staphylococcus* species, not *aureus* No fungi isolated	*Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected

Table 4.3-7 continued *Microorganisms isolated from Phase IIa crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit – Day 60
4	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Staphylococcus aureus* *Candida albicans*	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated	No bacteria isolated *Candida albicans*
	Urine	No bacteria isolated No fungi isolated	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected

Crewmember (Backup)	Sample	Pre-entry	Crewmember (Backup)	Sample	Pre-entry
5	Throat	*Neisseria* species *Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Enterobacter gergoviae* No fungi isolated	6	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Corynebacterium* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Micrococcus* species *Klebsiella pneumoniae* No fungi isolated		Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Staphylococcus aureus* No fungi isolated
	Urine	No bacteria isolated No fungi isolated		Urine	No sample collected
	Feces	Normal enteric flora No fungi isolated No ova or parasites seen		Feces	No sample collected

Table 4.3-7 continued *Microorganisms isolated from Phase IIa crewmembers*

Crewmember (Backup)	Sample	Pre-entry	Crewmember (Backup)	Sample	Pre-entry
7	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Staphylococcus* species, not *aureus* No fungi isolated	8	Throat	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Staphylococcus* species, not *aureus* No fungi isolated
	Nasal	*Staphylococcus aureus* *Staphylococcus* species, not *aureus*		Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species
	Urine	No bacteria isolated No fungi isolated			*Streptococcus* species, alpha-hemolytic No fungi isolated
	Feces	Normal enteric flora *Candida albicans*		Urine	*Lactobacillus* species *Staphylococcus* species No fungi isolated
				Feces	Normal enteric flora No fungi isolated

Phase III

Air, surfaces, and carpet were sampled immediately before entry (day 0), after 2, 25, 45, 65 days, and upon egress (day 91). A large number of bacterial species were detected on day 0 before closing the chamber but not isolated again throughout the study. These included several bacteria of medical importance, such as *Klebsiella* species*, Serratia marcescens,* and *Enterobacter gergoviae*.

In general, microbial levels from most of the surfaces were low. Dramatic changes in bacterial levels were exemplified by the third-floor air vent, third-floor sink, and first-floor urinal (Table 4.3-8). Fungal levels on tested surfaces fluctuated, although they remained low throughout the test. The only exception was a slight increase on day 65 at the third-floor sink. Carpet samples displayed relatively low bacterial counts on day 0, then rose rapidly at all sites, with counts remaining over 10^7 CFU/m^2 throughout the majority of the test. Fungal levels in carpet samples were initially high but decreased below 10^2 CFU/m^2 after day 45 and did not increase even upon egress (day 91). The bacteria identified from surface samples included a wide diversity of genera including *Bacillus, Corynebacterium, Staphylococcus,* and *Micrococcus*. No fungal genera appeared to dominate the surface flora. The wide variety of microbial flora collected was illustrated by analysis of the first-floor carpet (Table 4.3-9).

Table 4.3-8 *Microbial contamination (CFU/cm²) at selected surface sites during Phase III*

		Day					
Sample site		**0**	**2**	**25**	**45**	**60**	**91**
Third-floor air vent	Bacteria	15	30	0	0	8	1,500
	Fungi	0	0	0	0	0	15
Third-floor sink	Bacteria	30	1,600	170	680	1,700	210
	Fungi	0	0	75	NC	315	30
First-floor urinal	Bacteria	7	8	8	8	40	900
	Fungi	0	0	0	0	0	30

NC = not collected

Table 4.3-9 *Microbial diversity and occurrence identified from the first-floor carpet during Phase III*

	Day					
Bacteria	**0**	**2**	**25**	**45**	**65**	**91**
Bacillus sp.	X	X		X		X
Bacillus azotoformans					X	
Bacillus brevis	X	X	X			X
Bacillus coagulans				X		
Bacillus licheniformis	X	X		X	X	X
Bacillus megaterium	X	X				
Bacillus mycoides	X					
Bacillus pasturii	X					
Bacillus pumilus	X	X		X		
Bacillus sphaericus		X				
Bacillus subtilis varglobigii	X					
Chryseomonas luteola	X					
Corynebacterium sp.		X	X	X		X
Corynebacterium afermentanis				X	X	
Corynebacterium aquaticum A	X					
Corynebacterium pseudodiptherticium			X			
Enterobacter agglomerius grp 3B		X				
Enterobacter gergoviae	X	X				
Erysipelothrix rhasiophthiae/tonsialum						X
Kingell kingae		X				
Rothia dentrocarios		X				

Table 4.3-9 continued *Microbial diversity and occurrence identified from the first-floor carpet during Phase III*

	Day					
Bacteria	**0**	**2**	**25**	**45**	**65**	**91**
Serratia marcesans	X					
Staphylococcus capitis					X	
Staphylococcus capitis ss *ureolyticus*		X	X			
Staphylococcus caprae					X	
Staphylococcus epidermidis A			X	X		
Staphylococcus haemolyticas				X		
Staphylococcus simulans						X
Fungi						
Acremonium sp.		X		X		
Alternaria sp.	X					
Aspergillus sp.			X		X	
Aspergillus flaus					X	
Candida parapulois				X		
Cladosporium sp.	X	X			X	
Cryptococcus laurentii						X
Curvularia sp.			*X*			
Fusarium sp.			X			
Hyphomycete	X			X		
Penicillium sp.		X				
Rhodotorula sp.	X	X				
Unidentified yeast	X	X				

Throughout the testing period, air sampling indicated low bacterial counts, with the exception of a sharp increase at all sites on day 25. The only bacterial species common to all air samples on day 25 was *Staphylococcus hominis*, a common skin flora. Fungal counts in air samples displayed low levels except for the pre-entry samples on day 0. The identification of bacterial isolates indicated that most bacteria found in the air, surface, and carpet were from the genera *Bacillus, Corynebacterium,* and *Staphylococcus*. The total number of bacterial species in the chamber, as a whole, decreased rapidly after two days but leveled off after day 25. Individual bacterial species fluctuated. The fungal genera *Penicillium, Cladosporium,* and *Aspergillis* were predominant.

Burkholderia cepacia, the most common isolate from Phases I and IIa studies, was again detected in the potable water tank samples, but it was not the most common isolate. In Phase III, *Flavobacterium meningosepticum,* a bacterium that is nonpathogenic in adults, was detected most often. This bacterium was not isolated in other studies. Total heterotrophic counts were within specification during Phase III testing, with the exception of one slight overage of 108 CFU/100 ml (Figure 4.3-6). No coliforms or anaerobes were detected in any of the water samples analyzed during the testing period. On and after day 61, various *Bacillus* species were detected in the water system.

In crew microbiology results from Phase III, *Candida albicans* was recovered pre-entry from the throat swabs and feces of crewmembers 3 and 4 and from the feces of crewmember 8 (Table 4.3-10). On day 91 (exit), *Escherichia coli* and *Enterococcus faecalis* were recovered from the crewmember 2 urine sample in low numbers.

Table 4.3-10 *Microorganisms isolated from Phase III crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit - Day 90
1	Throat	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species No fungi isolated	*Neisseria* species *Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Corynebacterium* species No fungi isolated
	Nasal	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Cladosporium* species *Penicillium* species Hyphomycete	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Urine	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Lactobacillus* species No fungi isolated	*Corynebacterium* species *Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans* *Trichosporon* species	No sample collected

Table 4.3-10 continued *Microorganisms isolated from Phase III crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit - Day 90
2	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species No fungi isolated	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Corynebacterium* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Cladosporium* species *Streptomyces* species	*Neisseria* species *Streptococcus* species, alpha-hemolytic No fungi isolated
	Urine	*Lactobacillus* species *Staphylococcus* species No fungi isolated	*Escherichia coli* *Enterococcus faecalis* *Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora	Normal enteric flora with few *Staphylococcus aureus* No fungi isolated

Table 4.3-10 continued *Microorganisms isolated from Phase III crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit - Day 90
3	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Candida albicans*	*Neisseria* species *Streptococcus* species, alpha-hemolytic *Staphylococcus aureus* No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Cladosporium* species	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Aspergillus* species
	Urine	No sample collected	*Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected
4	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Candida albicans*	*Neisseria* species *Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Corynebacterium* species No fungi isolated
	Nasal	*Corynebacterium* species *Staphylococcus* species, not *aureus* No fungi isolated	*Staphylococcus aureus* *Corynebacterium* species No fungi isolated
	Urine	*Corynebacterium* species *Staphylococcus* species *Lactobacillus* species No fungi isolated	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected

Discussion

The information collected during the closed-chamber studies strongly indicates a connection between the concentration and diversity of the microbial flora and the presence of the crew and plant life. The presence and activity of the support personnel before or after each study caused microbial levels to fluctuate and often increase. In addition, the presence of medically significant bacteria before Phase III, but not during the study, suggests the contribution of microorganisms by human interaction before the study. Sharp fluctuations in microbial concentrations, such as the day 25 increase during Phase III, was quite possibly due to human activity, although an exact cause has not been established. The microbial flora during the Phase I study dramatically shows the influence of plants in an isolated environment, as the predominant bacterial species were mostly plant-associated bacterial genera such as *Clavibacter* and *Curtobacterium*. The connection between microorganisms and other life does not imply as great of an influence. While bacterial loads in both the habitation and plant growth chambers during Phase I increased with length of human presence, the fungal loads decreased during the same period. In addition, microbial loads in Phases II, IIa, and III followed unique patterns of changes in concentration over time. The trends in microbial concentration and diversity confirmed the need for standards to insure microbial control, but also suggested a futility in attempts to "sterilize" the environment prior to occupancy.

Surface samples taken from areas with direct contact with water, such as sinks, were compared to samples from air vents which remained relatively dry. While, intuitively, the wet areas should have higher microbial populations than the drier areas, neither of these sets of surfaces consistently maintained higher microbial counts based upon a comparison of Phase IIa and Phase III data (Table 4.3-11). Compared to the overall surface sample averages, certain wet sites did display high bacterial levels during both studies, such as samples from the sink which averaged 20.87 ± 9.26 CFU/cm^2 during Phase IIa and 41.50 ± 14.77 CFU/cm^2 during Phase III. Other wet sites displayed high levels in one study, but not both. For example, samples from the first-floor urinal displayed high levels during Phase IIa, averaging 34.10 ± 12.12 CFU/cm^2, but only averaging 0.63 ± 0.33 CFU/cm^2 during Phase III. The inconsistency of the comparison of wet and dry areas was probably the result of housekeeping patterns among the crews. Surface samples taken from carpet displayed high microbial levels, reinforcing the need for judicious use of carpeting in self-contained systems.

Table 4.3-11 *Average microbial concentration and diversity from surface samples during Phases IIa and III*

	Phase IIa		Phase III	
Site	**Bacteria**	**Fungi**	**Bacteria**	**Fungi**
Air Vents				
Average Level (CFU/cm^2)	11.5 ± 3.0	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2
Average Species or Genera (CFU/cm^2)	10.9 ± 1.6	3.1 ± 0.4	3.5 ± 1.1	1.9 ± 0.6
Wet Areas				
Average Level (CFU/cm^2)	16.7 ± 4.2	0.03 ± 0.02	10.1 ± 4.6	1.1 ± 0.7
Average Species or Genera (CFU/cm^2)	10.2 ± 0.9	0.6 ± 0.4	8.6 ± 2.4	2.0 ± 0.6

In both studies, certain bacterial species dominated both air and surface samples. Of the 42 species isolated in air and surface samples throughout all of Phase IIa, 15 were isolated at more than one site and 10 were isolated at more than two. Seven of these species were isolated on every floor of the chamber. Of the 31 species isolated in air and surface samples throughout all of Phase III, 12 were isolated at more than one site and six were isolated at more than two. Only four of these species were isolated on every floor of the chamber. Certain bacterial species, such as *Bacillus brevis* during Phase IIa, were isolated from surface samples on every floor, but never from air samples. Other bacteria, such as *Staphylococcus capitis* during Phase III, were found in air samples but not isolated from every floor. These findings indicate that bacteria are transient in nature and do not necessarily maintain their presence indefinitely.

The pattern of bacterial appearance during the studies varied. Of the common isolates, most could be detected in at least one site during each sampling period. However, certain bacteria were detected during only one sampling period, such as the detection of *Bacillus azotoformans* on all three floors during day 65 of Phase III. No particular microbial species were associated with either the dryer air vents or the wet areas. The pattern of bacterial dominance and unexpected appearance were likely the result of cleaning patterns and human traffic.

The identification of fungal isolates indicated a large variety of contaminants with no single genera dominating the environment throughout the test. However, the genera *Penicillium, Cladosporium,* and *Aspergillis* were common during all studies. The total number of genera decreased over time, although no particular

fungi appeared to endure better than the others. As with the pattern displayed by the bacteria, the presence of fungi over time appeared to be affected by cleaning patterns and human traffic. The efficiency of the air system to remove fungi from the air, and subsequently surface samples, may have also contributed to the decreased fungal levels.

The lack of proliferation of bacterial species and numbers may also be a function of their interaction within the community. A nonlinear relationship between bacterial concentration and diversity on surface samples during Phase IIa and Phase III suggests a repression of the proliferation of new species after bacterial concentration reaches a certain limit (Figure 4.3-7). For these studies, the concentration where repression began was 5 to 10 CFU/cm^2.

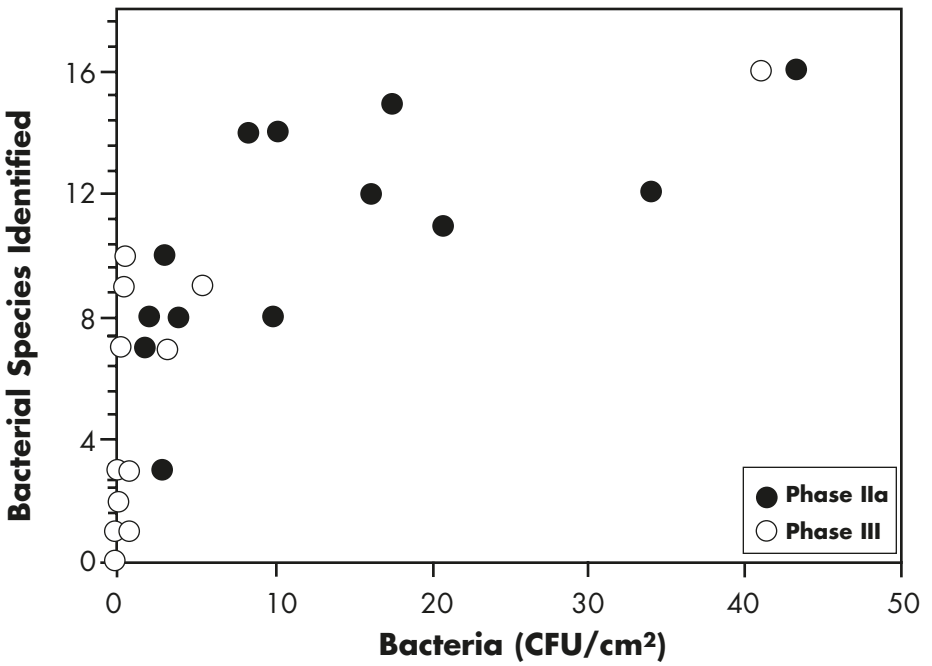

Figure 4.3-7 *Bacterial concentration (CFU/cm^2) versus diversity at surface sampling sites during Phases IIa and III*

Potable water concentrations were affected by varying disinfectant levels, and thus microbial trend analysis was difficult. Bacterial diversity in the potable water from the Phase III study was greater than other studies. For example, during Phase IIa *Burkholderia cepacia,* isolated in 39.4% of the samples, was the predominant bacterial contamination during the Phase IIa study. Only five other bacterial species were detected and appeared in 13.5% of the samples. During Phase III, *Flavobacterium meningosepticum* (found in 33.3% of the samples), *Stentrophomonas*

maltophilia (11.8%), *Burkholderia cepacia* (11.8%), and several species of *Bacillus* (11.8%) were most commonly detected. The identified *Bacillus* species, *subtilis*, *licheniformis*, and *pumilus*, were detected only after day 60. Since no gram-positive rods were detected during the 60-day Phase IIa water study, their presence in Phase III may be the result of either changes in the water systems, levels of disinfection, or the additional 31-day duration of the Phase III study.

Overall the microbiota isolated from the crew was characteristic of healthy individuals. Organisms such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are opportunistic pathogens that may cause disease in immunocompromised hosts but not healthy individuals. *Staphylococcus aureus* is frequently a resident of the nasal passages, and *Candida albicans* is a ubiquitous yeast that is found as normal flora of the alimentary tract and mucocutaneous membranes. No clinical symptoms were experienced by the crewmembers as a result of the presence of any of these organisms. No ova or parasites were seen in any of the crewmembers, and all viral cultures were negative. No significant changes were found in the body flora during Phases II, IIa, or III.

Several studies focused on potential biodegradation and methods of prevention. Coating the stainless steel coupons from Phase I with biocides temporarily controlled microbial biofilm formation. However, the coating did not eliminate biofilm formation. The major organism in all the samples taken during this study was *Methylobacterium*, a common water bacterium. During Phase II, biofilm-forming bacteria were identified using PLFA. These bacteria were controlled with iodine disinfection, although the iodine did not remove the biological materials from the coupon surface as measured with the lipid analysis.

In addition, the ability of viral contaminants to survive the water treatment system was evaluated using bacteriophages MS-2 and PDR-1. These viruses were chosen because of their similarity to the human enterovirus (MS-2) and rotavirus (PRD-1). Both of these viruses have been used in other municipal studies as they absorb poorly to flocculated material and thus must be removed by the filtration mechanisms within the water purification system. The advanced water purification techniques completely removed the viral particles from the recycled potable water to below the detection limit of this assay.

SIGNIFICANCE

These studies confirm a generally accepted relationship between microorganisms and other living organisms in a closed ecosystem. Disinfection and cleaning patterns changed this equilibrium as displayed by shifts in both microbial concentration and identity. In a small ecosystem, such as the chambers, the relationship is more dramatically affected by the actions of participants and thus more difficult to control. This understanding applies not only to NASA spacecraft, but also other small confined areas such as office buildings. Further complicating the ecosystem is the microbial interrelationship. On surfaces, these studies suggest a competitive

inhibition that limits bacterial diversity. This finding could be of great importance in decontamination, as a sterile surface may be a more fertile breeding ground than expected for different, possibly pathogenic microorganisms. The number of potential microorganisms at any given site is large, and thus continued sampling and analysis must continue to gain a better understanding of the diversity of microbial flora, their interrelationship, and the effect of human activities on the consortium.

These studies showed the ability of advanced water recovery systems to microbially purify water to potable quality, including the removal of viruses. However, the purification process appears to be susceptible to bacterial biofilm formation and potential corrosion problems. The appearance of gram-positive bacteria after 60 days during Phase III suggests a major flora change. Future work should focus on the effect of recycling water on microbial flora after extended periods of time.

Perhaps the most intriguing need for future research may be the changes that occur in the microorganism in a confined environment. Both bacteria and fungi adapt to changes in their environment, and genotypic and phenotypic alterations should be expected. Environmental stresses such as the addition of disinfectants or possibly the proximity of several microbial populations to each other could cause eventual changes in phenotypic characteristics including antimicrobial resistance and virulence. As questions of this nature are answered in closed chambers, the safety and health of the astronauts will be ensured for future long-term missions.

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4.4

Crew Food Systems

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SUMMARY

The Advanced Life Support (ALS) chamber tests provided a unique opportunity to evaluate future space food systems and to assess potential problems associated with conversion of chamber-grown plants to edible foods. The JSC Space Food Systems Laboratory (hereafter referred to as the Food Lab) provided food support for all of the life support tests. The objective for the first two tests was to provide a nutritionally sound and acceptable food system with the limited food preparation and storage facilities in the chambers. Crew sensory evaluations, supermarket surveys, menu planning, and nutritional analyses were completed to accomplish the objective. The 15-day Phase I food system consisted of shelf-stable food and a microwave oven. Phase II evaluated an abbreviated International Space Station (ISS) food system with microwave ovens, a freezer, and a refrigerator. The objective of the Phase IIa mission was to evaluate a more advanced ISS food system. The food system for the 91-day Phase III test evaluated a potential regenerative food system. A 50 percent plant-based diet was tested for 81 days, and a menu with 90 percent of the calories derived from the approved crops to be grown was evaluated for 10 days. All of the objectives of the food systems were met and/or exceeded for all the tests.

The results of these food system tests established that a 50 percent frozen food system with microwave heating for food preparation was an optimum combination of food preservation technologies for maintaining an acceptable food system. This concept will continue to be followed for the ISS food system.

The 10-day BIO-Plex test verified that a menu can be developed from the basic crop list. This menu is acceptable for a crew for 10 days, and most of the nutritional requirements are attained. The drawback involves the fact that a plant-based diet is very labor intensive with excessive waste. Comprehensive research is needed in the areas of food processing and preparation in an enclosed environment.

Introduction

Food systems for the chamber studies became more complex, with additional objectives for each progressive chamber test. The JSC Food Lab provided food support for all of the life support tests. The food system being planned for the ISS is 50 percent frozen/refrigerated with microwave/convection heating for food preparation. The ALS chamber tests provided a unique opportunity to verify this concept with an isolated crew in a closed environment. The basic objective for the first test was to supply a shelf-stable food supply for one person for 15 days. Additional crewmembers were added for the subsequent missions and additional requirements were placed on the food system. Menus were planned based upon crew interviews, food questionnaires, and available food. Most of the food was procured from local supermarkets and passed into the chamber on a routine schedule. Crewmembers performed sensory evaluations on the food while in the chamber, and crew debriefs were held after each test to gain feedback.

Methods

Early Human Test Initiative (EHTI) Phase I

The food system for the Phase I test consisted of shelf-stable foods that were heated in a microwave oven. The menu was based upon food sensory evaluations conducted with the prime and back up crewmembers, food questionnaires, and crew interviews. Food from local supermarkets and Shuttle vendors was used to make up the diet. Recommended dietary allowances (RDA) were used as the dietary requirements (1).

Early Human Test Initiative Phase II

Facilities, Nutritional Requirements, Sensory Evaluations, and Menu

A combination freezer/refrigerator and two microwave ovens (600 watts each) were included in the chamber for the Phase II food system. The main objective of the food system was to evaluate the use of frozen food and microwave heating in an isolated environment as was being planned for the ISS. The prime and back-up crews completed food frequency and food questionnaires, and the results of the questionnaires were used to develop a supermarket survey to determine available foods. Two sensory evaluations on potential foods were conducted with the crewmembers using a nine-point hedonic scale where nine equals “like extremely” and one equals “dislike extremely” (2). Data from the sensory evaluations were used to develop a menu based upon crew preferences. Caloric requirements were calculated for each crewmember based on the World Health Organization (WHO) equations and the duration of the daily exercise (3). Macronutrient percentages

(carbohydrate, protein, and fat) were calculated based on the nutritional requirements for ISS missions up to 360 days (4). The menu was designed as a 10-day cycle to repeat three times during the 30-day chamber test. Each crewmember had a standard menu with some minor deviations to accommodate personal preferences. The crew was allowed to make substitutions to the menu during the test if desired. The daily menu included breakfast, lunch, dinner, and two snacks. The breakfast meal consisted of milk or juice, tea/coffee, fruit, and cereal or a bread product. The lunch meal consisted of a salad, a microwaveable lunch item or lunch-style food, a bread product, and a beverage. The dinner meal included a microwaveable dinner entrée, a microwaveable vegetable, and a dessert. Snacks included fruit, nuts, popcorn, granola bars, and yogurt.

The menu, including planned snacks, was approximately 2,200 kcal for the male subjects and 2,000 kcal for the female subject. Approximately 12 to 15 percent of calories came from protein, 50 to 55 percent from carbohydrate, and 30 to 35 percent from fat. The four test subjects were provided copies of their menus with the nutritional content, a list of commercial products available for substitution, and a sensory evaluation form to be completed during the test.

The food was made up of 16 percent fresh, 49 percent refrigerated/frozen, and 35 percent shelf-stable foods. Most of the food was stored outside the chamber and passed into the chamber through the airlock daily.

Advanced Human Life Support Enclosed System Study Phase IIa

Chamber Test as a Ground-Based Analogue for the ISS Food System

The main objective for the Phase IIa food system was to evaluate the proposed ISS food system by emulating it as close as possible in the chamber test. The food preparation hardware inside the chamber was the same as the Phase II test except the microwave ovens were 1,000 watts each instead of 600. The menu was developed by identifying commercial products that were comparable to the ISS food list in supermarkets and in the Nutritionist III database (5). A standard menu was created using the ISS standard menu and the commercial products version of the ISS food list. Prior to the test, two sensory evaluations were held with the crew. Approximately 20 commercial foods were evaluated at each session. The crewmembers were provided the ISS standard menu, the food list, food questionnaire, and a copy of their sensory results. This information was used by each crewmember to develop an individual 20-day cycle personal preference menu for the Phase IIa test. A follow-up meeting was held with the crew to discuss their personal menus, make minor nutritional adjustments, and determine the pantry contents. The pantry was a 20 percent planned overage that the crew could use for substitution. Caloric requirements and macronutrient percentages were calculated as in Phase II.

Each crewmember had a personalized menu, but on three occasions the crew shared a common "theme" meal at dinner. Menu analysis was performed using the Nutritionist III database (4). The menus consisted of approximately 3,000 kcal for the male subjects and 2,100 kcal for the female subject. Approximately 12 to 15 percent of calories came from protein, 50 to 55 percent from carbohydrate, and 30 to 35 percent from fat. The subjects were provided copies of their menus with nutritional content, a list of commercial products available for substitution, and sensory evaluation sheets to be performed during the test.

The food was made up of 15 percent fresh, 50 percent refrigerated/frozen, and 35 percent shelf-stable foods. Most of the frozen food was stored outside the chamber and passed into the chamber through the airlock. Forty days of shelf-stable food was stored inside the chamber prior to start, and was replenished on day 40. Sensory evaluations using the nine-point hedonic scale were completed every Friday by the crewmembers. The evaluation forms were passed into the chamber weekly.

Lunar-Mars Life Support Test Project Phase III

The overall objectives of the food system for Phase III were to:

a) Emulate a food system for a long-duration space mission
b) Determine the acceptability of the menu over the 91-day duration of the test
c) Examine the ease and sufficiency of food preparation and processing
d) Provide a safe food system which supplies a nutritious menu for the crewmembers
e) Render a food system that may be monitored and controlled to support medical experiments
f) Satisfy the physiological needs and the psychological food-related needs of the crew
g) Test a 10-day BIO-Plex menu to identify its acceptance in a closed-system environment that simulates long-term habitation in the ALS test bed facilities.

The objectives of the 10-day BIO-Plex portion of the study were to measure acceptance and determine if the nutritional needs could be met with a plant-based diet that incorporated a large portion of the ALS crops into the food system.

The Phase III 91-day food system was divided into two sections, the 81-day food system (September 19 to December 19, 1997) and the 10-day food system (October 20-29, 1997). The 81-day portion of the test provided a 50 percent plant-based diet (four or less servings of meat per week) utilizing a food system comprised of fresh, frozen, and thermostabilized foods. The Growth Apparatus for the Regenerative Development of Edible Nourishment (GARDEN), from Quantum Devices, Inc., Barneveld, WI, provided fresh Waldman's green lettuce. The GARDEN supplied the crew with four heads of lettuce every 10 days throughout the 91-day test,

including one harvest of lettuce during the 10-day BIO-Plex menu test.

A full-size, side-by-side refrigerator/freezer (67.6 x 31.5 x 32.5 in.) was provided inside the chamber. The unit held three days of frozen food and a one-week supply of fresh food, as well as several miscellaneous refrigerated food items. A chest freezer (29.5 x 41.5 x 22.5 in.) was available outside of the chamber for a one-week supply of frozen food storage. Storage was available for shelf-stable food items and dishware in a stainless steel cabinet (24 x 30 x 18 in.) inside the chamber. This cabinet also housed the microwave/convection oven. Additional space was located behind the television (37 x 86 x 12 in.) on the first floor; this storage space was used for shelf-stable food items and paper goods. A bread maker, blender, stovetop burner, and microwave/convection oven were provided in the chamber.

In addition, the Variable Pressure Growth Chamber at Johnson Space Center provided wheat during the test. The wheat berries were harvested and dried prior to the 10-day BIO-Plex menu. The dried wheat berries were processed into flour, mixed with other ingredients, and individually bagged for use in three bread recipes (whole wheat, soy, and sweet potato bread) that were prepared by the crew. These bags were transferred into the chamber for use during the last half of the test. A second harvest of wheat berries occurred after the 10-day test, and those berries were also processed into flour and used by the crew for bread baking.

The Phase III food system was developed with the aid of food frequency and nutrition questionnaires. The questionnaires helped to determine the selection of food items, recipes, and menus, and also defined food preferences, allergies, and habits. The caloric requirements for the Phase III food system were calculated, using a moderate activity factor, based on the World Health Organization (WHO) equations as follows:

Men (30 - 60 years): 1.7 (11.6W + 879) = kcal/day required
Women (30 - 60 years): 1.6 (8.7W + 829) = kcal/day required
W = weight in kg

81-Day Menu Development

A preliminary menu was designed, and the prime and back-up crew evaluated 38 items in two food evaluation sessions to determine the acceptability of the menu. Evaluations were performed using a nine-point hedonic scale, in which acceptability was rated with nine being “like extremely” and one being “dislike extremely” (2). In addition, nine vegetarian Meal Ready-to-Eat (MRE) products were evaluated. Five of the nine MREs were rated acceptable for use (average of six or above on the nine-point hedonic scale).

In August, a prime crewmember was replaced with a back-up. Due to this adjustment, some items on the menu were changed to accommodate the new crewmember.

The 81-day caloric requirements were calculated for the standardized menu, and the menu was finalized. This menu used three types of foods: fresh, frozen, and shelf-

stable. The fresh food items included fruits, vegetables, fresh chamber lettuce, fresh bread, and various prepared food items. The frozen food supply included frozen food entrees, vegetables, fruits, desserts, and various beverages. Shelf-stable food items were vegetarian MREs, snacks, canned items, boxed mixes, beverages, and condiments. The 81-day menu was designed as a 20-day cycle (Table 4.4-1) that would repeat four times.

The crewmembers were given a standardized menu. This minimized the time required for food preparation and processing; however, the menu did provide fresh food items that required extra preparation and frozen items requiring thawing/heating ahead of time.

At the halfway point, Halloween, and on a crewmember's birthday, special treats were provided. A special holiday meal was prepared for the crew to be served on Thanksgiving (November 26). This meal included a roasted turkey that was prepared in the JSC Food Lab as well as numerous additional typical holiday foods.

The 81-day menu was analyzed using the Nutritionist III Database (4). The baseline menu without beverages or snacks provided approximately 1,900 kcal per day. On average, the menu supplied 13 percent of the calories from protein, 64 percent of the calories from carbohydrate, and 23 percent of the calories from fat.

The four crewmembers were provided with a copy of the menu for each 20-day cycle during the 81-day portion of the test. Each crewmember also received a sensory evaluation form every Friday during the test to evaluate the menu. The menu and sensory evaluation score sheets were sent to the crew via electronic mail.

Table 4.4-1 *Twenty-day sample of the 81-day menu*

DAY 1: Friday, Sept. 19	**DAY 2: Saturday, Sept. 20**	**DAY 3: Sunday, Sept. 21**
Cereal	Cereal	Belgian waffles (2 waffles)
English muffin (1 muffin)	**Toast	Fruit yogurt (4.4 oz container)
Margarine (1 t.)	Margarine (1 t.)	Margarine (1 t.)
Jelly/jam	Jelly/jam	Syrup (1/4 cup)
Milk/juice (8 oz)	Milk/juice	Milk/juice
Coffee/tea	Coffee/tea	Coffee/tea
Broccoli pasta salad (1 cup)	Macaroni and cheese	Spicy black bean burger (1 patty)
Grilled chicken sandwich	Broccoli spears (2 spears)	Sandwich bun (1 bun)
**Lettuce/tomato/onion	**Baby red potatoes (2 potatoes)	Baked potato chips (12 chips)
Kiwi slices (1 kiwi)	**Blushing pears (1/2 cup)	Dill pickle spear
Beverage	Beverage	**Salad
		Beverage
Cheese manicotti w/ tomato sauce	Turkey	Garlic buttered baked fish fillet (1 fillet)
Breadsticks (1)	Mashed potatoes	**Pasta accents, garlic herb (1 c. cooked)
Salad	Peas	**Soy bread
*Strawberry cheesecake (1/6 cheesecake)	**Whole wheat bread	Margarine
Beverage	Margarine	*Chocolate eclair (1 eclair)
	*Cherry cobbler (1/4 cobbler)	Beverage
	Beverage	

*Items need advanced thawing/heating
**Items need preparation

Table 4.4-1 continued *Twenty-day sample of the 81-day menu*

DAY 4: Monday, Sept. 22	**DAY 5: Tuesday, Sept. 23**	**DAY 6: Wednesday, Sept. 24**
Oatmeal (1 packet)	Cereal	Pancakes (3 pancakes)
*Strawberries (2/3 cup)	**Toast	*Blueberries (3/4 cup)
Milk/juice	Margarine (1 t.)	Margarine (1 t.)
Coffee/tea	Jelly/jam	Syrup (1/4 cup)
	Milk/juice	Milk/juice
	Coffee/tea	Coffee/tea
Won ton soup (1 container)	Tuna noodle casserole	Vegetable soup (~1 cup prepared)
Vegetable egg roll	Crinkle cut carrots (2/3 cup)	Saltine crackers (5 crackers)
Rice, white	Cantaloupe slices	**Grilled cheese sandwich
Snow pea pods (1/2 pkg)	Biscuit (1 biscuit)	Kiwi slices (1 kiwi)
Beverage	Margarine	Blue Bell cup (1 cup)
	Beverage	Beverage
Eggplant parmigiana	Vegetable bowl w/teriyaki rice	Roasted turkey w/gravy
*Garlic bread (1/6 loaf)	Apricot halves (1/2 cup)	Cornbread dressing
Salad	**Soy bread	Cut, whole carrots (3/4 cup)
*Lemon meringue pie (1/6 pie)	Margarine	Tropical fruit salad (1/2 cup)
Beverage	*Coconut cream pie (1/5 pie)	**Sweet potato bread
	Beverage	Margarine
		Beverage

*Items need advanced thawing/heating
**Items need preparation

Table 4.4-1 continued *Twenty-day sample of the 81-day menu*

DAY 7: Thursday, Sept. 25	**DAY 8: Friday, Sept. 26**	**DAY 9: Saturday, Sept. 27**
Cereal	Cereal	Cereal
Fruit yogurt (4.4 oz container)	English muffin (1 muffin)	Banana
Milk/juice (1 cup)	Margarine (1 t.)	Milk/juice
Coffee/tea	Jelly/jam	Coffee/tea
	Milk/juice (1 cup)	
	Coffee/tea	
Bean & cheese burrito (1)	Four-cheese pizza (1/4 pizza)	Fettuccine alfredo
Fiesta corn (1/2 cup)	Mandarin oranges (1/2 cup)	**Baby red potatoes (2 potatoes)
Tortilla chips (9 chips)	Salad	California style veggies (3/4 cup)
Picante sauce (2 T.)	Raspberry jelly roll	**Soy bread
Chocolate chip cookies (3)	Beverage	Margarine
Beverage		Beverage
Grilled lemon pepper fish fillet (1 fillet)	Steak or chicken	Beef fajita pocket or Turkey/broc/cheese pocket (1 pocket)
**Scalloped potatoes (1 cup)	**White beans and rice (3/4 cup)	Red beans & rice (1 MRE)
Mixed vegetables (2/3 cup)	Asparagus spears (8 spears)	Red grapes
Biscuit (1 biscuit)	Red grapes	*Chocolate chip cookie dough sundae
Margarine	**Soy bread	Beverage
*Key lime pie (1 slice)	Margarine	
Beverage	Beverage	

*Items need advanced thawing/heating
**Items need preparation

Table 4.4-1 continued *Twenty-day sample of the 81-day menu*

DAY 10: Sunday, Sept. 28	**DAY 11: Monday, Sept. 29**	**DAY 12: Tuesday, Sept. 30**
French toast (2 pieces)	Cereal	Cereal
Sliced peaches (1/2 cup)	Plum	Plain bagel (1)
Margarine (1 t.)	Milk/juice	Cream cheese (2 T.)
Syrup (1/4 cup)	Coffee/tea	Jelly/jam
Milk/juice		Milk/juice
Coffee/tea		Coffee/tea
Quiche Florentine (1 quiche)	Tomato bisque	Fried chicken w/gravy
**Pasta accents, garden herb (1 c. cooked)	(~1 cup prepared)	Mashed potatoes
Red grapes	Saltine crackers (5 crackers)	Corn
Beverage	**Grilled cheese sandwich	**Soy bread
	Tropical fruit salad (1/2 cup)	Margarine
	Beverage	Jell-O (1 Jell-o cup)
		Beverage
Harvest burger (1 patty)	Country roasted turkey	Cheese lasagna
**Lettuce/tomato/onion	Rice pilaf	California style veggies (3/4 cup)
Cheese (1 slice)	Pineapple chunks (1/2 cup)	**Whole wheat bread
Sandwich bun (1 bun)	Dinner roll (1)	Margarine
Western beans (1 MRE)	Margarine	*Flaky coconut layer cake
**Coleslaw (1 1/2 cups)	Fruit freeze (1)	Beverage
Blue Bell cup (1)	Beverage	
Beverage		

*Items need advanced thawing/heating
**Items need preparation

Table 4.4-1 continued *Twenty-day sample of the 81-day menu*

DAY 13: Wednesday, Oct. 1	**DAY 14: Thursday, Oct. 2**	**DAY 15: Friday, Oct. 3**
Cereal	Oatmeal (1 packet)	Cereal
**Toast	Fruit yogurt	*Raspberries (3/4 cup)
Margarine	Milk/juice	**Toast
Jelly/jam	Coffee/tea	Margarine
Milk/juice		Jelly/jam
Coffee/tea		Milk/juice
		Coffee/tea
Cream of broccoli soup	Won ton soup (1 container)	Corn on the cob (1/2 ear)
Harvest burger (1 patty)	Oriental rice w/ veggies (1 package)	Black-eyed peas & okra (1 MRE)
**Lettuce/tomato/onion	Broccoli spears (2 spears)	*Blueberries (3/4 cup)
Sandwich bun (1 bun)	Mandarin oranges (1/2 cup)	Angel food cake
Escalloped apples (1/2 package)	Blue Bell cup (1 cup)	Beverage
Beverage	Fortune cookies (3 cookies)	
	Beverage	
Bean & cheese burrito (1 burrito)	Bow tie pasta w/creamy tomato sauce	Steak or chicken
**Rice, Spanish (1 cup)	Asparagus spears (8 spears)	**Baked potato
Green beans (2/3 cup)	Pineapple chunks (1/2 cup)	Mixed vegetables
Sherbet (1/2 cup)	**Sweet potato bread	Dinner roll (1 roll)
Beverage	Fruit freeze (1)	Margarine
	Beverage	*Lemon meringue pie (1/6 pie)
		Beverage

*Items need advanced thawing/heating
**Items need preparation

Table 4.4-1 continued *Twenty-day sample of the 81-day menu*

DAY 16: Saturday, Oct. 4	**DAY 17: Sunday, Oct. 5**	**DAY 18: Monday, Oct. 6**
Pancakes (3 pancakes)	Cereal	Cereal
Cantaloupe slices	English muffin (1 muffin)	**Toast
Margarine	Margarine (1 t.)	Margarine (1 t.)
Syrup (1/4 cup)	Jelly/jam	Jelly/jam
Milk/juice	Milk/juice	Milk/juice
Coffee/tea	Coffee/tea	Coffee/tea
Cheese enchilada	Turkey, ham & cheese pocket	Minestrone soup (1 MRE)
Mexican rice & beans	**Sweet potato	Saltine crackers (5)
Tortilla chips (9 chips)	Salad	**Blushing pears (1/2 cup)
Picante sauce (2 T.)	**Strawberry shortcake	**Salad
Strawberry parfait royale	Beverage	Beverage
Beverage		
Vegetable lasagna	Macaroni & cheese	Cheese ravioli in marinara sauce
Red grapes	Cauliflower (2/3 cup)	Garlic bread
Salad	Tropical fruit salad (1/2 cup)	Green beans (1/2 cup)
Chocolate pudding (1 pudding cup)	**Whole wheat bread	*Peach cobbler (1/4 cobbler)
Beverage	Margarine	Blue Bell cup (1)
	Beverage	

*Items need advanced thawing/heating
**Items need preparation

Table 4.4-1 continued *Twenty-day sample of the 81-day menu*

DAY 19: Tuesday, Oct. 7	**DAY 20: Wednesday, Oct. 8**
Oatmeal	Belgian waffles (2 waffles)
Kiwi slices	Peaches, sliced, raspberry flavored
Milk/juice	Margarine (1 t.)
Coffee/tea	Syrup
	Milk/juice
	Coffee/tea
Country fried chicken	Blackened chicken
Mashed potatoes	Rice
**Soy bread	Corn
Margarine	Chocolate pudding (1)
Jell-O	**Soy bread
Beverage	Margarine
	Beverage
Fettuccine alfredo w/broccoli pieces	Vegetable pizza
Crinkle cut carrots (2/3 cup)	*Strawberries
Breadsticks (1)	Green beans (1/2 cup)
Margarine	Chocolate mousse (1/5 mousse)
*Raspberries (3/4 cup)	Beverage
Beverage	

*Items need advanced thawing/heating
**Items need preparation

10-Day Menu Development

The 10-day BIO-Plex menu was designed to meet the NASA requirements which specified that at least 90 percent of the calories be derived from the NASA crop list (Table 4.4-2). The remaining 10 percent consisted of resupply items, such as fruit, beverages, and spices. Recipes for the 10-day BIO-Plex menu were selected from recipes that met the requirements or could be modified to meet them. The first requirement was that the recipe had to consist of mostly NASA crops or crop products. Another requirement was minimal preparation time or few preparation steps. These recipes were then evaluated for overall sensory acceptability using a nine-point hedonic scale. A sensory score of six or greater was considered acceptable for the menu. Items that could be prepared in the chamber were assigned to the crew. The remaining food preparation and processing activities were performed in the JSC Food Lab. Most of the preparation with the raw products was performed outside of the chamber, and the crew received partially prepared foods in labeled containers. The development of the menu for the 10-day test was a two-step process (6, 7).

Step 1: Selection and Screening of Recipes

Recipe selection was based on the following criteria:

a) Majority of ingredients derived from the recommended crop list (Table 4.4-2)
b) Limit the number of ingredients that would require resupply
c) Overall taste panel acceptability of six or greater on hedonic scale
d) Non-labor-intensive recipe preparation

Table 4.4-2 *NASA crop list*

Wheat	White Potato	Salad mix:
Peanut	Sweet Potato	lettuce, tomato,
Soybean	Rice	green onion,
		green leafy
		vegetables, herbs

Sources for the recipe search came from vegetarian cookbooks, the Internet, and soybean cookbooks. Recipes were initially screened through a laboratory tasting session. Spices and flavorings contribute minimally to the amount of the resupply needed to support the menu; therefore it was assumed that these items would be available in the quantities required. Some of the selected recipes were reformulated to include ingredients that would be available in the resupply supply or recommended crop list or its derivatives. Once the reformulation of the recipe was completed, a taste panel evaluated the recipes for degree of acceptability.

Ten to 25 panel members consisting of NASA and contractor employees ages 20 to 60 years old participated in the sensory analysis. A nine-point

hedonic scale (where one is "dislike extremely" and nine is "like extremely") measured each product for the degree of acceptability with regard to six attributes: appearance, color, odor, flavor, texture, and overall acceptability (2). The final criterion used for recipe selection was preparation time.

Step 2: Develop Menu Based on Requirements

After the recipes were selected, the 10-day menu was designed. Emphasis was placed on foods familiar to a Western diet since these foods had higher acceptability scores with the pool of potential crewmembers. Several requirements were considered in the design of the menu. The first requirement was that 90 percent of the calories come from the baseline crops. Sugar, oil, and other products that can be derived through processing of the baseline crops were acceptable and contributed to the 90 percent requirement. Another requirement was that the final menu would, at a minimum, meet the WHO caloric requirements for each crewmember (3). The RDAs were the basis for the remainder of the nutritional requirements (1).

The nutritional analysis, using the Minnesota Nutrition Data System software, was used to adjust the menu to better provide for the nutritional needs of the crew (9). If deficiencies were noted, foods rich in that nutrient were added or increased in quantity. Emphasis was placed on foods that did not require fortification or flavor enhancers, since these would be considered resupply items and decrease the self-sufficiency of the system. The menus were individualized where possible to meet crewmembers' specific nutritional requirements and personal preferences (Table 4.4-3). The other crewmember had additional snacks added to the menu to meet a higher caloric requirement.

Table 4.4-3 *Nutritional information for the 10-day BIO-Plex menu, crewmembers 1, 2, and 4*

Day	kcal	CHO (g)	Protein (g)	Fat (g)	% kcal from fat	Na (mg)	Ca (mg)	Fe (mg)
1	2204	380	51	65	27	3052	634	22
2	2140	330	82	66	28	3602	1076	31
3	2292	364	48	79	31	7049	434	22
4	2375	422	95	49	19	6273	1031	31
5	2443	384	85	75	28	4336	1121	26
6	2522	518	45	44	16	2115	575	17
7	2547	391	80	87	31	4838	886	26
8	2118	354	65	57	24	3164	819	26
9	2680	443	94	75	25	5272	741	30
10	2249	334	68	83	33	2771	808	24
Average	**2357**	**360**	**71**	**68**	**26**	**4247**	**812**	**26**
estimated need	2280 WHO	50-60% total kcal RDA	63-68 RDA	max 76 RDA	≤ 30 RDA	500-3500 RDA	800 RDA	RDA: male:10 female:15

The 10-day BIO-Plex menu was a 10-day cycle (see Table 4.4-4). Except for beverages, fruit, and bread, the majority of foods did not repeat. Two menus were developed for the 10-day test to meet the caloric needs of the crewmembers (a 3,000 kcal menu and a 2,280 kcal menu). The 3,000 kcal diet consisted of second helpings and additional snacks. The menu was planned so that all four crewmembers would have the same foods in different quantities.

Table 4.4-4 *BIO-Plex 10-day menu for crewmembers 1, 2, and 4*

Meal		Day 1		Day 2
Breakfast	41 g	Soybread*	110 g	Coffee cake*
	38 g	Strawberry jelly (C)	240 ml	Chocolate soy milk (C)
	240 ml	Orange juice, reconstituted (C)	38 g	Strawberry jelly (C)
	opt	454 g Coffee, rehydrated (C)	240 ml	Orange juice, reconstituted (C)
	opt	15 g Soymilk, plain (for coffee) (C)	opt	454 g Coffee, rehydrated (C)
	opt	15 g Sugar (for coffee) (C)	opt	15 g Soymilk, plain (for coffee) (C)
			opt	15 g Sugar (for coffee) (C)
Snack	105 g	Strawberries (C)	124 g	Applesauce (C)
Lunch	241 g	Vegetable stew	275 g	Hot and sour soup
	123 g	Baked potato (red potato)	250 g	Vegetable peanut stir fry
			158 g	Oven rice
	60 g	Soybean & red pepper sauce	47 g	Egg roll (frozen, homemade)
	opt	Green onion, chopped (1 small)	opt	10 g Mustard
	41 g	Soy bread*	480 ml	Beverage w/art. sweetener (C)
	124 g	Pears (C), juice pack		
	240 ml	Beverage with sugar (C)		
	70 g	No-bake cookies		
Snack	10 g	Pretzel sticks		
	70 g	No-bake cookies		
Dinner	312 g	Spinach lasagna	130 g	Tofu basil pasta sauce
	45 g	Skillet garlic bread (soy)*	226 g	on whole wheat fettucine noodles (C)*
	236 g	Lettuce salad *		
	32 g	Dressing, garlic & herb (C)*	67 g	Wheat bread*
			78 g	Cooked spinach
	78 g	Mixed veg: potatoes/ carrot/peas (C)*	65 g	Peanut cake*
			480 ml	Beverage: instant tea (C)
	78 g	Carrot cake*		
	480 ml	Beverage: instant tea (C)		

LEGEND: Common condiments not listed. Beverages with sugar and with artificial sweetener were powdered, fruit-flavored commercial products.
(C) = commercially available product
* = assembled or cooked in chamber
opt = optional

Table 4.4-4 continued *BIO-Plex 10-day menu for crewmembers 1, 2, and 4*

Meal		Day 3		Day 4
Breakfast	63 g	Sweet potato bread*	64 g	Wheat bread*
	38 g	Strawberry jelly (C)	240 ml	Chocolate soy milk (C)
	240 ml	Grape juice, reconstituted (C)	38 g	Strawberry jelly (C)
			240 ml	Orange juice, reconstituted (C)
	opt	16 oz. Coffee rehydrated (C)	opt	454 g Coffee rehydrated (C)
	opt	15 g Soymilk, plain (for coffee) (C)	opt	15 g Soymilk, plain (for coffee) (C)
	opt	15 g Sugar (for coffee) (C)	opt	15 g Sugar (for coffee) (C)
Snack			124 g	Pears, juice pack (C)
Lunch	354 g	Potato soup	435 g	Garlic lentil soup
	190 g	Crunchy confetti salad	134 g	Whole wheat bread*
	113 g	Fruit cocktail (C)	125 g	Tempeh-rice salad
	240 ml	Beverage with sugar (C)	124 g	Peaches, juice pack (C)
			opt	Strawberry jelly (C)
			240 ml	Beverage with sugar (C)
Snack			10 g	Pretzel sticks
Dinner	114g	Tempeh sandwich filling*	3 = 135 g	Soybean soft tacos (assembly)*: Tortilla
	126 g	Sweet potato bread*	129 g	Soybead filling (.5 c divided into 3)
	opt	Lettuce, tomato, onion, mustard		Toppings: lettuce, tomato, onion
	198 g	Marinated tomato & onions	104 g	Pico de gallo (C)
	163 g	Peach cobbler	75 g	Pineapple cake*
	480 ml	Beverage: instant tea (C)	480 ml	Beverage: instant tea (C)

LEGEND: Common condiments not listed. Beverages with sugar and with artificial sweetener were powdered, fruit-flavored commercial products.
(C) = commercially available product
* = assembled or cooked in chamber
opt = optional

Table 4.4-4 continued *BIO-Plex 10-day menu for crewmembers 1, 2, and 4*

Meal		Day 5		Day 6
Breakfast	144 g	Peanut butter bread*	110 g	Coffee cake*
	240 ml	Chocolate soy milk (C)	38 g	Strawberry jelly (C)
	38 g	Strawberry jelly (C)	240 ml	Grape juice, reconstituted (C)
	240 ml	Orange juice, reconstituted (C)	opt	480 ml Coffee, rehydrated (C)
	opt	480 ml Coffee, rehydrated (C)	opt	15 g Soymilk, plain (for coffee) (C)
	opt	15 g Soymilk, plain (for coffee) (C)	opt	15 g Sugar (for coffee) (C)
	opt	15 g Sugar (for coffee) (C)		
Snack	75 g	Peanut butter cookies	124 g	Applesauce (C)
Lunch	157 g	Spinach quiche, no cheese	582 g	Vegetable pizza
	41 g	Soybread*	225 g	Chocolate pudding
	236 g	Green salad*	240 ml	Beverage w/art. sweetener (C)
	124 g	Peaches, juice packed (C)		
	75 g	Peanut butter cookies		
	240 ml	Beverage w/art. sweetener (C)		
Snack	72 g	Peanut butter bread		
Dinner	315g	Tempeh cacciatore	431 g	Sweet and sour tempeh
	226 g	Whole wheat noodles*	158 g	Rice
	90 g	Skillet garlic bread (soy)	47 g	Egg rolls (frozen, homemade)
	236 g	Lettuce salad*	opt	10 g Mustard
	32 g	Vinaigrette dressing: Italian (C)	133 g	Peas (C)*
	72 g	Cooked spinach	113 g	Fruit cocktail, juice packed (C)
	163 g	Lemon custard pie	480 ml	Beverage: instant tea (C)
	105 g	w/Strawberries (C) on pie		
	480 ml	Beverage: instant tea (C)		

LEGEND: Common condiments not listed. Beverages with sugar and with artificial sweetener were powdered, fruit-flavored commercial products.
(C) = commercially available product
* = assembled or cooked in chamber
opt = optional

Table 4.4-4 continued *BIO-Plex 10-day menu for crewmembers 1, 2, and 4*

Meal		Day 7		Day 8
Breakfast	54 g	Plain bagel	144 g	Peanut butter bread*
	38 g	Strawberry jelly (C)	38 g	Strawberry jelly (C)
	240 ml	Orange juice, reconstituted (C)	240 ml	Orange juice, reconstituted (C)
	opt	480 ml Coffee, rehydrated (C)	opt	480 ml Coffee, rehydrated (C)
	opt	15 g Sugar (for coffee) (C)	opt	15 g Soymilk, plain (for coffee) (C)
	opt	15 g Soymilk, plain (for coffee) (C)	opt	15 g Sugar (for coffee) (C)
			124 g	Pineapple, can, juice packed (C)
Snack	240 ml	Beverage w/sugar (C)	240 ml	Grape Juice, reconstituted (C)
Lunch	296 g	Vegetable chowder	300 g	Vegetable carrot stir fry
	78 g	Spicy black bean burger (C)	158 g	On rice
			47 g	Egg roll (homemade, frozen)
	82 g	Soybread*		
	10 g	Mustard	opt	2 tsp. Mustard
	opt	Lettuce, tomato, onion	240 ml	Beverage w/art. sweetener
	170 g	Red potatoes (C)*		
	124 g	Apricots, juice packed (C)	192 g	Strawberry sorbet (C)
	213 g	Peanut butter pie		
	240 ml	Beverage w/art. sweetener (C)		
Snack	10 g	Pretzel sticks (C)		
Dinner	184 g	Spaghetti sauce*	89 g	Soybean ragout
	226 g	Whole wheat spaghetti noodles (C)*	158 g	Steamed rice, white
			133 g	Peas (C)
	45 g	Skillet garlic bread (soy)*	119 g	Spinach salad*
			32 g	Vinaigrette dressing (C)
	72 g	Cooked spinach	122 g	Choc-strawberry tofu trifle
	236 g	Salad with tomato & onion		
	32 g	Dressing: Italian (C)	480 ml	Beverage: instant tea (C)
	213 g	Peanut butter pie		
	480 ml	Beverage: instant tea (C)		

LEGEND: Common condiments not listed. Beverages with sugar and with artificial sweetener were powdered, fruit-flavored commercial products.
(C) = commercially available product
* = assembled or cooked in chamber
opt = optional

Table 4.4-4 continued *BIO-Plex 10-day menu for crewmembers 1, 2, and 4*

Meal		Day 9		Day 10
Breakfast	64 g	Wheat bread*	41 g	Soybread*
	3 Tbs.	Peanut butter, no salt, old-fashioned (C)	48 g	Peanut butter, no salt, old-fashioned (C)
	2 Tbs.	Strawberry jelly (C)	240 ml	Chocolate soy milk
	240 ml	Orange juice, reconstituted (C)	38 g	Strawberry jelly (C)
	opt	480 ml Coffee, rehydrated (C)	240 ml	Orange juice, reconstituted (C)
	opt	15 g Soymilk, plain (for coffee) (C)	opt	240 ml Coffee, rehydrated (C)
	opt	15 g Sugar (for coffee) (C)	opt	15 g Soymilk, plain (for coffee) (C)
			opt	15 g Sugar (for coffee) (C)
Snack	124 g	Pears, juice packed (C)		
Lunch	78 g	Spicy black bean burger (C)	180 g	Roasted garlic soybean hummus
	138 g	Wheat bread*	82 g	Soybread*
	10 g	Mustard	opt	Sandwich toppings: lettuce, tomato, green onion
	opt	Sandwich toppings: lettuce, tomato, green onion	opt	10 g Mustard
	130 g	Baked soybeans	204 g	Tabouli salad (C)
	124 g	Peaches, juice pack (C)	40 g	Dried apples (C)
	240 ml	Beverage w/art. sweetener (C)	233 g	Baked rice casserole
	137 g	Chewy brownies (C)	240 ml	Beverage w/art. sweetener (C)
Snack			137 g	Chewy brownies
Dinner	1 =	Chili bean burrito (assembly)*:	606 g	Pepper pizza
			236 g	Lettuce salad w/croutons
	45 g	Tortilla	163 g	Lemon custard pie
	159 g	Chili bean burrito filling	105 g	Strawberries (C) on pie
			480 ml	Beverage: instant tea (C)
	170 g	Red potatoes (C)*		
	104 g	Pico de gallo		
	236 g	Green salad*		
	32 g	Dressing: Italian (C)		
	480 ml	Beverage: instant tea (C)		

LEGEND: Common condiments not listed. Beverages with sugar and with artificial sweetener were powdered, fruit-flavored commercial products.
(C) = commercially available product
* = assembled or cooked in chamber
opt = optional

The crew completed sensory evaluation scores for each food item on a food tracking data sheet. The crew also utilized the nine-point hedonic scale for these evaluations. The crew provided estimates of plate waste on the food tracking data sheet. The crew circled which fraction, in one-quarter intervals, best described the amount of food remaining on his/her plate at the end of the meal. Food waste was either weighed by the crew during the 10-day test and then discarded, or it was passed out of the chamber to be weighed. The data was collected, and the trends were summarized. Time studies were done to determine the amount of time spent on preparation activities, which included cleaning, removing inedible biomass, chopping, slicing, and gathering ingredients (see Figure 4.4-1).

Discussion

Early Human Test Initiative Phase I

The Phase I food system for the crew of one consisted of shelf-stable foods that were heated in a microwave oven. Since there was only one test subject, limited data was available for analysis. The crewmember was satisfied with the diet for that length of time, but expressed a desire for more variety in the choice of foods.

Early Human Test Initiative Phase II

More food preparation equipment was included in the Phase II test to meet the food system objective of evaluating a 50 percent frozen/refrigerated food mix in an isolated environment. In general the food system was well accepted by the crew. The higher quality frozen dinners were more desirable, and beverages and juices were preferred to mask the taste of iodine in the water. Even though two microwave ovens were provided, food preparation took longer than expected. The crew maintained one common meal at dinner and enjoyed the special request items, both of which helped to improve morale. Recommendations for future missions were to increase the microwave capability and the frozen food storage in the chamber.

Advanced Human Life Support Enclosed System Study Phase IIa

The main objective of the Phase IIa food system was to evaluate the ISS food system by emulating it as close as possible in the chamber test. The microwave ovens were upgraded to 1000 watts to improve food preparation time. Overall the crew was very satisfied with the food system. The ethnic variety of the food selections was good. The crew reported that they missed toast and carbonated beverages. The frozen foods were bagged by day for transfer into the chamber, and this saved time. They would have preferred more fresh vegetables. The only fresh vegetables were prepackaged modified-atmosphere salads. They would have preferred that more of the frozen vegetables be served plain so that each individual could decide

what sauce to add. They always ate dinner together since it was easier to prepare one entrée for more than one person. The results from the weekly sensory evaluations inside the chamber were very positive, averaging between 7 (like moderately) and 8 (like very much). The overall daily average for all subjects was 7.85.

Lunar-Mars Life Support Test Project Phase III

The multiple objectives of the Phase III food system resulted in a much more detailed involvement in the test. The objective of the 81-day menu was to simulate a BIO-Plex mission where 50 percent of the food would be derived from chamber-grown crops (see Table 4.4-2). This 50 percent was defined as four or less servings of meat per week. The 10-day menu had 90 percent of the calories derived from the potential chamber-grown crops.

81-Day Menu Crew Debrief – Crew Assessment of Food System and Food Choices

A formal crew debrief was held for the 81-day menu. Three crewmembers (one male and two female) attended the debriefing. The general consensus of the crew was that the food system was very good. Some food fatigue was experienced toward the end of the test, especially toward the frozen food entrees. They would have preferred a menu that was a true 20-day cycle with fewer repeating food items. They would have preferred higher quality frozen food entrees. There were too many bean burritos and grilled cheese sandwiches. Overall, the crew followed the menu fairly closely, although they did make some changes. They did cook and prepare all items on the menu, but some substitutions were made by switching foods either to different times or to different days. One crewmember had a problem with low-fat entrees and felt they were not very tasty and required supplementation with butter. Some found themselves craving and using more salt than usual. Another member was concerned about the fat content of the menu and did not always eat according to the menu. Clearly these comments indicate that past eating habits influenced the crewmembers' perception of the chamber food system.

Cooking and preparing tended to be performed by one crewmember on a rotating basis. The crew usually ate meals together, especially at dinner. The presentation of the food was very important to the crew. There was a negative impact to all the paper, plastic, and cardboard that accompanied almost all the food.

The holiday meals were very gratifying, and surprises for long-duration missions will be very important because they boost morale. Thanksgiving was a very important meal because it gave the crewmembers a time to relax and enjoy their meal. Making the food items as "real" as possible is needed for future long-duration missions.

Different varieties of texture were missed. It would have been nice to have different varieties of cheese or chips that offered different textures. Snacks were craved quite frequently and associated with comfort food. However, not many snacks were eaten because snacks were not kept out nor were they readily available all of the time. The crew tended to snack together during certain periods such as movie time.

Large quantities of beverages were consumed because the relative humidity in the chamber was 40 percent and it was very dry. Typically 25 percent of the beverages were consumed as plain water and the rest as juice or fruit-flavored beverages. No one really liked the Ultra High Temperature (UHT) milk, and it was mentioned several times that real milk was missed. The UHT milk was mostly used for coffee and cereal. The crew did prefer the chocolate soymilk that was used during the 10-day menu more than the chocolate milk offered during the 81-day menu.

The menu had more than an adequate amount of desserts. It was suggested to alternate a low-fat dessert with a high-fat dessert. Another suggestion was to offer one large dessert that could be enjoyed for a couple of days. Bread was also an issue. Fresh bread was enjoyed, but it repeated too often in the menu; it was therefore not prepared as often as indicated by the menu. The sweet potato bread and wheat bread were enjoyed the most. The crew expressed that there was too much soy bread as they had developed taste fatigue after the 10-day BIO-Plex menu. The average sensory score for the 81-day menu was 7.3 (like moderately).

During the chamber test, three weighed-food record periods were conducted. The crew tended to finish all the food on their plates so they would not have to weigh leftovers. During this time they did not eat salad, bread, or anything that called for preparation because of the extra weighing. Snacking was also limited because of the weighing. The crew felt this biased their food records because their typical food consumption was altered.

The crew suggested a reevaluation of using the WHO (3) equation to calculate caloric requirements for the menu. They would rather have something that reflected the amount of calories that would be typically or realistically consumed, because the menu contained more calories than they felt comfortable eating.

10-Day Menu Crew Debrief

The 10-day menu crew debriefing was held via teleconference. The BIO-Plex menu overall was very acceptable. They reported that they enjoyed it and would miss it. The BIO-Plex meals were a pleasant change from the frozen entrees provided in the 81-day portion of the test.

The majority of the food items were familiar to the crew. They enjoyed the home-cooked nature of the meals. They missed items like milk, sour cream, butter spread, and steaks, and having a large variety of snack foods. They would have liked more sauces for the vegetables. The crew recommended different forms of bread rather than sliced bread all the time. One crewmember said that hamburger buns would have been a nice change.

All crewmembers indicated that variety was important to the menu. Variety was adequate for the 10 days, but, for a longer test, increased variety is recommended.

The crew did not experience food fatigue from the overall menu. Prior to the test, breakfast items such as waffles were replaced by various breads on the menu at the

request of the crew to decrease food preparation time in the mornings. Although this change was implemented, the crew recommended more variety with the breakfast items.

The limited choices of snack foods (no-bake cookies and egg rolls) contributed to food fatigue for these items. To improve variety, some of the crew would have liked to see snacks such as cereal and different types of vegetables.

Although one crewmember was aware of the NASA nutritional requirements and nutritional recommendations, none of the crewmembers used the requirements for their meal selection. One crewmember was concerned about the fat content of the menu. As a result, that crewmember avoided certain food items that were perceived to be high fat and thus influenced the consumption.

Crewmembers were not aware of the substitution options on the last page of the menu. The crew indicated that they would have liked the ability to substitute foods at will and to manage their own resources. This would have given them the opportunity to adjust the recipes to reflect their personal preferences. Also, the crew recommended that the menu be flexible enough to give them a choice to eat the leftovers, thereby utilizing the food more efficiently. The serving sizes were often too large.

The crew was satisfied with the BIO-Plex menu, and they did not feel underfed or feel any discomfort. The overall physical comfort level met the crew's expectations. There was a noticeable increase in the methane level and solid waste. However, this did not affect the crew's ability to complete their tasks. The crew commented that the measured volume of fecal waste was approximately doubled during the BIO-Plex menu test.

Overall, the crew acceptance of the food system was good. There were no significant changes in acceptability of the food throughout the 91-day test. The average sensory score for the 10-day menu was 7.0 on a hedonic scale of 1 - 9 (lowest acceptability to highest acceptability).

Nutritional Analysis

The nutritional analysis on the menu showed that the 10-day BIO-Plex menu met the caloric, carbohydrate, protein, and fat needs of the crew (see Table 4.4-3). Despite the lack of dairy products, the current recommended dietary allowance for calcium was met through other calcium-rich foods such as tofu and soybeans. However, it has been recommended that the RDA for calcium be raised to 1000 mg/day, and this menu would not meet this increased requirement (8).

The iron provided by the menu was higher than the respective RDAs for men and women. Iron overloading from this menu, however, may not be a problem due to poor absorption of iron from plant foods (10). Since iron overloading is a potential issue for space flight due to decreased turnover of red blood cells in microgravity, the iron bioavailability should be carefully considered during further development of the BIO-Plex menu (11).

The sodium content of the menu exceeded the nutritional recommendation for space flight, which is less than 3,500 mg/day (5). This level is recommended due to concerns related to the effect of high sodium intake on calcium metabolism. In addition to the sodium provided by the foods, the crew was allowed to add salt and pepper at the point of service. It was noted in the crew debriefing that at least one crewmember had used significant amounts of salt and soy sauce, which would have further increased the sodium levels in his/her diet. The issue of sodium content must be considered in the further development of the BIO-Plex diet, since this represents a potentially negative impact on the health of crewmembers.

All of the other RDAs were met except for vitamin D, vitamin B_{12}, and zinc. The 10-day menu provided 28 percent of the recommended vitamin D, 85 percent of vitamin B_{12}, and 74 percent of the recommended zinc. These are traditionally nutrients of concern for vegetarians. However, because of the short duration of the test, it was not considered critical to meet the requirements for these three nutrients. It is important to note that a final BIO-Plex menu developed for long-duration space flight, or for surface habitats on the Moon or Mars, would have to meet these requirements by use of fortified foods or other means. Vitamin D intake becomes even more important in situations such as these where the crewmember will not be exposed to sunlight.

Menu Preparation Times

Preparing meals for the BIO-Plex menu took longer than for the 81-day menu. One possible reason for the added preparation times was that the frozen microwaveable meals in the 81-day menu were very easy to prepare. Since the crew's experiment schedule was based on a five-day work week to match the supporting personnel outside of the chamber, the crew recommended weekends for food processing activities. The cooking was not difficult, but the cleaning was nearly impossible due to the water and cleaning equipment limitations inside the chamber. As a result, dishes and transport containers were cleaned in the JSC Food Lab. Food activities, like cooking pasta, used a large amount of water. It was recommended to consider water usage in future food system tests.

Table 4.4-5 contains the raw data collected for the time analysis. The data from this menu test indicates that a crew of four would spend an average of 4.6 crew hours per day in preparation and clean-up activities. This assumes the crew starts with ready-to-use ingredients. In the BIO-Plex facility and in other situations where a crew will be growing its own food, the time required for the actual processing of the crops into useable ingredients must be taken into account as well. Crop processing, food preparation, and clean-up activities will have an enormous impact on crew time, and efforts must be made to minimize the time required for all aspects of the food system. Bulk production of menu items and the automation of food processing and meal preparation are likely candidates to aid in time reduction.

Table 4.4-5 *Time required for total meal preparation in the 10-day BIO-Plex menu*

	CLEAN UP	MEAL PREPARATION		
	Total	Food Lab	20 ft Chamber	Total
Day/Meal	Time* (min)	Time* (min)	Time* (min)	Time (min)
PRETEST ACTIVITIES				
Food pre-preparation (egg rolls & cookies)	**15**	**122**		**122**
Daily food preparation activities for day 1	10	25		25
DAY 1				
Breakfast (all in 20 ft chamber)			25	25
Lunch	10	78	5	83
Dinner	15	30	45	75
Daily food preparation activities for day 2	15	72		72
Food pre-preparation (flour milling)	**15**	**45**		**45**
20 ft chamber end of the day clean up	20			
DAY 2				
Breakfast (all in 20 ft chamber)			20	20
Lunch	17	59	10	69
Dinner	10	08	50	58
Daily food preparation activities for day 3	11	58		58
20 ft chamber end of the day clean up	20			
DAY 3				
Breakfast (all in 20 ft chamber)			10	10
Lunch	10	30		30
Dinner	05	35	20	55
Daily food preparation activities for day 4	05	40		40
Food pre-prep. (tortillas)	**05**	**60**		**60**
20 ft chamber end of the day clean up	20			
DAY 4				
Breakfast (all in 20 ft chamber)			20	20
Lunch	06	39	5	44
Dinner	12	60	15	75
Daily food preparation activities for day 5	41	119		119
20 ft chamber end of the day clean up	20			

*These are partial times; meal preparation was split up between the Food Lab and the 20 ft. chamber

Table 4.4-5 continued *Time required for total meal preparation in the 10-day BIO-Plex menu*

	CLEAN UP	MEAL PREPARATION		
	Total	Food Lab	20 ft Chamber	Total
Day/Meal	Time* (min)	Time* (min)	Time* (min)	Time (min)
DAY 5				
Breakfast (all in 20 ft chamber)			10	10
Lunch	05	20	30	50
Dinner	05	35	35	70
Daily food preparation activities for day 6	07	90		90
Food pre-prep. (bagels)	**05**	**09**		**09**
20 ft chamber end of the day clean up	20			
DAY 6				
Breakfast (all in 20 ft chamber)			20	20
Lunch (all in 20 ft chamber)			45	45
Dinner	10	26	20	46
Daily food preparation activities for day 7	05	34		34
20 ft chamber end of the day clean up	20			
DAY 7				
Breakfast (all in 20 ft chamber)			10	10
Lunch	03	20	30	50
Dinner (all in 20 ft chamber)			40	40
Daily food preparation activities for day 8	05	65		65
20 ft chamber end of the day clean up	20			
DAY 8				
Breakfast (all in 20 ft chamber)			10	10
Lunch	30	30	15	45
Dinner	15	80	15	95
Daily food preparation activities for day 9	05	40		40
Food pre-prep. (tortillas)	**05**	**55**		**55**
20 ft chamber end of the day clean up	**20**			

*These are partial times; meal preparation was split up between the Food Lab and the 20 ft. chamber

Table 4.4-5 continued *Time required for total meal preparation in the 10-day BIO-Plex menu*

	CLEAN UP	MEAL PREPARATION		
	Total	Food Lab	20 ft Chamber	Total
Day/Meal	Time* (min)	Time* (min)	Time* (min)	Time (min)
DAY 9				
Breakfast (all in 20 ft chamber)			20	20
Lunch	05	35	20	55
Dinner	06	45	15	60
Daily food preparation activities for day 10	30	81		81
20 ft chamber end of the day clean up	20			
DAY 10				
Breakfast (all in 20 ft chamber)			15	15
Lunch	15	40	30	70
Dinner (all in 20 ft chamber)			40	40
20 ft chamber end of the day clean up	20			
MENU ENDING TOTALS	513	1294	645	1939
FOOD PRE-PREPARATION				
FOOD PRE-PREP TOTAL		291		
FOOD PRE-PREP CLEAN UP TOTAL	45			

*These are partial times; meal preparation was split up between the Food Lab and the 20 ft. chamber

Plate Waste Data

Figure 4.4-1 shows the waste data compiled by day. Total waste was comprised of preparation waste, plate waste, and leftovers. All percentages were determined in relation to the finished weight of the menu items. Preparation waste was the amount of waste generated each day during preparation of the menu items, and these percentages remained at low levels throughout the menu test. Plate waste data was determined by comparing what the crewmember actually ate to the planned serving size. The plate waste data shows wide day-to-day variation. Larger amounts of plate waste could be attributed to lower acceptability of menu items served on that day or excessive serving size(s). The high percentage of leftovers on many days suggested a need for better scaling of the recipes to the crew size.

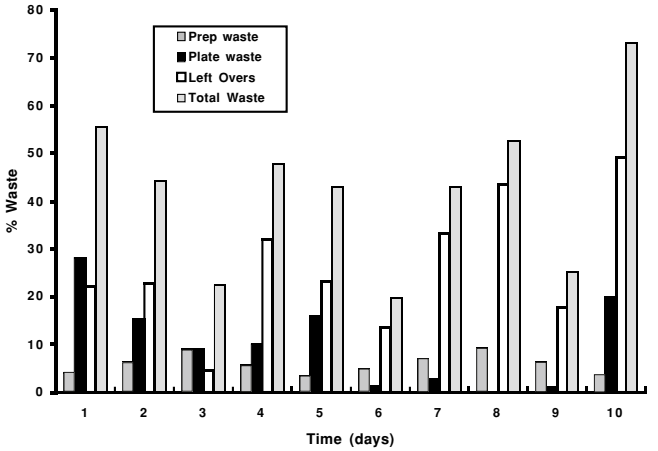

Figure 4.4-1 *Quantities of the different sources of waste monitored during the 10-day BIO-Plex diet*

Phase III Conclusions and Assessments

All of the objectives of the food system were met and/or exceeded for the entire 91-day test. This test rendered a food system that was monitored and controlled to support medical experiments, while satisfying the physiological and psychological food-related needs of the crew. This test also successfully incorporated a 10-day BIO-Plex menu, which integrated some food processing and meal preparation activities, into the 91-day test to increase closure of the recycling loop within the chamber system. Also, the test was successful in providing an acceptable plant-based menu for 10-day habitation in a closed-system environment. The nutritional needs were met, except for three nutrients (vitamin D, zinc, and vitamin B_{12}). These nutrients are typically low in vegetarian diets, therefore the need for fortified foods and/or supplementation of these nutrients may be necessary for long-duration tests.

Microbiological safety must be a consideration for transfers in and out of the chamber for future tests. It is a possible hazard to transfer food with body fluids and/or trash. Food should have dedicated transfer bins, rather than simply using the bin in which other items were transferred. Daily transfers were routinely limited, but separate transfer or separate bins for transfer must be evaluated for food products.

The amount of trash generated is a significant concern in a closed system, and this must be closely evaluated in future tests. The food packages and containers should be reevaluated to eliminate excess food-related trash.

The appropriateness of using the WHO equation to calculate caloric requirements must be evaluated. Specific requirements, constraints, and goals should be established three months prior to a test for future tests with food systems similar to the 10-day BIO-Plex test. Activities must be identified and scheduled to include rehearsals two months prior to a test so that the system will be complete before the beginning of the test.

The menu should be expanded from 10 days to 30 days to increase variety, and it also should be flexible enough to allow crews to utilize leftovers. A larger meal substitution base should be developed to prevent taste fatigue. Attention should be given to the participation of the crew in menu planning. A possible suggestion is that the crewmembers be able to plan their own menus under the direction of a registered dietitian using a preplanned menu as a guideline.

Recipes need to be reformulated for appropriate meal serving sizes in order to reduce leftovers. Realistic and manageable goals for handling the waste obtained from food processing, meal preparation, plate waste, and leftovers must be determined.

Education for the crew regarding the food system is essential. Nutrition education would be beneficial to prevent changes in diet or consumption based on misconceptions of nutrition. In future tests, when crews will be doing more extensive food preparation, it might also be useful to train the crew in basic cooking techniques to improve the quality of their meals.

The Importance of Ground-Based Analogues to Developing Food Systems for Space flight

The ALS chamber tests provided a unique opportunity to evaluate future space food systems and to assess potential problems associated with conversion of chamber-grown plants to edible foods. The results of these food system tests verified that a food system similar to the planned ISS Assembly Complete food system utilizing approximately 50 percent frozen and 50 percent shelf-stable food preservation technologies was at least moderately acceptable to a crew for an extended duration (81 days).

The 10-day BIO-Plex test confirmed that a menu could be developed from the basic crop list. This menu is acceptable for a crew for 10 days and meets most of the nutritional requirements; it is, however, a very labor-intensive diet with excessive waste. Comprehensive research is needed in the areas of food processing and preparation in an enclosed environment.

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Acknowledgments

The following are recognized for their outstanding effort in supporting the Phase I through Phase III food systems:

Lisa Bye, Lockheed Martin
Mike Fohey, Lockheed Martin
Bobbi Friebele, Lockheed Martin
Paula Hall, Lockheed Martin
Eric Lane, University of Houston
Lisa Lubin, Lockheed Martin
Donna Nabors, Lockheed Martin
Connie Oertli, Lockheed Martin
Lisa Ristow, Lockheed Martin
Kathy Ruminsky, Bowling Green State University
Carol Stiller, Lockheed Martin

5.1

Nutritional Status Assessment During Phases IIa and III of the Lunar-Mars Life Support Test Project

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SUMMARY

The studies described here were designed to assess nutritional status during the chamber stays and to validate a new tool for estimating dietary intake during space flight. Comprehensive nutritional assessments were conducted before, during, and after the chamber studies. Dietary intake was assessed using three techniques: traditional weighed dietary records, and two Food Frequency Questionnaires both designed for use with space food systems but administered to obtain either daily or weekly intake estimates. These were compared with each other to assess variability between techniques.

Introduction

Nutrition is a critical concern for extended-duration space missions (11). Loss of body weight is a primary consequence of altered nutrition and is frequently observed during space flight (11). Other existing dietary concerns for space flight include excessive intakes of sodium and iron and insufficient intakes of water and vitamin D (11). Furthermore, dependence on closed or semiclosed food systems increases the likelihood of inadequate intakes of key nutrients. This is a significant concern for extended-duration space missions.

Space nutrition research often necessitates detailed recording of all food consumption. While this yields extremely accurate data, it requires considerable time and effort, and thus is not suitable for routine medical monitoring during space flight. To alleviate this problem, a food frequency questionnaire (FFQ) was designed to provide a quick and easy, yet reasonably accurate, method for crewmembers to provide dietary intake information to the ground support crew.

We report here a study which was designed to assess nutritional status before, during, and after the 60-day and 91-day chamber stays. An additional goal of the study was to validate a food frequency questionnaire designed specifically for use with space flight food systems.

Subjects and Methods

Subjects

Subject characteristics are described elsewhere. All procedures were reviewed by the Johnson Space Center Institutional Review Board to ensure ethical use of human subjects. Informed consent was obtained from all subjects.

Dietary Intake Assessment

The subjects completed a standard food frequency questionnaire, entitled Block95 (1), prior to entering the chamber to assess usual diet over the past year. During the chamber stay, a specialized food frequency questionnaire (described below) was completed to assess intake either over 24-hour (FFQ 24-h) or seven-day (FFQ 7-d) periods. The FFQ 24-h was administered three times per week on weeks 4 and 7 of the 60-day Phase IIa study, and weeks 1, 4, 6, 9, and 12 of the 91-day Phase III study. The FFQ 7-d was administered once per week on weeks 1, 3, 6, and 8 of the 60-day study, and weeks 2, 5, 8, 10, and 13 of the 91-day study. Five-day weighed food records were completed on weeks 2 and 5 of the 60-day study and on weeks 3, 7, and 11 of the 91-day study. During the weighed record sessions, subjects were provided a digital scale and log book and were instructed to weigh and record all food, fluids, vitamin/mineral supplements, and medicines consumed. A research dietitian (BLR) met with the subjects before the prechamber data collection session to provide training for all diet intake assessment methods.

Three of the Phase IIa subjects reported occasional use of vitamin/mineral supplements, while one Phase III subject reported daily supplement use. Intake data contained herein represent total nutrient intake (i.e., intake from both the foods consumed as well as supplements).

Food Frequency Questionnaire (FFQ)

The food frequency questionnaire used in the chamber was constructed by one of the authors (GB) based on the key nutrient contents of the more than 200 food items on the menu list. Nutrient data for all foods (except milk and dried cereals for the 60-day study, see below) were obtained using the Nutrition Data System (NDS-R, Version 4.01/29 developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 29 released Dec. 1996). For the 60-day study, nutrients in milk and dried cereal were obtained using values provided by Block et al. Specific nutrients studied included energy, protein,

calcium, sodium, iron, and water. Two versions of the chamber food frequency questionnaire were presented, one asking about dietary intake for the past 24 hours, the other for the past seven days. Responses for these questionnaires were handwritten.

Biochemical Assessment of Nutritional Status

A complete biochemical nutritional assessment profile was developed for use with flight crews on extended-duration space missions. This assessment profile was used in these ground-based studies to determine the impact of the semiclosed, space-like food system on crew nutritional status. Specific tests and analytical methods are shown in Table 5.1-1, are described in more detail in JSC#28566 (Nutritional Status Assessment for Extended Duration Space Flight, Rev. 1, 2000), and have been reported elsewhere (13).

Table 5.1-1 *Analytical methods used for biochemical analyses*[1]

Protein status	
Retinol binding protein (S)[2]	*radial immunodiffusion*
Transthyretin (S)	*nepholometry*
Protein electrophoresis (S)	*electrophoresis*
3-methylhistidine (U)	*ion exchange chromatography*
Water-soluble vitamin status	
RBC transketolase stimulation (WB)	*spectrophotometric*
RBC glutathione reductase (WB)	*spectrophotometric*
RBC NAD/NADP (WB)	*spectrophotometric*
N-methyl nicotinamide (U)	*HPLC*
2-pyridone (U)	*HPLC*
RBC transaminase (WB)	*spectrophotometric*
4-pyridoxic acid (U)	*HPLC*
Red cell folate (WB)	*radioreceptor assay*
Vitamin C (S)	*HPLC*

[1]Details of most methods have been published in reference 13. Detailed descriptions of all tests are available in JSC #28566 (Nutritional Status Assessment for Extended-Duration Space Flight, Rev 1, 2000)

[2]Sample types are indicated in parentheses: S = serum or plasma, WB = whole blood or erythrocytes, U = urine, RBC = red blood cells

[3]Abbreviations of analytical methods: ELISA = enzyme-linked immunosorbent assay, HPLC = high-performance liquid chromatography, ICP-MS = inductively coupled plasma emission mass spectrometer, IRMA = immunoradiometric assay, ISE = ion-selective electrode, RIA = radioimmunoassay

Table 5.1-1 continued *Analytical methods used for biochemical analyses*[1]

Calcium/bone status	
25-hydroxyvitamin D (S)	*RIA*[3]
1,25-dihydroxyvitamin D (S)	*RIA*
Parathyroid hormone, intact (S)	*IRMA*
Osteocalcin (S)	*RIA*
Calcium (S)	*ISE*
Alkaline phosphatase:	
Total (S)	*spectrophotometry*
Bone-specific (S)	*ELISA*
Ionized calcium (S)	*ISE*
N-telopeptide (U)	*ELISA*
Pyridinoline (U)	*ELISA*
Deoxypyridinoline (U)	*ELISA*
Hematology	
Hemoglobin (WB)	*spectrophotometry*
Hematocrit (WB)	*calculation*
Mean corpuscular vol. (WB)	*electronic pulse measurement*
Transferrin receptors (S)	*ELISA*
Transferrin (S)	*microparticle immunoassay*
Ferritin (S)	*enzyme immunoassay*
Ferritin iron (S)	*antibody isolation, ICP-MS*
Antioxidant status	
Total antioxidant capacity (S)	*spectrophotometry*
Superoxide dismutase (WB)	*spectrophotometry*
Glutathione peroxidase (WB)	*spectrophotometry*
Malondialdehyde (S)	*spectrophotometry*
4-OH-alkenal (S)	*spectrophotometry*
8-OH-deoxyguanosine (U)	*HPLC*

[1]Details of most methods have been published in reference 13. Detailed descriptions of all tests are available in JSC #28566 (Nutritional Status Assessment for Extended-Duration Space Flight, Rev 1, 2000)
[2]Sample types are indicated in parentheses: S = serum or plasma, WB = whole blood or erythrocytes, U = urine, RBC = red blood cells
[3]Abbreviations of analytical methods: ELISA = enzyme-linked immunosorbent assay, HPLC = high-performance liquid chromatography, ICP-MS = inductively coupled plasma emission mass spectrometer, IRMA = immunoradiometric assay, ISE = ion-selective electrode, RIA = radioimmunoassay

Table 5.1-1 continued *Analytical methods used for biochemical analyses*[1]

Mineral status	
Iron (S)	*ICP-MS*
Zinc (S,U)	*ICP-MS*
Selenium (S,U)	*ICP-MS*
Iodine (S,U)	*ICP-MS*
Phosphorus (U)	*spectrophotometry*
Magnesium (U)	*spectrophotometry*
Fat-soluble vitamin status	
Retinol (S)	*HPLC*
Retinyl palmitate (S)	*HPLC*
ß-carotene (S)	*HPLC*
∝-carotene (S)	*HPLC*
Serum phylloquinone (S)	*HPLC*
∝-tocopherol (S)	*HPLC*
γ-tocopherol (S)	*HPLC*
γ -carboxyglutamic acid (U)	*HPLC*
tocopherol:lipid ratio (S)	*calculation*
General	
Aspartate aminotransferase (S)	*enzymatic rate reaction*
Alanine aminotransferase (S)	*enzymatic rate reaction*
Sodium (S)	*ISE*
Potassium (S)	*ISE*
Chloride (S)	*ISE*
Cholesterol (S)	*spectrophotometry*
Triglyceride (S)	*spectrophotometry*
Creatinine (S,U)	*spectrophotometry*

[1]Details of most methods have been published in reference 13. Detailed descriptions of all tests are available in JSC #28566 (Nutritional Status Assessment for Extended-Duration Space Flight, Rev 1, 2000)
[2]Sample types are indicated in parentheses: S = serum or plasma, WB = whole blood or erythrocytes, U = urine, RBC = red blood cells
[3]Abbreviations of analytical methods: ELISA = enzyme-linked immunosorbent assay, HPLC = high-performance liquid chromatography, ICP-MS = inductively coupled plasma emission mass spectrometer, IRMA = immunoradiometric assay, ISE = ion-selective electrode, RIA = radioimmunoassay

Bone densitometry and body composition were determined using dual energy X-ray absorptiometry techniques (Hologic QDR 2000). Total body water (TBW) was determined using isotope (^{18}O) dilution, as described previously (8). Sodium bromide was used to measure extracellular fluid volume (ECF) (3). Body weight was determined weekly using a standard scale.

Biosample Collection

For the 60-day test, blood samples were collected six days prior to entering the chamber (designated CD-6) and four days after completion of the chamber stay (designated R+4). For the 91-day study, blood samples were collected once before (CD-9), twice during (chamber day 30, designated CD30, and CD40), and once after (R+4) the chamber stay. The CD30 and CD40 blood collections were immediately before and after implementation of the BIO-Plex diet (described in Chapter 4.4).

Fasting blood samples were collected immediately after awakening, at the same time of day, in order to minimize the effect of diurnal changes in endocrine and biochemical markers. For the 60-day chamber study, a total of 52 mL of blood were collected over approximately 70 days. For the 91-day chamber study, a total of 98 mL of blood were collected over approximately 100 days.

Urine was collected for two 24-hour periods before, every day during, and two 24-hour periods after the chamber studies; pre- and postchamber urine collections began on the day of blood collection. Complete urine analysis was conducted once (on CD32) during the 60-day study and three times (CD30, CD40, and CD60) during the 91-day chamber study.

All urine samples were collected as individual voids. During the chamber studies, urine samples were stored in a refrigerator in the chamber and were transferred to the outside in one of the two to three daily exchanges through the airlock. Urine samples were processed in the laboratory daily, 24-hour pools were created, and aliquots were either analyzed immediately or were frozen for batch analysis upon completion of the study.

Statistical Analysis

Dietary data were analyzed using repeated-measures analysis of variance. The class variable was assessment tool (FFQ 24-h, FFQ 7-d, Weighed Records), and the dependent variables were the nutrients of interest. Prechamber dietary intake data are presented, but these were not included in the statistical analyses, as the differences between prechamber and in-chamber intakes were not a primary research question.

Biochemical analyte data for the 60-day study were analyzed using paired t-tests, except when in-chamber analyses were available. In these cases, and for the 91-day chamber study, data were analyzed using repeated-measures analysis of variance. The class variable was study phase (prechamber, in-chamber, postchamber), and

dependent variables were the indices measured. This analysis identified effects of the semiclosed food system on indices of nutritional status. Because of the repeated-measures design of this study, each subject served as his or her own control. The only exception to this analysis was for the RBC transketolase assay for thiamin status. Since this is qualitative rather than quantitative, statistical analyses were not performed.

Findings

Results of the dietary intake studies are shown in Table 5.1-2. Energy and protein intakes were similar for the three intake assessment techniques during both studies. Caloric intakes were 94 ± 16% and 85 ± 16% of the World Health Organization (WHO) recommendations for the subjects in the 60-day and 91-day tests, respectively. Subjects in both tests maintained their body weights within 3% of their pretest values on exit from the chamber.

During the 60-day study, questionnaire estimates of calcium and iron intakes were lower than those of the weighed diet records (Table 5.1-2). Subsequent analysis revealed that these differences were related to differences in the nutrient content

Table 5.1-2 *Dietary intake data*[1]

	60-Day Chamber Study			
	Pre[2]	FFQ 24-h	FFQ 7-d	Weighed Records
Energy				
MJ/d	9.38 ± 1.45	10.51 ± 0.45	9.97 ± 0.65	10.76 ± 0.43
kcal/d	2243 ± 347	2511 ± 108	2384 ± 156	2571 ± 102
Protein, g/d	104.9 ± 18.9	80.5 ± 4.6	70.4 ± 6.3	75.8 ± 3.7
Calcium, mg/d	907 ± 185	910 ± 145[a]	943 ± 127[ab]	1120 ± 112[b]
Iron, mg/d	18.0 ± 0.4	19.4 ± 2.7[a]	23.6 ± 4.3[ab]	26.7 ± 4.2[b]
Sodium, mg/d	3603 ± 580	4100 ± 347	3752 ± 287	3890 ± 330
Water, mL/d	[3]	1689 ± 232[a]	1953 ± 277[b]	2430 ± 232[c]

[1]Data are mean ± SEM and represent the average of the four individual subject averages for each assessment technique. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other

[2]Prechamber data were not included in statistical analyses

[3]Data not available – the prechamber questionnaire was not designed to estimate water intake

Table 5.1-2 continued *Dietary intake data*[1]

	90-Day Chamber Study			
	Pre[2]	FFQ 24-h	FFQ 7-d	Weighed Records
Energy				
MJ/d	8.57 ± 2.03	8.72 ± 0.46	7.41 ± 0.32	9.20 ± 0.83
kcal/d	2048 ± 485	2083 ± 109	1770 ± 77	2199 ± 198
Protein, g/d	84.4 ± 21.9	59.4 ± 2.5	51.8 ± 4.3	58.5 ± 3.2
Calcium, mg/d	1116 ± 374	1052 ± 322	937 ± 349	1126 ± 162
Iron, mg/d	16.4 ± 3.9	21.0 ± 7.5	17.2 ± 5.8	20.1 ± 5.7
Sodium, mg/d	3252 ± 902	3845 ± 267[a]	2876 ± 287[b]	3332 ± 170[ab]
Water, mL/d	[3]	2730 ± 721	2626 ± 747	3217 ± 471

[1]Data are mean ± SEM and represent the average of the four individual subject averages for each assessment technique. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other
[2]Prechamber data were not included in statistical analyses
[3]Data not available – the prechamber questionnaire was not designed to estimate water intake

data used for two foods (milk and cereal) between the nutrient databases used to analyze the weighed diet records and the food frequency questionnaire. When the databases were synchronized for nutrient content of these food items, no differences were observed (data not presented). This problem was identified prior to the initiation of the 91-day study and was thus avoided in that study.

Sodium intake assessment yielded similar results for the three techniques during the 60-day chamber study. However, the FFQ 24-h sodium intakes were higher than those for FFQ 7-d questionnaires during the 91-day study.

Water intake assessment during the 60-day study was different for all three assessment techniques. Conversely, no differences were observed during the 91-day study.

Body weight did not change during the chamber studies (Figure 5.1-1). No changes in total body water were observed in either chamber study (Figure 5.1-2). Markers of lean body mass, urinary creatinine (Figure 5.1-3) and 3-methylhistidine (data not presented) were unchanged during the chamber studies. Extracellular fluid volume (ECFV) was measured using a 1.2 g dose of sodium bromide in capsule form for the 60-day study. One subject experienced gastric distress and subsequently did not receive the bromide dose after the chamber. ECFV did not change in the other three subjects (Figure 5.1-2). Modifications to the ECFV protocol resulted in administration of 1.5 g of sodium bromide as a ~50 mL liquid solution for the 91-day study. This form of the dose was better tolerated, and ECFV was similarly unaffected during the longer chamber study (Figure 5.1-2).

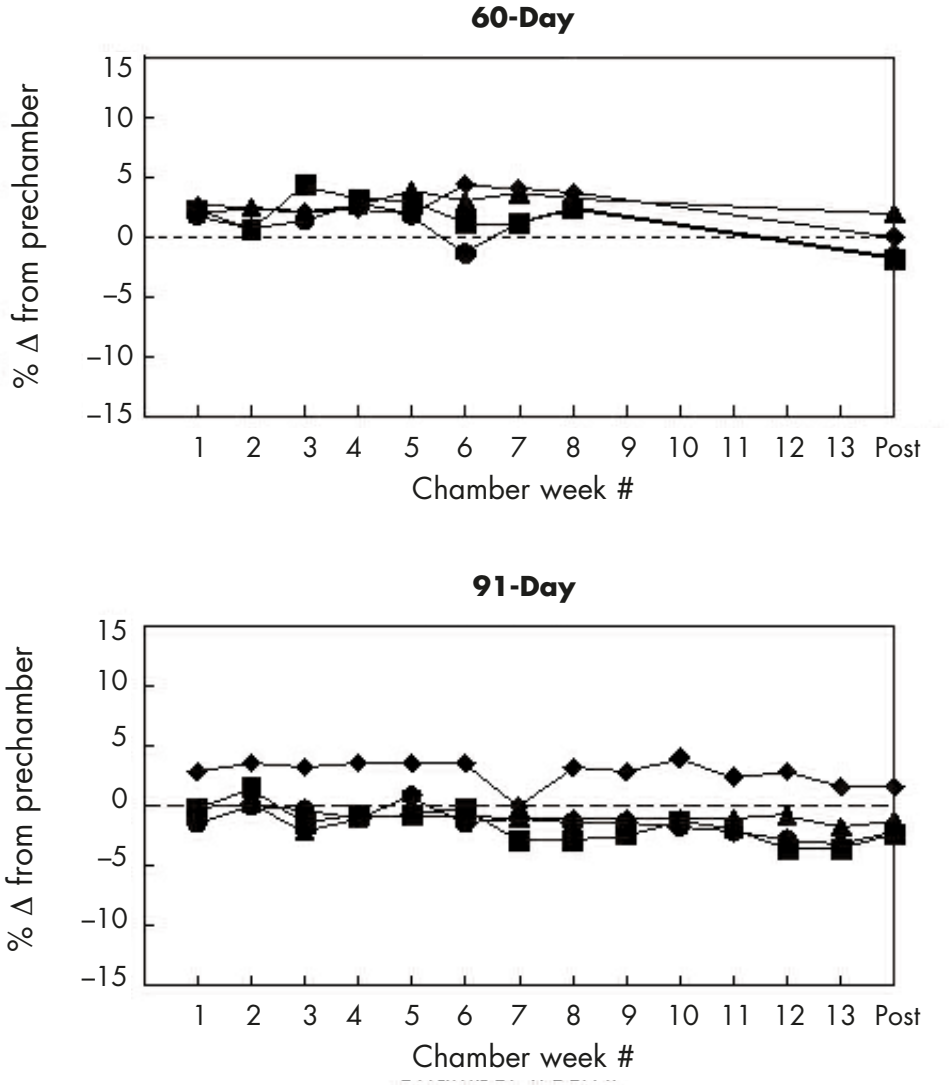

Figure 5.1-1 *Body weight data for the 60-day and 91-day chamber tests. Data are expressed for each individual as a percent change from their prechamber body weight*

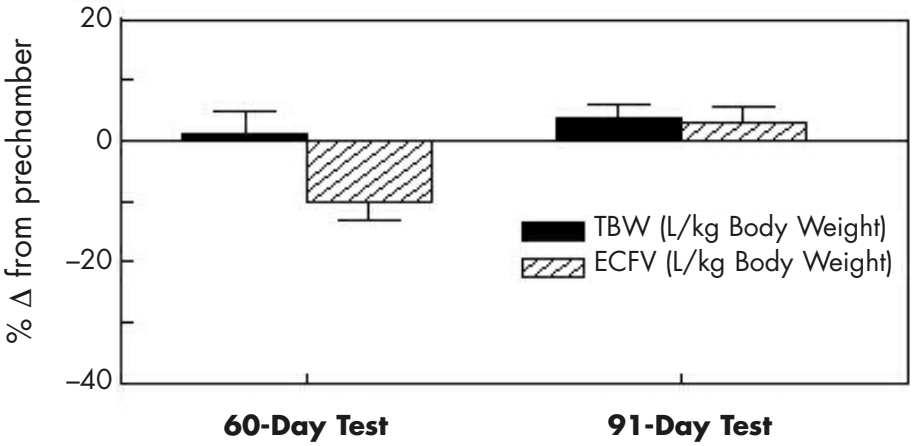

Figure 5.1-2 *Fluid compartments (TBW, ECVF) for the 60-day and 91-day chamber tests. Data are expressed for each individual as a percent change from their prechamber measurement*

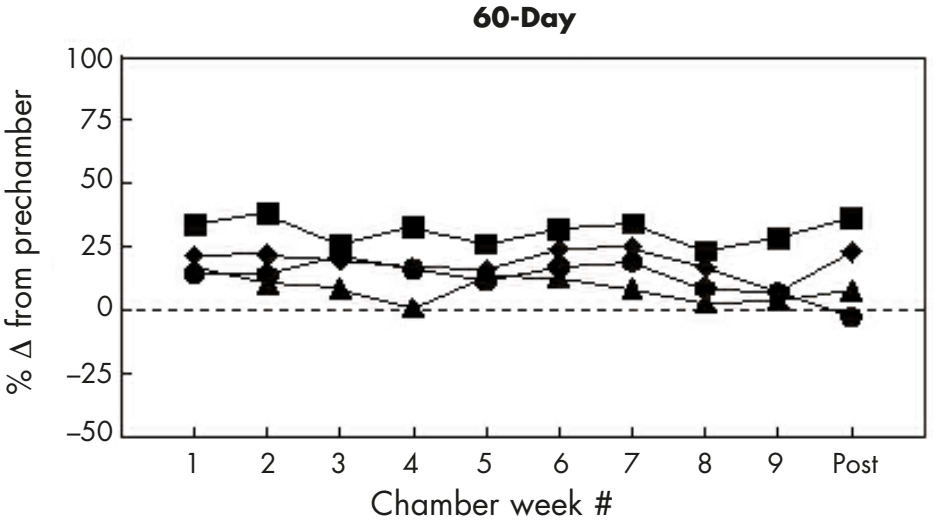

Figure 5.1-3 *Urinary creatinine excretion for the 60-day and 91-day chamber tests. Data are expressed for each individual as a percent change from their prechamber data*

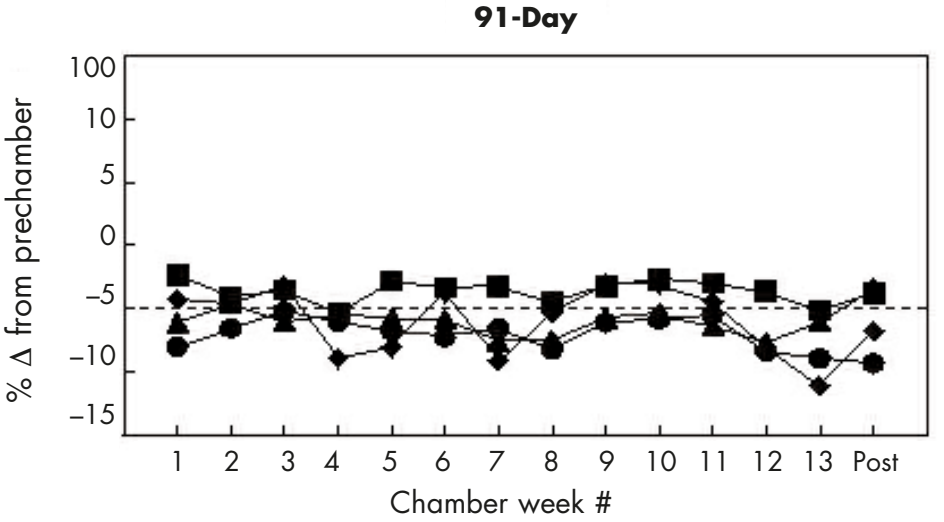

Figure 5.1-3 continued *Urinary creatinine excretion for the 60-day and 91-day chamber tests. Data are expressed for each individual as a percent change from their prechamber data*

Iron status tended to be negatively influenced throughout both studies (Table 5.1-3, Figure 5.1-4), despite high dietary iron intake (Table 5.1-2, Figure 5.1-4). Serum ferritin decreased by 21 ± 13 μg/L ($p = 0.054$) after the 60-day test, and by 29 ± 22 μg/L ($p < 0.05$) after the 91-day test. All subjects had iron intakes in excess of NASA recommendations. Most other hematological parameters (Table 5.1-3) tended to decrease.

There was a steady decline in serum 25-hydroxyvitamin D concentrations noted throughout the 91-day study, with final concentrations being significantly lower than prechamber values (Table 5.1-4, Figure 5.1-5). There was a tendency for both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations to decline in both studies (Figure 5.1-5). Vitamin D intake (Figure 5.1-5) was below the NASA recommendation of greater than 10 mg/day in six of the eight subjects, although dietary vitamin D intake was higher in the 60-day study compared to the 91-day study (Figure 5.1-5). There was also a small but statistically significant decline in serum calcium at CD30, although all data during the 91-day study were within clinical normal ranges (Table 5.1-4). Bone-specific alkaline phosphatase was increased at the end of the 60-day study but not the 91-day study (Table 5.1-4). Other indices of bone and calcium metabolism were unchanged (Table 5.1-4).

Table 5.1-3 *Hematological and iron status indices*[1]

	60-Day Chamber Study		91-Day Chamber Study			
	Pre	Post	Pre	CD30	CD40	Post
Hemoglobin (g/L)	149 ± 13[2]	146 ± 11	134 ± 4	130 ± 8	127 ± 7	126 ± 5
Hematocrit	0.44 ± 0.05	0.42 ± 0.04	0.39 ± 0.01	0.38 ± 0.03	0.37 ± 0.02	0.37 ± 0.01
Mean corpuscular vol (fL)	93 ± 3	92 ± 3	90 ± 4[ab]	90 ± 3[ab]	91 ± 3[a]	89 ± 4[b]
Serum ferritin (µg/L)	119 ± 20	98 ± 31[2]	77 ± 57[a]	68 ± 53[ab]	66 ± 56[ab]	49 ± 36[b]
Ferritin iron µg Fe/L % saturation	 20.7 ± 6.2 17.5 ± 5.1	 16.6 ± 4.5 17.5 ± 4.9	 [3] 	 [3] 	 [3] 	 [3]
Transferrin (g/L)	2.27 ± 0.20	2.22 ± 0.35	2.73 ± 0.37	2.53 ± 0.22	2.53 ± 0.27	2.73 ± 0.26
Transferrin receptors (mg/L)	3.6 ± 0.9	3.5 ± 1.6	3.8 ± 0.9	4.0 ± 1.1	4.2 ± 0.8	3.4 ± 0.5

[1]Data are mean ± SD. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other
[2]$p = 0.054$
[3]Analyses not available

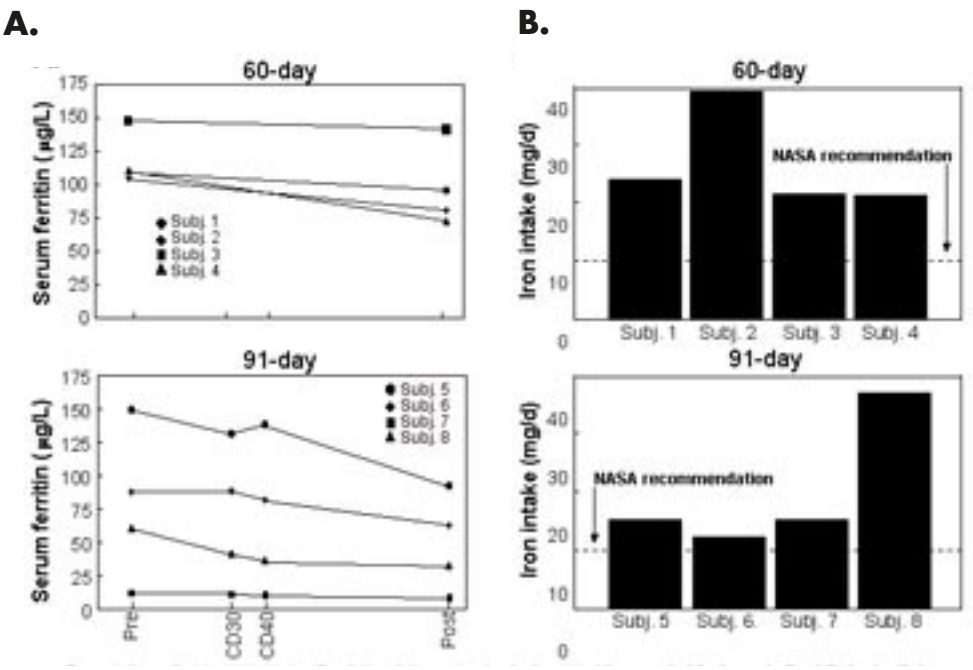

Figure 5.1-4 *Serum ferritin concentration (Panel A) and dietary iron intake determined from weighed food records (Panel B) for the 60-day and 91-day chamber tests*

Table 5.1-4 *Serum calcium and bone metabolism markers*[1]

	60-Day Chamber Study		91-Day Chamber Study			
	Pre	Post	Pre	CD30	CD40	Post
Calcium						
Total (mmol/L)	2.54 ± 0.06	2.54 ± 0.12	2.43 ± 0.11[a]	2.26 ± 0.09[b]	2.35 ± 0.14[ab]	2.35 ± 0.07[ab]
Ionized (mmol/L)	1.27 ± 0.01	1.27 ± 0.02	1.27 ± 0.04	1.26 ± 0.04	1.26 ± 0.05	1.27 ± 0.02
Parathyroid hormone (ng/L)	26.9 ± 9.3	25.8 ± 7.3	21.8 ± 12.9	18.6 ± 9.1	28.6 ± 16.5	22.3 ± 7.5
25-(OH)-vitamin D (nmol/L)	45.9 ± 6.3	43.5 ± 6.3	76.3 ± 14.4[a]	58.9 ± 13.2[ab]	54.9 ± 17.1[ab]	44.2 ± 23.1[b]
1,25-$(OH)_2$-vitamin D (pmol/L)	56.2 ± 38.5	60.9 ± 31.2	74.1 ± 29.0	59.2 ± 20.2	65.7 ± 22.3	47.0 ± 30.3
Alkaline phosphatase						
Total (μkat/L)	0.8 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	1.1 ± 0.4	1.1 ± 0.3	1.1 ± 0.3
Bone-specific (μkat/L)	0.18 ± 0.04[a]	0.24 ± 0.06[b]	0.16 ± 0.06	0.16 ± 0.09	0.16 ± 0.09	0.16 ± 0.08
Osteocalcin (ng/mL)	12 ± 3	11 ± 4	10.3 ± 4.8	12.1 ± 5.3	12.9 ± 5.4	11.3 ± 6.7

[1]Data are mean ± SD. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other

Table 5.1-5 *General chemistry indices*[1]

	60-Day Chamber Study		91-Day Chamber Study			
	Pre	Post	Pre	CD30	CD40	Post
Total protein (g/L)	72 ± 3	69 ± 1	71 ± 4[a]	65 ± 4[b]	65 ± 5[b]	68 ± 4[ab]
Albumin (g/L)	44 ± 2	43 ± 4	45 ± 3	43 ± 3	44 ± 4	45 ± 3
Transthyretin (mg/L)	[2]	[2]	274 ± 45	250 ± 55	255 ± 85	240 ± 67
Creatinine (μmol/L)	104 ± 15	97 ± 13	82 ± 11	77 ± 15	75 ± 17	73 ± 20
Cholesterol (mmol/L)	4.53 ± 0.76	4.25 ± 0.84	4.56 ± 0.94	4.63 ± 1.12	4.20 ± 0.97	4.56 ± 1.31
Triglycerides (mmol/L)	0.7 ± 0.2	0.87 ± 0.13	0.89 ± 0.67	0.94 ± 0.44	1.06 ± 0.64	0.95 ± 0.74
Sodium (mmol/L)	142 ± 1[a]	140 ± 1[b]	139 ± 2[a]	140 ± 0[ab]	141 ± 1[b]	139 ± 0[a]
Potassium (mmol/L)	3.9 ± 0.3	3.7 ± 0.1	3.9 ± 0.4	3.7 ± 0.1	3.7 ± 0.2	3.5 ± 0.1
Chloride (mmol/L)	108 ± 3	104 ±	106 ± 1	107 ± 3	107 ± 2	107 ± 2
Aspartate transaminase (U/L)	25 ± 3	26 ± 6	20 ± 3	20 ± 4	19 ± 1	18 ± 3
Alanine transaminase (U/L)	18 ± 4	22 ± 10	17 ± 6	16 ± 2	13 ± 2	13 ± 3

[1]Data are mean ± SD. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other
[2]Analyses not available

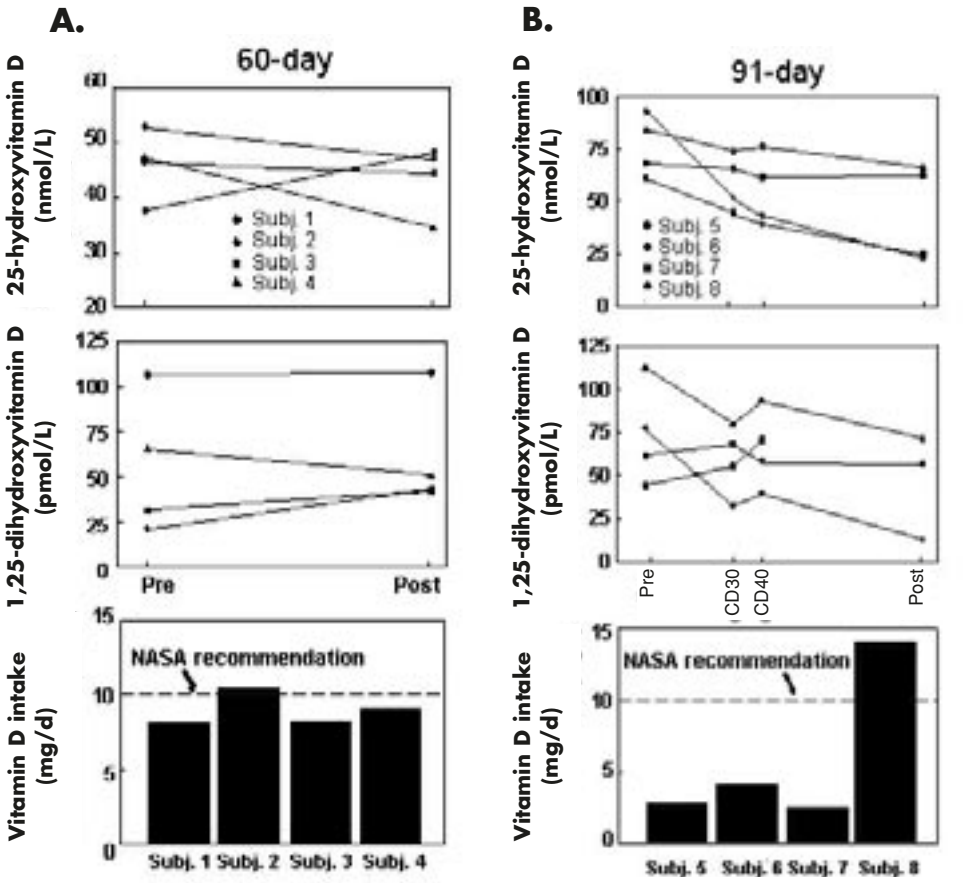

Figure 5.1-5 *Serum vitamin D metabolite concentrations and dietary vitamin D intake determined from weighed food records for the 60-day (Panel A) and 91-day (Panel B) chamber tests*

Note: There was insufficient sample to complete 1, 25-dihydroxyvitiamin D determinations on Subject 5

General clinical chemistry (Table 5.1-5) and antioxidant-related measurements (Table 5.1-6) were relatively unchanged during the two chamber studies. There was a negligible, albeit statistically significant, decrease in serum sodium concentration during the 60-day study. Serum sodium was slightly elevated on CD40 during the 91-day study. Serum total protein concentrations were slightly decreased on CD30 and CD40 and returned to prechamber levels after the 91-day study. Glutathione peroxidase activity was slightly elevated during the 91-day chamber study. Urinary calcium and collagen crosslink (n-telopeptide, pyridinium crosslinks, and deoxypyridinoline) excretion did not change during either of the chamber studies (Figure 5.1-6).

Table 5.1-6 *Vitamin status antioxidant/oxidative damage indices*[1]

60-Day Chamber Study			
	Pre	CD32[2]	Post
RBC transaminase (% activation; vitamin B_6)	113 ± 13		121 ± 18
RBC glutathione reductase (% activation; riboflavin)	17.8 ± 6.5		10.9 ± 1.5
RBC folate (nmol/L)	928 ± 54		1092 ± 167
RBC superoxide dismutase (U/g Hb)	592 ± 40		659 ± 43
RBC glutathione peroxidase (U/g Hb)	26.3 ± 3.1		25.2 ± 1.9
Oxygen radical absorbance capacity (mmol/L)	1.13 ± 0.09		1.18 ± 0.13
8-OH-2'-deoxyguanosine (µmol/mol creatinine)	1.16 ± 0.14	1.18 ± 0.50	1.20 ± 0.34

90-Day Chamber Study					
	Pre	CD30	CD40	CD60[2]	Post
RBC transaminase (% activation; vitamin B_6)	89.6 ± 11.8	93.8 ± 20.1	95.2 ± 18.5		88.3 ± 11.0
RBC glutathione reductase (% activation; riboflavin)	31.6 ± 24.8	32.8 ± 29.3	28.9 ± 20.2		25.2 ± 18.7
RBC folate (nmol/L)	1662 ± 532[a]	1763 ± 571[ab]	1796 ± 531[ab]		1907 ± 610[b]
RBC superoxide dismutase (U/g Hb)	986 ± 143	943 ± 122	986 ± 90		1050 ± 92
RBC glutathione peroxidase (U/g Hb)	46.6 ± 14.9[ab]	56.8 ± 11.9[a]	53.6 ± 15.8[ab]		44.3 ± 14.3[b]
Oxygen radical absorbance capacity (mmol/L)	1.17 ± 0.08	1.10 ± 0.10	1.13 ± 0.11		1.23 ± 0.13
8-OH-2'-deoxyguanosine (µmol/mol creatinine)	1.37 ± 0.32	1.34 ± 0.43	1.24 ± 0.41	1.38 ± 0.50	1.23 ± 0.49

[1]Data are mean ± SD. For each study, data in the same row with different letter superscripts are significantly ($p < 0.05$) different from each other

[2]Urine samples were collected and analyzed at CD32 of the 60-day study and on CD60 of the 91-day study; however, blood samples were not

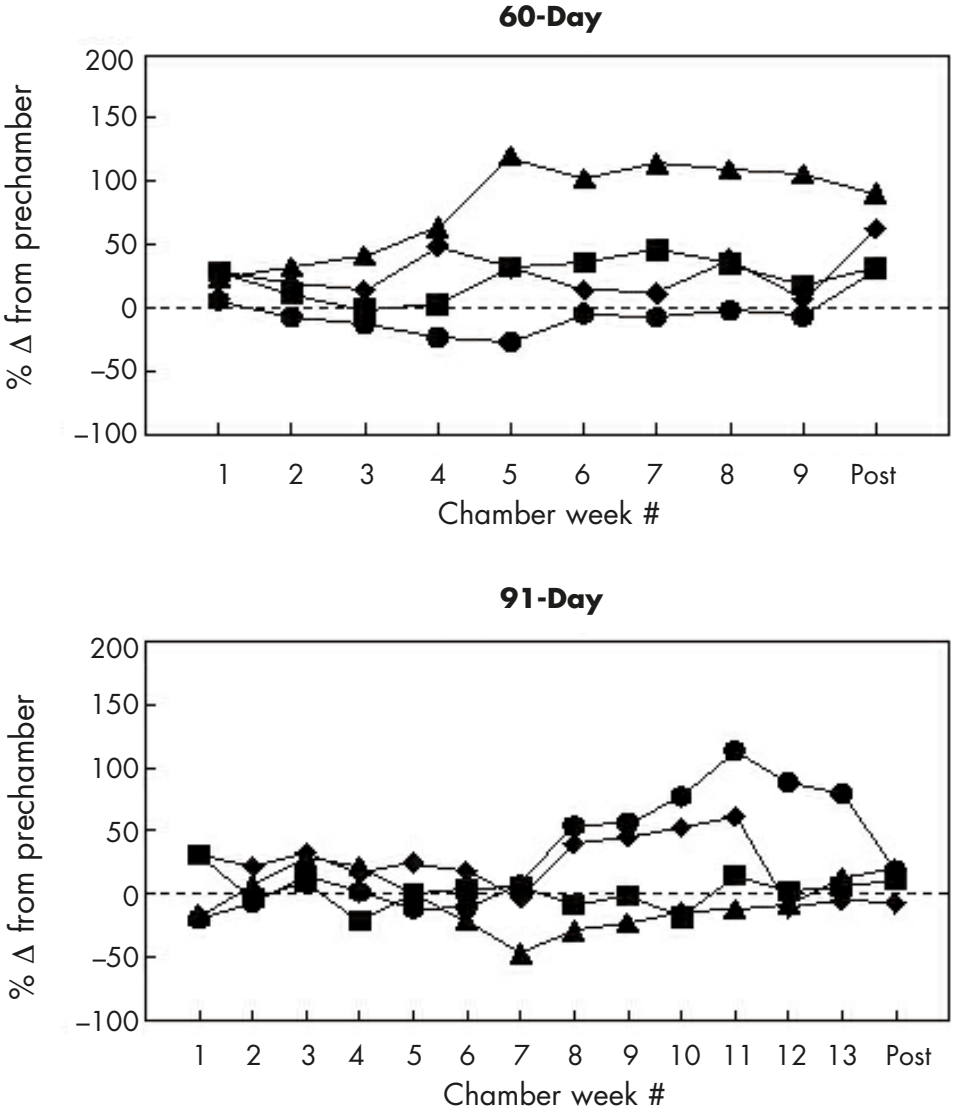

Figure 5.1-6 *Urinary collagen crosslink excretion for n-telopeptide for the 60-day and 91-day chamber tests*

Folate status, as assessed by the concentration of RBC folate, increased by more than 16% in three subjects during the 60-day study and increased by more than 17% in three subjects during the 91-day study (Figure 5.1-7a, Table 5.1-6). Folate intake, as determined during the weighed diet sessions, was generally above standard recommendations (Figure 5.1-7b).

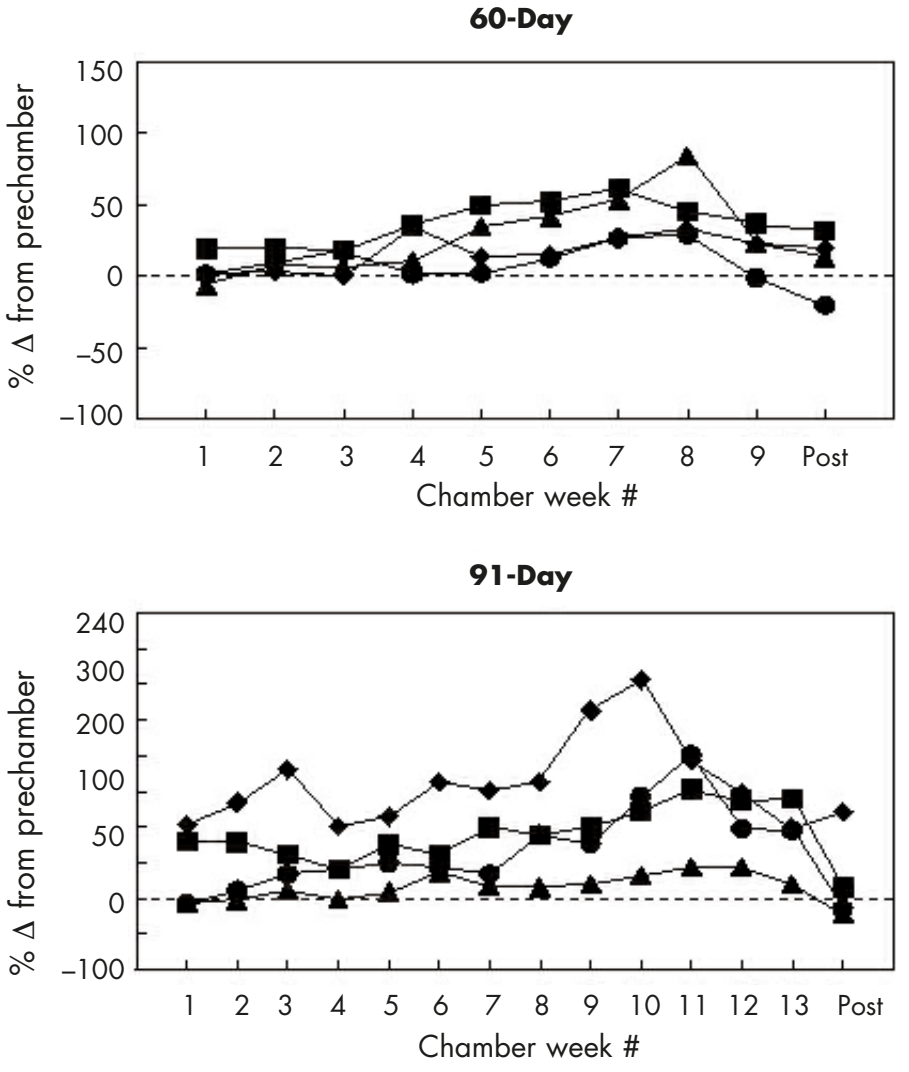

Figure 5.1-6 continued *Urinary collagen crosslink excretion for pyridinium crosslinks for the 60-day and 91-day chamber tests*

Vitamin B_6 and riboflavin status were unchanged during the chamber studies (Table 5.1-6). Thiamin status, as assessed by erythrocyte stimulation of transketolase by thiamin pyrophosphate, did not change from prechamber levels during the 91-day study (data not presented). Thiamin data were not available for the 60-day study.

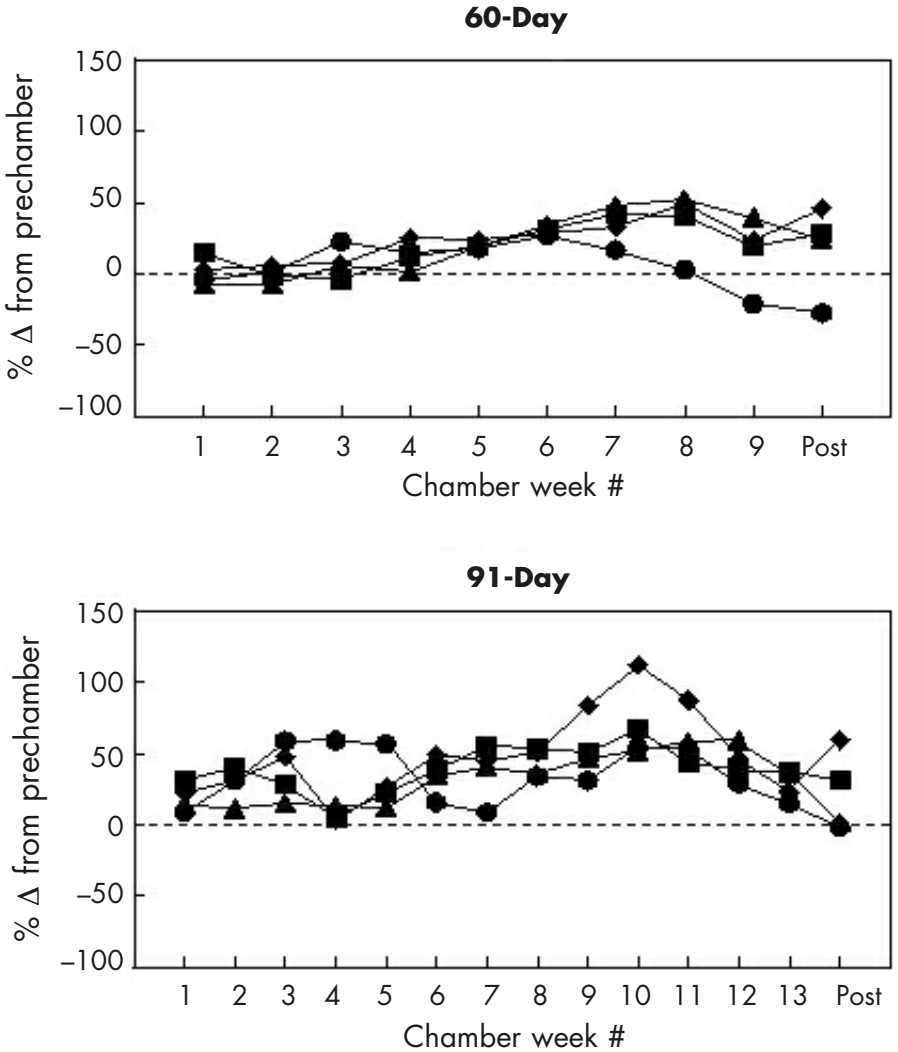

Figure 5.1-6 continued *Urinary collagen crosslink excretion for deoxypyridinoline for the 60-day and 91-day chamber tests*

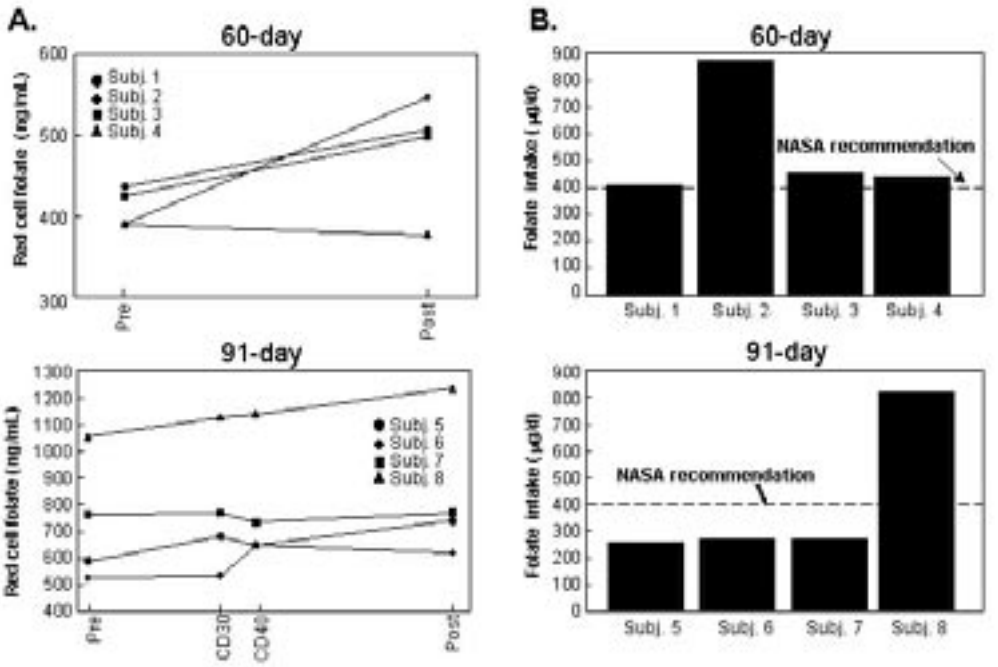

Figure 5.1-7 *Red blood cell folate concentration (Panel A) and dietary folate intake determined from weighed food records (Panel B) for the 60-day and 91-day chamber tests*

Discussion

The study described here provided a valuable opportunity to test a nutritional assessment profile and a unique food frequency questionnaire in an environment similar to that found on a space station, without the constraints of an actual space mission. The results indicate that a specially designed food frequency questionnaire can be used to reliably estimate individual dietary intake. These studies confirm that a semiclosed food system can support nutritional requirements over a short period of time (i.e., two to three months).

The comprehensive nutritional status assessment profile described here (with minor modifications) has been implemented by NASA as a medical requirement for extended-duration (i.e., International Space Station) space travelers. The anthropometric, biochemical, clinical, and dietary assessment components each contributes valuable information to the total picture of nutritional status. The intent is to provide a preflight assessment of crew nutritional status to assure optimal status prior to flight, a real-time means of monitoring dietary intake during flight, and a nutritional component for the postflight rehabilitation program.

Inadequate dietary intake is a significant concern during space flight. Skylab crewmembers consumed the amount of energy prescribed (7) due to experimental constraints which required adequate intake. This demonstrated that it is indeed possible to meet the dietary recommendations during space flight. Subjects in the studies provided here consumed adequate amounts of energy and maintained body mass. The FFQ developed and tested here will provide the ability to monitor and make recommendations to the crewmembers about dietary intake while on orbit.

Fluid compartments were unaffected after both chamber studies as determined by isotope dilution methods. ECFV determined using the liquid bromide dose was better tolerated in the 91-day study, however the determinations were higher than expected. ECFV, which is approximately 40% of total body water (6), was 62 ± 4% of measured total body water in the 91-day study compared to 33 ± 5% in the 60-day study. Although ECFV and total body water are typically highly correlated (6), neither the capsule nor liquid forms of the sodium bromide correlated well with total body water measurements (R = 0.42 and 0.18, respectively) in these studies. A previous evaluation of the liquid dosing regimen was conducted with 10 subjects, where ECFV was determined by both bromide dilution and bioimpedance techniques (2). These ECFV measurements were similar (bromide: 20.9 ± 5.1 L, BIA: 20.3 ± 4.5 L) and correlated well with BIA determination of total body water (R = 0.89). These observations suggest that additional modifications may be needed for routine determination of ECFV by bromide dilution.

Bone mineral loss during space flight results in increased urinary crosslink (12) and calcium excretion (9, 10). Hypercalciuria contributes to the increased risk of renal stone formation associated with space flight (14). Vitamin D is of concern during space flight due to absence of endogenous production related to the lack of ultraviolet light exposure (4) and also due to its importance in bone and calcium metabolism. Vitamin D stores were decreased in the 91-day chamber study but were unchanged in the 60-day study.

Iron status appeared to decline during the course of the studies (e.g., decreased ferritin, and a tendency for decreased hemoglobin and hematocrit). This occurred despite relatively high iron intakes. However, in examining individual diet records for the source of this iron, much of the intake was associated with (low bioavailability) fortified cereals. Conversely, limited intakes of other micronutrients may be of concern when individuals are dependent upon a closed or semiclosed food system for truly extended periods (i.e., years).

Although nutritional status was generally adequate in the 60-day and 91-day tests, micronutrient status is of concern in a semiclosed food system. Three subjects in the 91-day test had inadequate folate intakes, and three subjects in each test had inadequate vitamin D intakes. However, 10 days of the vegetarian BIO-Plex diet did not affect any of the biochemical indices examined during the 90-day test.

SIGNIFICANCE

This study was important for evaluating the space flight food frequency questionnaire and also for assessing a food system similar to that planned for the International Space Station. The International Space Station food system is still in development, and the data collected here will be important in further defining and refining this system in order to assure optimal health during long-duration flights.

Acknowledgments

We thank the Lunar-Mars Life Support Test Project crewmembers for their time, effort, and dedication to the success of these studies. The Nutritional Status Assessment profile reported here was defined and refined with the help of a panel of extramural experts. We gratefully acknowledge the advice and guidance offered by Gladys Block, Stephen Coburn, Michael Holick, Carl Keen, Donald McCormick, James Olson, and Connie Weaver.

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5.2

Exercise Countermeasures Demonstration Projects During the Lunar-Mars Life Support Test Project Phases IIa and III

Stuart M.C. Lee, Ph.D., Mark E. Guilliams, Suzanne M. Schneider, Ph.D.

SUMMARY

The purpose of this demonstration project was to assess the compliance of crewmembers to perform exercise countermeasures similar to those planned for use during stays aboard the International Space Station (ISS) and to assess the outcomes of performing these countermeasures. During the 60-day Phase IIa project, crewmembers exercised for six consecutive days alternating between aerobic and resistive exercise, and rested on the seventh day. On the aerobic exercise days, subjects exercised for 30 minutes on an electronically braked cycle ergometer using an interval protocol. On the resistive exercise days, crewmembers performed five major multijoint resistive exercises (bench press, seated press, lat pull, squats, and heel raises) in a concentric-only mode, targeting those muscle groups and bones that are believed to be most severely affected by space flight. Subjects performed maximal efforts with each repetition. Both exercise protocols were well tolerated by the subjects, demonstrated by 98% compliance with the aerobic exercise prescription and 91% adherence to the resistive exercise prescription. After 60 days, the crewmembers improved their peak aerobic capacity by an average of 7%. Strength gains during all exercises were noted.

During the 91-day Phase III project, the frequency of the exercise countermeasures was increased to include both aerobic and resistive exercises each day for six days, with rest on the seventh day. For aerobic exercise, the cycle protocol was performed three days/week similar to the Phase IIa project. However a steady-state treadmill protocol was added on the remaining three exercise days. The same resistance exercise protocol was performed as in Phase IIa, except that the upper- and lower-body exercises were divided and performed on separate days. Three of the four subjects tolerated the aerobic exercise training well. One crewmember developed knee pain in the final third of the chamber test and did not perform further cycle or lower-body resistive exercises for the remainder of the study. The three

crewmembers who participated in all countermeasures and postchamber testing had an average increase in aerobic capacity of 14%. Of these remaining three crewmembers, one consistently refrained from resistive exercise one day per week. Strength gains were not consistently obtained during this study. These results, showing little or no change in muscle strength while demonstrating some improvement in aerobic capacity, may be consistent with an overtraining syndrome.

Taken together, the results from these two studies suggest that the prescribed aerobic and resistive exercises generally were well tolerated. However, combining both resistive and aerobic exercises with only one day of rest each week may result in a decreased benefit of strength training. Periodization of exercise protocols and/or reduction of exercise intensity or frequency may be desired to obtain optimum increases in both aerobic and resistive exercise capacities.

Introduction

Four crewmembers participated in each of two chamber tests. Phase IIa was a 60-day chamber test while the Phase III test had a duration of 91 days. Previous chamber studies were conducted in which exercise was performed, but these two projects were the first in which specific exercise prescriptions were developed for the crewmembers and the outcomes of the exercise protocols were measured. These two chamber studies served as ground-based test beds for exercise countermeasure procedure development in support of future activities for the crews of the International Space Station (ISS).

The objectives of the Exercise Countermeasures Demonstration Projects were:

1) to assist in the development and evaluation of exercise testing and prescription methods being considered for ISS; and
2) to provide realistic perturbations of carbon dioxide production and oxygen utilization as anticipated during ISS to challenge the environmental control systems.

In each project, eight crewmembers were selected for participation, four as prime and four as back-up. Crewmembers were screened for health status by means of a modified Air Force Class III Physical and a graded treadmill exercise test to volitional fatigue with 12-lead electrocardiogram. Subjects received written and verbal explanation of the procedures specific to the exercise countermeasures demonstration project and signed informed consent documents confirming their understanding and acceptance. All testing procedures and protocols were reviewed and approved by the NASA Johnson Space Center Institutional Review Board. All pre and postchamber testing, as well as prechamber training, were performed in the NASA Johnson Space Center Exercise Physiology Laboratory. Eventually, four crewmembers entered the chamber in each study and remained there for the duration of the test project. In Phase IIa, the crew consisted of three men and one woman. In Phase III, the crew consisted of two men and two women. Data presented here are from the four prime crewmembers only from each of the two chamber tests.

Phase IIa Methods

Study Overview

The characteristics of the prime crewmembers, three males and one female, were: age – 31 ± 4 years; height – 175 ± 5 cm; and body mass – 70.4 ± 10.9 kg. Prior to entry into the chamber, crewmembers completed both a graded maximal cycle exercise test to volitional fatigue and a submaximal cycle ergometer exercise protocol. Crewmembers also received training on the aerobic and resistance exercise countermeasures prior to chamber entry. At the conclusion of the chamber test, the four crewmembers repeated the maximal cycle exercise test.

During the chamber test, on alternate days the crewmembers completed the aerobic and the resistive countermeasures three times per week. In several instances, exercise was delayed or cancelled due to malfunction of the environmental control systems. Three times during the 60-day period, on days 15, 30, and 58, crewmembers completed the submaximal exercise test in place of the aerobic exercise training to assess their training status.

The aerobic exercise testing protocols chosen for this project are similar to those proposed for use in the Space Medicine Project (SMP) on the ISS as a means to monitor crew health. Similarly, the exercise countermeasures, both aerobic and resistive, were similar to those suggested for use on the ISS to maintain crew health. However, no pre to postchamber resistance exercise testing was performed during the Phase IIa test.

Maximal Cycle Exercise Test

Crewmembers completed a maximal exercise test on a cycle ergometer to quantify their individual fitness levels and to aid in the prescription of the aerobic exercise countermeasure. Data from this test were used also to develop the exercise prescription for the submaximal exercise test. Crewmembers performed the maximal cycle exercise test both before chamber entry and after chamber exit.

Crewmembers pedaled on an electronically braked cycle ergometer in the upright position at a constant pedaling cadence of 75 rpm. Expired gases were collected and analyzed using a Quinton Qplex Metabolic Cart (Quinton Industries, Seattle, WA) interfaced with a mass spectrometer (MG-1100, Marquette, Inc., Minneapolis, MN). Heart rate (HR) was monitored using a three-lead ECG configuration (Quinton Q5000 Stress Test System, Quinton Industries, Seattle, WA). The maximal cycle test began with three 3-minute stages of increasing workloads. For male subjects, these workloads were 50, 100, and 150 watts. Female subjects completed workloads of 50, 75, and 100 watts. Thereafter, for both subject groups the workload was increased in 25-watt increments each minute until volitional fatigue. Peak oxygen consumption (VO_2pk) was accepted as the mean of the last two 30-second measurements of oxygen consumption (VO_2). HR was recorded in the last 15 seconds of each minute, and Rating of Perceived Exertion (RPE; Borg's revised 10-point scale) (10) was recorded in the last 20 seconds of each

stage. Systolic (SBP) and diastolic blood pressures (DBP) were measured manually by the auscultatory method in the last 30 seconds of each three-minute stage.

Submaximal Cycle Exercise Test

The submaximal cycle exercise test was prescribed individually for each subject according to the level of performance of each subject in the maximal cycle exercise test. Subjects completed three 5-minute exercise intensities of 25, 50, and 75% of VO_2pk on the same electronically braked upright cycle ergometer. Subjects recovered by cycling for five minutes at 25% of VO_2pk. The pedaling cadence was maintained at 75 rpm. Expired gases were collected as the subjects exercised using a Quinton Qplex Metabolic Cart interfaced with a mass spectrometer. HR was measured using a HR monitor (Polar Vantage XL, Polar, Inc., Stamford, CT), previously validated in our laboratory (13). HR data were saved in 15-second intervals. Means of both VO_2 and HR measured in the last two minutes of each stage were calculated. This test was performed twice prior to chamber entry and repeated on days 15, 30, and 58 of the chamber stay. Testing days were chosen to be similar to those anticipated for crewmembers aboard the ISS. Tests conducted prior to the chamber stay were performed with metabolic gas analysis. The other tests were self-administered by the subjects in the chamber without metabolic gas analysis.

Aerobic Exercise Countermeasure

Based upon the results of the VO_2pk exercise test, an exercise countermeasure (Figure 5.2-1) that has been used previously to maintain exercise capacity in bed rest subjects (4) was prescribed. This exercise protocol was performed on the same cycle used during the prechamber testing at a constant pedaling cadence of 75 rpm. Each crewmember's individual aerobic exercise prescription was preprogrammed into the cycle ergometer. HR data were recorded each 15-sec during the exercise countermeasure using a HR monitor. The data were downloaded on a weekly basis and added to each individual's database.

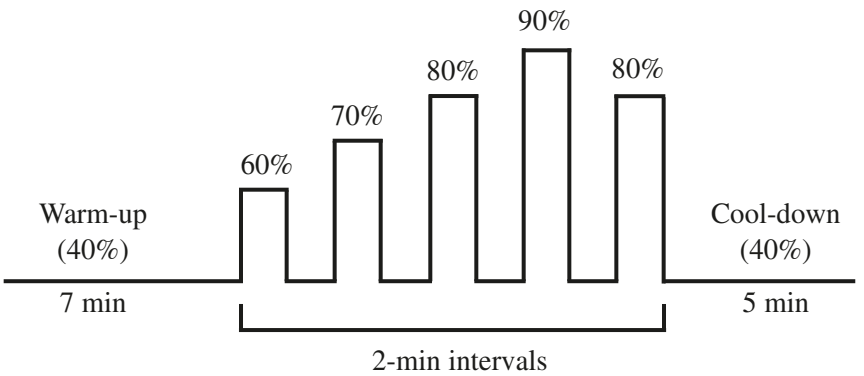

Figure 5.2-1 *Aerobic exercise countermeasure protocol*

Resistance Exercise Countermeasure

Crewmembers trained isokinetically three days per week on a computer-controlled resistive exercise device (Computerized Exercise System (CES), Ariel Life Systems, Inc., San Diego, CA). The CES consists of a single, multifunction exercise station, using passive hydraulic resistance, integrated with a laptop computer. This multifunction station allows for the performance of several multijoint exercises. Crewmembers performed bench press, seated shoulder press, lat pull, squats, and heel raises. All training was in the concentric mode only.

Throughout the study, subjects performed four sets of each exercise, one warm-up set at approximately 50% of their maximum effort followed by three sets of maximal effort with each repetition. The first week of resistance training in the chamber was treated as a familiarization period. Each day of the first week, crewmembers performed four sets of 10 repetitions of each exercise at 40°/sec with the exception of the heel raise that was performed at 15°/sec. From weeks two to nine, crewmembers performed a miniperiodization of resistance exercise within each week. The number of sets was maintained at four throughout, one warm-up and three at maximal effort, but the velocity of movement, number of repetitions per set, and amount of muscle tension developed varied across the week (Table 5.2-1). On the first day of resistance training within the week, the bench press, lat pull, seated shoulder press, and squats were performed at a slow speed (LO) of 20°/sec for six repetitions per set. The second day of training was performed at the fastest speed (HI) of 50°/sec for 12 repetitions, and the third day was performed at a moderate speed (MED) of 35°/sec for eight repetitions. Crewmembers performed the same number of repetitions for the heel raises as the other exercises, but the velocities of movement were 10°/sec on LO, 15°/sec on MED, and 20°/sec on HI. By performing maximal efforts with each repetition on each day, the subjects generated the greatest muscle forces on the first day (LO) during the slow speed of movement, the least muscle tension on the second day (HI) during the fastest movement speed, and a moderate amount of muscle tension during moderate movement speed (MED).

Table 5.2-1 *Movement velocity and repetitions for each resistive exercise day*

Movement Speed	Calf Raise		Others	
	Speed (°/sec)	Repetitions	Speed (°/sec)	Repetitions
LO	10	6	20	6
MED	15	8	35	8
HI	20	12	50	12

The torque profile for each repetition performed during the resistance training was automatically stored on the laptop computer for later analysis. Variables of interest in this demonstration project were peak torque, average peak torque, and total work. Peak torque was taken as the highest torque output from a single repetition measured in each individual set averaged across the three sets. Average peak torque was the average of the peak torque from every individual repetition from all three sets. Total work was the summation of work performed in all three sets. The data from the warm-up set and from the first week of training were not included in this analysis.

Data Analysis

All data are expressed as mean ± standard error (SE), unless otherwise noted. Although the sample size is small, the data from the aerobic exercise tests were analyzed statistically to provide objective information regarding the trends in the data. Pre- to postmaximal aerobic exercise data were statistically analyzed using dependent t-tests. Pre to postsubmaximal exercise data from the VO_2pk exercise test were analyzed using repeated measures ANOVA.

An ANOVA revealed no difference in HR at each of the workloads during the duplicate prechamber submaximal aerobic exercise tests. Therefore, the data from the two prechamber tests were averaged as a baseline measurement. Previous experience with other data sets (6) has suggested that the HR response to the higher workloads is affected most by changes in training status. Therefore, an ANOVA was performed on the HR response to the third submaximal exercise stage, 75% VO_2pk, across the four test times.

The efficacy of the resistive exercise countermeasure protocol was assessed in this project by examining the daily resistive exercise records for each subject. Peak torque, average peak torque, and total work data from the CES were calculated at early (week two), mid- (week five), and late (week eight) chamber stay. Only peak torque is reported here. Because of the varying amount of compliance within subjects, data were not statistically analyzed.

Phase IIa Results

Aerobic Exercise Countermeasure

Over the course of the nine weeks of the chamber confinement, crewmembers were prescribed to perform a total of 23 aerobic exercise countermeasure sessions. The range of compliance to this prescription was from 91 to 100% with a mean of 98 ± 4%. Two crewmembers completed all requested exercise sessions. Reasons for other crewmembers not completing all exercise sessions included work scheduling and failure of environmental control systems. Each subject attained the desired exercise intensities for this countermeasure protocol (Figure 5.2-2).

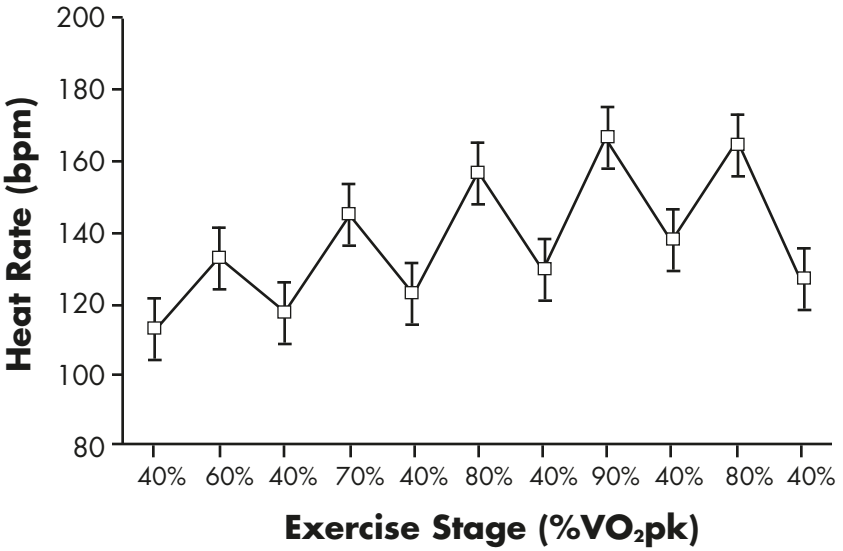

Figure 5.2-2 *Mean (± SE) heart rate response to aerobic exercise countermeasure across chamber confinement across all crewmembers*

Resistive Exercise Countermeasure

Crewmembers were prescribed to perform a total of 26 resistive exercise countermeasure sessions. The range of compliance for completing all or part of the daily resistive exercise prescription ranged from 81 to 100% with a mean of 91 ± 10%. Two subjects completed all the exercises prescribed each day, and one subject completed all the exercises on 21 out of the 26 resistance training days. No specific reason was given as to why this subject did not exercise. The fourth subject completed the upper-body exercises on 22 of the 26 resistance exercise days, but due to recurring back pain completed the squats and heel raises during only 58% of the exercise sessions. This subject had a previous history of back injury. Because of the varying amount of compliance within subjects, data were not statistically analyzed. However, visual inspection of these data suggest that crewmembers who completed the resistance exercise training exhibited increased strength across the chamber stay (Figure 5.2-3).

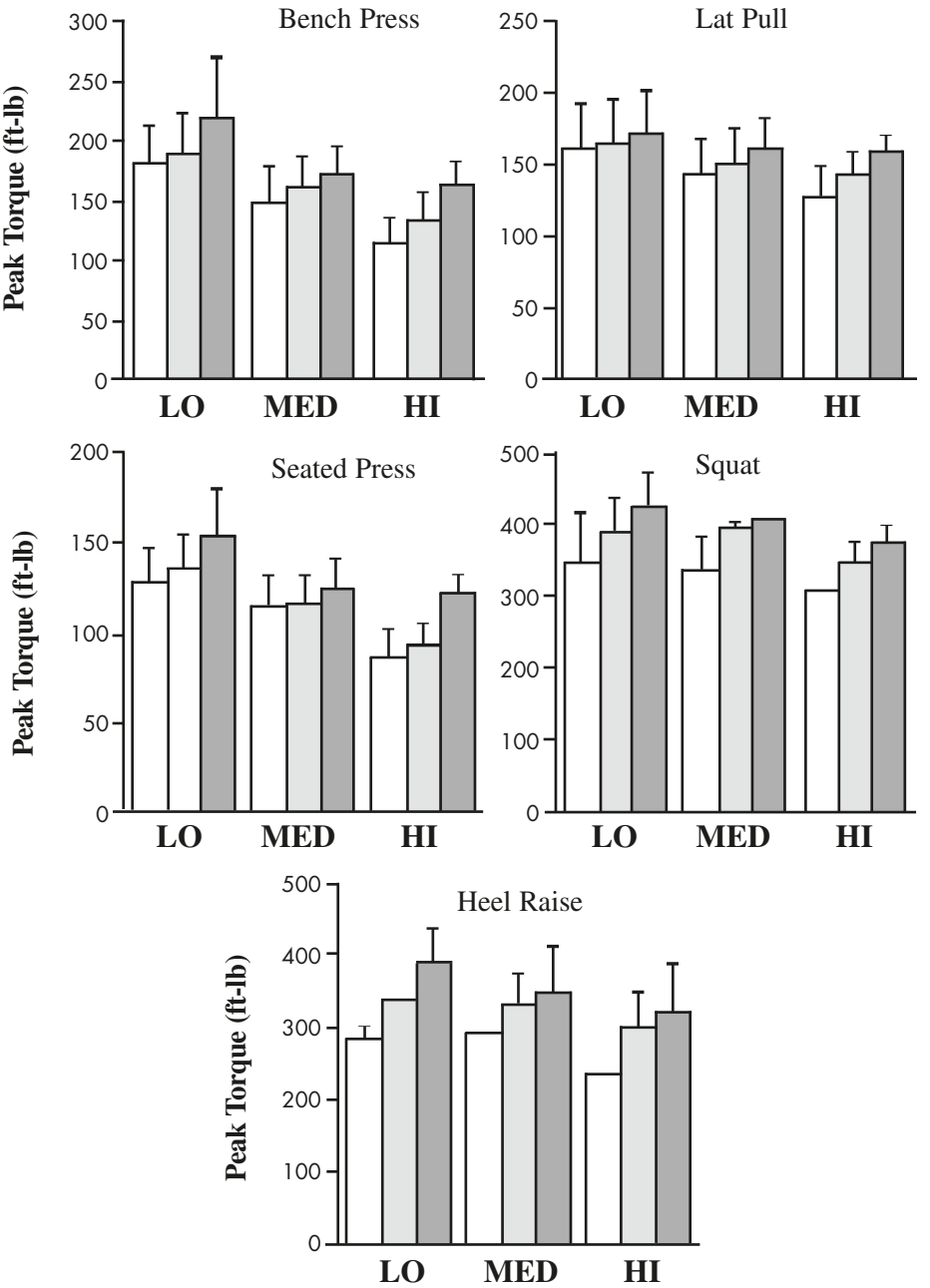

Figure 5.2-3 *Peak torque developed during bench press (n=3), seated press (n=3), lat pull (n=3), squat exercise (n=2), and heel raise (n=2) exercises across time at each training speed. Open bar is early (week 2), light gray bar is mid- (week 5), and dark gray bar is late (week 8) chamber stay.*

Pre to Postchamber Maximal Aerobic Exercise Test Results

The crewmembers' mean (± SE) VO_2pk was 2.82 ± 0.32 L/min (39.9 ± 5.5 ml/kg/min) before entering the chamber. This corresponded to a mean test time of 13.0 ± 0.5 min and a peak workload of 238 ± 22 watts. After the chamber stay, crewmembers significantly ($p < 0.05$) increased their total test time (13.9 ± 0.4 min) and the peak workload achieved (269 ± 24 watts). Although this resulted in a mean increase in VO_2pk of 7%, the improvement in VO_2 pk was not statistically significant when expressed as either absolute ($P = 0.06$) or relative ($P = 0.11$) VO_2. Mean peak HR was not changed from before (190 ± 2 bpm) to after the chamber stay (190 ± 3 bpm).

The mean submaximal HR, SBP, and DBP responses during the maximal exercise test were analyzed (Figure 5.2-4). The HR response to the first two submaximal exercise workloads was unchanged from pre to postchamber. However, the HR response at the third submaximal exercise stage was significantly less

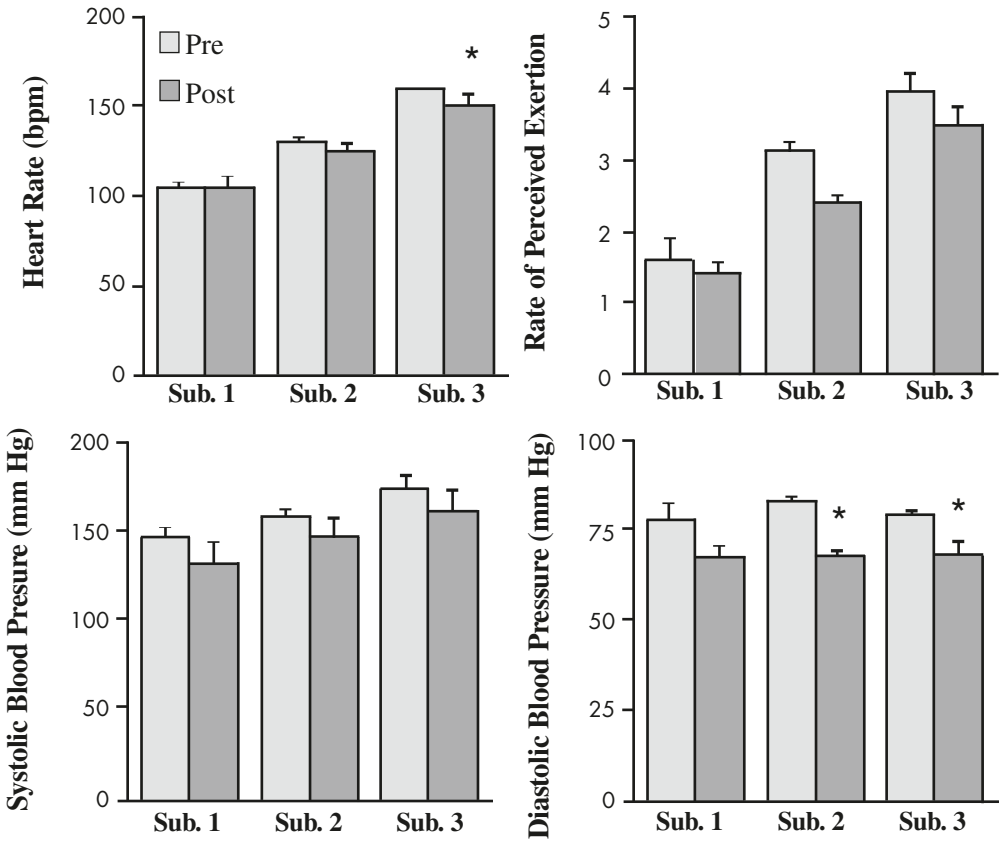

Figure 5.2-4 *Mean (± SE) heart rate, rating of perceived exertion, and blood pressure responses to submaximal exercise stages*
**Significantly different from prechamber*

($P < 0.02$) after the exercise training in the chamber (159 ± 3 vs. 149 ± 6 bpm). There was a main effect of time, pre to postchamber, on SBP, but pressures were not significantly different pre to postchamber at any particular submaximal exercise stage. The DBP during the submaximal exercise stages were significantly lower during the second (82 ± 2 vs. 67 ± 2 mm Hg) and third stages (78 ± 2 vs. 68 ± 3 mm Hg). There was also a main effect of time on the RPE reported during the submaximal exercise stages, but similar to SBP, there was no specific submaximal exercise stage in which the RPE were significantly different from pre to postchamber.

Pre- to In-Chamber Submaximal Aerobic Exercise Tests

All four prime crewmembers completed five submaximal exercise tests, two prior to and one each on day 15, day 30, and day 58 of the chamber stay. Although there was a trend ($P = 0.12$) towards a decrease in HR across time, there was no significant difference between the HR during the submaximal exercise test from prechamber to day 58 (Figure 5.2-5).

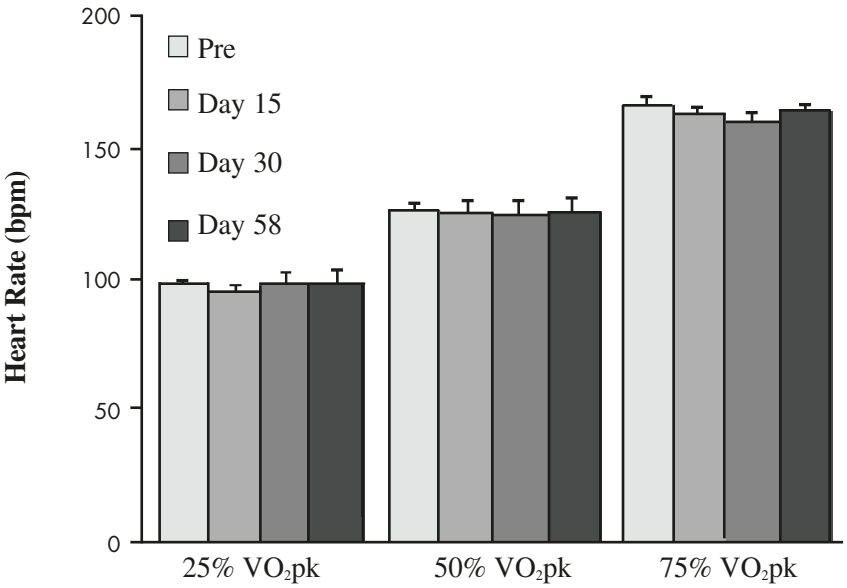

Figure 5.2-5 *Mean heart rate (± SE) during submaximal aerobic exercise tests for prime crew across chamber confinement*

Phase IIa Discussion

Maximal Cycle Exercise Test

The performance of this exercise test prior to and after the chamber stay appears to have been well tolerated. The duration of the exercise test allowed for adequate

warm-up by the subject prior to reaching higher exercise intensities. All four crewmembers demonstrated an increase in peak workload achieved, total test time completed, and improvements in submaximal exercise responses during the maximal exercise test. However, the increase in VO_2pk was not statistically significant.

Submaximal Cycle Exercise Test

One crewmember showed a "classical" training response of decreasing HR at each submaximal exercise test over test times. Two subjects showed a decline in HR on days 15 and 30, but the heart rate response on day 58 was unchanged from the prechamber test. One subject showed essentially no change in HR across testing times. There was no apparent difference between the subjects in relation to their exercise prescription adherence that would explain the differences in the individual responses. All four subjects trained at the same relative exercise intensity during the aerobic exercise countermeasure.

It is interesting to note that lower submaximal HR responses were seen during the maximal exercise test after the chamber test than before it. It is possible that the active work schedules and disrupted sleep patterns of the subjects influenced the HR responses during the submaximal exercise tests in the chamber. The more rigorously controlled atmosphere of the laboratory for the VO_2pk exercise test after the chamber test had been completed may have provided for better data acquisition to assess responses to submaximal exercise intensities.

Aerobic Exercise Countermeasure

Crewmembers from previous chamber tests participated in regular exercise, but this was the first time that an exercise was prescribed for each crewmember on an individual basis with respect to the aerobic exercise protocol anticipated for use on the ISS. Further, although previous crews believed that they increased their fitness through the exercise training (15), this was the first time during the chamber studies that improvements in aerobic capacity were objectively quantified.

Crewmembers increased their VO_2pk by an average of 7% with a range of 1 to 20%. The subject who experienced the least improvement in aerobic capacity as a result of the training performed within the chamber had the highest aerobic capacity prior to the study. Conversely, the subject with the lowest aerobic capacity had the greatest improvement. From this limited data set, it appears that the performance of these exercise countermeasure protocols most benefits less fit subjects.

Resistive Exercise Countermeasure

Data from the resistive exercise countermeasure are difficult to interpret in two of the four subjects. However, it appears that muscular strength was increased in all subjects who performed the exercise requested. Improved subject motivation, increased variety in exercises performed, and more objective testing protocols may improve results from future demonstration projects.

Phase III Methods

Study Overview

The Phase III test provided an opportunity to evaluate potential exercise countermeasure and testing procedures during an even longer, 91-day chamber stay. In Phase III, two men and two women served as crewmembers with a mean (± SD) age of 34 ± 6 years, height of 173.5 ± 11.9 cm, and body mass of 68.3 ± 10.4 kg.

The testing and monitoring procedures were similar to those used in Phase IIa, except that isokinetic tests of muscle strength and endurance were added to the testing regime. Prechamber testing included maximal cycle and treadmill tests to volitional fatigue, two submaximal cycle ergometer exercise tests, training sessions for the cycle and treadmill aerobic exercise countermeasures, and two isokinetic muscular strength and endurance tests. Subjects also received two training sessions on the CES, the same resistance exercise training device used in Phase IIa. During the chamber stay, crewmembers performed the submaximal cycle exercise test biweekly on the seventh day of the week. After chamber stay, crewmembers returned to the laboratory for the maximal cycle and isokinetic strength and endurance tests.

Peak Aerobic Exercise Tests

VO_2pk was assessed in each subject on both the cycle ergometer and the treadmill. The maximal cycle test was used to prescribe exercise and to assess changes in aerobic capacity before and after the chamber test. The protocol was identical to that used during the Phase IIa testing.

The maximal treadmill test was used to aid in the prescription of the treadmill countermeasure and was performed before the chamber test. Based upon the results of the previous treadmill test performed during the subject screening and upon the feedback of the subjects, exercise intensities were prescribed individually for each crewmember. Expired gases were collected and analyzed using a Quinton Qplex Metabolic Cart (Quinton Industries, Seattle, WA) interfaced with a mass spectrometer (MGA-1100, Marquette, Inc., Minneapolis, MN). Heart rate was monitored using a three-lead ECG configuration (Quinton Q5000 Stress Test System, Quinton Industries, Seattle, WA). The test began with three 3-minute stages of increasing speed while level running (i.e., 5, 6, and 7 mph). Thereafter, the treadmill speed was held constant, treadmill grade was increased in increments of 3% until volitional fatigue. VO_2pk was accepted as the mean of the last two 30-second measurements of oxygen consumption. HR was recorded in the last 15 seconds of each minute, and RPE was recorded in the last 20 seconds of each stage. HR and VO_2 were averaged in the last minute of each submaximal exercise stage to develop a regression that would be used for the determination of the treadmill speed during the countermeasure exercise.

Submaximal Cycle Exercise Tests

Submaximal cycle tests were performed twice before and every two weeks during the chamber stay using the same protocol described in the Phase IIa test.

Isokinetic Muscle Strength and Endurance

Crewmembers were tested on three occasions: two prechamber tests and one postchamber test. Concentric and eccentric isokinetic strength of the knee, ankle, and trunk, and muscular endurance of the knee were assessed during both flexion and extension (Table 5.2-2).

Table 5.2-2 *Isokinetic testing protocols*

Joint	Mode	Speed	Reps	Range of Motion
Knee	Isometric	0°/sec	4	60°
Knee	Concentric	60°/sec	6	10 to95°
Knee	Concentric	120°/sec	5	10 to 95°
Knee	Eccentric	60°/sec	5	20 to 95°
Knee	Concentric	120°/sec	21	10 to 95°
Ankle	Concentric	30°/sec	5	-20 to 25°
Ankle	Concentric	60°/sec	5	-20 to 25°
Ankle	Eccentric	60°/sec	5	-20 to 25°
Trunk	Concentric	60°/sec	5	75 to 130°
Trunk	Eccentric	30°/sec	5	75 to 130°

All testing at the knee was performed with the subject in the upright, seated posture. Isometric testing at the knee was performed with the knee at 60° of knee flexion. Subjects performed four repetitions of five-sec maximal isometric contractions separated by one minute of rest between efforts in each specific direction of movement.

Exercise Countermeasures

The major differences in the exercise countermeasures between this study and Phase IIa were that the frequency of aerobic exercise sessions was increased from three to six days each week by adding three additional 30-minute treadmill sessions. The interval cycle exercise countermeasure was identical to the protocol used during Phase IIa. The treadmill protocol consisted of five minutes of warm-up at 40%, 20 minutes of exercise at 70%, and five minutes of cool-down at 40% of VO_2pk measured during the treadmill maximal exercise test. Treadmill and cycle ergometer exercise were performed on alternating days. In addition, the resistive exercise protocol used in Phase IIa was divided such that crewmembers performed the upper- and lower-body exercises on separate days (Table 5.2-3). Exercise countermeasures were generally performed in the order prescribed, but crewmembers were allowed to perform additional exercise based upon personal preference.

Table 5.2-3 *Schedule of exercise countermeasures*

Day of the Week	Aerobic Exercise	Resistance Exercise
1	Cycle	Upper Body
2	Treadmill	Lower Body
3	Cycle	Upper Body
4	Treadmill	Lower Body
5	Cycle	Upper Body
6	Treadmill	Lower Body
7	Rest or Submaximal Test	Rest

Scheduling of other activities, failure of environmental control systems, and personal preferences occasionally resulted in deviances from this schedule.

Beginning 30 days prior to chamber entry until the chamber exit, all subjects maintained daily exercise logs and completed activity questionnaires every two weeks. Additionally, all crewmembers monitored their exercise intensity using a HR monitor during aerobic exercise prechamber. During the chamber stay, the crewmembers continued to utilize the HR monitors during the aerobic exercise countermeasures.

Data Analysis

Data analysis for Phase III was similar to that performed for Phase IIa with respect to the common testing and countermeasures. Pre to postchamber measures of aerobic capacity were not compared statistically because only three subjects completed all these tests.

During isokinetic testing, peak torque was determined in each strength test for both extension and flexion. For data analysis of the endurance test at the knee, the first repetition was disregarded. Thereafter, total work, work at repetitions 1-3, work at repetitions 9-11, and work at repetitions 18-20 were determined from the endurance test data. The highest values obtained in each test prechamber were used for comparison to postchamber testing. All four subjects participated in these tests both pre and postchamber. Statistical comparisons of isokinetic variables were made with paired t-tests.

Phase III Results

Aerobic Exercise Countermeasures

In general, crewmembers adhered to the aerobic exercise countermeasure prescription. However, one crewmember did not participate in all in-chamber exercise due to

knee discomfort experienced in the later third of the study. On two separate occasions all exercise was delayed or suspended due to environmental control concerns.

Cycle countermeasure exercise was prescribed 39 times from chamber entry to exit. As noted above, environmental control concerns impacted prescribed exercise, including one day during which exercise was cancelled altogether. Mean (± SD) compliance with the exercise prescription was 90 ± 12%, with a range from 72% in the injured crewmember to 97% in two crewmembers. The fourth crewmember completed 95% of the prescribed cycle exercise countermeasure sessions. Only one crewmember performed extra cycle exercise in addition to the prescribed cycle exercise sessions.

Treadmill countermeasure exercise was prescribed 39 times from chamber entry to chamber exit. Crewmembers ranged in compliance to this prescription from 72% in the injured crewmember to 104% in one crewmember. The other two crewmembers were 100% compliant with the prescription. Because crewmembers frequently extended the time they spent exercising on the treadmill at the end of the prescribed countermeasure, mean (± SD) duration of this exercise countermeasure was 34 ± 6 min. All crewmembers participated in at least one treadmill session in addition to that prescribed. One crewmember performed only one additional 30-minute session, but another crewmember exercised 40 additional times. This crewmember often performed treadmill walking after the cycle countermeasure session. The other crewmembers performed 6 and 17 additional treadmill exercise sessions.

Resistive Exercise Countermeasure

Overall compliance with the resistive exercise countermeasure was 88% across all four crewmembers. However, the crewmember that experienced knee discomfort did not complete lower-body resistive exercises after week 10. In addition, resistive exercise was unable to be performed for a period of five days because of electrical problems with the hardware. The problem was present again at a later time but was resolved before exercise schedules were impacted. Average compliance within crewmembers ranged from 78 to 97%.

To assess changes in strength across the length of chamber stay, peak torque developed during the performance of the LO speed exercises was examined (Figure 5.2-6). Although some crewmembers showed increased strength in individual exercises, as a group these crewmembers did not demonstrate a consistent increase in strength. Similar results were found for the HI and MED speed exercises.

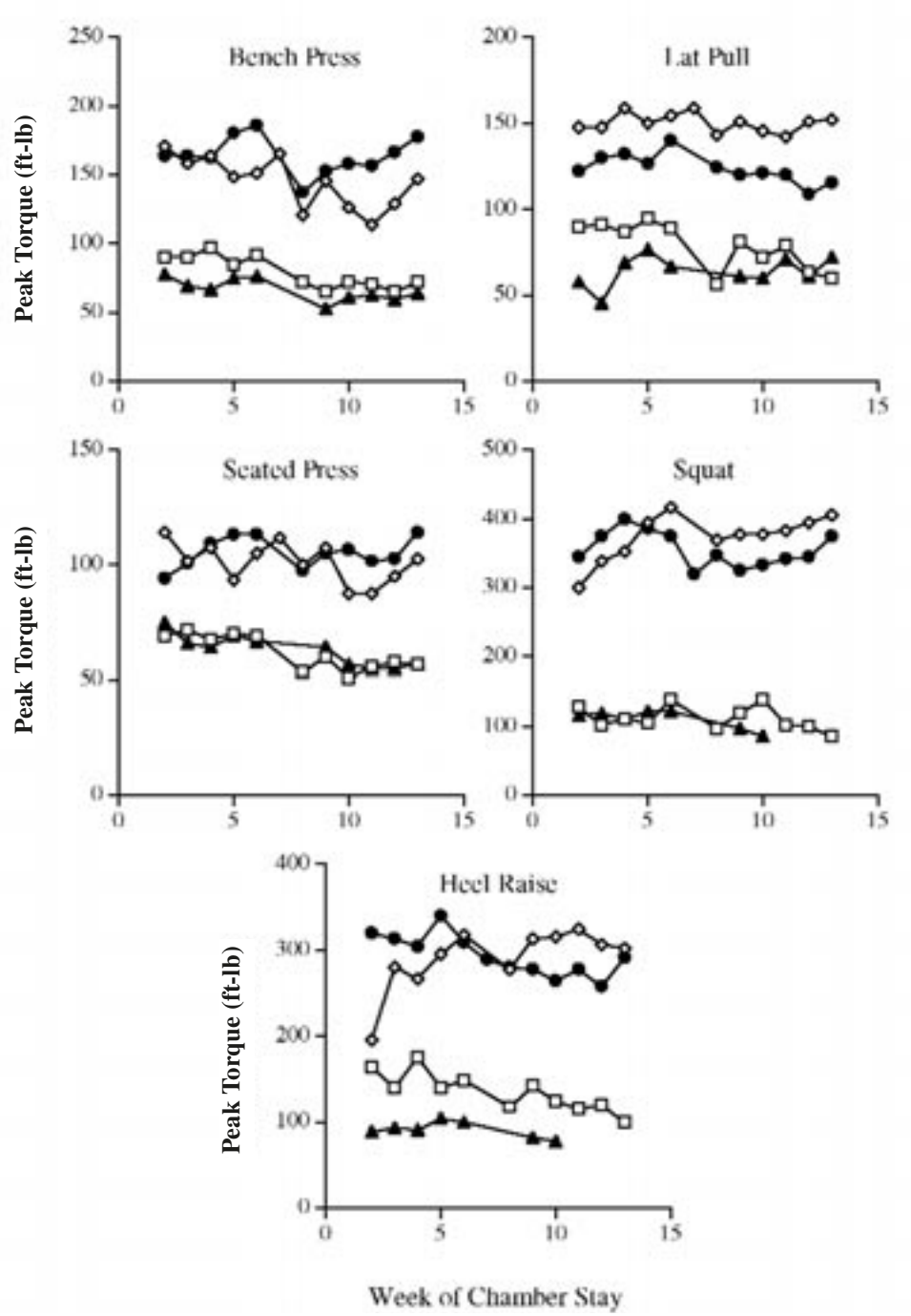

Figure 5.2-6 *Individual peak torque values obtained during isokinetic resistance exercise training at low (LO) speed of movement. Similar results were found for the HI and MED exercise speeds*

Maximal Cycle Exercise Test

Due to knee discomfort experienced by one subject, only three of the subjects participated in the postchamber cycle VO_2pk exercise test. Two of the three subjects attained higher exercise intensities (+50 watts) and longer VO_2pk test durations during postchamber testing (Table 5.2-4). VO_2pk in each of these two subjects increased by 0.5 L/min (8-9 ml/kg/min), an increase of approximately 20%. The VO_2pk of the third subject increased by 4%.

Table 5.2-4 *Mean (± SE) cycle VO_2pk exercise test results in three subjects*

	Prechamber	Postchamber
VO_2pk (l/min)	2.51 ± 0.16	2.89 ± 0.28
VO_2pk (ml/kg/min)	36.9 ± 3.4	43.2 ± 5.2
Peak Exercise Intensity (watts)	208 ± 8	242 ± 22
Total Test Time (min)	11.8 ± 0.4	12.9 ± 0.4

In the three crewmembers that completed post-chamber testing, both HR and the respiratory exchange ratio (RER) during submaximal and maximal exercise appeared to be reduced compared to prechamber testing (Figure 5.2-7).

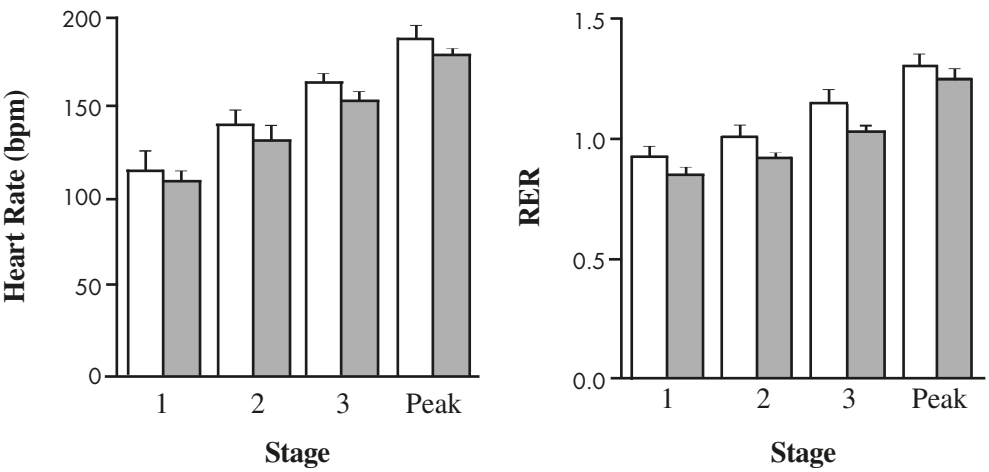

Figure 5.2-7 *Mean (± SE) HR and RER during pre (open bars) and postchamber (shaded bars) maximal cycle testing in three subjects*

Pre- to In-Chamber Submaximal Aerobic Exercise Test Results

The heart rate response during the submaximal exercise tests remained fairly constant during the first two submaximal exercise levels (25 and 50% VO_2pk) throughout the chamber stay. However, during the 75% VO_2pk exercise level, the average heart rate response decreased across the weeks of training (Figure 5.2-8).

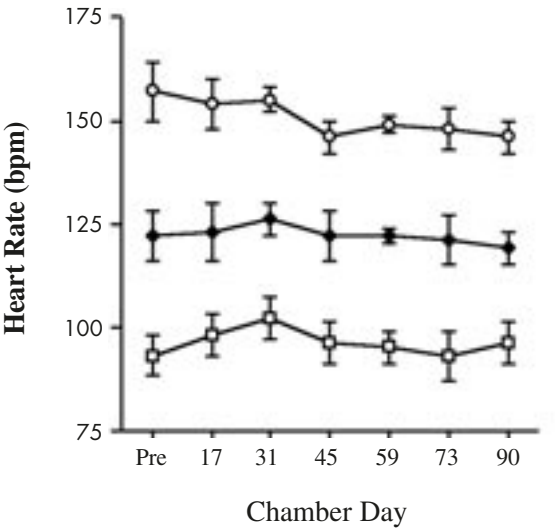

Figure 5.2-8 *Heart rate response during in-chamber submaximal exercise testing during each exercise stage at 25% (open squares), 50% (closed diamonds), and 75% VO_2pk (open circles)*

Pre to Postchamber Muscle Strength and Endurance

There was no observed difference in muscle strength during either extension or flexion at the joints tested pre to postchamber (Table 5.2-5).

Table 5.2-5 *Isokinetic strength and endurance (N-m) pre to postchamber*

Joint	Mode	Speed	Type	Movement	Pre	Post
Knee	Isometric	0°/sec	Strength	Extension	186 ± 31	195 ± 29
				Flexion	92 ± 11	95 ± 10
Knee	Concentric	60°/sec	Strength	Extension	157 ± 26	158 ± 37
				Flexion	91 ± 14	87 ± 17
Knee	Concentric	120°/sec	Strength	Extension	125 ± 21	134 ± 27
				Flexion	84 ± 13	81 ± 13
Knee	Eccentric	60°/sec	Strength	Extension	213 ± 44	217 ± 44
				Flexion	110 ± 15	104 ± 15
Knee	Concentric	120°/sec	Endurance	Extension	2,322 ± 277	2,344 ± 302
				Flexion	1,544 ± 187	1,323 ± 211
Ankle	Concentric	30°/sec	Strength	Extension	114 ± 17	109 ± 19
				Flexion	34 ± 3	32 ± 3
Ankle	Concentric	60°/sec	Strength	Extension	87 ± 23	86 ± 16
				Flexion	26 ± 4	26 ± 3
Ankle	Eccentric	60°/sec	Strength	Extension	155 ± 29	152 ± 25
				Flexion	53 ± 5	47 ± 5
Trunk	Concentric	60°/sec	Strength	Extension	215 ± 45	207 ± 55
				Flexion	116 ± 15	113 ± 20
Trunk	Eccentric	30°/sec	Strength	Extension	519 ± 59	508 ± 77
				Flexion	126 ± 18	125 ± 24

There appeared to be no change in total work performed during the knee endurance test during either extension or flexion from pre- to post-chamber stay in either group (Figure 5.2-9). Further, there was no difference in work performed at 1-3, 9-11, and 18-20.

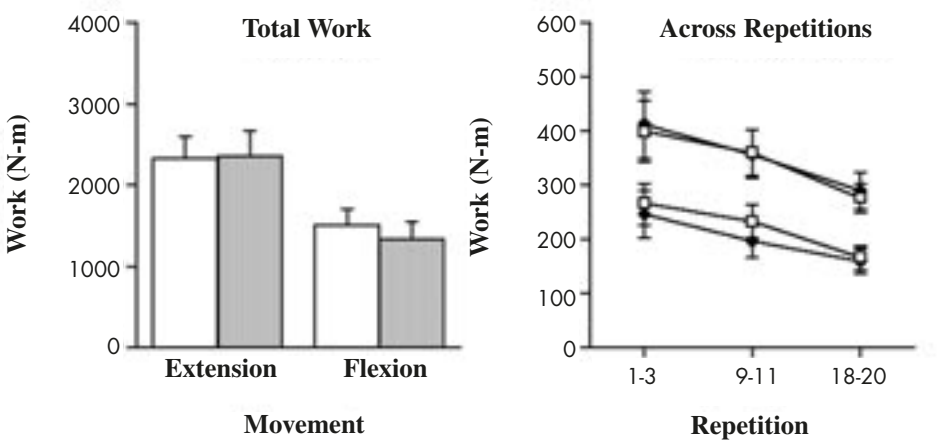

Figure 5.2-9 *Pre (open bar) to postchamber (shaded bar) total work during knee endurance testing (120°/sec, 20 repetitions) in the four subjects. Also, pre (open squares) to postchamber (solid diamonds) sum of work performed during repetitions 1-3, 9-11, and 18-20 of knee endurance testing (120°/sec, 20 repetitions)*

Phase III Discussion

Maximal Exercise Testing

Maximal exercise testing was performed prechamber stay using each exercise modality, cycle and treadmill, for which countermeasures were to be prescribed. In this way, we were able to develop modality-specific exercise prescriptions. It has been suggested that for future chamber studies and for space flight that the amount of testing should be reduced, such that only one maximal exercise test, treadmill or cycle, be performed. There are several problems with this approach:

- If the choice is made to utilize a treadmill maximal exercise test, it is likely that the protocol used for this test will be either a Bruce or modified Cunningham protocol. Both of these tests were developed for a low fit or cardiac rehabilitation population, and therefore may not be appropriate for a more physically fit group such as the astronaut corps. The profile of these protocols may not be appropriate for crewmembers to reach their "true" exercise capacity.

- It is common for VO_2pk measured on a treadmill to be 10-20% greater than the VO_2pk measured on a cycle ergometer (9). In this demonstration project, the measured VO_2pk during treadmill testing was significantly greater (19%) than that achieved during the cycle ergometry testing. Determining the desired exercise intensities for the cycle ergometer protocol using the treadmill VO_2pk value therefore would likely result in a prescription of cycle exercise intensities that may be greater than attainable by the crewmember. Conversely, using the results of a cycle ergometer test may result in the prescription of treadmill exercise intensities that are too low.
- In addition, not using metabolic data specific to the exercise modality for which a prescription is being developed would require the use of normative equations. The American College of Sports Medicine has stated that intrasubject variability of measurements of VO_2 may have a standard error of as high as 7%, and the variability of prediction equations may be even greater (10). Use of generally accepted equations may result in errors of up to 16% (16). Therefore, it would be preferable to make exercise prescriptions based upon modality-specific data obtained from the individual crewmember.

In general, maximal exercise testing was well tolerated in this group of highly motivated subjects. However, concerns with subject disqualification related to monitored changes in 12-lead EKG, whether specifically diagnostic or not, may limit the long-term use of these testing protocols as more subjects decline to participate. Therefore, it has been suggested that maximal exercise capacities be estimated from submaximal values. In our own experience, this is not desirable. Dependent upon the method used, prediction of maximal heart rates during cycle ergometer testing may range in error from -10 to +26% and prediction of maximal oxygen consumption can be ± 50% for some individual subjects.

Submaximal Exercise Testing

It was unexpected that the submaximal exercise responses during the chamber study appeared to differ slightly from the submaximal exercise responses during the postchamber maximal cycle test. While the chamber results indicated a reduction in heart rate during the chamber stay only at the highest exercise intensity (75% VO_2pk), the postchamber heart rate data was reduced at each of the three submaximal exercise levels. This difference may be due to the less stringent testing conditions during the chamber tests. This situation would be quite similar to the testing conditions during an actual space flight. At the lower exercise intensities, extraneous inputs from the environment and the self-collection of data and running the test may influence heart rate especially at the lower exercise intensities. Only at the highest exercise level may the exercise heart rate response become a true indicator of the training status.

Muscular Strength and Endurance Testing

Two methods were used to evaluate changes in muscle strength: first, the change in isokinetic resistances during the daily training sessions and second, data from the isokinetic strength tests performed before and after the chamber stay. Both sets of data in this study are consistent and support the fact that there was little increase in muscle strength during the chamber stay. When the peak torque measured during the heel raise exercise were compared with the peak torque measured during the plantar flexion portion of the isokinetic test, the values measured appear to move in similar directions from pre to postchamber in three of the four crewmembers (Figure 5.2-10). When similar comparisons were made from squat exercise and knee extension data, three of the four crewmembers displayed similar responses. These data suggest that the isokinetic testing provided valid results, but an examination of a larger database is necessary to confirm this.

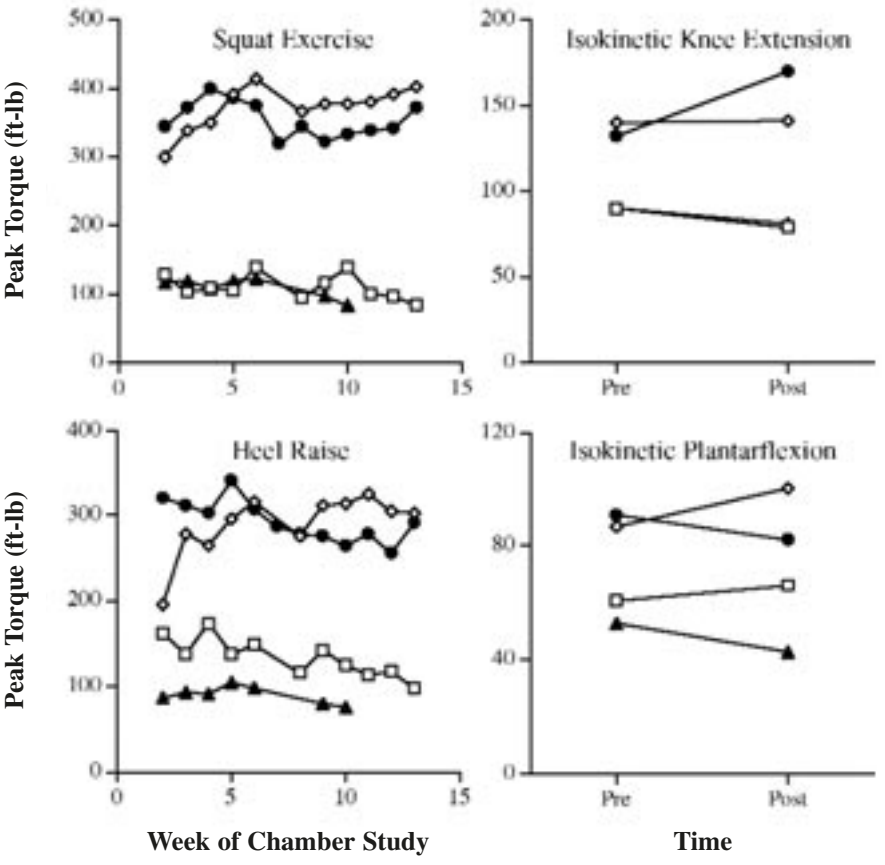

Figure 5.2-10 *Pre (open bar) to postchamber (shaded bar) total work during knee endurance testing (120°/sec, 20 repetitions) in the four subjects. Also, pre- (open squares) to postchamber (solid diamonds) sum of work performed during repetitions 1-3, 9-11, and 18-20 of knee endurance testing (120°/sec, 20 repetitions)*

Aerobic Exercise Countermeasures

The mean (± SD) time spent performing aerobic exercise protocols was 32 ± 10 min per day in these four crewmembers, ranging from 26 to 47 min across crewmembers. Therefore, crewmembers spent an average of 2941 ± 895 min exercising across the 91-day chamber stay, ranging from 2405 to 4224 min, and totaling for all four crewmembers 11,762 min. This total includes the time spent performing exercise in addition to that for performing the submaximal cycle exercise test.

The aerobic countermeasures performed in this demonstration appear to have been effective in increasing the aerobic capacity of the crewmembers. This suggests that these countermeasures also may be effective in preventing the decrements in aerobic capacity observed following space flight. Although similar interval exercise protocols have been effective during 5, 14, and 30-day bed rest studies (4, 6, 7, 17), only data from space flight studies will confirm this. Compared to the Phase IIa study, treadmill exercise was added and the frequency of exercise increased from three to six times per week. In the three subjects who completed both pre and postchamber testing, the average in increase in VO_2pk was greater in Phase III, but it is not possible to predict with certainty whether the fourth subject would have exhibited a similar improvement.

The interval protocol for the cycle ergometer and the continuous protocol for the treadmill were chosen to train for different tasks. The interval protocol with its high intensity stages was chosen to maintain high exercise capacities, involving both aerobic and anaerobic energy systems, in case high intensity work was required either during flight or immediately after flight. The continuous protocol was chosen to maintain work endurance for long periods of effort, which might be required during extravehicular activities (EVA) or intravehicular activities (IVA) while during flight or during extraterrestrial exploration. While the crewmembers found these protocols to be challenging at first, they expressed a desire for an increased variability in the protocols prescribed to reduce any staleness experienced in the repetition of these protocols over a long period of time. In addition, consideration must be given for future LMLSTP studies, as well as space flight countermeasures, to develop a periodization protocol to decrease the likelihood of overtraining, which may have been observed in one Phase III crewmember.

Although both cycle and treadmill exercise countermeasures were prescribed equally in number, when crewmembers chose to exercise longer or more frequently than prescribed, they chose to perform treadmill exercise. Only one crewmember on a single occasion performed an additional exercise session on the cycle ergometer. This preference also has been reflected in the performance of exercise during long-duration space flight. Postflight debriefs with U.S. astronauts who lived aboard the Russian space station, Mir, revealed a strong preference for treadmill exercise. Reasons given for this preference included desire to ambulate, positive feelings from the compression of the harness restraint system during treadmill exercise, and less boredom experienced during the performance of treadmill

exercise. Perhaps the addition of virtual reality or computer games linked to the performance of exercise will improve the desire to exercise on both the cycle, treadmill, or during participation in any other exercise modality.

Interestingly, the crewmember that performed the greatest total volume of exercise was the one who showed no improvement in VO_2pk from pre- to post-chamber. These data suggest that there was no additional benefit from performing exercise greater than that prescribed. However, this crewmember performed primarily low-level (walking) exercise in addition to the daily exercise countermeasures. It could be argued that this crewmember was the one who participated in the greatest amount of exercise prior to chamber entry, but the in-chamber exercise was of substantially greater intensity. It is more likely that this subject was not experiencing sufficient rest between high-intensity bouts of exercise such that performance on the maximal cycle ergometer test was unchanged from prechamber and/or declined as the end of the chamber stay approached (5). This subject reported increased fatigue as the demonstration project neared completion and began to decrease the amount in excess of the exercise prescription. In addition, similar to the crewmember that experienced knee discomfort, this crewmember also had consistent decreases in muscle strength by the end of the study as suggested by decreased peak torques during isokinetic training.

The interval protocol on the cycle ergometer was prescribed because it was felt that the prescription of such a varied and high intensity protocol for the treadmill, while not an issue during LMLSTP, may be problematic during space flight. The loss of gravitational forces during cycle ergometry is unlikely to result in significant alterations in metabolic responses to cycle exercise. For example, in our experience, it does not appear that heart rate responses to cycle exercise during short-duration space flight are significantly different from that experienced during normal gravity (11), primarily because the mass of the body is supported during both normal and micro-gravity. In contrast, during treadmill exercise in normal gravity the body mass must be supported, but this is not the case during exercise in the microgravity environment. The z-axis component of treadmill exercise during microgravity exposure is wholly dependent upon the loading system that restrains the subject. During Skylab, Space Shuttle, and Mir missions, whole-body loading of the crewmembers has been accomplished through the use bungee cords, or a spring-based system in line with bungee cords, attached to a torso harness system. The design of these systems has been such that the loading carried by the crewmember is distributed similarly to that when carrying a large backpack, with the load placed on the hips and shoulders. This type of loading has been reported to be uncomfortable such that crewmembers typically load to a level of only one-half to two-thirds of their body weight. Thus, crewmembers may vary their loading dependent on their comfort levels. As a result, the exercise responses to treadmill exercise at a specific belt speed may vary depending upon the amount of loading, and the attainment of a specific metabolic load equivalent to normal gravity ambulation may require high treadmill speeds which may not be practical for treadmill

construction (size of motor required, wear on treadmill parts, etc.) or may be unsafe for crewmembers. Although treadmill running in microgravity at different loads has yet to be systematically investigated, Boda and co-workers (1) have reported that metabolic loads can be attained during treadmill exercise with the body in the horizontal position using lower-body negative pressure that approximates the metabolic loads encountered during normal upright treadmill exercise. In this configuration, the interval exercise protocol during bed rest has been successfully employed (6, 7, 17).

Resistive Exercise Countermeasure

The resistive exercise countermeasure prescribed in this chamber test was similar to that used during Phase IIa. Crewmembers performed the same exercises in Phase IIa and Phase III, but in Phase IIa all the resistance exercises were performed each of three days during the week. In contrast, during Phase III the exercises were divided into upper- and lower-body exercises and performed on different days such that crewmembers were performing resistance exercise six days per week. During Phase IIa, this countermeasure appeared to improve strength in three of the four crewmembers. This was not true in Phase III. Crewmembers had inconsistent changes in strength during Phase III, as evidenced by strength training records, which was manifested in no change in strength or endurance between pre- to post-chamber measurements made during isokinetic testing. An explanation of the divergent results from the two studies may be that the manner in which the training was performed by the crewmembers differed. In Phase IIa, crewmembers completed their resistive exercise training with minimal rest between sets (~two min). In Phase III, crewmembers increased the rest period between sets, often performing other tasks and duties while resting from the previous test. The rest periods of the Phase III crew may not have been optimal for the development of muscle strength through resistive exercise training. Future protocols will address this issue.

In addition, there may be some degree of incompatibility of strength and aerobic training that could have a negative effect on strength development. However, the majority of the studies that have demonstrated this effect have employed high volumes and intensities of both strength and endurance training (2). In the Phase IIa crewmembers this may not have been problematic. Although the in-chamber exercise was at a greater level than that in which crewmembers usually engaged, the frequency was similar to that which has been shown to result in little interference in strength development (8). However, the increase in total exercise volume (resistance and aerobic) employed during Phase III may have been sufficiently greater than Phase IIa so as to induce some overtraining (2). Perhaps a periodization of both strength and endurance training may alleviate this problem and result in more consistent strength gains.

Overtraining

All four crewmembers performed the cycle ergometer countermeasure protocol at the same intensity as prescribed prior to chamber entry through day 66. On day 67, the intensity of exercise was increased in all four subjects. This increase was indicated based upon the results of the submaximal aerobic exercise tests that suggested that each had increased their aerobic fitness. Three of the four crewmembers tolerated this increase well, but the fourth reported minor discomfort in the left knee following the performance of this increased workload.

The peak workload for the injured subject increased from 165 to 174 watts. The peak HR attained during this exercise countermeasure session was 177 bpm, similar to that attained during the prechamber protocol practice session (176 bpm) and to that attained during the first two exercise sessions upon chamber entry (171 and 173 bpm). The cause of the knee discomfort in this subject was unclear, but the subject performed the first, less intense protocol for the subsequent cycle ergometer countermeasure session. The knee discomfort appeared not to lessen, and therefore cycle ergometer exercise was discontinued.

The inability of the crew surgeon to perform a physical examination with this subject interfered with our ability to determine the cause of knee discomfort in this subject. In our opinion it is unlikely that this small increase in peak exercise intensity is responsible. However, since this subject was not highly physically active prior to chamber entry, it is possible that the subject was nearing a point of overtraining and that the increase in workload accelerated this process. This indicates the need to allow a longer period of lower intensity, or active rest, for the subjects periodically when performing these countermeasures across a long period of time. Allowing crewmembers a week of less intense countermeasures every three weeks may reduce the incidence of such problems. It is difficult to reach this conclusion though since this is the first report of this nature under these conditions.

OVERALL CONCLUSIONS AND SIGNIFICANCE

These LMLSTP studies have allowed preliminary evaluation of potential exercise testing and countermeasure procedures. Lessons learned from these projects may be applied to space flight with the important consideration that training responses in a 1-g environment may not be exactly representative of space flight; exercise in the chamber projects were intended to increase exercise capacity, while exercise countermeasures in a microgravity environment are intended to maintain overall conditioning. Additionally, exercise performed within the constraints imposed by microgravity (e.g., subject loading during treadmill exercise) or with the actual flight hardware may provide a different training stimulus. Further, thresholds for training and over training may differ between the two conditions and vary among the target organ systems. Data collected during actual space flights will be required to provide the final confirmation of our countermeasure programs.

The data collected during these chamber studies also must be considered extremely preliminary due to the small number of subjects. In many cases, appropriate statistical analyses could not be performed due to the limited sample size.

With these considerations, preliminary observations from these studies are:

- The aerobic and resistive countermeasures tested in these projects provided a training stimulus when performed on separate days (Phase IIa). Further work is needed to explore a possible negative effect on strength training when aerobic exercise was performed on the same day as resistive training (Phase III).
- Most crewmembers preferred treadmill exercise over cycle exercise. This has been reported also by long-duration space flight crews. However, due to effects of microgravity on treadmill exercise loading, exercise prescriptions and testing protocols can be more accurately applied on a cycle.
- Compliance to our exercise prescriptions was very good, but some discontent was evident from the postchamber debriefs. Increasing the variety of exercise protocols, exercise devices and addition of virtual reality head gear or other forms of entertainment during exercise may improve exercise compliance.
- Almost all subjects reported a desire for more variety of exercise prescriptions.
- The lack of strength increase may suggest that the rest periods between sets were too long or that there was an overtraining response in the Phase III crew with the increased aerobic exercise volume. In future studies and during space flight, crew exercise time should be protected to optimize the effect of training. Also, the addition of muscle damage markers to future training studies may help to elucidate this issue.
- The exercise logging materials used in this study and the feedback from the subjects has been used in developing the computer-based flight logs currently planned for the ISS.

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ACRONYMS

ANOVA	analysis of variance
bpm	beats per minute
CES	computerized exercise system (Ariel Life Sciences, Inc.)
DBP	diastolic blood pressure
ECG	electrocardiogram
EVA	extravehicular activity
HI	high speed
HR	heart rate
ISS	International Space Station
IVA	intravehicular activity
l/min	liters per minute
LMLSTP	Lunar-Mars Life Support Test Project
LO	low speed
MED	medium speed
ml/kg/min	milliliters per kilogram per minute
RER	respiratory exchange ratio
RPE	rating of perceived exertion
rpm	revolutions per minute
SBP	systolic blood pressure
SD	standard deviation
SE	standard error
SMP	Space Medicine Project
VO_2pk	peak oxygen consumption
VO_2	oxygen consumption

5.3

Reactivation of Latent Viruses

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ABSTRACT

Reactivation of latent viruses may pose an important health risk for people living and working in extreme environments, such as space and Antarctica. Stress-induced changes in immune function under such conditions may increase the incidence and duration of viral reactivation and shedding. We studied viral shedding and reactivation patterns in 8 subjects (5 male and 3 female) participating in 60-day (Phase IIa) and 91-day (Phase III) chamber studies of the Lunar-Mars Life Support Test Project (LMLSTP) at NASA Johnson Space Center. Saliva, blood, and urine samples were collected from the 8 subjects before, during, and after chamber isolation. Using a polymerase chain reaction assay, saliva samples were processed for Epstein-Barr virus (EBV) DNA and urine samples were analyzed for cytomegalovirus (CMV) DNA detection. EBV DNA was detected in 35% of the total saliva samples from both chamber studies, 13% (range 0 to 31%) of samples from the 60-day study, and 51% (range 33 to 81%) of the samples from the 91-day study. Detection frequency was highest prior to chamber isolation. Although CMV DNA was not detected in any of the urine samples collected from these subjects before and after the studies, antibody titers to CMV were significantly increased over control values. Interestingly, EBV antibody titers did not differ significantly from the controls. Our current finding of increased viral reactivation in closed-chamber study subjects agrees with our previous Space Shuttle and Antarctic data. These results support and extend our previous observations that latent viral reactivation increases during space flight, which is consistent with a stress-induced decrease in immune function.

Introduction

Herpes viruses are the most readily recognized latent viruses and the leading infectious cause of blindness in the United States (10). The establishment of viral latency and subsequent reactivation are not well understood, but decreased cellular immunity is known to increase the incidence and duration of reactivation and shedding of some latent viruses (1, 2). Reactivation of latent viruses may cause no symptoms or may produce significant illness (e.g., shingles). These viruses are

carried by astronauts into space and may pose an important health risk in a closed-loop environment. The risk of reactivation almost certainly increases as the duration of space missions increases. Risks associated with many infectious agents are reduced by the preventive measures of the health stabilization program before flight. However, reactivation of latent viruses is unaffected by such actions. A variety of stress factors may cause reactivation of these viruses, such as psychosocial stress, trauma, sunlight, respiratory infection, and fever.

Epstein-Barr virus (EBV), a DNA virus, infects more than 90% of the adult population worldwide and is the etiologic agent of infectious mononucleosis (4, 9). EBV is highly infectious and can be transmitted by microdroplets and by direct contact with saliva. Normally, primary infection is a self-limiting disease leading to a lifelong persistence of EBV in the B lymphocytes or epithelial cells of the oropharynx of infected individuals. EBV is also associated with serious illnesses including Burkitt's lymphoma, nasopharyngeal carcinoma, diffuse oligoclonal B-cell lymphoma, Hodgkin's disease, AIDS-associated lymphoma, and post-transplant lymphoproliferative disease. EBV can be found in saliva after reactivation, which can be triggered by a variety of factors, perhaps including space flight. The physical and psychological stresses associated with the launch and landing of spacecraft, and living and working in the crowded, closed environment in microgravity, may result in viral reactivation and shedding. The effects of stress on viral reactivation are probably mediated through the endocrine-immune axis (1, 2, 3, 5).

Numerous studies have shown altered immune function among individuals living and working together in space and in selected ground-based analogs of space flight. These alterations include phenotypic changes within leukocyte populations, impaired neutrophil function, decreased natural killer (NK) cell activity (15), reduced T-cell proliferation to mitogenic stimulation, altered cytokine production (13), and depressed cutaneous delayed-type hypersensitivity response (12, 16). Reduction in cell-mediated immunity (CMI) may lead to viral reactivation, which may be expressed as a) asymptomatic viral shedding, b) a localized clinical infection (e.g., herpetic lip lesion) limited by the cell-mediated immune system to a cell-to-cell transfer of virus, or c) a generalized local infection or a more severe disseminated infection.

Previously, we investigated latent EBV as a candidate virus for latent viral reactivation and reported a greater frequency of EBV DNA shedding by astronauts before space flight than during and after flight (11). Reactivation of EBV occurred before flight, and eight-fold or greater increases in anti-EBV antibodies were observed during flight (14). Recently, in a ground-based space analog study conducted in Antarctica, we reported increased salivary EBV shedding with diminished CMI response during long periods of isolation (7). The results of these studies suggested that decreased cellular immune function leads to reactivation and shedding of potentially infectious viruses. Thus, a major concern associated with space flight-induced immunosuppression is the possibility of infectious diseases posing an unacceptable medical risk to subjects.

The limited access to space requires the use of ground-based analogs of space flight. Environmental chambers have been used since the Skylab program (1973-1974) to simulate specific aspects of space flight. In the present study, the polymerase chain reaction (PCR) was used to identify EBV DNA in saliva and CMV DNA in urine as a measure of viral shedding in response to 60-day (Phase IIa) and 91-day (Phase III) chamber studies of the Lunar-Mars Life Support Test Project (LMLSTP) at NASA Johnson Space Center.

Materials and Methods

Subjects

Eight subjects (5 males and 3 females) participated in the two chamber studies, one of 60 days and the other of 91 days (4 subjects each). Their ages ranged between 28 and 42 years. The control group was composed of 11 healthy age-matched adults (10 males and 1 female).

Samples

Saliva, blood, and urine were collected from each subject participating in the LMLSTP Phase III study. As shown in Table 5.3-1, saliva was collected every other day upon arising, and blood and urine were collected once before and once after the study. Saliva samples were processed for EBV DNA, whereas urine samples were processed for CMV DNA detection.

Table 5.3-1 *Schedule of sample collection from subjects during the 60-day and 91-day chamber studies*

Sample	Study Phase			
	Prechamber		In-Chamber	Postchamber
Saliva (2-3 ml)	60-day study	MWF for 2 weeks immediately before entry	MWF	MWF for 2 weeks immediately after exit
	91-day study	MWF for 4 weeks beginning 8 weeks before entry	MWF	MWF for 2 weeks immediately after exit
Blood (10 ml)	Once, immediately before entry		None	Once, immediately after exit
Urine (3 ml)	Once, immediately before entry		None	Once, immediately after exit

MWF = Monday, Wednesday, and Friday

Saliva samples were collected with Salivette kits (Sarstedt, Inc., Newton, NC), which consist of a cotton roll in a polypropylene vial. Subjects placed the roll in their mouth until it became saturated, and then returned the roll to the vial. Saliva samples were centrifuged immediately after collection and stored frozen at -70°C. All samples collected from a given subject were analyzed simultaneously. Saliva (2 to 3 ml) and urine (3 ml) specimens were concentrated with a 100-KD filtration unit (Filtron Technology Corp., Northborough, MA). DNA was extracted by a nonorganic extraction method (Qiagen Inc., Chatsworth, CA), and EBV DNA was detected as described earlier (6, 11) and described here briefly as follows. The PCR primers were directed at the EBV polymerase accessory protein gene (BMRF1): P1, 5'-GTC CAA GAG CCA CCA CAC CTG (The Midland Certified Reagent Co., Midland, TX), and P2, 5'-biotin CCC AGA AGT ATA CGT GGT GAC GTA GA (Digene Diagnostics, Gaithersburg, MD). These primers were used at a concentration of 200 µM with 10 µM deoxynucleic acid triphosphates (Perkin Elmer, Branchburg, NJ). PCR was optimized using buffer II (Perkin Elmer) with 2.5 mM $MgCl_2$. DMSO (Sigma, St. Louis, MO) was added to a final concentration of 5%. AmpliGold (2.5 units per 100 µl reaction mixture) (Perkin Elmer) was added, and 5 µl of the purified DNA was added to 20 µl of the reaction mixture. The cycle parameters were 95°C for 9 min, followed by 40 cycles of 94°C for 15 sec, 61°C for 15 sec, and 72°C for 15 sec, with a final extension step at 72°C for 5 min.

CMV DNA was detected using primers that target the major immediate early gene (P1, 5'-TGT CCT CCC GCT CCT C, and P2, biotin 5'-ATG AAG GTC TTT GCC CAG TA). All reactions were carried out using a Perkin-Elmer GenAmp system 9600. An initial denaturation step of 94°C for 9 min was followed by 40 cycles of 94°C for 30 sec, 69°C for 15 sec, 72°C for 30 sec, 72°C for 7 min, and a 4°C hold. The amplified product was analyzed for the presence of EBV or CMV using the Digene Sharp Signal System (Digene Diagnostics Inc., Gaithersburg, MD) according to the manufacturer's instructions.

Measurement of Antiviral Antibody Titers

EBV and CMV antibody titers were determined by indirect immunofluorescence assay. Commercially prepared substrate slides and control sera were used for determining antibody (IgG) titers for viral capsid antigen (VCA) and early antigen (EA) of EBV, CMV, and measles (Bion Enterprises, Park Ridge, IL). Four-fold dilutions of plasma were prepared with phosphate-buffered saline. The endpoint titer was determined as the highest dilution of serum in which immunofluorescent-positive cells could be detected. All specimens were batch analyzed and read blind-coded.

Samples from 11 healthy age-matched adults were collected as controls. Saliva, urine, and blood samples (one of each) were collected from each of these subjects on day 1, day 7, and day 22 of the tests. This collection schedule closely approximates the pre- and postflight collection schedule of a 12-day Shuttle flight. One-time urine samples were also collected from 30 additional healthy age-matched subjects for CMV analysis.

Data Analysis

The frequency of viral shedding in saliva and viral antibody titers in plasma from chamber study subjects and controls were tested for normality. One-way analysis of variance was performed to study significant differences across different times during the study.

The method of generalized estimating equations with a logit link was used to find significant difference between the phases (pre-, in-, and postchamber). The differences between sampling periods were considered significant if $P < 0.05$.

Findings

A total of 418 saliva samples were collected from the eight subjects participating in the two chamber studies. These samples were analyzed for EBV DNA using PCR.

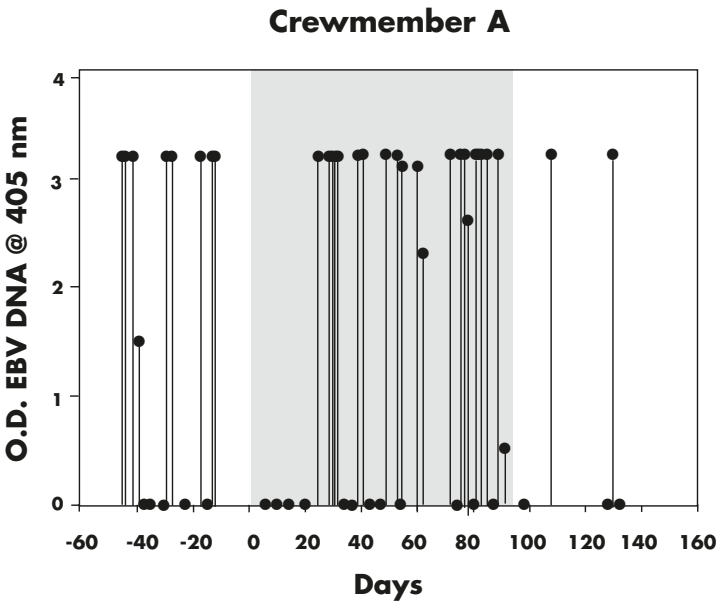
Overall, EBV DNA was detected in 35% of the 418 total saliva samples collected from these subjects. The subjects in the 60-day chamber study had EBV DNA in 13% (range 0 to 31%) of their samples, and the subjects in the 91-day study

Table 5.3-2 *EBV DNA presence in saliva from chamber subjects*

	Crewmember	Saliva Samples		
		Number of Samples	Number of Samples Positive for EBV DNA	% Positive
91-Day Chamber Study	A	62	50	81
	B	56	32	57
	C	67	22	33
	D	62	22	35
60-Day Chamber Study	E	48	15	31
	F	42	2	5
	G	41	5	12
	H	40	0	0
TOTAL	8	418	148	35
Control	11	27	1	4

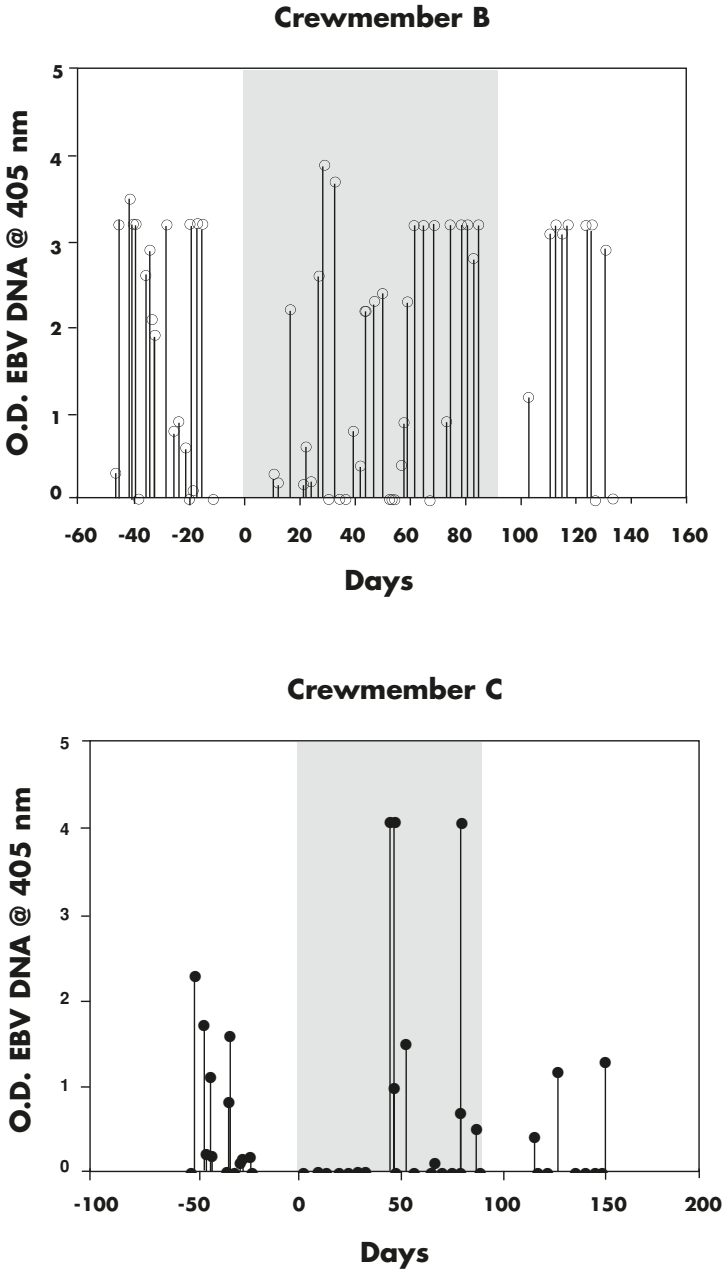
had EBV DNA in 51% (range 33 to 81%) of their samples (see Table 5.3-2). The individual EBV DNA shedding patterns are shown in Figure 5.3-1. During both studies, all but one subject (in the 60-day chamber study) shed EBV. Subject H did not shed EBV in 40 saliva specimens collected over the 88-day collection period. EBV shedding frequency by two of these subjects (A and B of the 91-day chamber study) was very high (57% and 81% EBV-positive saliva specimens), while the other 6 subjects had low to moderate shedding frequencies (see Table 5.3-2). The average shedding frequency of EBV in saliva samples (collected before, during, and after chamber) was significantly greater for the chamber subjects (35%) than

the control group (4%) ($P < 0.005$). Although a higher shedding rate (51%) was observed in the 91-day study than in the 60-day study (13%), the difference between the shedding frequencies was not statistically significant. Subsequent analyses were, therefore, performed on the pooled data from both studies. The incidence of positive EBV findings for the chamber subjects and the control subjects was compared across the three phases (prechamber, in-chamber, and postchamber). The incidence rates for the three phases were determined as follows: prechamber, 37%; in-chamber, 31%; and postchamber, 25%. Using the method of generalized estimating equations with a logit link, we found no significant difference between the phases at a test level of $P = 0.05$. However, when comparing the postchamber and prechamber phases, we observed a P value of 0.069. This suggests the incidence of EBV shedding may actually be lower after chamber exposure, but because of the small number of subjects in the study, we were not able to reject the hypothesis of no difference at $P = 0.05$. Also, the EBV shedding patterns of the 60-day subjects and 91-day subjects were not significantly different.



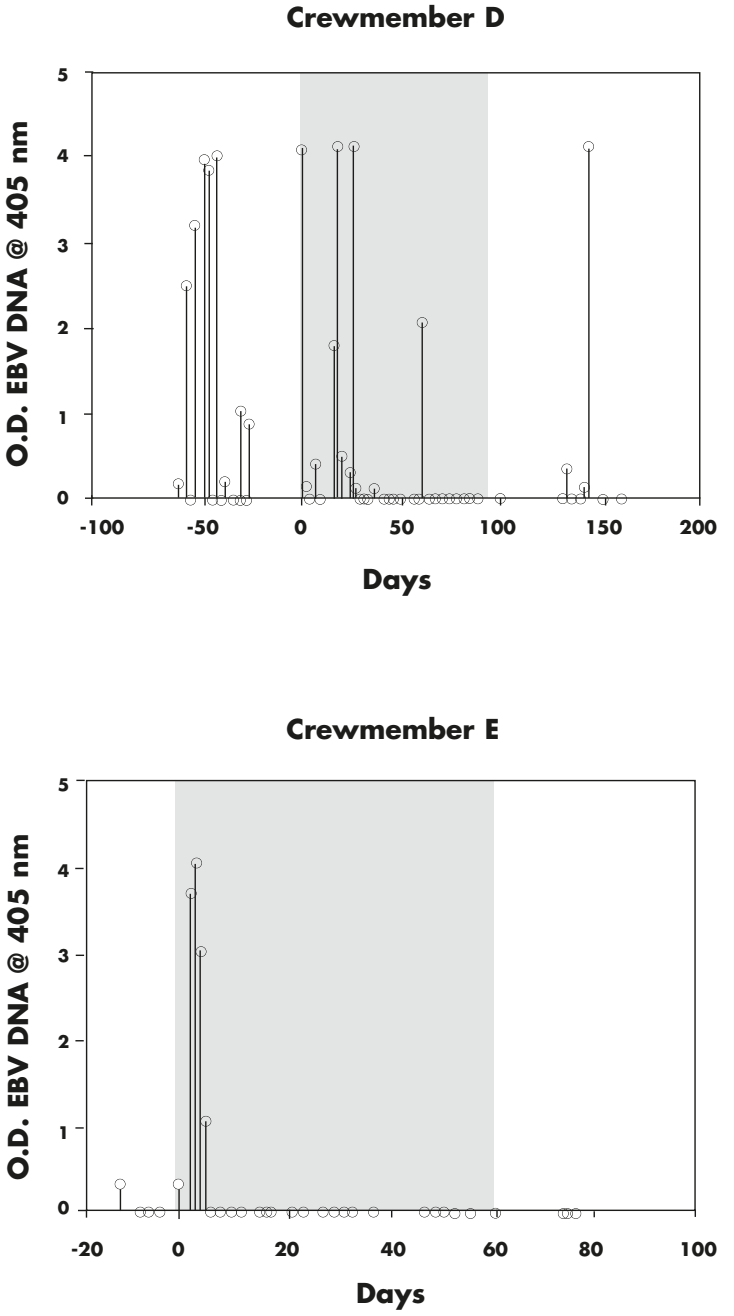
Shaded area represents in-chamber sampling period.
O.D.= optical density

Figure 5.3-1 *Individual EBV DNA shedding patterns for test subjects*



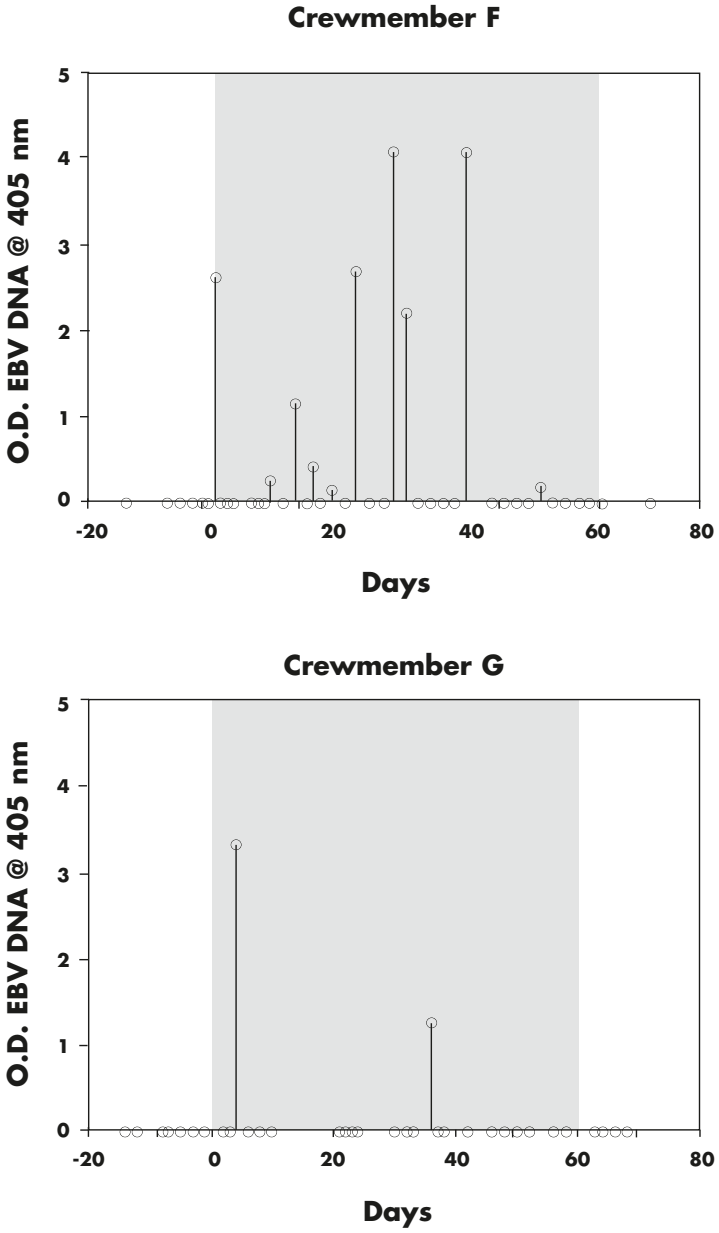
Shaded area represents in-chamber sampling period.
O.D.= optical density

Figure 5.3-1 continued *Individual EBV DNA shedding patterns for test subjects*



Shaded area represents in-chamber sampling period.
O.D.= optical density

Figure 5.3-1 continued *Individual EBV DNA shedding patterns for test subjects*



Shaded area represents in-chamber sampling period.
O.D.= optical density

Figure 5.3-1 continued *Individual EBV DNA shedding patterns for test subjects*

Sixteen urine samples collected from the subjects of both chamber studies before and after the in-chamber phase were analyzed for CMV DNA. Interestingly, not a single sample showed evidence of CMV DNA. The presence of CMV DNA in urine from the control group was also rare (1/81 samples).

Viral antibody titers for EBV and CMV, measured in samples from all 8 subjects before and after the study, are given in Table 5.3-3. Because viral antibody titers of subjects in the two studies were not significantly different, the data were pooled for further analysis. No significant differences in EBV VCA, EBV EA, or CMV IgG antibody titers were observed before and after either of the studies. EBV titers did not differ from those of the control group. CMV antibody titers of subjects were greater than those of the control group before and after the study.

Table 5.3-3 *Viral antibody titers before and after the 60-day and 91-day chamber studies*

Viral Antibody	Antibody Titer (Mean ± SE)				
	Chamber Study		Control Group		
	Pre-	Post-	Day 1	Day 7	Day 22
EBV VCA IgG[1]	7.32 ± 0.32	7.52 ± 0.34	6.12 ± 0.65	6.22 ± 0.52	6.12 ± 0.50
EBV EA IgG[2]	5.02 ± 0.41	4.44 ± 0.12	5.52 ± 0.31	5.96 ± 0.35	5.98 ± 0.32
CMV IgG[3]	6.82 ± 0.18[4]	6.94 ± 0.18[4]	3.09 ± 0.31	2.91 ± 0.31	2.91 ± 0.31

[1]Antibody for viral capsid antigen of Epstein-Barr virus
[2]Antibody for early antigen of Epstein-Barr virus
[3]Antibody for cytomegalovirus
[4]Statistically significant as compared to control ($P < 0.05$)

Discussion

This is the first study of EBV and CMV reactivation in subjects in a closed chamber, serving as a ground-based space analog. Current data show significant reactivation and shedding of EBV DNA by PCR occurred before, during, and after chamber isolation. With the exception of two subjects, the saliva specimens containing EBV DNA were 3 to 20 times higher than a healthy control group. Even though no CMV DNA was detected in urine by PCR, elevated CMV antibody titers indicated reactivation had occurred before isolation and perhaps continued during the isolation phase. This is consistent with stress being the initiator. These results are similar to our previous findings in Antarctic expeditioners (7) and astronauts (6, 8, 11, 14). However, some differences were found. For example, we frequently detected CMV in astronaut urine unlike the chamber studies. Also, progressively

increasing levels of antibodies to the viral capsid antigen of EBV were found in astronauts before, during, and after space flight (8, 14). No quantitation of viral DNA was conducted in the current chamber studies, whereas eight-fold increases in EBV DNA were observed in Space Shuttle crewmembers (6). Moreover, the current results in chamber subjects support and extend our previous observations that latent viral reactivation increased during space flight and Antarctic winter-over.

Recently, we demonstrated increased salivary shedding of EBV following diminished CMI response during the 8 to 9 months of isolation in the Antarctic (7). These findings are consistent with reductions in CMI response observed by Drs. Sams, D'Aunno, and Feeback during the 91-day chamber study (reported in Chapter 5.4 of this publication). Their results demonstrate a reduction in the ability of the subjects to respond to selected recall antigens, indicating a diminished CMI. Their findings are similar to our findings in Antarctic expeditioners (7).

SIGNIFICANCE

Space flight represents a unique environment for humans to work and live in, and astronauts experience numerous forms of stress from variable gravitational forces, isolation, confinement, and a variety of psychosocial factors. Stress associated with space flight results in increased levels of stress hormones and decreased cellular immunity, and now we have demonstrated increased EBV and CMV reactivation and shedding in astronauts and closed-chamber subjects. These findings are consistent with the stress model showing the effects of stress being mediated through the hypothalamus-pituitary-adrenal axis (3).

Future studies should be expanded to include behavioral assessment and study of selected stress hormones and additional latent viruses (e.g., human herpes virus 6, herpes simplex virus types 1 and 2, and varicella-zoster virus). Quantification of shed viruses will be included to determine if the number of shed viral copies increases during decreased CMI.

Based on the viral shedding and viral antibody response, the chamber isolation model serves as a good ground-based analog for space flight viral reactivation studies. Reduced cellular immunity and increased reactivation of EBV and CMV associated with chamber isolation are consistent with the Antarctic winter-over stress model and space flight experiences. The chamber isolation analog has proved to be a cost-effective model for studies of space flight-associated stress and the resulting cascade of human physiological effects.

Acknowledgments

We thank Dr. Jane Krauhs of Wyle Laboratories, Inc. for comments on the manuscript. The statistical analysis was performed by Al Feiveson, Life Sciences Research Laboratories, JSC, Houston, Texas. Funding for this project was provided by NASA Grant 111-30-10-03.

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5.4

The Influence of Environmental Stress on Cell-Mediated Immune Function

Clarence Sams, Ph.D., Dominick D'Aunno, M.D., Daniel L. Feeback, Ph.D.

SUMMARY

An experimental protocol of repeated skin testing with several challenge antigens was utilized to assess the status of cell-mediated immunity (CMI) in all four Phase III crewmembers before, during, and after a 91-day duration stay in the chamber. An identical protocol was used on the same days in four age- and sex-matched control subjects who were not isolated within the chamber. By chamber day 45, all chamber subjects showed either an attenuated response or no response to all of the skin test antigens as determined by a decrease (hypoergy) or absence (anergy) in the CMI score. By chamber day 90, all four chamber subjects had an anergic response to all seven challenge antigens. Control subjects' responses changed variably from baseline, as expected, throughout the entire test period, but the average CMI score did not change significantly. Statistical analyses revealed a significant reduction (48.7% ± 10.1 SEM) in the CMI score in chamber subjects compared to control subjects. The CMI score of chamber crewmembers at 30 days following the period of chamber isolation was slightly reduced (13.1% ± 13.05 SEM), but the reduction was not statistically significant compared to control values. These results indicate that human subjects may suffer a decrease in cell-mediated immune responsiveness when challenged by moderate (91 days) duration isolation within an enclosed chamber. Additionally, the results support the utility of such chamber studies as a test bed for long-duration space missions including lunar/Mars exploration-class and Earth-orbiting space station missions and may further serve as an experimental model for determining the mechanisms underlying the attenuation of CMI function in extended-duration isolation.

INTRODUCTION

Objectives of Experiment

This investigation had two specific aims: (1) to determine if isolation of human subjects within the closed chamber would adversely affect function of the cell-mediated arm of the immune system as assessed by a delayed-type hypersensitivity (DTH) skin response to purposely introduced foreign antigens, and (2) to determine if the closed-chamber test bed is an appropriate ground-based analogue to further investigate the potential effects of isolation on underlying mechanisms that may alter cell-mediated immune function during long-duration space flight or extended stays on an Earth-orbiting space station facility.

Background

The human immune system is composed of multiple interacting elements including contributions from both the humoral and cell-mediated arms. These elements play unique roles and interact in various ways with each other in maintaining the optimum immune status and health of humans. CMI involving sensitized T-lymphocytes is important in defense against certain infectious agents (e.g., viruses and fungi), in surveillance against neoplastic cells, and in regulation of immune function. CMI function testing has traditionally been done by skin testing with cutaneous placement of recall antigens (delayed cutaneous hypersensitivity). By introducing an antigen to which an individual has been previously exposed, the capacity of T-lymphocytes to respond to an antigen in memory can be assessed.

Measurement of cutaneous DTH responses to a battery of commonly encountered antigens is a generally accepted and preferred means of assessing CMI function. In the past, such DTH testing suffered from lack of standardization of testing techniques, number and characterization of reactions, doses employed, and interpretation of reactions and results. A commercially available system (Multitest® CMI device; Pasteur Mériéux Serums et Vaccins, SA, Lyon, France) has solved these problems by providing simultaneous and reproducible application of seven standardized recall antigens as a means of measuring DTH in assessment of CMI. Because of its properties, widespread clinical acceptance, ease of use, and availability of scientific studies from other investigators (2, 3, 4), this system was adopted for this study.

In this investigation, repeated skin testing was utilized in order to determine the functional state of the chamber crew's CMI system over time and compare it to a control group of subjects not exposed to the environmental stress of isolation within the closed chamber.

This process of skin testing and evaluation of cell-mediated immune function has been used in other extreme environments such as Antarctic expeditions (6, 9),

tours of duty in submarines, and during both short- (7, 8) and long-duration (5) space flights. All of these studies have shown that stress can have a negative impact on CMI function. The exact mechanisms underlying these changes are not yet fully understood.

Methods and Materials

Human Subjects

There were two subject groups in this study. The experimental (chamber) group consisted of the four chamber occupants and the control group consisted of four sex- and age-matched volunteers. The test protocol, layman's summary, and informed consent documents were approved by the NASA Johnson Space Center Institutional Review Board prior to commencement of the study. All human subjects (chamber and control) received an informed consent briefing detailing the experimental protocol and risks and signed the informed consent documents before the start of the study. All individuals completed a training session on the proper application of the skin test device and measurement of the results.

CMI Device Description and Procedure

Multitest® CMI (Pasteur Mériéux Serums et Vaccins, SA, Lyon, France) is a disposable applicator made of acrylic resin. It has eight heads with nine tines on each head, linked by a support and loaded with seven different antigens (Tetanus Toxoid, Diptheria Toxoid, Streptococcus Group C, Tuberculin (Old), Candida, Trichophyton, Proteus) and a glycerin control with one antigen or the control per head. The following procedure was used for application of the testing device at each time point:

1) The volar surface of a forearm is cleansed with an alcohol pad and allowed to dry
2) The test device is then placed against the forearm and firmly pressed into the skin. The prongs at the tip of each arm enter the skin and deliver the antigen
3) A rocking motion is used to ensure adequate delivery of the antigens and control
4) The test device is removed, and the area is allowed to dry for 5 minutes
5) A permanent marker is used to outline the skin area tested to allow later observation of the proper sites
6) After 48 hours, each antigen site is evaluated for induration and calipers are used to measure the diameter of the induration along the vertical and horizontal axes
7) The number of antigens that reach at least 2 mm in diameter are considered positive. The sum total millimeters of induration and the number of positive antigens are recorded and used to determine a "CMI score" according to the formula: CMI Score = Sum of Mean Indurations ÷ Number of Positive Antigens.

Test Protocol

At 30 days prior to chamber entry, all subjects had seven specific antigens and one control placed subcutaneously on the volar surface of a forearm utilizing the Multitest® CMI device according to the manufacturer's instructions. Forty-eight hours after antigen placement, the number of positive responses to the seven antigens and to the negative control was observed, the level of induration for each positive antigen was measured by a physican evaluator (Dr. D'Aunno), and the results were recorded.

On day 45 of the chamber stay, the Multitest® CMI device was used to apply the antigens in all subjects. Forty-eight hours later, skin responses in the control group were measured by the physician evaluator. The chamber crew used the Telemedicine Instrumentation Pack to transmit the images of the skin responses to the physician evaluator who coached the chamber crew on measurement of the indurations.

Forty-eight hours prior to the end of the chamber stay, all subjects had a repeat placement of the antigens with the Multitest® CMI device. Upon completion of the 91-day test in the chamber, skin responses were measured by the physician evaluator in both the chamber crewmembers and in the control group.

One month after the chamber study, all subjects had a repeat placement of antigens with the Multitest® CMI device. The results were interpreted 48 hours later by the physician evaluator.

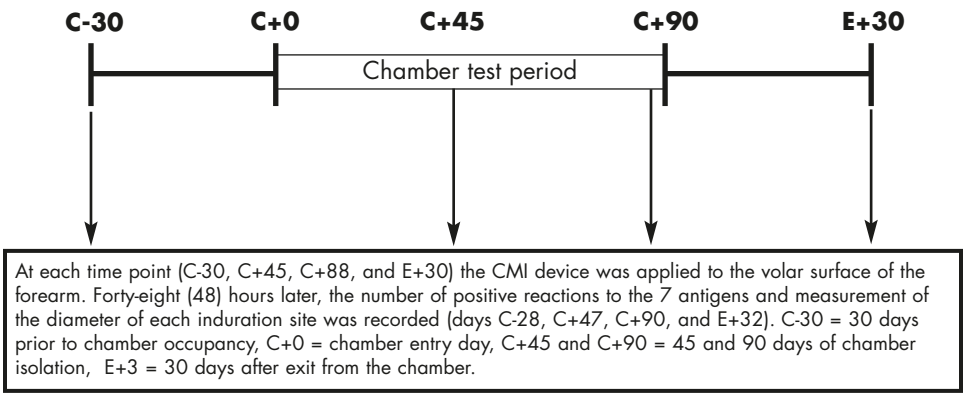


Figure 5.4-1 *Assessment of cell-mediated immunity protocol timeline*

Table 5.4-1 *Experimental test protocol*

Day	C-30	C-28	C+45	C+47	C+88	C+90	E+30	E+32
Action	Antigens Placed	Skin Results Evaluated	Antigens Placed	Skin Results Evaluated	Antigens Placed	Skin Results Evaluated	Antigens Placed	Skin Results Evaluated
Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects	4 Chamber Crew 4 Control Subjects

RESULTS

Tables 5.4-2 and 5.4-3 summarize the results in each of the chamber and control subjects at each study time point. The number of antigens that produced a measurable induration, the sum of mean induration measurements for each time point, and the calculated CMI score are given in tabular form for each subject for both the chamber and control groups.

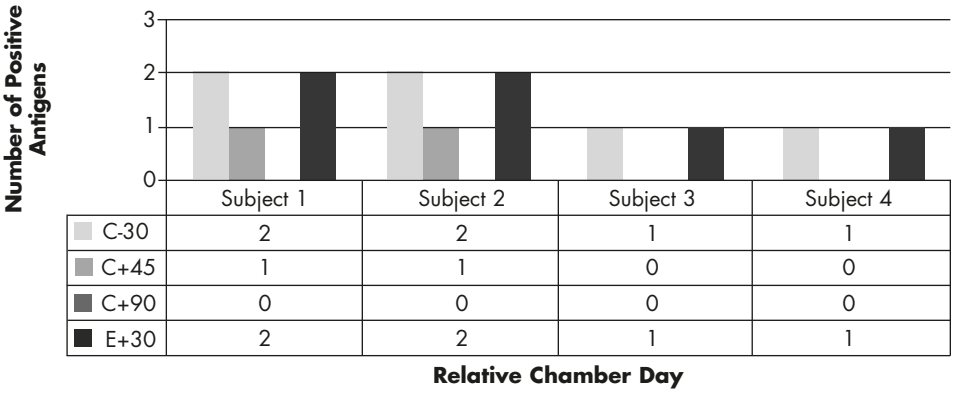
Table 5.4-2 *CMI measurements in control and chamber subjects at C-30 and C-45 days*

	C-30			C+45		
Chamber Group	# of + Antigens	Sum of Mean Indurations	Calculated CMI Score	# of + Antigens	Sum of Mean Indurations	Calculated CMI Score
Subject #						
1	2	6	3	1	2	2
2	2	8	4	1	2	2
3	1	2.3	2.3	0	0	0
4	1	5.5	5.5	0	0	0
Control Group						
Subject #						
5	3	11.7	3.9	3	9.2	3.1
6	4	14.4	3.6	4	14.8	3.7
7	2	12	6	2	12	6.0
8	4	10.8	2.7	6	21.3	3.6

Table 5.4-3 *CMI measurements in control and chamber subjects at C+90 and E+30 days*

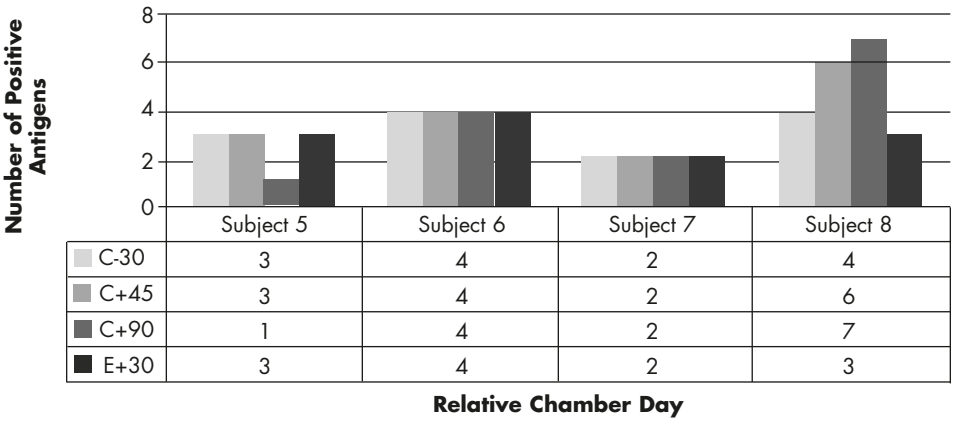
	C+90			E+30		
Chamber Group	# of + Antigens	Sum of Mean Indurations	Calculated Score	# of + Antigens	Sum of Mean Indurations	Calculated CMI Score
Subject #						
1	0	0	0	2	6	3
2	0	0	0	2	7.5	3.75
3	0	0	0	1	2.4	2.4
4	0	0	0	1	4.1	4.1
Control Group						
Subject #						
5	1	4.1	4.1	3	10.6	3.5
6	4	14.1	3.5	4	15	3.8
7	2	11.9	6.0	2	12.2	6.1
8	7	23.5	3.4	3	10.6	3.5

The number of positive reactions to the seven antigens is shown for each of the chamber (Figure 5.4-2) and control (Figure 5.4-3) subjects at each of the study time points. The chamber subjects had fewer responses to the seven antigens at the C-30 time point compared to the control subjects. By chamber day 45 (C+45), the chamber subjects showed hypoergic responses, and by chamber day 90 (C+90) all chambers subjects exhibited anergy to the seven challenge antigens. The chamber subjects had returned nearly to their prechamber baselines within 30 days of exiting the chamber. The control group subjects responded to more antigens at the C-30 time point and showed variable responses but only minor changes throughout the study period.



	Subject 1	Subject 2	Subject 3	Subject 4
C-30	2	2	1	1
C+45	1	1	0	0
C+90	0	0	0	0
E+30	2	2	1	1

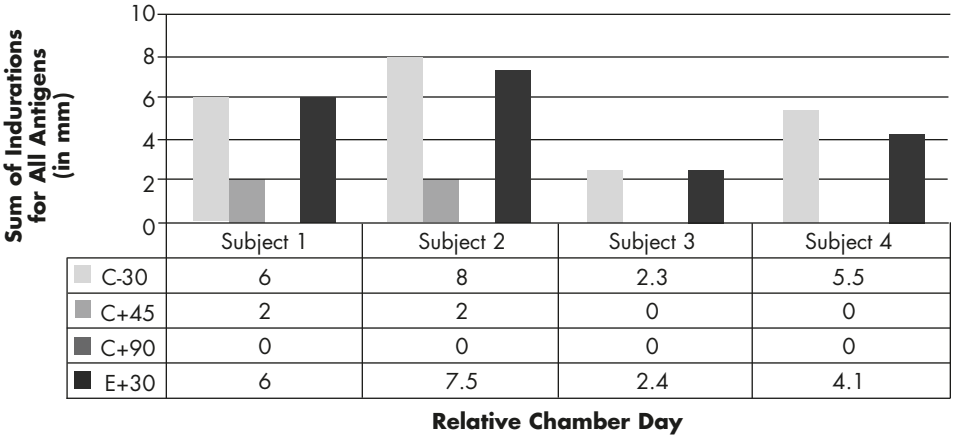
Figure 5.4-2 *Number of positive reactions to seven antigens in four chamber subjects by relative chamber day*



	Subject 5	Subject 6	Subject 7	Subject 8
C-30	3	4	2	4
C+45	3	4	2	6
C+90	1	4	2	7
E+30	3	4	2	3

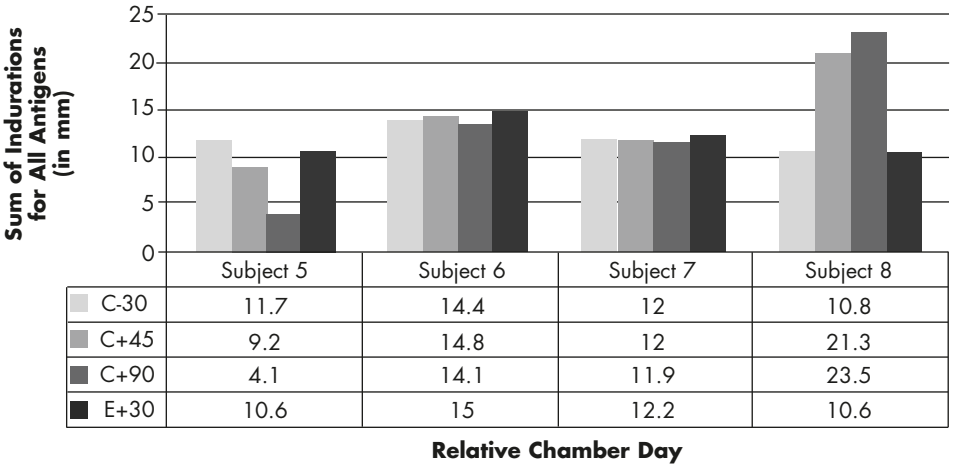
Figure 5.4-3 *Number of positive reactions to seven antigens in four control subjects by relative chamber day*

The sum of indurations of positive responses to the seven challenge antigens is shown for the chamber (Figure 5.4-4) and control (Figure 5.4-5) subjects. Since the chamber subjects responded initially (C-30) to fewer antigens, the baseline value for the sum of the indurations in the chamber subjects is less than that for the control group. The chamber subjects showed a loss of reactivity to most of the antigens by chamber day C+45 and had no response to any of the antigens and thus no measurable indurations at chamber day C+90. By 30 days after exit from the chamber (E+30), the measured sum of indurations had returned to near baseline level in the chamber subjects. The control subjects showed a variable response throughout the study. One control subject exhibited a slightly attenuated response on chamber day C+45 while another showed increased responses at chamber days C+45 and C+90. On day E+30, all control subjects had values similar to pre-chamber baseline measurements.



	Subject 1	Subject 2	Subject 3	Subject 4
C-30	6	8	2.3	5.5
C+45	2	2	0	0
C+90	0	0	0	0
E+30	6	7.5	2.4	4.1

Figure 5.4-4 *Sum of indurations (in mm) from all positive skin reactions to seven antigens in four chamber subjects by relative chamber day*



	Subject 5	Subject 6	Subject 7	Subject 8
C-30	11.7	14.4	12	10.8
C+45	9.2	14.8	12	21.3
C+90	4.1	14.1	11.9	23.5
E+30	10.6	15	12.2	10.6

Figure 5.4-5 *Sum of indurations (in mm) from all positive skin reactions to seven antigens in four control subjects by relative chamber day*

The most interesting and meaningful results (Average CMI score) for both the control and chamber groups are summarized in Figure 5.4-6. The average CMI score for the control subjects varied little throughout the entire study period (range 4.05 to 4.23). However, the chamber subjects as a group showed a profound decrement in their average CMI scores on chamber days C+45 and C+90 with no response to any of the seven challenge antigens noted in any of the four chamber subjects on chamber day C+90. The average CMI score of the chamber group had returned to near the baseline level at 30 days postchamber (E+30).

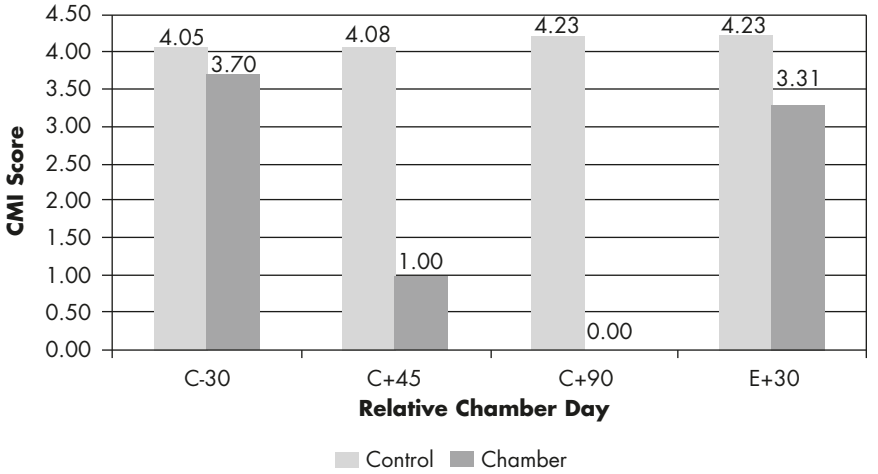


Figure 5.4-6 *Mean CMI scores in 4 chamber and 4 control subjects by relative chamber day. The mean CMI score for control subjects was relatively unchanged throughout the study period*

The control and experimental groups in this study were small (n = 4 for each group), and since the CMI score was a calculated value (CMI Score = sum of diameters of induration/number of positive reactions to antigens) based on whether a skin reaction occurred resulting in an area of induration, then the case in which there were no skin reactions to any of the seven antigens was problematic in that the mathematical calculation was not defined due to division by zero. For calculation of a CMI score when there was no response to any of the antigens, the CMI score was recorded as 0. These factors required a thoughtful approach in order to provide useful statistical comparisons. An expert statistician was consulted for guidance, and the approach taken was to regard the study as having a single perturbation—isolation within the chamber. A statistical model was developed in which data were combined from both the control and chamber groups for the subjects not confined to the chamber (n = 20; all measurements for the control group at all study time points plus the prechamber measurements from the chamber group; these data comprised the control data set). The data obtained on both in-chamber study time points (C + 45 and C + 90) were combined for the chamber group to comprise a data set of values (n = 8) measured during chamber isolation; the in-chamber data set). Finally, a third data set consisted of the data from measurements made postchamber on the chamber group 30 days after exit from the chamber (E + 30; n = 4; the postchamber data set). The three data sets were analyzed for variance and the variance expressed as a percent change ± SEM from the control data set calculated from measurements made in subjects not isolated in the chamber. Figure 5.4-7 shows the results of these statistical comparisons. The chamber subjects had a nearly 49% decrease in their CMI scores during the chamber

stay which was statistically different from the control data set at p = 0.002. The CMI scores of the chamber subjects were still decreased from control values by approximately 13% at time point E+30 (30 days after exiting the chamber), but the difference was not significant.

DISCUSSION

The effects of stress on the human immune system have been studied in numerous environments including Antarctic expeditions (6, 9) and in spacecraft during both short- (7, 8) and long-duration (5) Earth-orbital missions. These studies have collectively shown decrements in human immune function associated with these environments including decreased cell-mediated immune function.

The primary aim of the current study was to determine if isolation of human subjects within a closed chamber over a period of 91 days would adversely affect function of the cell-mediated arm of the immune system as assessed by delayed-type hypersensitivity (DTH) skin responses to specific test antigens utilizing a commercially available and scientifically validated cutaneous test system (2, 3, 4). The CMI scores were significantly decreased (-48.7% ± 10.1 SEM; p= 0.002; n = 8) for the chamber subjects during chamber isolation. The CMI scores were decreased below the control level (-13.1% ± 13.05 SEM) for the isolated chamber subjects at 30 days after exit from the chamber but were not statistically different from the control values at this time point.

Based on previous studies (5, 6, 7, 8, 9) in analogue environments, the decrement in CMI function during chamber isolation was not unexpected. An interesting aspect of this particular study was that the subjects also participated in an exercise study (see Chapter 5.2: Exercise Countermeasures Demonstration Projects During the Lunar-Mars Life Support Test Project Phases IIa and III). For Phase III, the chamber subjects completed a battery of exercise countermeasures including both aerobic and resistive exercises each day for six days, resting on the seventh day. For aerobic exercise, a cycle protocol was performed three days per week and a steady-state treadmill protocol was added on the remaining three exercise days. Additionally, an upper- and lower-body resistance exercise protocol was performed. The benefits of exercise on the immune system are well documented, and thus it would be predicted that the negative effects of chamber isolation should be at least partially offset by participation in daily exercise. However, the type, level, and duration of exercise seems to be important in achieving increased immune responsiveness, and excessive levels of certain types of exercise may contribute to decrements in immune function (1).

The short-term and long-term effects of decreased CMI in isolated human subjects are not known. Since CMI plays important roles in combating infectious agents (e.g., viruses and fungi), in surveillance against neoplastic cells, and in regulation of immune function, possible consequences include increased susceptibility to acute and chronic infections, increased cancer risk, and immune dysregulation. The level of

these increased risks associated with decreased CMI function in conjunction with isolation and other still poorly understood environmental, physiological, and psychological factors is not known. Additional prospective and retrospective longitudinal studies are required to better understand underlying mechanisms and the level of risks associated with decreased CMI function in persons living in isolated environments. The role of exercise and the specific types, intensity levels, and duration in modulating the immune response during isolation requires further investigation.

The final aim of the project was to determine if the closed-chamber test bed is an appropriate ground-based analogue to further investigate the potential effects of isolation on underlying mechanisms that may alter cell-mediated immune function during long-duration space flight or extended stays at an Earth-orbiting space station facility. Experience and knowledge gained by this study supports the use of closed chamber studies for this purpose.

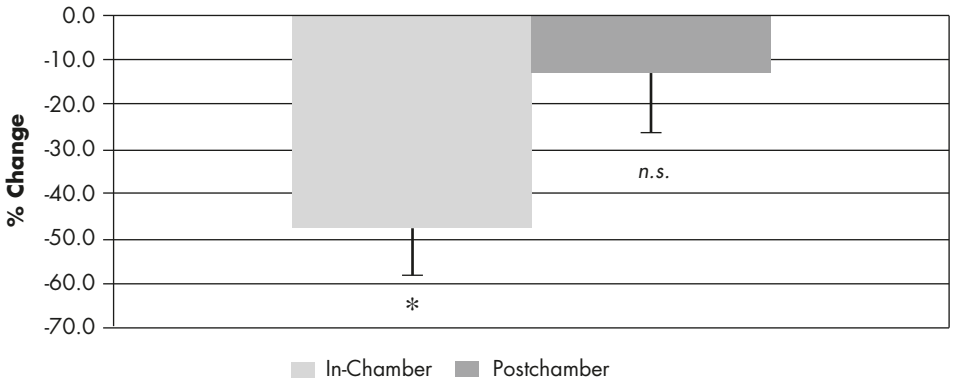


Figure 5.4-7 *Change (in percent ± SEM) from control (n=20) in composite CMI score for in-chamber measurements (n=8) and postchamber measurements (n=4). *Significant difference (p=0.002). n.s. = not significant*

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5.5

Physiological Effects of Iodinated Water on Thyroid Function

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SUMMARY

Iodine has been used as a potable water treatment and storage bactericidal agent by NASA for over three decades beginning with the Apollo program, and was a critical factor for the achievement of human space flight. Iodine is currently used for Space Shuttle potable water microbial control and is planned for the U.S. component of the International Space Station (ISS). Human consumption of iodinated water is known to transiently affect thyroid-related hormone levels (acute Wolff-Chaikoff effect) and potentially may result in acute and/or chronic thyroid dysfunction in susceptible individuals. NASA's ongoing health surveillance program includes the measurement of thyroid-related hormone levels as part of the medical assessment of thyroid function in all astronauts throughout their careers and natural lives; a summary of these findings and those from an age- and sex-matched peer group is presented. An investigation was undertaken during three ground-based, closed-chamber tests (Phases II, IIa, and III of 30, 60, and 91 days' duration, respectively) to examine thyroid function and hormone levels in chamber subjects who consumed potable water containing iodine. The iodine concentration in the water was within the range planned for use on the ISS. Crewmembers' thyroid function was monitored pre- and postchamber for Phases II and IIa, and pre-, during, and postchamber for Phase III. Although all crewmembers remained free of symptoms and signs of thyroid dysfunction, the data demonstrated that with high levels of iodine intake (4 to 20 mg/day depending on dietary water intake levels), two of the eight crewmembers had notable changes in thyroid-stimulating hormone (TSH) levels, and all crewmembers showed elevations (up to 10-fold in some cases) in urinary iodine levels indicative of the increased iodine intake. Removal of iodine from the drinking water at day 35 in the 91-day chamber test reduced urinary iodine concentrations, but urinary iodine levels remained greater than prechamber values, indicating either possible contamination during urine collection and processing, or persistent iodine exposure of crewmembers from sources other than drinking water.

Urinary iodine levels returned to prechamber baseline levels immediately after crew departure from the chamber. A follow-up study demonstrated that urine collection and processing methods were not the source of the persistent 1.0 to 1.5 mg/day urinary iodine levels. Although no definitive source was identified for these continually elevated urinary iodine levels, there may have been multiple contributing sources. Eventually, TSH levels of all crewmembers returned to prechamber values. As a result of both this study and the recommendations of a NASA-solicited panel of expert thyroidologists, NASA has established an upper limit for daily iodine consumption of 1.0 mg/day from all sources (food and potable water) during space flight. Since iodine still must be used to achieve microbial control, iodine removal technologies have been developed that extract iodine from the spacecraft potable water supply before human use. Independent of this study and NASA's decision to establish a maximum level of daily iodine intake, the U.S. National Academy of Sciences Food and Nutrition Board subsequently established an upper safe limit for iodine intake from all sources at 1.1 mg/day for the general population.

Background

Iodine and Thyroid Function

Iodine, essential for mammalian life, is a component of the two major thyroid hormones, thyroxine (T4) and triiodothyronine (T3), comprising 65 and 59% of their respective weights (7, 10). These hormones regulate many key biochemical processes, especially protein synthesis and enzymatic activity (7). Major target organs are the developing brain, muscle, heart, pituitary, and kidney, but nearly all somatic cells possess nuclear thyroid hormone receptors. Most ingested iodine is reduced in the gut to iodide, which is absorbed almost completely (16). Once in circulation, iodide is removed primarily by the thyroid gland and kidney. The thyroid selectively concentrates iodide with the excess excreted into the urine. Other organs and tissues that can concentrate iodine include the major salivary glands, the mammary glands, the choroid plexus of each brain ventricle, and the gastric mucosa. Thyroid-stimulating hormone (TSH), produced in the anterior lobe of the pituitary gland, regulates the release of thyroid hormones from the thyroid gland into the blood. TSH secretion from the pituitary gland increases when the levels of circulating thyroid hormones decrease. An increased serum TSH level may indicate subclinical (normal serum T3 and T4) or clinical (elevated serum T3 and/or T4) hypothyroidism, while a decreased serum TSH level is usually associated with subclinical or clinical hyperthyroidism. The thyroid responds to the ingestion of elevated quantities of iodine by a transient decrease in thyroid hormone synthesis, termed the acute Wolff-Chaikoff effect (2, 4, 7, 24).

TSH levels have circadian phases that change when sleep/wake cycles are disrupted. Allan and Czeisler (1) found TSH levels normally peak in the late evening, fall precipitously at sleep onset, and remain low throughout the morning

and afternoon. For subjects (n = 15) that remained awake on a 40-hour constant routine (missed a single night of sleep), TSH levels remained elevated over a longer period of time extending into the morning hours. They further demonstrated that both the output of the human circadian pacemaker as well as the inhibitory effect of sleep contribute to the regulation of TSH secretion. Furthermore, a lack of corresponding circadian rhythmicity in circulating levels of thyroid hormones (T3, T4) seen under the same experimental conditions indicates that the relatively large peripheral pools of thyroid hormone are not acutely altered by the normal daily rhythmicity in TSH levels.

The U.S. National Academy of Sciences Food and Nutrition Board (9, 10) Recommended Dietary Allowance (estimated average requirement + 2 standard deviations) is 0.150 mg/day for adult men and women, and the Food and Agriculture Organization (25) of the United Nations World Health Organization (WHO) recommendation is 0.130 and 0.110 mg iodine/day for men and women, respectively. Based on the Food and Drug Administration Total Diet Study (10), iodine intake from food in the United States is approximately 0.190 to 0.300 mg/day. Iodine intake from dietary supplements varies but is approximately 0.140 mg/day (9). Urine contains 90% of the excreted iodine with the remainder in stool and sweat, and is a good indicator of recent iodine consumption. From U.S. data (NHANES I and III), urinary iodine ranges in concentration from 0.0110 to 0.0155 mg/dL (10).

Since 1922, iodine has been used as a method of emergency water treatment for U.S. military personnel (8). Subsequent studies in military personnel on the effects of iodinated water intake at various concentrations for periods as short as one week to as long as six months have documented thyroid enlargement and changes in blood levels of thyroid hormones and TSH (6, 13, 15). However, none of these studies documented any acute clinical manifestations with the exception of mild thyromegaly or any long-term sequelae contributable to consumption of iodinated drinking water. In a particularly relevant study (13), seven men and one woman ingested four tablets of tetraglycine hydroperiodate in water per day for 12 weeks (84 days), providing 32 mg total iodine per day to each subject. Serum inorganic iodide rose from a baseline level of 2.7 to approximately 100 μg/dL, while urinary iodide excretion increased 150-fold from a pretreatment mean of 0.276 to 40 mg/day. As expected, mean T4 and T3 levels declined after seven days. The mean T4 level remained below baseline throughout the study but T3 had recovered by the end of the 13-week (91-day) period. Serum TSH and the subject's response to TRH rose significantly after seven days and remained elevated at three months. Thyroid volume determined by ultrasonography increased an average of 37%, but clinical signs of neither hyperthyroidism nor hypothyroidism were observed (15). In another study, prisoners (22) consumed 0.5 to 1.0 mg iodine/L of drinking water with no significant clinical effects. However, 44 (46%) of 96 Peace Corps volunteers, consuming approximately 50 mg iodide/day over a period of up to one year from contamination of water purification units, had enlarged thyroid glands, while 30 of

those had normal thyroid function tests and 33 (34%) of the 96 had thyroid dysfunction (11). In another study (20), no differences were found in T3 and T4 levels with administration of iodine as I^- (iodide) versus I_2 (iodine) in humans. However, elevations were observed in TSH with both forms, which raised concerns about the impact of consumption of iodine in drinking water for spacecraft. There are published reviews of various iodide toxicity population and case studies (2, 19).

The consequences of iodide supplementation on thyroid function in normal subjects are reported for two studies in Table 5.5-1 (14). TSH concentrations were studied in 30 adult males who received 0.5, 1.5, and 4.5 mg/day as supplemental iodide for two weeks (5). The subject's average prestudy urinary iodine was 0.29 mg/day, and food sources represented approximately 0.3 mg/day. Although still within the normal range, the mean basal serum TSH concentration increased significantly in those receiving the two higher doses of iodine. In a similar study (18), nine men and 23 women received iodine supplementation at 0.25, 0.5, or 1.5 mg/day. Baseline urinary iodine was approximately 0.191 mg/day, and calculated dietary intakes were 0.2 mg/day. Those receiving 1.5 mg/day of iodide showed a significant increase over baseline in their TSH levels, with no effects seen at the two lower doses. In a 28-day study of 225 adult women (treatment plus controls), there were significantly elevated TSH concentrations with iodine intakes of 0.75 mg/day or more (3).

Table 5.5-1 *Effects of low-dose iodide supplementation on thyroid function in normal subjects*[a]

Investigator (ref #)	Iodide (mg/day)	TSH	TSH Response to TRH[b]	T4	Free T4	T3
Paul (15)	0.25	No effect	No effect	No effect	No effect	No effect
Paul (15)	0.5	No effect	No effect	No effect	No effect	No effect
Gardner (5)	0.5	No effect	↑	No effect	No effect	No effect
Paul (15)	1.5	↑	↑	↓	↓	↓
Gardner (5)	1.5	↑	↑	↓	↓	No effect
Gardner (5)	4.5	↑	↑	↓	↓	No effect

[a]Reference 14

[b]Thyroid-releasing hormone

Thyroid dysfunction is more likely to appear with prolonged consumption of pharmacological doses of iodine in susceptible individuals. Generally, most individuals with normal underlying thyroid function remain euthyroid during periods of iodine consumption (2, 3, 19). Individuals who have autoimmune thyroid disease and/or iodine deficiency as well as individuals living in areas of low iodine intake may respond adversely to high iodine intakes. These responses include thyroiditis, goiter, hypothyroidism, and hyperthyroidism. Signs of acute iodine poisoning include burning mouth, throat, and stomach, abdominal pain, fever, nausea, vomiting, and other symptoms (11, 21). Thus, the Food and Nutrition Board (10) reported that adults have no observed adverse effect at levels of 1 to 2 mg/day and a lowest observed adverse effect at a level of 1.7 mg/day for iodine. The Food and Nutrition Board currently recommends an upper limit for total iodine intake from combined food and water sources be less than 1.1 mg/day (10).

History of Iodine in U.S. Space Flight Programs

Iodine has been used for the last 30 years, beginning with the Apollo program, as an effective bactericidal, virucidal, and amebicidal agent for drinking water (20, 23); this includes the potable water system of spacecraft, training galleys, and NASA closed-chamber tests. Microbial quality of potable water first became an issue during the Gemini program because the water consumed by the astronauts had to be stored before the flight and throughout the duration of the flight. This storage would potentially allow proliferation of hazardous microorganisms in the stored water. Of even greater concern was the threat of a microbial hazard from the potential cross-contamination between the potable water supply and the urine disposal system. A variety of chemical disinfectants were used with varying success during the Gemini program. On some missions, a quaternary ammonia compound (Roccal®) that had an undesirable taste was employed while chlorine in the form of hypochlorite, which was effective for only a few days, was used on other missions. There was no capability to add additional chlorine either on the ground during preparation for flight or in flight during missions of up to 14 days, thus limiting the utility of chloride as a disinfectant for space flight.

With the recognition of the microbial hazard in the potable water during the Gemini program, the development of the Apollo Command Module and Lunar Module incorporated the requirement to maintain a biocide in the potable water. The Command Module used sodium hypochlorite for microbial control in combination with sodium dihydrogen phosphate for pH control and sodium nitrate for corrosion control. Because of chlorine depletion and the dilution of the stored water with water produced by the fuel cells, nitrate, chlorine, and phosphate had to be added manually by the crew daily. This system provided the required microbial control but was time consuming for the crew and periodically caused adverse chlorine tastes because of spiking of the chlorine level at the time of its addition. In contrast, the Lunar Module water that was stored on board before launch was iodine treated. The configuration of the launch vehicle required that this water be loaded

into the Lunar Module tanks about 30 days before launch. Testing of the system demonstrated that the active reduced form of iodine remained effective throughout the combined water storage period and length of the mission. The successful use of iodine in the Lunar Module of the Apollo program led to its being the agent of choice in the subsequent Skylab program.

The Skylab program, consisting of three serial missions (Skylab 2, 3, and 4) of increasing lengths (28, 56, and 84 days, respectively) over a period of approximately one year, required that all water be stored on board the vehicle before launch. Iodine was selected because of its water system materials compatibility – a flight-compatible (colorimetric starch-iodine reaction) method of monitoring levels to determine when it needed to be replenished – and an in-flight addition method utilizing a $KI:I_2$ stock solution. To better ensure system materials compatibility, the water system was fabricated of stainless steel. Because reduced iodine in time eventually converts to iodide, which is not an effective disinfectant, iodine stock solution was periodically added which resulted in a total iodine content in the potable water of up to 72 mg/L during the longest (84-day) Skylab mission.

The Space Shuttle, like the Apollo Command Module, uses hydrogen and oxygen fuel cells to generate electrical power with continuously produced water as the by-product (23). Prior to the Shuttle program, an iodinated anion-exchange resin system was developed that reliably introduces effective levels of iodine into the fuel cell water as it flows through a packed resin bed into the water storage tanks. Shuttle crewmembers' mean iodine exposure levels have varied from 3.8 to 5.9 mg/L water (Table 5.5-2). This system has proven to be successful as it reliably provided the required level of microbial control during periods between missions and throughout the duration of flight.

Table 5.5-2 *Exposure to iodine during space flight*

Mission	Mean Exposure Time (days)	Mean Iodine Level (mg/L of water)
Skylab 2, 3, 4	57.2	11.2
Shuttle (flights 1-25)[a]	6.4	5.9
Shuttle (flights 26-63)[b]	9.5	3.8

[a]STS-1 to STS-51L

[b]STS-26 to STS-87

The U.S. water recovery system for the ISS is being developed to use iodine for potable water microbial control utilizing an iodinated resin system. The major aspect of the ISS that differs from previous U.S. missions is that for the first time there will be reclamation of water from urine, wash water, and humidity condensate for use as potable water. This will place additional demands on the water disinfection system because of the increased microbial contaminant content of the source waters.

Thyroid Function Among Astronauts as Compared to Controls

As part of the astronaut longitudinal epidemiological research program (Longitudinal Study of Astronaut Health), thyroid function is monitored and compared to age-, sex-, and career-matched controls (14). Since 1994, thyroid peroxidase and thyroglobulin antibody testing has been performed as part of astronaut candidate selection, but such testing has not been done in the comparison population (14). For this longitudinal study, subclinical hypothyroidism is defined as elevated serum TSH values in the presence of normal serum T3 and T4 levels at three or more annual medical physical examinations. As discussed earlier in this chapter, astronauts flown over the history of the U.S. space program have had a variety of exposures to iodine (Table 5.5-2). Calculated iodine water intake was based on an average water intake of 1.9 L/day multiplied by the length of the mission.

There was no documented increased incidence of thyroid disease in the 270 astronauts studied as compared to the peer group (n = 258), and there was also no increased incidence of thyroid dysfunction related to total iodine exposure during space flights (14). While astronauts flying on longer-duration missions, such as Skylab or on multiple Shuttle flights, had increased iodine exposure compared to those who have not flown or who flew only on a single Shuttle flight, there was no association between the incidence of thyroid disease and the number or duration of space fights flown. There was no statistically based evidence that space flight or iodine exposure increased the incidence of subclinical or clinical thyroid disease in U.S. astronauts (14).

Purpose of the Thyroid Function Study

The planned ISS U.S. water system will recycle water from urine and other wastewaters for human consumption and requires long-term storage. Iodine is planned as the disinfectant at 2 to 4 mg/L (I_2) concentration with a total iodine concentration of 3 to 6 mg/L (I_2 and I^-). With the proposed iodine exposure levels and the crewmembers originating from many countries including regions of low iodine intake, additional concerns have been raised regarding the use of iodine in ISS potable water. The purpose of this study was to document the thyroid functional biochemical measurements (n = 12), urine iodine levels (n = 8), and any clinical signs or symptoms of thyroid dysfunction in the 12 crewmembers who participated in the chamber studies.

Methods

Characteristics of Ground-Based Test Subjects and the Chamber Tests

Of the 12 crewmembers, four were female, and all were under 42 years of age. Each selected crewmember was considered healthy. All had normal weight per height, and as part of the selection process each passed a class III Air Force medical examination, had normal thyroid function, and successfully completed an exercise stress test.

The chamber tests, in order of occurrence, were 30 days, Phase II; 60 days, Phase IIa; and 91 days, Phase III. Each chamber test had four crewmembers with one, one, and two women in Phases II, IIa, and III, respectively. In all three chamber tests, after the water recycling process was completed, 3.5 to 5.2 mg iodine/L was added to the water as the disinfectant. In Phase II (30 days) and Phase IIa (60 days), thyroid hormonal status (TSH and thyroid hormone levels) was assessed both in the pre- and postchamber period. In Phase III, the 91-day study, TSH, thyroid hormones, and urinary iodine levels were measured before and several times in chamber (first measurement at the 30-day mark). These were followed up with several more measurements in the postchamber period upon the subjects' exit from the chamber at the completion of the study. Subjects were monitored until all indices of iodine/thyroid status had returned to prechamber levels.

For the Phase III study (91 days), due to changes in TSH levels at the 30-day mark, iodine was removed from the potable water before consumption. This was accomplished by installing an iodine-removal device and a microbial filter (0.2 μm) at the point of use of the water on chamber day 35. This device removed the iodine (I_2 and I^-) from the drinking water but did not affect the iodine levels in the remainder of the water used, i.e., for hygiene activities. Urinary iodine levels (argon plasma mass spectrometer, Mayo Clinic Laboratories, MN) were spot-checked to determine iodine exposure. Anti-thyroidal (anti-thyroglobulin and anti-TPO) antibodies were determined (12) in only the four Phase III test crewmembers.

Methods of Analysis

Table 5.5-3 lists the analyses performed on water, urine, and blood samples. Several methods were used over the course of the chamber studies, reflecting updating of the methodologies. Reference ranges are provided with the results (Tables 5.5-4 through 5.5-6).

Table 5.5-3 *Methods of analysis*

Measurement	Chamber Phase	Method
Urinary iodine	IIa, III	Method described in detail in Appendix Inductively Coupled Plasma Mass Spec (ICP-MS)
T3	II, IIa, III	Microparticle Enzyme Immunoassay (MEIA)
T4	II, IIa, III	Microparticle Enzyme Immunoassay (MEIA)
Free thyroxine index (FTI)	II, IIa, III	Calculated from total T4 and T-uptake
TSH	II, IIa, II	Microparticle Enzyme Immunoassay (MEIA) II and IIa used first-generation test TSH Abbott IMX, Last measure on IIa and all of III used second-generation hTSHII Abbott IMX
Free T4	IIa, III	Radioimmunoassay by direct equilibrium dialysis
Anti-TPO	III	Chemiluminescence
Thyroglobulin AB	III	Chemiluminescence

Classification of Thyroid Dysfunction

Subclinical hypothyroidism is defined as a state of mild thyroid hormone deficiency characterized by elevated TSH levels and normal thyroid hormone levels (21). Subclinical hyperthyroidism is defined as depressed TSH levels and normal thyroid hormone levels. Symptoms and signs monitored were those of thyroid hormone hyperfunction including nervousness, restlessness, tachycardia, tremor, weight loss, and heat intolerance (19). Clinical hypothyroidism is diagnosed in an individual with symptoms of hypothyroidism (muscle cramps, dry skin, cold intolerance, constipation, poor energy, and easy fatigability), elevated TSH, and low thyroid hormone levels (T3 and/or T4).

Table 5.5-4 *Thyroid and iodine levels for the Phase II crewmembers*

			Prechamber Testing 8 days prechamber	Postchamber Testing		
	Laboratory Measurement	**Reference Range**	**0 days iodinated water**	**2 days postremoval iodinated water**	**19 days postremoval iodinated water**	**42 days postremoval iodinated water**
Subject 1	TSH	0.0-6.0 μIU/mL	1.5	2.0	1.8	nd
	Total T4	4.5-13.0 μg/dl	7.5	7.4	7.6	nd
	Free T4	0.89-1.80 ng/dl	-	-	-	nd
	T3 uptake	0.70-1.07	0.98	1.0	0.90	nd
	FTI	5.00-12.00	7.65	7.40	8.44	nd
	Total T3	57-170 ng/dl	107	90	94	nd
Subject 2	TSH	0.0-6.0 μIU/mL	1.4	2.5	1.6	1.2
	Total T4	4.5-13.0 μg/dl	5.4	5.5	5.6	6.7
	Free T4	0.89-1.80 ng/dl	-	-	-	1.22
	T3 uptake	0.70-1.07	0.77	0.73	0.79	1.22
	FTI	5.00-12.00	7.01	7.53	7.09	8.48
	Total T3	57-170 ng/dl	112	76	90	76
Subject 3	TSH	0.0-6.0 μIU/mL	0.8	1.2	0.6	1.0
	Total T4	4.5-13.0 μg/dl	5.2	6.0	5.8	6.5
	Free T4	0.89-1.80 ng/dl	-	-	-	1.13
	T3 uptake	0.70-1.07	0.73	0.79	0.69	0.75
	FTI	5.00-12.00	7.12	7.59	8.41	8.67
	Total T3	57-170 ng/dl	105	101	74	90
Subject 4	TSH	0.0-6.0 μIU/mL	1.9	2.3	1.6	1.0
	Total T4	4.5-13.0 μg/dl	7.9	7.1	7.3	8.5
	Free T4	0.89-1.80 ng/dl	-	-	-	1.15
	T3 uptake	0.70-1.07	0.86	0.88	0.92	0.92
	FTI	5.00-12.00	9.19	8.07	7.93	9.24
	Total T3	57-170 ng/dl	124	100	108	81

nd = no data collected

Table 5.5-5 *Thyroid and iodine levels for the Phase IIa crewmembers*

			Prechamber Testing 8 days prechamber	Postchamber Testing		
	Laboratory Measurement	Reference Range	0 days iodinated water	2 days postremoval iodinated water	19 days postremoval iodinated water	42 days postremoval iodinated water
Subject 1	TSH	0.0-6.0 μIU/mL	3.1	3.6	1.7	2.9
	Total T4	4.5-13.0 μg/dl	8.2	9.2	10.5	8.9
	Free T4	0.89-1.80 ng/dl	-	nd	-	-
	T3 uptake	0.70-1.07	1.10	1.17	1.24	1.07
	FTI	5.00-12.00	7.45	7.86	8.47	8.32
	Total T3	57-170 ng/dl	126	127	106	123
Subject 2	TSH	0.0-6.0 μIU/mL	2.2	3.1	1.2	1.87
	Total T4	4.5-13.0 μg/dl	5.4	7.4	6.7	6.5
	Free T4	0.89-1.80 ng/dl	-	-	-	1.13
	T3 uptake	0.70-1.07	0.74	0.79	0.71	0.68
	FTI	5.00-12.00	7.30	9.37	9.44	9.56
	Total T3	57-170 ng/dl	93	115	87	86
Subject 3	TSH	0-6.0 μIU/mL	0.6	1.6	0.7	0.87
	Total T4	4.5-13.0 μg/dl	7.9	8.3	7.8	8.0
	Free T4	0.89-1.80 ng/dl	-	-	-	1.03
	T3 uptake	0.70-1.07	0.89	0.89	0.92	8.99
	FTI	5.00-12.00	8.88	9.33	8.48	8.99
	Total T3	57-170 ng/dl	132	143	114	122
Subject 4	TSH	0-6.0 μIU/mL	4.5	1.0	14.9	6.1
	Total T4	4.5-13.0 μg/dl	5.0	6.8	3.4	4.8
	Free T4	0.89-1.80 ng/dl	-	-	-	0.90
	T3 uptake	0.70-1.07	0.62	0.72	0.74	0.66
	FTI	5.00-12.00	8.06	9.44	4.59	7.27
	Total T3	57-170 ng/dl	97	121	81	72

nd = no data collected

Table 5.5-6 *Thyroid and iodine levels for the Phase III crewmembers*

			Prechamber Testing	In-Chamber Testing						Postchamber Testing	
			8 days prechamber	31 days chamber	33 days chamber	41 days chamber	60 days chamber	91 days chamber		2 days postchamber	
	Laboratory Measurement	Reference Range	0 days iodinated water	31 days iodinated water	33 days iodinated water	7 days postremoval iodinated water	28 days postremoval iodinated water	59 days postremoval iodinated water	61 days postremoval iodinated water		
Subject 1	TSH	0.047-5.01 µIU/L	1.00	4.06	nd	2.69	2.70	1.85	nd		
	Total T4	4.5-13.0 µg/dl	8.5	7.1	nd	7.7	7.8	7.6	nd		
	Free T4	0.89-1.80 ng/dl	1.15	1.02	nd	1.12	1.11	1.32	nd		
	T3 uptake	0.70-1.07	0.92	0.82	nd	0.72	0.85	0.78	nd		
	FTI	5.00-12.00	9.24	8.66	nd	10.69	9.18	9.74	nd		
	Total T3	57-170 ng/dl	81	86	nd	82	108	106	nd		
	Anti-TPO AB	0.0-2.0 IU/ml	nd	< 2.0	nd	nd	nd	nd	nd		
	Anti-thyroglobulin AB	0.0-2.0 IU/ml	nd	< 2.0	nd	nd	nd	nd	nd		
	Urine volume	760-2500 ml	nd	1905	nd	1635	1900	750*	1363		
	Urine iodine	100-460 µg/24hr	nd	9458	nd	2367	975	698*	269		
	Cum. iodine intake	in mg	0	388	413	429	431	432	432		
Subject 2	TSH	0.047-5.01 µIU/L	1.90	3.34	4.06	3.27	2.70	2.89	nd		
	Total T4	4.5-13.0 µg/dl	8.5	6.5	7.2	7.1	7.5	7.6	nd		
	Free T4	0.89-1.80 ng/dl	1.09	0.83	1.03	0.93	0.99	1.14	nd		
	T3 uptake	0.70-1.07	0.93	0.9	0.88	0.79	0.86	0.82	nd		
	FTI	5.00-12.00	9.14	7.22	8.18	8.99	8.72	9.27	nd		
	Total T3	57-170 ng/dl	85	73	79	74	93	102	nd		
	Anti-TPO AB	0.0-2.0 IU/ml	nd	< 2.0	nd	nd	nd	nd	nd		
	Anti-thyroglobulin AB	0.0-2.0 IU/ml	nd	< 2.0	nd	nd	nd	nd	nd		
	Urine volume	760-2500 ml	nd	4105	nd	2475	3790	3381	3357		
	Urine iodine	100-460 µg/24hr	nd	16857	nd	733	1020	1467	1249		
	Cum. iodine intake	in mg	0	446	467	481	484	485	485		

*Data may not represent complete 24-hour collection
nd = no data collected

Table 5.5-6 continued *Thyroid and iodine levels for the Phase III crewmembers*

			Prechamber Testing	In-ChamberTesting					Postchamber Testing	
			8 days prechamber	31 days chamber	33 days chamber	41 days chamber	60 days chamber	91 days chamber	2 days postchamber	
	Laboratory Measurement	Reference Range	0 days iodinated water	31 days iodinated water	33 days iodinated water	7 days postremoval iodinated water	28 days postremoval iodinated water	59 days postremoval iodinated water	61 days postremoval iodinated water	
Subject 3	TSH	0.047-5.01 µIU/L	0.80	3.07	nd	2.00	1.41	0.75	nd	
	Total T4	4.5-13.0 µg/dl	6.9	6.8	nd	7.6	6.6	7.6	nd	
	Free T4	0.89-1.80 ng/dl	1.30	1.1	nd	1.33	1.16	1.55	nd	
	T3 uptake	0.70-1.07	0.71	0.76	nd	0.74	0.75	0.71	nd	
	FTI	5.00-12.00	9.72	8.95	nd	10.27	8.80	10.70	nd	
	Total T3	57-170 ng/dl	94	97	nd	108	89	111	nd	
	Anti-TPO AB	0.0-2.0 IU/ml	nd	< 2.0	nd	nd	nd	nd	nd	
	Anti-thyroglobulin AB	0.0-2.0 IU/mnd	nd	< 2.0	nd	nd	nd	nd	nd	
	Urine volume	760-2500 ml	nd	830	nd	725	1625	269*	1579	
	Urine iodine	100-460 µg/24hr	nd	7192	nd	755	986	287*	276	
	Cum. iodine intake	in mg	0	259	280	295	297	298	298	
Subject 4	TSH	0.047-5.01 µIU/L	1.40	2.28	2.31	2.03	2.50	2.30	nd	
	Total T4	4.5-13.0 µg/dl	7.0	6.8	7.1	7.2	7.4	7.3	nd	
	Free T4	0.89-1.80 ng/dl	0.96	0.78	0.96	1.02	0.89	1.13	nd	
	T3 uptake	0.70-1.07	0.93	0.98	0.99	0.89	0.97	0.83	nd	
	FTI	5.00-12.00	7.53	6.94	7.17	8.09	7.63	8.80	nd	
	Total T3	57-170 ng/dl	97	104	88	93	113	114	nd	
	Anti-TPO AB	0.0-2.0 IU/ml	nd	< 2.0	nd	nd	nd	nd	nd	
	Anti-thyroglobulin AB	0.0-2.0 IU/ml	nd	< 2.0	nd	nd	nd	nd	nd	
	Urine volume	760-2500 ml	nd	3130	nd	3555	3010	3761	1944	
	Urine iodine	100-460 µg/24hr	nd	16764	nd	1113	1261	1892	303	
	Cum. iodine intake	in mg	0	587	621	641	644	646	646	

*Data may not represent complete 24-hour collection
nd = no data collected

Results

Water, Urinary, and Dietary Iodine Levels

While individual water consumption was not determined for Phases II and IIa crewmembers, analysis of diet records and the systematic evaluation of water usage indicates the crewmembers consumed from 4 to 10 mg total iodine/day. For Phase IIa (60 days), drinking water contained approximately 2 to 4 mg I/L (Figure 5.5-1) and subjects excreted approximately 4.5 to 14 mg/day (Figure 5.5-2). Intake measurements were only completed twice during the study (Figure 5.5-2).

The actual individual water iodine intakes and urinary losses were quantified for the Phase III, 91-day test (Figures 5.5-3, 5.5-4). Figure 5.5-3 shows that the total iodine in the water varied from approximately 3 to 8 mg/L with an average level for the first 35 days around 5 mg total iodine/L. Urinary iodine levels reflect recent iodine consumption (Figure 5.5-4). In all subjects, urinary iodine levels increased about 10-fold during the first 30 days of the Phase III chamber study, with iodine consumption levels ranging from 8 to 20 mg/day. Urinary excretion means for subjects ranged from 9.4 to 16.9 mg I/day (Table 5.5-6). The variation in iodine intake between crewmembers was related to the individual volume of potable water consumed.

Following installation of the iodine removal device at the galley sink, the total iodine levels measured less than 0.05 mg/L in the drinking water but remained in full concentration in the shower and wash water. Urinary iodine levels decreased 5- to 10-fold after cessation of the water iodine exposure but remained above baseline values (Table 5.5-6 and Figure 5.5-4). By the end of the 91-day study, 56 days after cessation of drinking water iodine, the urinary values continued to remain above baseline values in three of the four subjects (Table 5.5-6, Figure 5.5-4). In three of the crewmembers, urine iodine levels fell to an average of 0.283 mg/24 hrs by three days after test completion. One crewmember (Subject 2) consumed a bundant iodine-rich seafood on multiple occasions after test completion and had a 24-hour urine iodine level of 1.2 mg/day two days after exiting the chamber (Table 5.5-6).

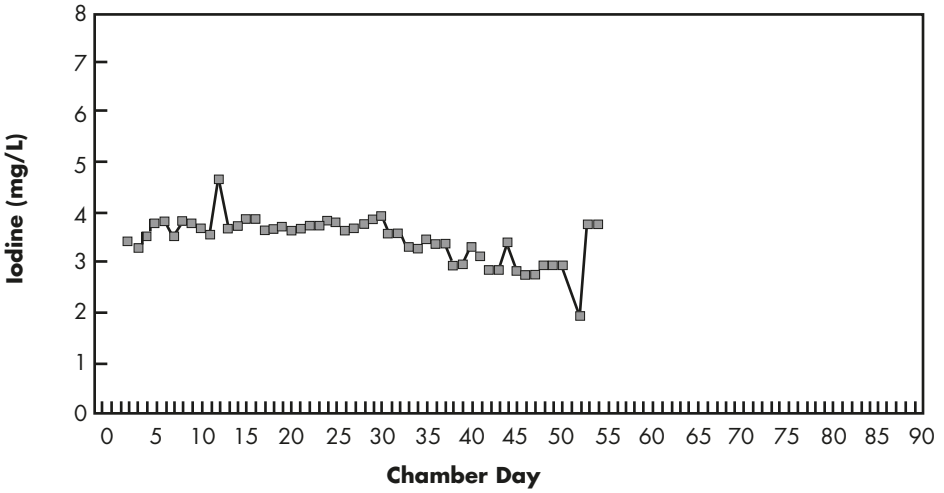

Figure 5.5-1 *Iodine levels in drinking water during Phase IIa by chamber day*

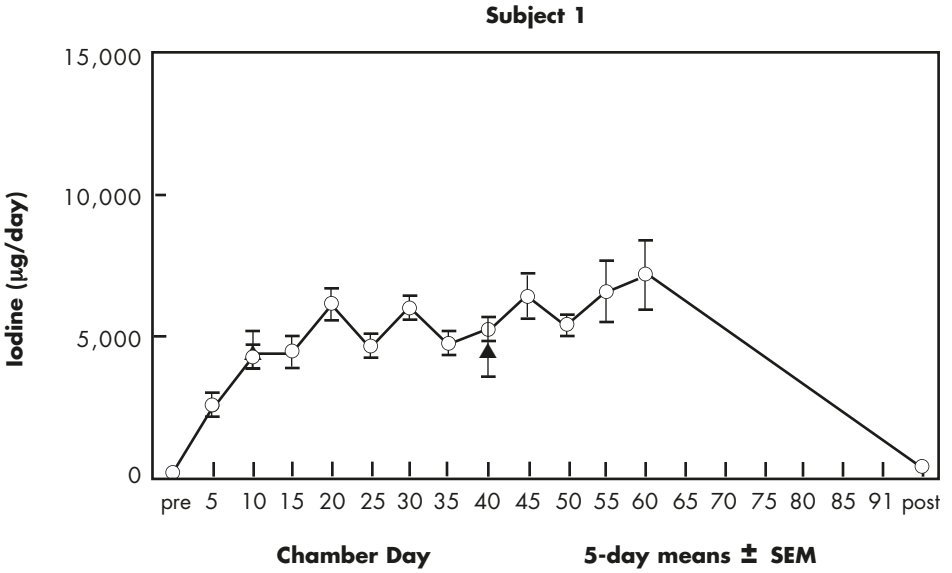

Figure 5.5-2 *Iodine intake from water (▲) and urinary iodine excretion (○) of crewmembers during Phase IIa*

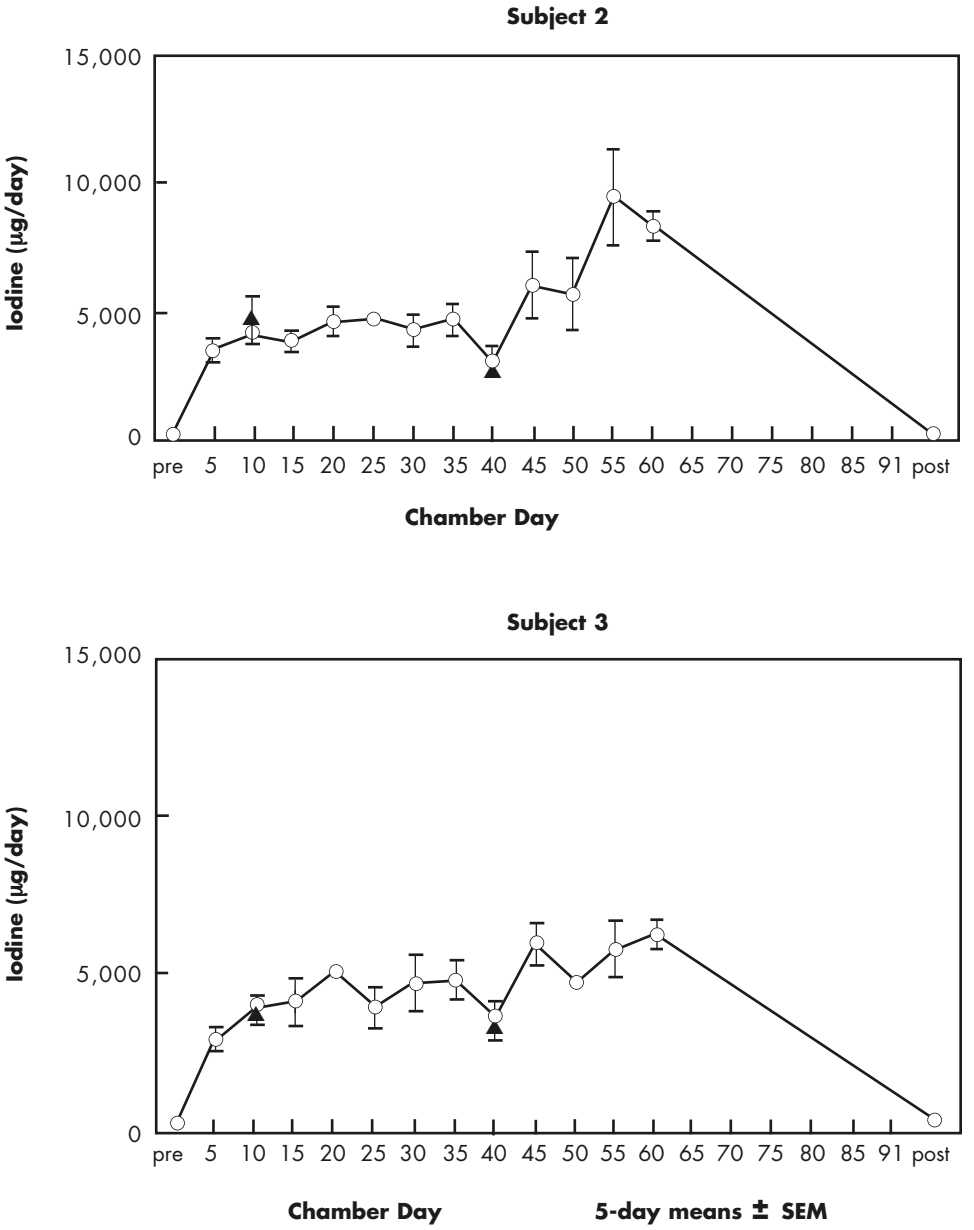

Figure 5.5-2 continued *Iodine intake from water (▲) and urinary iodine excretion (○) of crewmembers during Phase IIa*

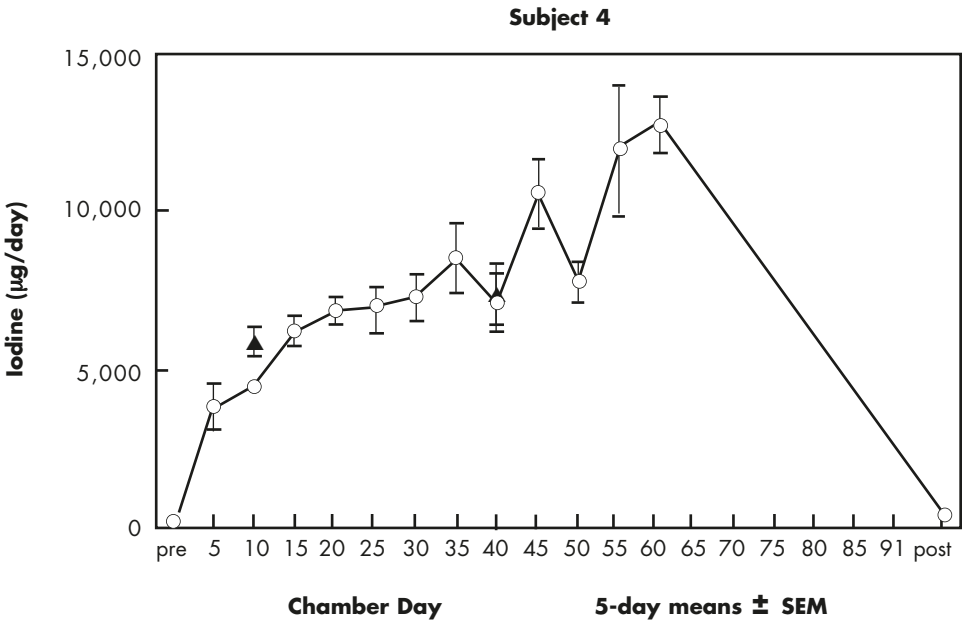

Figure 5.5-2 continued *Iodine intake from water (▲) and urinary iodine excretion (○) of crewmembers during Phase IIa*

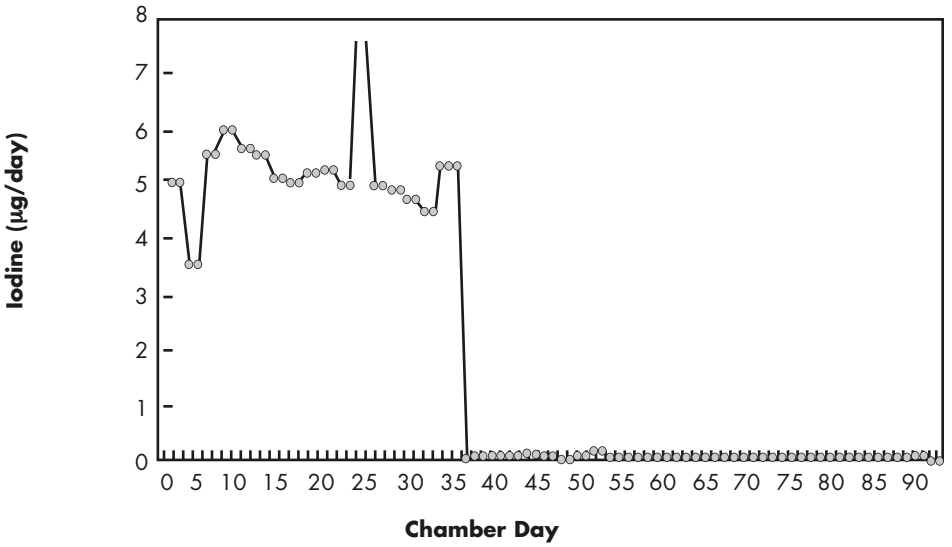

Figure 5.5-3 *Iodine levels in drinking water (mg/L) during Phase III by chamber day*

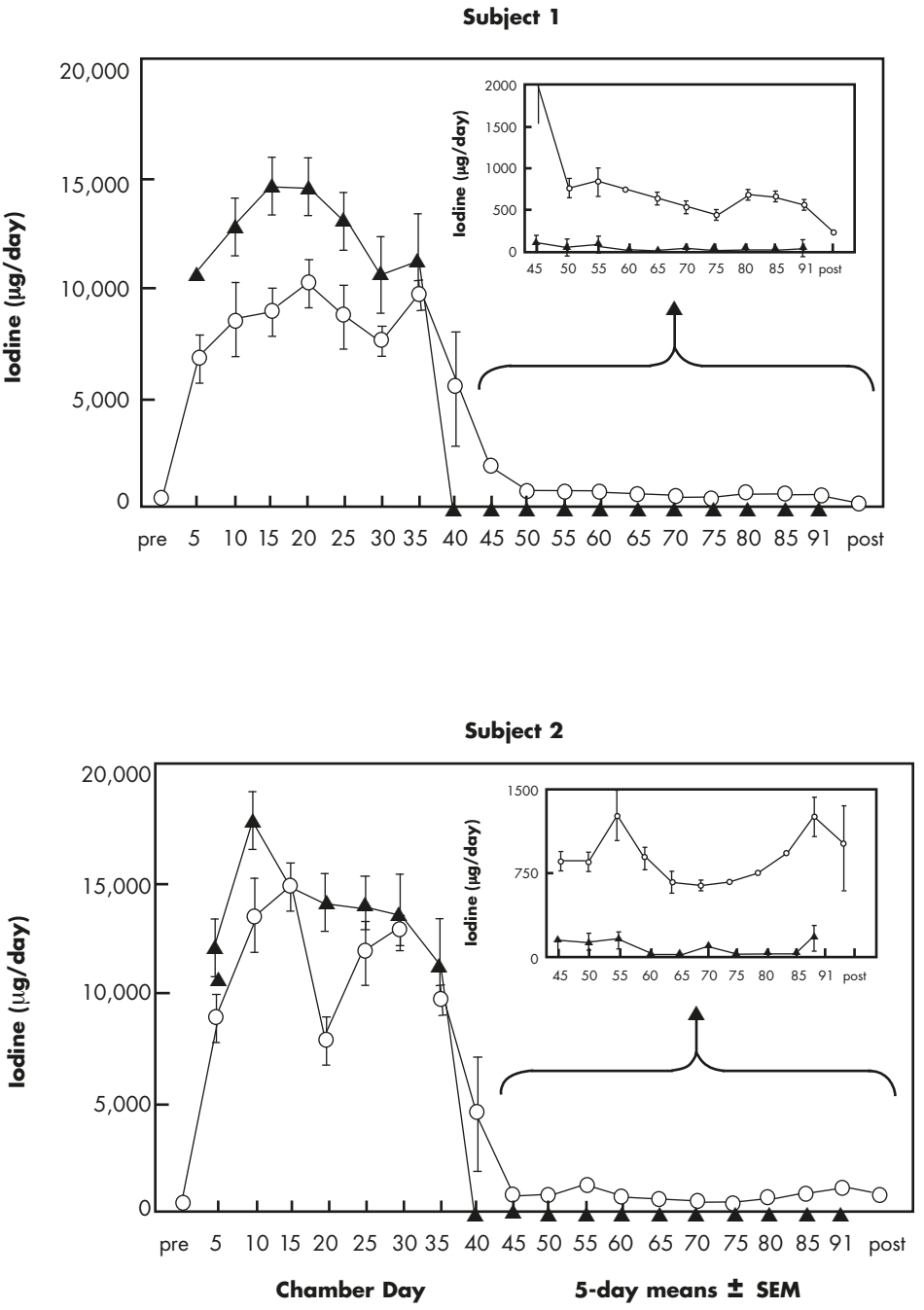

Figure 5.5-4 *Iodine intake from water (▲) and urinary iodine excretion (○) of crewmembers during Phase III*

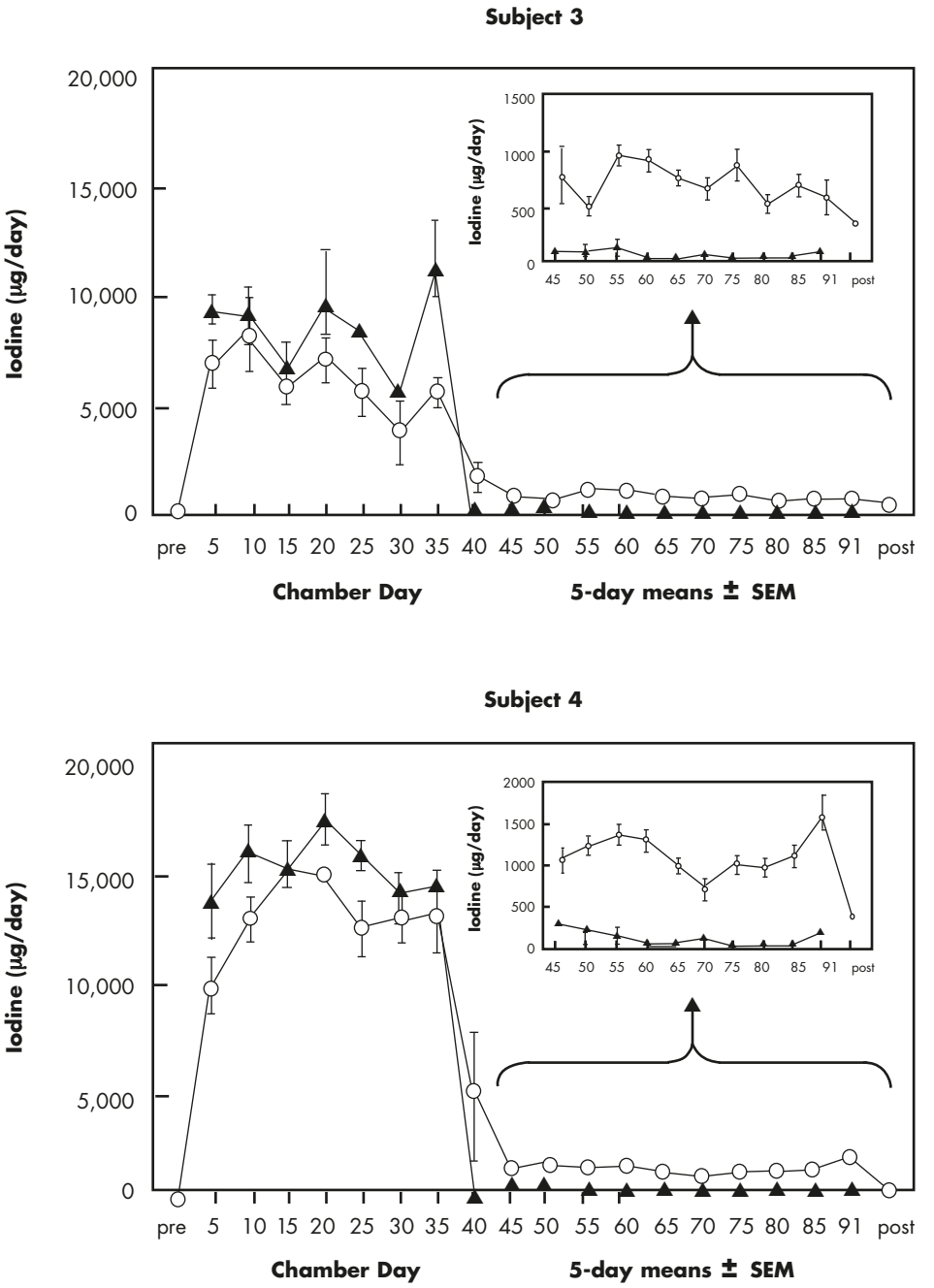

Figure 5.5-4 continued *Iodine intake from water (▲) and urinary iodine excretion (○) of crewmembers during Phase III*

Thyroid Function Assessment

Although mean TSH levels remained within normal range throughout the studies, there was a statistically significant increase in TSH levels in the Phase II and III studies (Tables 5.5-6 and 5.5-7). For the Phase II study, the values returned to baseline levels in all subjects within two weeks after the subjects exited the chamber. In the 60-day Phase IIa study, although not a statistically significant change, serum TSH in three of the four subjects had increased by the end of the study (Table 5.5-5). Subject 4, who had a baseline TSH value close to the upper limit, had a decreased TSH value below the baseline value at the end of the chamber study (Table 5.5-5). This subject had the highest iodine consumption and urinary levels (Figure 5.5-2) of the four subjects in the Phase IIa test. At day 62 postchamber, this subject had a very high TSH level and was referred for medical evaluation by an endocrinologist. There were no other biochemical or clinical signs of thyroid disease, and the TSH returned to near baseline value several months later (Table 5.5-5).

In the 91-day study, the mean serum TSH level in the four test subjects increased significantly above baseline values 30 days after entering the chamber (Table 5.5-6). After the iodine was removed from the drinking water on day 35 (concentration < 0.05 mg/L), the TSH values gradually decreased but did not completely return to baseline during the subsequent 56 days in the chamber (Table 5.5-7).

For Subject 4 in the Phase III study, the TSH remained higher than the baseline value but within the normal reference range (Table 5.5-6) throughout the chamber study period with no decrease in the level after removal of iodine from the drinking water. Figure 5.5-4 shows that the iodine consumption of this individual crewmember was highest of any of the Phase III crewmembers, between 15 and 23 mg/day throughout the 35 days. Elevated urinary iodine levels reflected this high consumption level. This crewmember had no detectable levels of TSH at 7 and 8 months after cessation of iodine consumption. The TSH levels remained low for a year after completion of the chamber study. After one year the TSH levels returned to prechamber baseline levels. All other measures of thyroid function remained within normal ranges. The consulting endocrinologist found no other biochemical changes or clinical signs indicative of thyroid disease and diagnosed the crewmember with iodine-induced subclinical hyperthyroidism.

Table 5.5-7 *Thyroid-stimulating hormone (TSH) levels for the Phases II, IIa, and III tests as mean ± standard deviation*[a]

Test Duration (days)	Phase	Iodine Exposure (days)	Prechamber	Postchamber[b]
			TSH[c] (μIU/L)	TSH[c] μIU/L)
30	II	30	1.4 ± 0.4	2.0[d] ± 0.6
60	IIa	60	2.6 ± 1.7	2.3 ± 1.2
91	III	30	1.3 ± 0.5	3.2[d] ± 0.7

[a]Each phase had four subjects

[b]Phases II and IIa reflect values from last day of the chamber test, but Phase III reflects values obtained on day 30 of the 91-day chamber study. Phase III drinking water contained iodine until day 35

[c]Normal ranges for TSH were 0.47-5.01 μIU/L

[d]After 30 days of iodine exposure there was a significant increase in TSH. Student's paired t-test, $p < 0.05$

Urinary Iodine Contamination Study

For the Phase III study, urinary iodine levels remained at approximately 1 mg/day after iodine removal from drinking water at day 35 (Figure 5.5-4), but returned to baseline levels within three days of exiting the chamber. One potential source of contamination involved the methods for collection, volume measurement and aliquoting procedures for the urine voids, and void volume determinations. A study (for details see Appendix) was completed to determine if this was the cause of the persistence of urinary iodine levels above baseline. The data demonstrated that contamination during urine collection and processing was not the source of the continued elevated urine iodine levels.

Other potential sources of iodine contamination were discussed and considered, however, no plausible explanation was identified. A few of the possibilities included release of iodine into the atmosphere from the clothes dryer (which vented into the chamber), ingestion of water from brushing teeth, showering, or residual iodine from washing of dishes (glasses, pots, pans, etc.) with iodinated water. There is a small potential for increased content of iodine in the crops grown in situ. While none of these seem to have major implications in iodine contamination, it is possible that there were multiple sources, and that small contributions from each contributed to the elevated level of excretion.

Discussion

The results from the three chamber tests demonstrated that the levels of iodine in the potable water system proposed for the ISS were too high. Since these studies were concluded, the Food and Nutrition Board (10) has established an upper limit for iodine intakes from all sources as 1.1 mg/day. The subjects in the chamber studies exceeded this level and consequently, for two subjects, TSH levels were significantly changed by the end of the chamber test. Chronic daily intake at the initially proposed levels of iodine to be provided by the U.S. ISS water system was determined to exceed these guidelines (7, 10, 17). Nutritional recommendations for the astronauts and the chamber test subjects are a minimum water intake of 2 L/day from food and fluids. Approximately 50 to 80% of that water is provided by the spacecraft water with the remainder from food sources. Thus, 1 to 2 L of iodinated water would be consumed and this would exceed the Food and Nutrition Board recommendations for daily iodine intake (10). Like the subjects in these chamber studies, astronauts will be required to participate in an intensive exercise program requiring water consumption for hydration. Thus, total iodine must be well within the 1.0 mg/day for 2 to 4 L of water consumed per day. Since food consumption provides about 0.25 mg/day, the requirement was set at a maximum of 0.25 mg of iodine/L in U.S.-supplied ISS water. This would limit water consumption to a maximum of 3 L/day to maintain daily intake levels from all sources at less than the limit established by NASA of less than 1.0 mg/day of iodine. Consequently, there remains concern that the maximum level of 0.25 mg/L of iodine in the spacecraft water will not provide sufficient microbial control thus increasing the risk to ISS crewmembers.

Thyroid dysfunction is more likely to appear with prolonged consumption of pharmacological doses of iodine, as evidenced by the transient subclinical hypothyroidism and subclinical hyperthyroidism states exhibited in some subjects and by the fluctuation of TSH levels (first high and then low) in the 60- and 91-day studies. TSH levels follow circadian rhythms that must be addressed in these types of evaluations. For the chamber studies, blood samples were collected in the morning to reduce the circadian effects as the crew was on a normal schedule. However, similar to the findings by Allan and Czeisler (1), these chamber subjects had periods of sleep deprivation and reported significant reduction of sleep time during their chamber stay. Changes in circadian patterns occur with sleep deprivation, and TSH peak levels and nadirs may occur at different times during such periods. Such changes can complicate the interpretation of TSH levels during iodine exposure.

Another concern was the persistently elevated urinary iodine in all crewmembers after the removal of the iodine from the drinking water. Because iodine excretion levels returned promptly to the normal range after leaving the chamber, it appears that the iodine within the chamber may have served as a source of contamination that maintained urinary iodine above baseline levels. It is highly improbable that the

source of urinary iodine was from the thyroid gland or other storage depots because thyroid iodine turnover is slow and would not decrease abruptly with exit from the chamber. Since the source of the contamination remains unknown, future chamber studies need to carefully review procedures and monitor crew iodine levels.

COMMENTARY

Upon recognition that the initially proposed water iodine levels for the ISS were excessive and because of the subsequent Food and Nutrition Board recommendation of the upper limit of 1.1 mg iodine/day, NASA established a limit for drinking water iodine levels with a requirement for concurrent maintenance of the microbiological standards. To achieve these requirements, NASA reduced the maximum concentration of water iodine significantly by the removal of iodine at the water port to the galley (point of use). These changes led to modification of the spacecraft water system. An iodine removal cartridge containing an anion-exchange resin was installed to remove the iodine from the drinking water prior to consumption. To obtain the levels for a maximum consumption of 3 L of water/day, and assuming the food iodine at 0.25 mg/day, the total water iodine concentration must be below 0.25 mg/L of water. Iodine containing supplements are not allowed for flight crewmembers.

Although there has been no epidemiological association between space crews' consumption of iodine in spacecraft drinking water and thyroid disease, NASA has instituted a surveillance program for thyroid health including periodic monitoring for thyroid autoantibodies and other measures of ensuring thyroid health.

Ongoing research continues in an effort to determine the most effective and safe means of disinfecting the potable water. Solutions to disinfecting the potable water in spacecraft and closed chambers may also lead to development of more effective community water systems in developing countries, military field deployment use, and wilderness and expedition water treatment systems and facilities.

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APPENDIX

Iodine Analysis

Urinary iodine determinations were conducted using an Elan 6000 Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Perkin-Elmer, Inc., Norwalk, CT) equipped with the Perkin-Elmer AS90 autosampler. Prior to each day of analysis, the ICP-MS was adjusted to achieve optimal operating conditions.

All solutions were prepared with redistilled nitric acid and with reagent-grade deionized water. Single element calibrators of iodide and indium, at concentrations of 1000 mg/L each, were obtained from High Purity Standards, Inc. (Charleston, SC). The iodine calibration standards were prepared in 0.1% HNO_3 with concentrations of 0.1, 1.0, 2.5, 5.0, and 10 μg/dL. Urine samples were diluted in 0.1% HNO_3 at various dilution factors (range 1:20 to 1:200) in order to read within the calibration curve. An internal standard (indium) was used to correct for nonspectral interferences and for signal shifts. Indium was chosen because it is close to the mass of iodine, it is not present in significant amounts in urine, and it is free from spectral interference. A 100 μg/dL stock solution of indium was prepared in 0.1% HNO_3 and added to the blank, calibration standards, and urine sample dilutions for a final concentration of 5 μg/dL.

A comparative study was conducted by preparing three aliquots of 16 samples with iodine concentrations to cover the range of the standard curve (specifically, ~20-900 μg/dL). One set of the 16 samples was sent to a commercial reference laboratory, another set was sent to a research laboratory, and a third set was analyzed by the Nutritional Biochemistry Laboratory at NASA Johnson Space Center. Results demonstrated excellent correlation (NASA vs. Research Lab, $R^2 = 0.98$; NASA vs. reference laboratory, $R^2 = 0.97$), indicating acceptable agreement between methods.

Reproducibility was assessed by using three urine pools with concentrations covering the range of the standard curve (iodine concentrations = 0.60, 4.76, and 9.04 μg/dL). The within-assay precision was assessed by analyzing five replicates of the controls in a single assay, repeated for five consecutive assays. For the three control pools, the within-assay CVs were 1.4%, 1.0%, and 0.7%, respectively. The between-assay CVs, from separate assays (n = 5), were 3.6%, 3.7%, and 3.4%, respectively.

Contamination Study

For the Phase III study, urine was collected in graduated cylinders, an aliquot was removed, and the samples were stored frozen until analysis. After each collection the cylinders were rinsed with 225 ml iodinated water (approximately 3.5 or 5.2 mg iodine/L in the 60-day and 91-day studies, respectively). Cylinders were placed upright until the next collection. Estimates of micturition rate, void volume, and iodine content indicated that it would take approximately 25 ml of this water to raise the urinary iodine content to the level seen in the urine of subjects drinking iodinated water. Nonetheless, a contamination study was designed to determine if washing the collection cylinder with water containing iodine might cause iodine contamination of the next collected urine sample.

Two urine pools were prepared, along with an iodinated rinse water. Aliquots were removed from both urine pools and the iodinated rinse water at the start of the study for later analysis. Aliquots of rinse water were collected each time it was used. Every hour for eight hours, 350 ml of urine was poured into a graduated cylinder (one for each pool), and an aliquot was removed. The cylinders were rinsed with 225 ml of iodinated water, placed upright, and allowed to stand until the next collection. To test different collection techniques, collections 1 through 4 included the residual rinse water which had pooled in the bottom of the cylinder while remaining upright. Collections 5 through 8 had the excess rinse water poured out of the cylinder before addition of the urine. Results (Figure 5.5-5) indicate that any iodine contamination from rinsing the cylinder had little effect on the urinary iodine concentration.

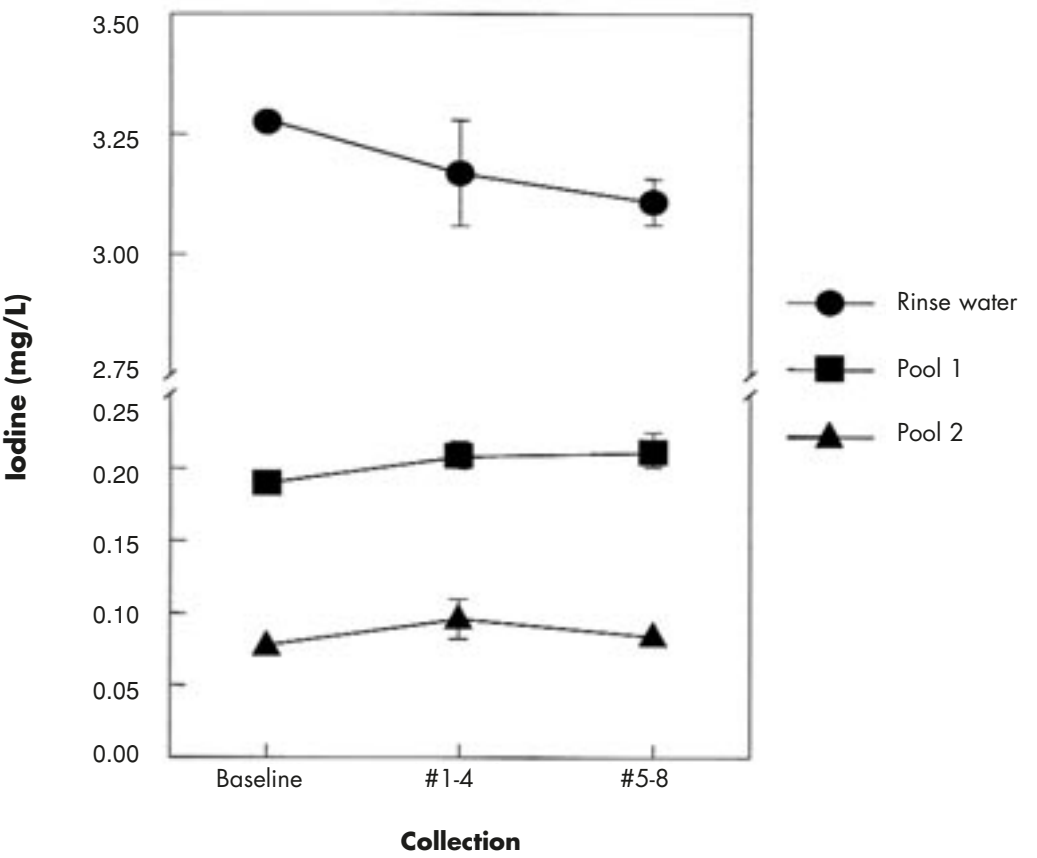

Figure 5.5-5 *Levels of urine contamination with different cylinder washings*

6.1

Telemedicine During Lunar-Mars Life Support Test Project Phase III

Jeffrey A. Jones, M.D., Patrick J. McGinnis, M.D., Douglas R. Hamilton, M.D., Dominick D'Aunno, M.D., Scott Simmons, Shannon Melton, Cheri Armstrong

ABSTRACT

Evaluation of crewmembers participating in previous closed-loop life support tests have revealed symptoms/signs (e.g., mucous membrane irritation) in crewmembers from elevated concentrations of noxious chemicals off-gassed from support structures. Improved means of monitoring, evaluating, and treating the isolated crew was sought for the Lunar-Mars Life Support Test Project (LMLSTP) Phase III test, in addition to providing support for several planned medical experiments. The Telemedicine Instrumentation Pack (TIP) was utilized to provide: 1) real-time medical care, 2) medical monitoring, and 3) science evaluation during the LMLSTP Phase III 90-day study. The TIP was found to be effective in assessment of the medical findings, resulting in prompt recommendations for management of conditions/injuries sustained during operations, and was highly effective in monitoring the crew for signs of contained atmosphere effects and acquiring medical science data.

Introduction

For humans to venture to other planetary bodies for extended-duration missions, a life-sustaining environment must be brought with, or pre-positioned for, the space travelers. There are no known extraterrestrial locations that provide all of the essential ingredients, in an immediately usable form, for human existence. Therefore man must build a habitat to provide the elements of life during his journeys and upon his arrival to the planetary body. In providing a space-qualified home for crews that closes the atmospheric, water, and energy loops (i.e., a closed-mass system), the habitat must provide: the proper balance of oxygen/nitrogen, carbon dioxide scrubbing/recycling, sustainable nutrition, potable water, waste elimination/recycling, etc. This closed-mass system must operate indefinitely without resupply from Earth – so it must recycle or regenerate all necessary elements for life and ensure these elements are safe for human existence.

There is the potential for accumulation of toxic levels of many substances within the confines of the habitat. These noxious and possibly harmful substances may be components of the habitat structure that become volatile with pressure changes or over time, or they may be produced by the recycling/reclamation process itself. It is the responsibility of the space medical team to ensure that the environment is routinely monitored for levels of possible harmful agents and that the crewmembers are monitored for signs of toxicity. The medical team also conducts routine health evaluations of the crew to maximize its performance in the extreme environments of planetary exploration.

Two goals of the medical operations participation in the LMLSTP Phase III project include:

1) the evaluation of training and use of the Telemedicine Instrumentation Pack (TIP) (Figure 6.1-1) by the Crew Medical Officer (CMO) and NASA flight surgeons for isolated long-duration crews, and
2) the use of the TIP, during isolated chamber stays, to support medical and life science research evaluations requiring serial crew physical examinations.

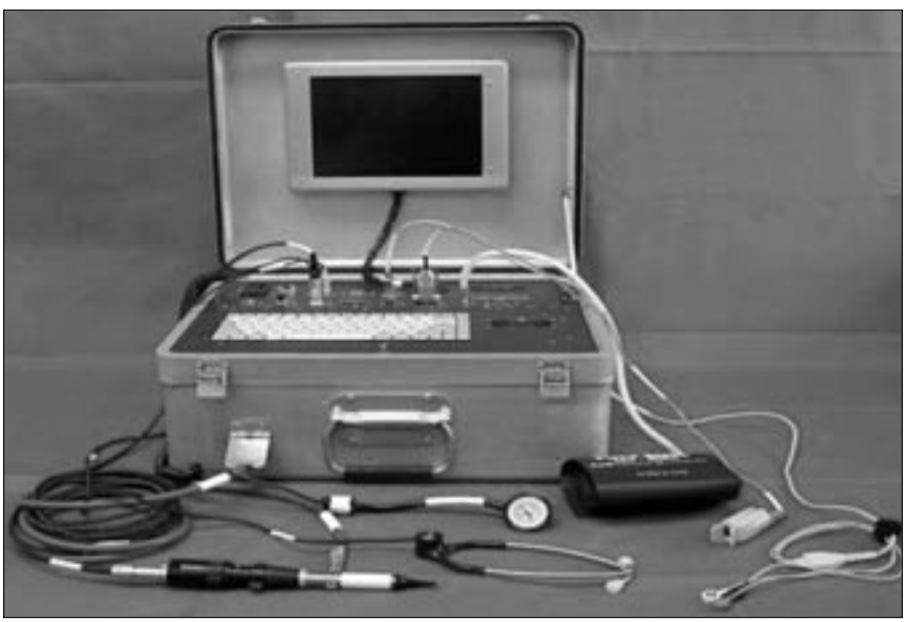

Figure 6.1-1 *The Telemedicine Instrumentation Pack*

The objectives of the TIP telemedicine evaluations by CMO to flight surgeon are:

- To evaluate the CMO training plan for TIP use in an isolated environment
- To evaluate the quality of the TIP physical examination imaging capabilities performed by nonphysician CMOs, as a preparation to DSO 334 which was scheduled to fly aboard STS-89 (January 1998)
- To understand the flight surgeon interaction requirements with the CMO, in order to obtain diagnostic or high-clinical utility images
- To test the limit of diagnostic accuracy of the TIP hardware by screening for signs of airborne mucous membrane irritation, injuries, and other clinical events
- To evaluate the use of the TIP in evaluating and following contingency medical condition (e.g., skin wounds) during periods of chamber isolation.

The objectives of the use of the TIP in supporting medical research in isolated crew chamber studies are:

- To assess the TIP's utility in accurately assessing skin responses for immune function testing, as a prelude to a follow-on study of immune status proposed for long-duration space flight
- To assess common indications of external physical signs of nutritional status using the TIP.

Planned medical operations research evaluations during LMLSTP Phase III are:

- Cell-mediated immunity in an isolated environment
- Space Flight Cognitive Assessment Tool and behavioral health assessment tool evaluation
- Behavioral trends and adaptation during space analogue missions
- Assessment of sleep quality during space flight simulations
- Habitability review using "SOIRT," the Space Operations Issues Reporting Tool
- Monitoring latent virus reactivation and shedding
- Evaluations of nutrition, noise, bone metabolism, exercise countermeasures, food systems, and bacterial biofilms.

Other objectives include:

- Comparisons of methods for remote training
- Portable Clinical Blood Analyzer – just-in-time training evaluation
- ISS Medical Kit, training, Telemedicine Instrumentation Pack/techniques validation.

Finally an assessment of the requirements for future chamber-type test bed medical support is to be acquired as a result of lessons learned from this LMLSTP study.

Methods

TIP Prechamber Entry Training

A two-hour session with the crew and trainers was conducted, led by Dr. D'Aunno reviewing immune response study design and rationale, procedures for subcutaneous application of antigens, and response measurement technique. No time was scheduled to provide the nonmedical CMOs with training for recognition of pathological conditions of the human body or even for normal immune responses to antigenic challenge in the integument. Therefore the CMOs were trained in observation techniques and were instructed how to visualize and palpate expected findings on the skin and mark the findings with a supplied nonpermanent marker.

A two-hour session was conducted with Drs. McGinnis and Jones, Ms. Cheri Armstrong, and Mr. Scott Simmons to train on deployment, configuration, operation, and stowage of the TIP and techniques for utilizing the various lenses, ophthalmoscope, and otoscope attachments. The CMOs were shown the techniques for:

- acquisition and verification of the nature of images of:
  - the skin – with the macrolens feature
  - the exterior eye and conjunctiva
  - the interior eye – lens and retina
  - the external ear canal and tympanic membrane
  - the nasal mucosa
  - the buccal mucosa, palate, and uvula
- placement of ECG electrodes and activation of the tracing acquisition software
- placement of pulse oximeter for obtaining O_2 saturation readings
- placement of the electronic stethoscope in appropriate anatomic locations for acquiring physiologic heart and lung sounds
- basic first aid provision

Examinations of the crewmembers were conducted on two occasions for general health assessment and to evaluate the possible effects of the atmospheric environment on chamber days 30 and 60. The skin examinations for immune function were also performed simultaneously with the general health examinations.

Evaluations of the TIP hardware performance, the video image quality, and the utilization protocols were conducted concurrently with the examinations. The CMOs performed the TIP examinations on one another on chamber day 30 in the wardroom area, as shown in Figure 6.1-2.

Figure 6.1-2 *The CMOs and TIP in chamber; deploying the macrolens*

Contingency examinations were to be conducted as needed but, in this study, were performed concurrently with the general examinations.

Flight surgeons and technical support personnel set up a telemedicine workstation in Building 7, external to the LMLSTP chamber, and monitored the examinations with direct video monitors from both the mounted internal chamber cameras and the video images generated by the TIP camera chip. Audio connections provided real-time feedback between the flight surgeons and CMOs (see Figure 6.1-3).

Figure 6.1-3 *The flight surgeons at the workstation outside the chamber*

Findings

The CMO training sessions were brief, but due to the user-friendly nature of the hardware and the well-formulated protocols for utilization, the CMOs rapidly acquired the skills required for effective deployment and operation of the TIP. Queries of the CMOs revealed that they felt well prepared for conducting examinations utilizing the TIP. They also felt well prepared to perform the periodic immune system study evaluations and were at no time uncomfortable with the procedures or observation techniques that they learned.

Acquisition of Data/Images in Performing the Evaluations

Image quality – The images obtained during the hardware evaluation and while performing the immunologic examinations in general were very good and were considered by the flight surgeons to be of diagnostic quality. There were several incidents of interference lines, generated by simultaneously operating electronic equipment, obscuring the video images. Interference suppression was felt to be important in preventing future such obscurations (see Figure 6.1- 4).

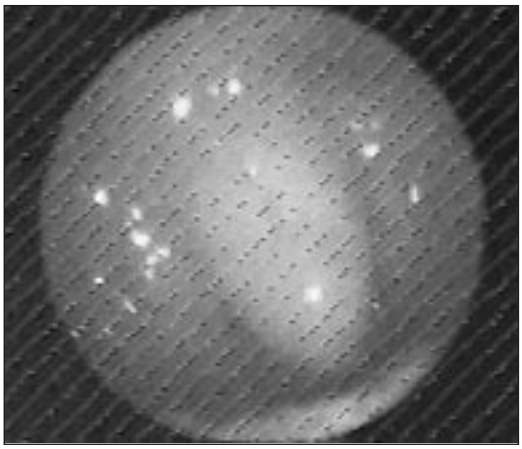

Figure 6.1-4 *The uvula and palate with significant interference in the NTSC, or video, signal*

Flight Surgeon/Crew Medical Officer interaction – A key to the ability of the crew to provide the flight surgeons with quality imaging, with the limited CMO training, was real-time feedback from transmitted images and data to the CMO performing the examinations. The flight surgeons had no delay in communication in this evaluation and therefore were able to provide instant feedback for position of camera lenses, oto- and ophthalmoscopes, and TIP instrument settings. The flight surgeons' feedback to the CMO allowed acquisition of images that were of maximal diagnostic value, without the need for later review and reacquisition of images that may have been suboptimal, e.g., if a "store and forward" methodology had been utilized. An example of the assisted image acquisition can be seen in Figure 6.1-5, where the tympanic membrane is visualized in motion during a Valsalva maneuver.

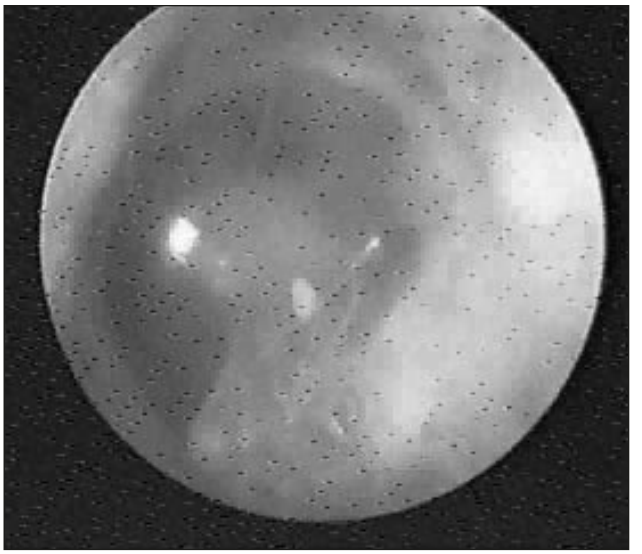

Figure 6.1-5 *The tympanic membrane, during the CMO otoscope examinatio*n

Mucous membrane assessment – The flight surgeons felt that they were able to adequately view the crew's mucous membranes for signs of injury or toxic effect from the video images provided by the CMO via the TIP. In the Phase III test, no signs of adverse effect from off-gassed agents were detected by the medical surveillance. However, several superficial linear lesions were noted in the nasal mucosa of one crewmember examined, which were felt secondary to the chamber's reduced relative humidity during the period preceding the examination (see Figure 6.1-6).

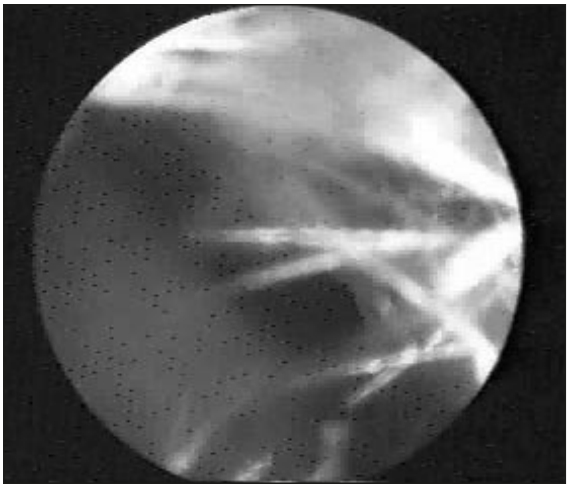

Figure 6.1-6 *The mucous membrane showing a small area of hemorrhage*

Contingency medical management – A superficial laceration of the mid-anterior tibia was sustained in one crewmember as a result of striking the lower leg on the metal stairs between levels in the chamber. The wound was evaluated by the macrolens of the TIP and found by flight surgeons not to require suturing. It was treated with steristrips, topical antibiotic ointment, and dressing (see Figure 6.1-7). The wound was followed on a subsequent exam and found to be healing well, with no evidence of infection. The TIP provided excellent images for inspection, assessment, and treatment recommendations for this minor medical contingency situation.

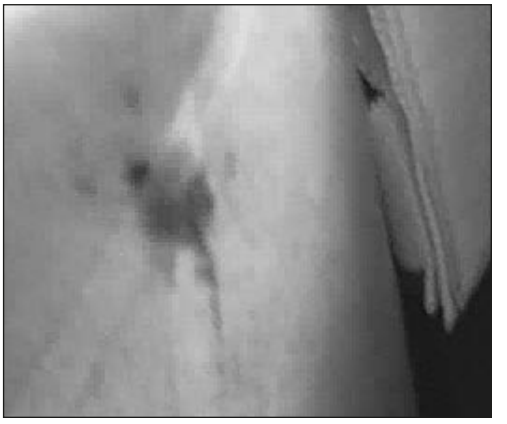

Figure 6.1-7 *A skin wound (shown here after treatment) incurred during chamber operations*

Immune function evaluation – The flight surgeons were easily able to visualize the sites of antigen application on the ventral forearm of all study crewmembers (see Figure 6.1-8). Erythema, when present, was easily discernable. The flight surgeons relied on the palpation skills taught to the CMOs for assessment of induration, but they were able to give real-time feedback to the measurements of both erythema and induration through the video and audio connections at the flight surgeon's external workstation.

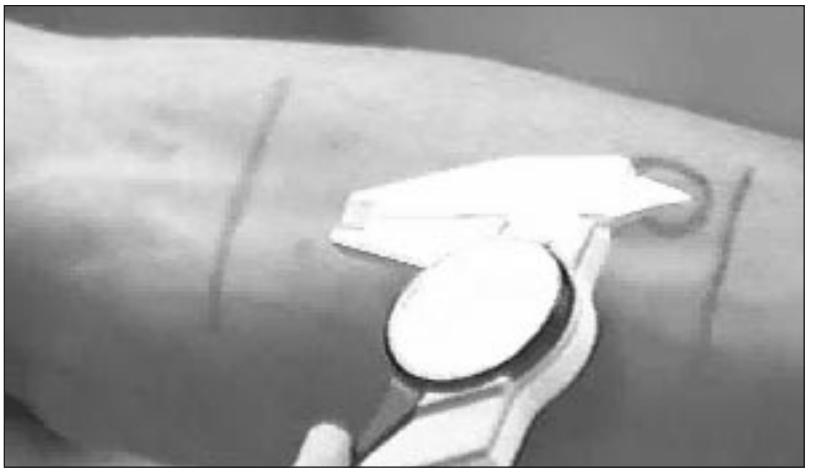

Figure 6.1-8 *Immunization site surveillance*

Nutritional assessment – Views of triceps skinfold measurements, as well as inferior conjunctiva for signs of anemia, were easily accomplished with the aid of the CMO-operated TIP.

Difficulties Encountered

Due to conflicting requirements in developing the flight hardware for STS-89, the 2X filter was not available to the Phase III chamber crew at the time of testing in the chamber, which made focusing on the skin surface more challenging for the CMOs.

The retinal images, with dimmed lighting but no pupillary mydriatics, are difficult to obtain, even with a highly trained user of the TIP ophthalmoscope. This hardware item may require additional modification to make the device more user-friendly, as the images were not as sharp and useful compared with the other examination images obtained during the study.

Episodic interference occurred with broadcast video imaging.

Conclusions

Accurate skin response measurement data was obtained with high confidence by the investigator, because the TIP was used to supervise the measurements real-time from a remote flight surgeon workstation.

The CMOs performed above expectations in operating the TIP hardware, especially considering the limited training, and were able to provide the flight surgeons with quality physical exam information.

Real-time or near real-time audio exchange capability was invaluable in directing the CMO while performing the examination in order to obtain the desired images.

The images obtained were adequate to make real-time diagnostic decisions based on visual appearance of the mucous membranes (e.g., an intranasal hemorrhage lesion was identified following a night of low-humidity sleep, tympanic membrane motility could be observed during a Valsalva maneuver, etc.).

RECOMMENDATIONS

The video interference problem should be evaluated and corrected for telemedicine support of future advanced life support studies and other test beds. In addition, with a permanent crew already aboard the International Space Station, real-time or near real-time video and audio communication capability with flight surgeons should be provided for crew health evaluation including contingency physical examinations. Plan to have a dedicated medical workstation inside future test bed facilities to provide a more robust medical evaluation and treatment capability for long-duration isolated chamber crews, and to serve as a test bed for newly developed medical equipment technologies and for validation of devised protocols for medical evaluation and care delivery. Finally, operational evaluations of the "store-and-forward" methodology for image acquisition should be performed, with a communication delay for feedback on the images, as would be required in an actual Mars mission medical scenario.

6.2

In Situ Training Project: LMLSTP Phase III Report

Barbara Woolford, Cynthia Hudy, Mihriban Whitmore, Ph.D., Andrea Berman, Jim Maida, Ph.D., Abhilash Pandya

INTRODUCTION

Objectives of Experiment

Long-duration space flight will require crewmembers to perform tasks they were not specifically trained for prior to flight. The length of the missions, on the International Space Station (ISS) or on future exploration missions, will prohibit detailed training in every possible activity and for every possible contingency. To ensure crew safety and productivity, efficient methods of providing rapid training without ground support are required.

Various forms of computer-based training are the best solution to this problem. The training material can be stored on CD-ROM, enabling shelves of manuals and drawings to be stored in a few cubic inches. Computers will be readily available on the spacecraft for a multitude of purposes. The status of computer-based training (CBT) using multimedia is far enough advanced that commercial authoring tools are available to enable nonprogrammers to create lessons rapidly, with multiple links to audio, video, drawings, and text.

The purpose of this study, carried out in the 20' chamber, was to evaluate the usefulness of specific features of multimedia training, including a two-dimensional task simulation.

History of Project

In the Phase IIa test, multimedia training was compared with two-way audio/video communication, which is similar to current methods of mission controllers talking a crewmember through a procedure. In that study, it was found that if the task only needed to be done once, the subjects preferred being "talked through" the task, and performed it in shorter time periods. However, comments during the debriefing indicated that the crew felt they would retain very little of the training received that way, compared to training learned from multimedia.

METHODS/OBJECTIVES

List and Description of Methods and Protocols

The protocol required each volunteer to be trained on two tasks. One task was primarily a physical task: assembling a Graphical Retrieval and Information Display (GRiD) computer from its components. The other was primarily cognitive/perceptual: operating a relatively complicated instrument called a ScopeMeter, which combines features of a multimeter and an oscilloscope. The training material was presented as material organized in a Web page manner.

The independent variables were task type (physical vs. cognitive) and multimedia (MM) training type (self-test (Enhanced MM) vs. no self-test (MM)) in the training. The dependent variables were time to perform the task, number of errors, number of times features were revisited, and subjective ratings of the usefulness of features of the training material. The subjects were videotaped while performing the task to enable later review of task performance.

A total of four subjects voluntarily participated in this in-chamber demonstration project. These participants were the LMLSTP Phase III chamber crew. Of the four participants, two rated themselves as expert Windows 95® and World Wide Web users and two said they were intermediate-to-novice users.

Prechamber: The participants were brought into the Usability Testing and Analysis Facility (UTAF) at Johnson Space Center for prechamber training. They were familiarized with the use of the Web-based multimedia application. The multimedia application was similar to the version used during the actual project sessions, in that it showed text instructions, photographs, diagrams, and video. Due to time constraints on software development, however, the prechamber training did not include a prototype of the interactive self-test. Furthermore, the participants were not shown any of the task hardware to be used during the sessions so as to not confound the in situ training process.

In-chamber: While in the chamber, each subject participated in two sessions scheduled a minimum of two weeks apart. Each session was performed in two phases: participants trained on a task and then performed the task without referring to the training materials. Participants were given two hours to complete both the training and task. During the test sessions, the test conductor was present in the viewing room; however, the crew was encouraged to perform the task using only the multimedia application provided in the chamber. They were advised to ask questions regarding the use of the training application only, not the task itself.

For each of the training sessions, participants were provided with introductory text, which explained the nature of the task to be performed and reminded them how to use the training software. Participants were instructed to view whichever multimedia features they preferred, in whatever order they preferred, and as often as they wanted. In the Enhanced MM session, participants were instructed to access the interactive self-test once they felt that the task had been learned completely through viewing the other multimedia features.

The tasks and multimedia training types were counterbalanced across participants. For three of four participants, training for one session was with MM and the other was with the Enhanced MM. The fourth participant inadvertently skipped the self-test, and thus performed both training sessions with MM.

After each training/task combination, participants completed questionnaires which examined the usability and acceptability of each training technique and its available features. In addition, a comprehensive questionnaire concerning the comparison of different techniques for different tasks, as well as the background knowledge of each participant, was administered upon completion of all the sessions. Finally, participants were invited to individual debriefing sessions with the test conductors to understand the background of this demonstration, as well as to provide any other comments that they had about the training methods or tasks.

Postchamber: It was discovered that two crewmembers had participated in audio-visual communication for an unplanned chamber maintenance procedure. This provided an unexpected opportunity for comparison of the multimedia training with an actual real-time training situation performed with audio-visual communications but no prepared training materials. These two crewmembers provided comments comparing and contrasting the training in this experiment with the real-world case.

List and Description of Hardware Used

An IBM ThinkPad 755CX laptop computer with an Internet connection (to access a local server) was used for all sessions. Netscape 3.0® for Windows 95® was installed on the ThinkPad in order to view the training applications. QuickTime and Macromedia Shockwave plug-ins were installed within Netscape® to allow the video clips and self-tests to be accessed and viewed.

The multimedia training applications were interactive, button-driven World Wide Web sites developed in HyperText Markup Language (HTML) and JavaScript. The World Wide Web format was used to present the multimedia applications since the experimenters were experienced in Web development and because the training software had the ability to track usage statistics through the server log (such as which multimedia features were accessed, the order of access, and the time spent on each feature).

The multimedia included text procedures, shortened text procedures called "cue cards," video clips, diagrams, photos, and software help. The Enhanced multimedia included all features, plus the addition of an interactive self-test, which asked the participant to perform the task "virtually" on the computer screen using the mouse to manipulate the task objects. The Enhanced multimedia self-test was developed in Macromedia Director.

To allow participants to view multimedia features while reading the text procedures, each training application consisted of a screen divided into three parts:

1) a series of buttons and pull-down menus that led to training materials;
2) a multimedia feature window; and
3) a procedure window.

An illustration of the screen layout and available features is shown in Figure 6.2-1.

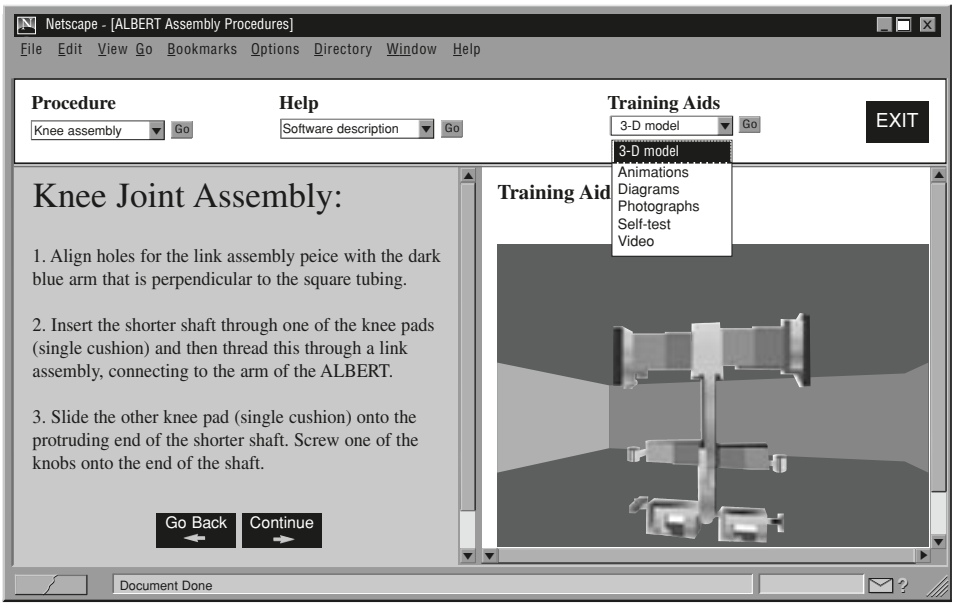

Figure 6.2-1 *Sample screen for the multimedia training material*

The two test objects – the GRiD computer and the ScopeMeter – are shown in Figures 6.2-2 and 6.2-3. The chamber crewmembers did not have any previous experience or training with these items or tasks. They were selected to provide "novel" but realistic tasks that might be performed in space flight. The GRiD laptop computer had served as a Payload and General Support Computer (PGSC) on the Shuttle. The participants received the computer disassembled and were tasked with assembling various components and connections. The Fluke ScopeMeter is a versatile device which can function as an oscilloscope as well as a meter for various scientific readings (voltage, current, etc.). The device consists of the ScopeMeter unit itself, a power supply, and two probes. The crewmembers were tasked with performing a specific set of procedures, using the ScopeMeter and accessories, to take a voltage self-diagnostic.

The task performance was observed and recorded using the video camera loca ed on the ground floor of the 20' chamber. This camera was permanently available throughout the 91-day test and was not unique to this experiment.

RESULTS

List of Pre-, In-, and Postchamber Anomalies

Prechamber anomalies:

None - all subjects participated in training sessions.

In-Chamber anomalies:

1. One subject forgot to utilize the self-test option when it was available.
2. One test session was interrupted temporarily.

Postchamber anomalies: No anomalies, but two subjects participated in an additional debrief.

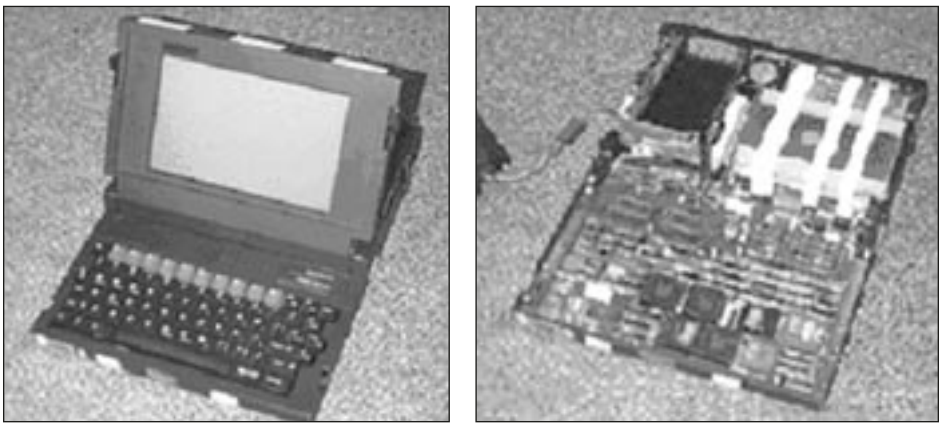

Figure 6.2-2 *The GRiD computer. The left photo shows the assembled computer. The right shows a stage midway through the assembly*

Table of Method/Protocol

Table 6.2-1 shows the planned protocol. Although one subject failed to use the self-test option when it was present, this table shows the number of subjects in each of the four conditions.

Table 6.2-1 *Protocol for Testing*

	GRiD (physical)	ScopeMeter (cognitive)
Enhanced MM (Self-Test)	2	2
MM (no Self-Test)	2	2

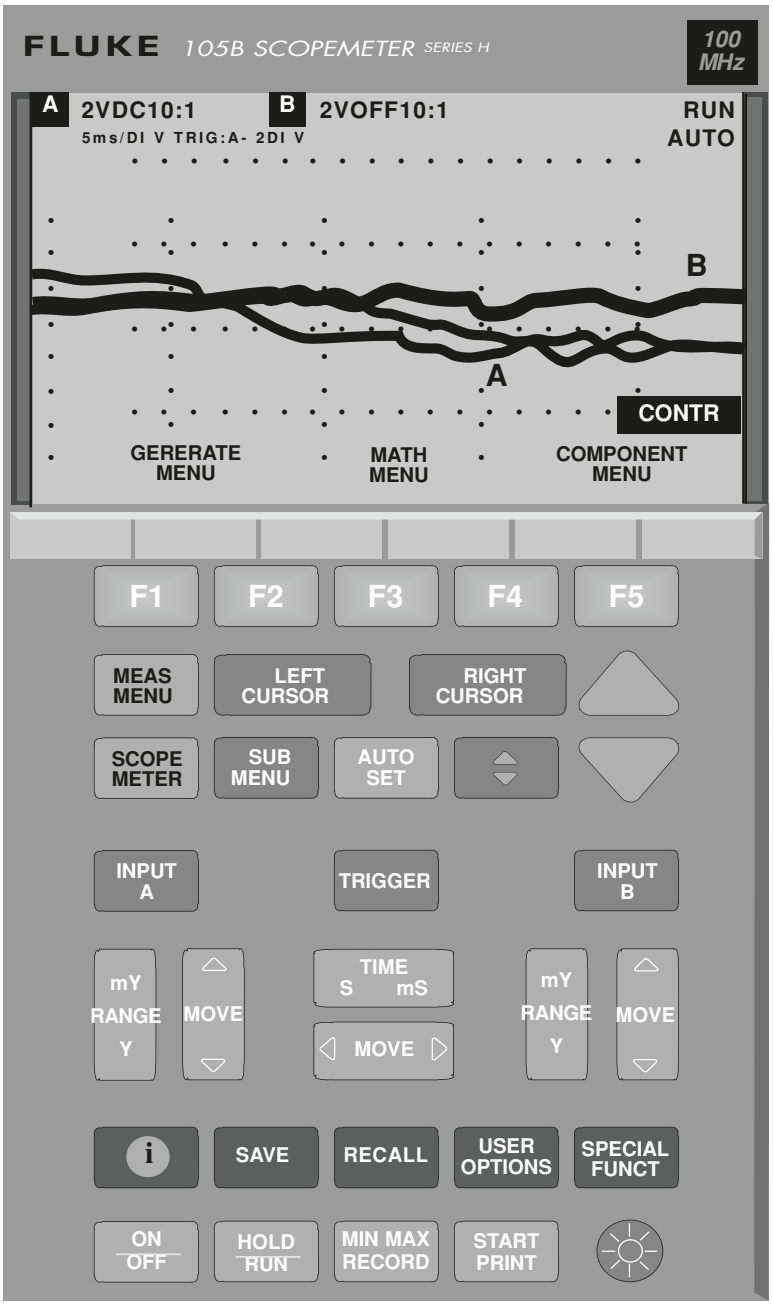

Figure 6.2-3 *The Fluke ScopeMeter, an electrical diagnostic instrument*

Completeness/Quality of Data

With only four subjects, this study by itself does not provide enough data for a robust statistical analysis. However, the usage data collected from the server and subjective debrief comments were very useful in confirming related studies and in refining the procedures for a larger laboratory study comparing virtual reality (VR) and multimedia for remote training.

Objective Results

There was remarkably little between-subject variation in time and number of errors for each of the two tasks. Time to complete the task (not including the time spent in training) was a dependent measure of great interest. Although the two tasks had approximately the same number of steps, the ScopeMeter task was performed much more quickly than the GRiD assembly. This is probably a result of the nature of the task: the GRiD assembly required many more types of physical operations, while the ScopeMeter task primarily required pushing keys and verifying information on the display.

ScopeMeter task times ranged from 3 to 4 minutes and did not seem to depend on the type of multimedia training received. GRiD task times (not including training) ranged from 15 to 27.5 minutes (see Table 6.2-2). Task times for the GRiD also did not depend on whether the subject had the self-test (Enhanced MM) or not. Analysis of the video data revealed that the task time for Subject 3 was not due to a deficiency in training, but rather a difficulty in performing some of the task steps (i.e., tucking cables in so the cover could close completely).

The second objective measure of interest was number of errors while performing the tasks. The subjects were not allowed to refer to the training material during the task performance, and did not have any type of cue cards or procedures to serve as memory aids. No errors were observed during performance of the ScopeMeter

Table 6.2-2 *Time (in minutes) to complete the GRiD assembly (physical) task for the MM and Enhanced MM training*

Subject Number*	Multimedia	Enhanced Multimedia
S1		15
S2	15	
S3		27.5
S4	18	

*NOTE: These subject numbers are not the same numbers assigned to the chamber crew in other studies

task. The camera view recorded during task performance was optimized to provide as close a view as possible of the subject's activities. Due to the small size of the ScopeMeter display, it was not possible to track performance of each step of that task. However, all four subjects reported correct delta-voltage readings to the test conductor at task completion and therefore were able to correct any errors they may have committed prior to finishing the task. Two subjects made errors during GRiD assembly: one subject made two errors, and the other made three errors. None of the errors were committed by more than one subject. The number of errors made did not depend on training type. However, it should be noted that the subject who made three errors was distracted during the GRiD assembly and took a short break, which may have contributed to the number of errors committed.

The third set of objective data collected was the frequency with which the different multimedia features were used. This data was automatically captured by the Web site software. A majority of the participants browsed through each of the multimedia features at least once during their training, rather than focusing on one or two features in particular. For both the GRiD and the ScopeMeter tasks, the animation/video clips were the most, photos were second, and diagrams were third (see Figure 6.2-4).

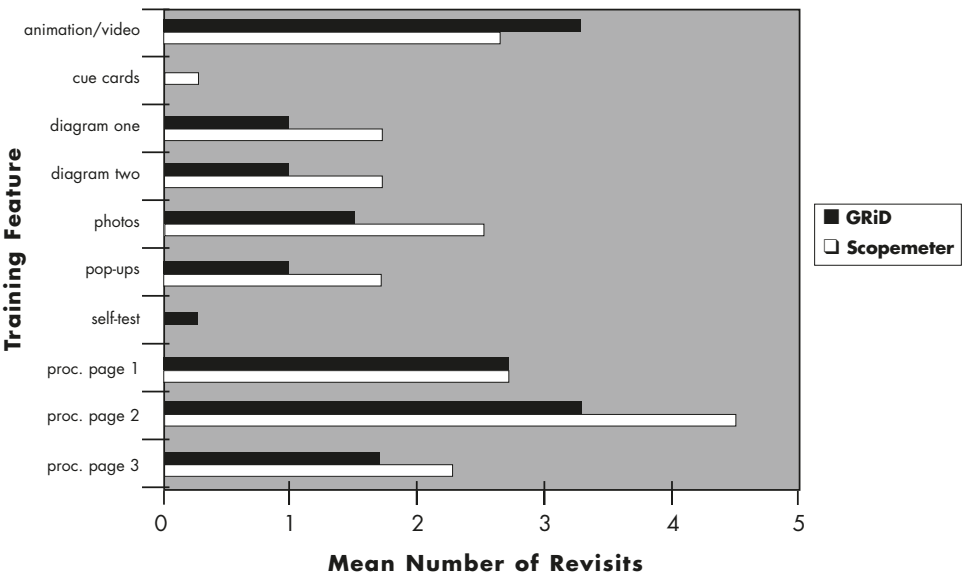

Figure 6.2-4 *Mean number of revisits to each of the multimedia features and each of the three procedure pages*

SUBJECTIVE RESULTS

The general categories investigated in the subjective questionnaires included:

- Usefulness of the various multimedia features, including the self-test
- Ease of navigation (between and within pages and multimedia features)

- Proficiency with Windows 95® and World Wide Web browsing
- Acceptability of training (i.e., amount of information provided and difficulty level)

All questions were posed with a 7-point Likert scale. For most questions, a response of 7 meant that the subject found no shortcomings on that specific item. However, for some questions, a middle rating of 4 meant that the subject rated that issue "Just Right," in between the two extremes of "Too Long" and "Too Short" or "Too Much Information" and "Too Little Information."

Figures 6.2-5 and 6.2-6 show the questionnaire ratings for each task. For those questions where 7 represented a response of "Completely Acceptable," such as the usefulness of the various multimedia features, the self-test, and ease of navigation, no subject responded with a rating below the "Neutral" value of 4. In other words, all items were rated between "Acceptable" and "Completely Acceptable." For those questions where 4 represented the "Just Right" response, such as the amount of information provided and difficulty level, ratings were between 3.5 and 4.5.

Furthermore, responses to questionnaire items correlated well with debrief responses and objective data. For example, the interactive self-test was one of the highest rated items in the questionnaires, with a mean rating of 5.3 on the GRiD task and 6.3 on the ScopeMeter task. During their debriefs, subjects explained that the self-test helped them perform both tasks; however, they found the content of the ScopeMeter self-test to be more helpful than that of the GRiD. Another strong correlation was found with the Cue Card training feature – an abbreviated list of the procedure steps. Especially in the GRiD questionnaire, this item was rated low relative to other training features, although it did indeed receive a rating of "Acceptable." In the objective data collected during training, it is evident that only one subject used the Cue Cards extensively during training.

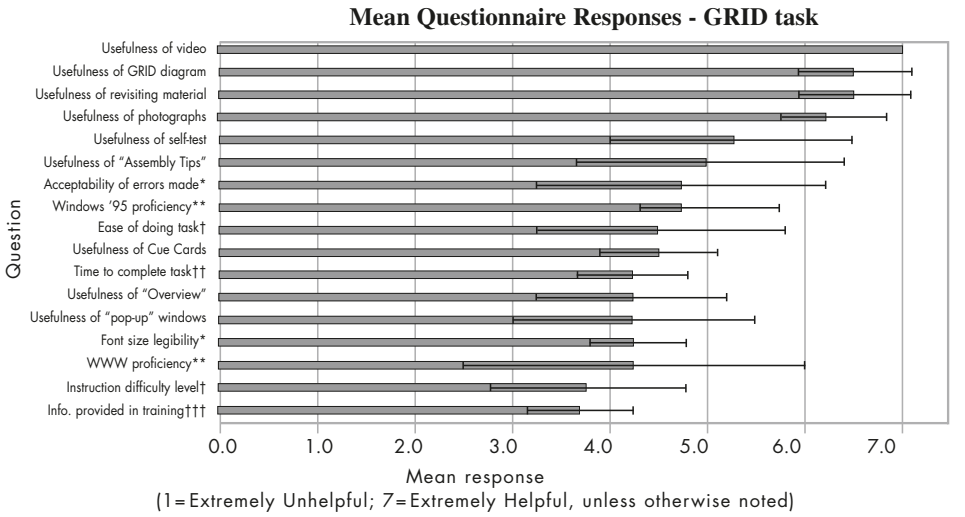

Figure 6.2-5 *Mean questionnaire responses for training and performing the GRiD task*

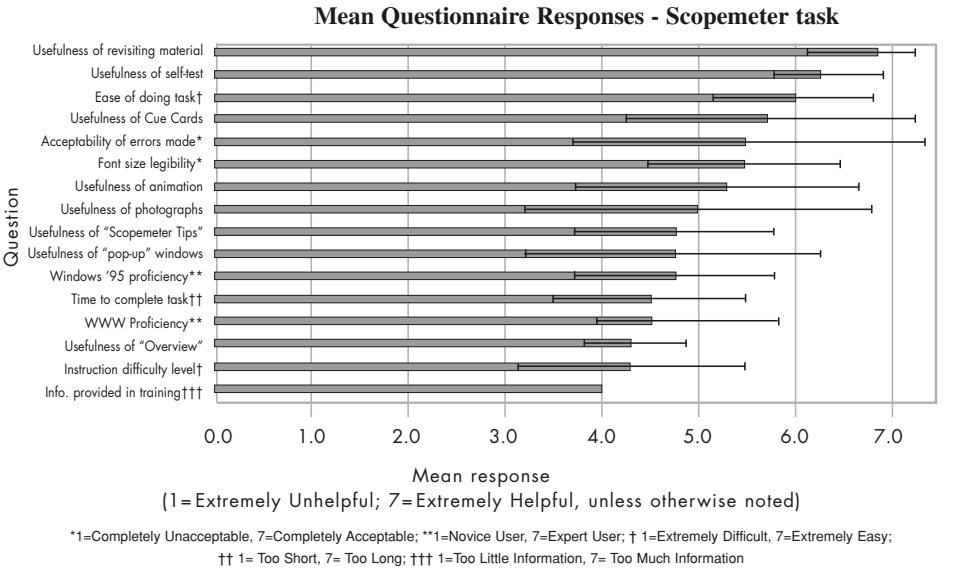

Figure 6.2-6 *Mean questionnaire responses for training and performing the ScopeMeter task*

DISCUSSION

Conclusions

When asked if they would prefer a multimedia training tool or real-time audio/video communication for learning various scenarios, most participants would prefer a multimedia training tool with a variety of features that they could have available as a reference. All of the participants felt that the interface was intuitive. They liked the separate windows for the text and the images, so that these features could be accessed simultaneously. A majority of the participants browsed through each of the multimedia features at least once during their training, rather than focusing on one or two features in particular.

Although task performance times did not differ between the MM and Enhanced MM conditions, the participants felt that the inclusion of the self-test improved their knowledge of the task. This is important for use in future training applications so that the crew can monitor how well they have learned a critical task before performing it. For example, exploration missions may have a time lag that is too great for crewmembers to rely on mission control for answers to questions or correction of a mistake; they must decide for themselves when they are ready to perform the task.

Many of the participants reported that the demonstration was well done and was a fun project. One participant commented, "Multimedia is an excellent option for consolidating and standardizing training." Another commented, "Different people learn in different ways and you've covered all the avenues."

Future work will compare these same multimedia training programs with simulation capability, with a virtual reality version of these training programs. The procedures and steps will be the same in both modes, but the simulation in the VR with be truly three-dimensional and the subject will be immersed in the system, rather than using mouse clicks on a flat picture.

7.1

Habitability and Environmental Factors: the Future of Closed-Environment Tests

Helen W. Lane, Ph.D., Daniel L. Feeback, Ph.D.

The previous chapters have reported the accomplishments and results from four chamber life support tests completed at NASA Johnson Space Center. Collectively, these illustrate the various interactions of the human with both the habitat and life support systems. Some studies evaluated habitable space as well as air revitalization, water recycling, and advanced technologies such as sensors. In others, the internal environment was evaluated with respect to specific parameters, including noise and human factors. The role of good health practices such as social/psychological, human factors, and food and nutrition was studied in a collective manner. Remote training methods for humans isolated or distanced from traditional instructional techniques and use of telemedicine systems were evaluated. Five major themes emerged from these four tests that were common to those of previous closed-system human life support test projects (1, 2):

- Interdependence of life support systems, habitable space, internal environments, and the human inhabitants
- Importance of testing engineering prototype hardware and advanced technology with "humans in the loop"
- Advances in spacecraft design due to integration of life support testing with human factors, behavior and performance, medical care, training, and life sciences
- Effectiveness of life sciences research with these types of "humans-in-the-loop" ground-based chamber tests; and
- Earth benefits from these types of tests (technology utilization for non-NASA applications).

Interdependence of life support systems, habitable space, internal environments, and the human inhabitants

An example of the interdependence of the water and air advanced life support systems with other activities and systems is illustrated in Figure 7.1-1.

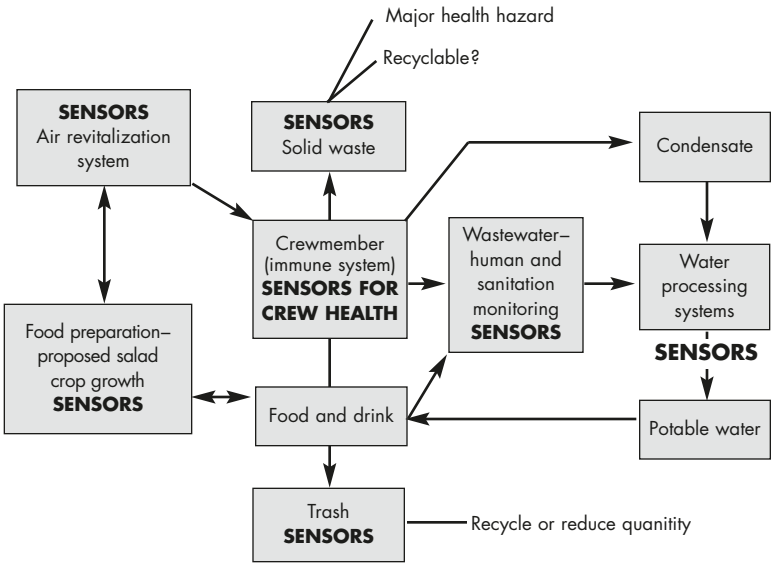

Figure 7.1-1 *An example of the interdependence between systems*

A closed system does not allow for resupply or replenishment of air and water from external sources, thus requiring development and use of technologies for total air revitalization and water recycling. To challenge these systems, the humans participated in a multitude of activities required for normal, healthy living such as physical exercise and food preparation. As NASA plans for very long duration missions, one scenario requires that at least some of the food be grown within the spacecraft using a system designed specifically for such a purpose. NASA has affectionately dubbed one such envisaged system the "salad machine" (Figure 7.1-2). It would be capable of growing foods that could be consumed with almost no preparation. NASA has also proposed utilizing other food systems such as growth of wheat and other grains for use in bread baking. Such activities have both psychological and nutritional benefits for humans in closed life support systems. In the Phase III test, the salad machine and bread baking produced changes in levels of air and water contaminants.

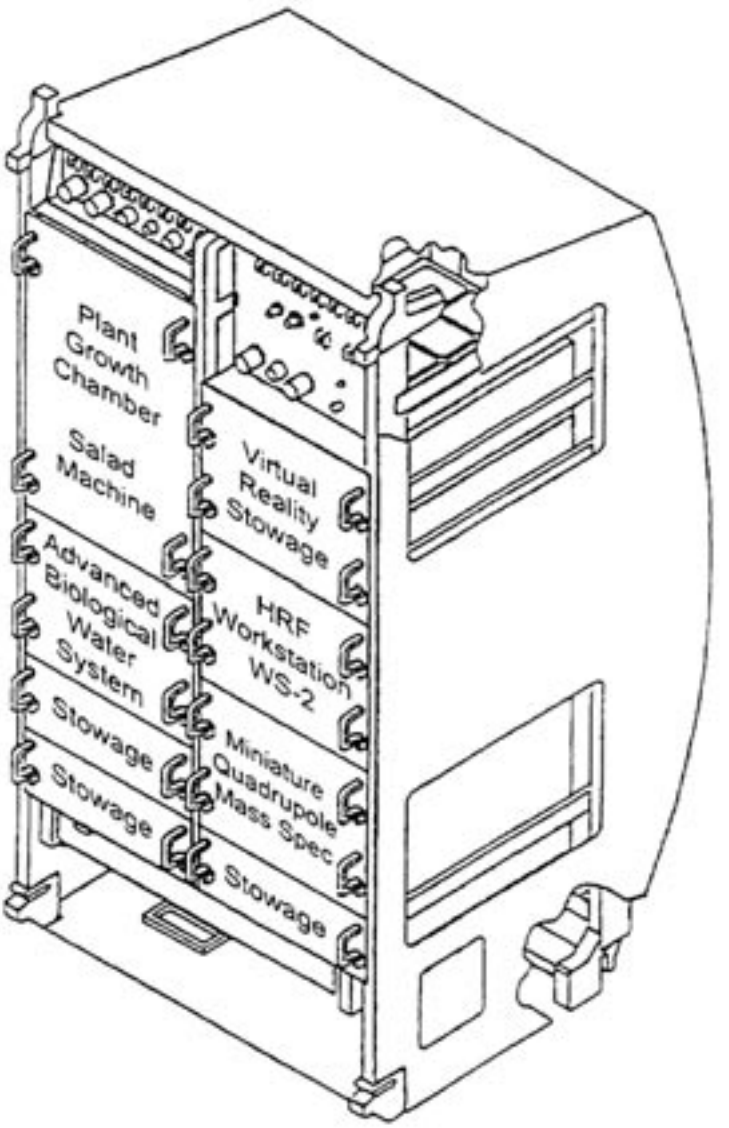

Figure 7.1-2 *The salad machine*

Exercise will be required of all crewmembers participating in long-duration flights. Exercise impacts thermal conditions and air quality (increased heat generation, oxygen consumption, and carbon dioxide generation) as well as increasing water condensate production. The Phase I test demonstrated that exercise enhanced air quality for growing wheat, and at the same time the plants removed some of the carbon dioxide. If major problems occur, such as crewmember noncompliance with the exercise protocol (Chapter 5.2: Exercise Countermeasures Demonstration Projects During the Lunar-Mars Life Support Test Project Phases IIa and III) either

voluntarily or due to injury, or in the case of failure of the food system, then the air and water systems must be able to compensate for such events and the resultant environmental changes. Conversely, the pollution of water and air can have deleterious effects on both the plants and crewmembers. Humans also vary their daily schedules. For instance, the crewmembers in the Phases II, IIa, and III tests changed their sleep/wake cycles (Chapter 3.4:Assessment of Sleep Dynamics in a Simulated Space Station Environment), but the engineers supporting the control center outside of the chamber did not. This complicated the crewmembers' psychological/social interactions with the control center personnel, impairing the performance of both (Chapter 3.5: Operational Psychology Countermeasures During the Lunar-Mars Life Support Test Project). Thus, a set of cascading events may affect both the functional capabilities of the life support systems and the crewmembers' ability to effectively and efficiently complete mission objectives. Closed life support chamber tests are effective and practical tools to study such interactions.

Importance of testing engineering prototype hardware and advanced technology with humans in the loop

From the very beginning of these types of tests (1), the influence of the human on the hardware was paramount. These early tests documented the myriad air contaminants that are generated due to long-term human presence. For example, because of bacterial flora in the human intestinal tract, humans are methane producers and as a result methane is a major but only one of many organic contaminants of such closed systems. Human habitation introduces a different set of microbiological contaminants as illustrated in another chapter in this book (Chapter 4.3: Microbiology). Use of sensor technology for environmental monitoring was challenging due to the complexity of the types of compounds encountered. For example, increasing levels of methane and hydrogen strained the sensor's capability to detect other compounds such as formaldehyde (Chapter 4.1: Air Quality). Removal and utilization of water from human wastes continues to be a focus for research (Chapter 4.2: Water Chemistry Monitoring). Humans vary in their level of hydration and this, in turn, affects urine concentration and consequently its specific gravity. Engineering systems must be capable of dealing with extreme variations in urine concentration and specific gravity. Additionally, urine may contain variations in levels and types of nitrogenous compounds as well as metabolites of pharmacological agents prescribed by the medical care team. At the same time, the environmental control system hardware must efficiently use limited resources including nonreplenishable chemicals and energy. Thus, use of these complex closed-loop systems is required in order to make advances in engineering hardware design and to provide an integrated test bed for functional verification.

Advances in spacecraft design due to integration of life support testing with human factors, behavior and performance, medical care, training, and life sciences research

These integrated test beds provide an important analog for advanced technology and research testing. This is clearly documented in the results reported in this book as well as in those from numerous other tests both in the United States and Russia. Operational activities such as space flight, basic research, and advanced life support closed-chamber tests all interact in an interdependent manner as illustrated in Figure 7.1-3.

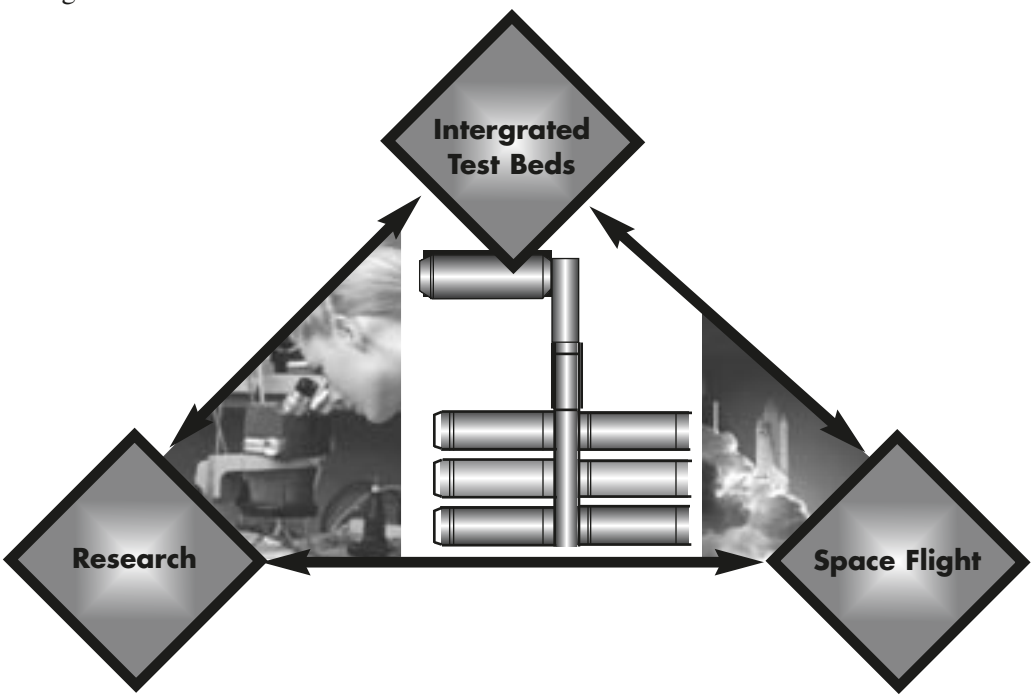

Figure 7.1-3 *Role of ground-based test beds with research and space flight*

The obvious approach to overcoming current barriers is to conduct basic research that will lead to advanced technologies which are first evaluated in ground-based test facilities, then with success become part of operational equipment for space flight. However, space flight operations often redirect research efforts away from original objectives to address more immediate and critical needs. For instance, early in the space program, body weight and bone mass losses were documented. These observations prompted research into the use of pharmacological agents, nutrition, as well as exercise as potential countermeasures (all of which can be partially tested in these life support closed test beds (Chapter 5.1: Nutritional Status Assessment During Phases IIa and III of the Lunar-Mars Life Support Test

Project and Chapter 5.2: Exercise Countermeasures Demonstration Projects During the Lunar-Mars Life Support Test Project Phases IIa and III). Furthermore, the studies from these tests often lead to additional research efforts. For instance, the results from the Phase I and III tests showed that food production, processing, and preparation could be part of the advanced life support systems (Chapter 4.4: Crew Food Systems). However, additional research efforts are needed that focus on the processing of hydroponically grown crops that could be used to recycle air and water. Present food processing practices use enormous amounts of water, a limited resource during space exploration. Basic research is required in production and processing of foods with limited water, and within the necessarily restricted volume and energy resources of spacecraft. Furthermore, foods grown under these conditions may have different physical properties (e.g., level of gluten from flour and nutritional qualities such as mineral components) than otherwise identically Earth-grown foods. This, in turn, requires additional basic research efforts to optimize food production processes to yield foods with appropriate nutritional content.

These test beds also provide an opportunity for human factors research that must consider the limitations imposed by spacecraft volume available for human habitat and its design. Thus, different components of human factors can be evaluated in a totally integrated fashion such as the living necessities of sleeping, eating, working, and use of leisure time (Chapter 3.2: Habitability: an Evaluation). Most human factors studies are directed toward component understandings, but within the environment of these advanced life support tests, such components can be integrated and verified. An example that has been evaluated is the importance of ambient noise level (Chapter 3.3: Acoustic Noise During the Phase III Chamber Test), sleeping space conditions, and personal space requirements (Chapter 3.7: Sociokinetic Analysis as a Tool for Optimization of Environmental Design). The human factors team must consider the combination of engineering and architectural design solutions that provides the bases for these types of research efforts.

These types of test beds also provide a chance to test various procedures in a safe and closely monitored environment. The medical support team can evaluate telemedicine hardware and procedures (Chapter 6.1: Telemedicine During Lunar-Mars Life Support Test Project Phase III), and the effectiveness of just-in-time training (Chapter 6.2: In Situ Training Project: LMLSTP Phase III Report) can be assessed. In the Phase III test, there was a minor medical event that was resolved utilizing the telemedicine and crew training processes (Chapter 2.2: Chamber Studies Medical Care Overview: Medical Officer's Report). Psychologists have used these test beds to evaluate crew teamwork training efforts and as a result were able to improve their astronaut team training, an outcome that is vital for long-duration space flight mission success (Chapter 3.5: Operational Psychology Countermeasures During the Lunar-Mars Life Support Test Project).

To improve understanding of human health and the essential support technologies, NASA Life Sciences teams have developed Critical Path Roadmaps to provide

the research programs necessary for improved spacecraft design as well as capabilities to improve crew health. As part of this effort, research and technology development has been categorized into several levels of technology readiness (Table 7.1-1). Within the Critical Path Roadmap, issues and questions are assigned to a specific level of technology development. If basic research is needed, then the efforts are assigned a low-level of technology readiness and the program emphasis is enabling research. Other more mature technologies are assigned a higher level of technology readiness. As important technologies are developed, the program determines whether these technologies should be tested in the laboratory or in a relevant environment such as in these ground-based test beds. Finally, after verification in the relevant environment, a subsystem prototype can be tested in a space environment or implemented as part of standard spacecraft operations.

Table 7.1-1 *Technology readiness levels*

Level	Definition
TRL 1	Basic principles observed
TRL 2	Technology concept and/or application formulated
TRL 3	Analytical and experimental critical function/proof-of-concept
TRL 4	Component and/or breadboard validation in lab
TRL 5	Component and/or breadboard in relevant environment
TRL 6	System/subsystem model or prototype demonstration in relevant environment
TRL 7	Subsystem prototype in a space environment
TRL 8	System completed and flight qualified through demonstration
TRL 9	System flight proven through mission operations

Effectiveness of life sciences research with these types of human-in-the-loop ground-based chamber tests

Life sciences research has benefited immensely both from ground-based chamber tests and from use of other types of test beds as scientific analogs to space flight and of other semi-isolated conditions such as polar ice stations, submarines, submersibles, etc. For instance, the findings from previous studies have shown changes in human sleep cycles, immune function, and psychological adaptations. Thus, certain features are shared between operational scenarios and ground-based chamber tests in the human participants. However, differences may exist as well. For example the exercise protocols used in Phases IIa and III had a different outcome than in a non-enclosed environment. Specifically, the exercise protocol tested in the Phase III test resulted in an overuse injury that is usually associated

with training for athletic events. Thus, the psychological and social roles of exercise may differ depending on the environment in which the exercise is performed. Determining both the shared and different features that exist between ground-based analogues and operational environments is important for future studies. More emphasis can then be placed on acquiring a better understanding of changes in human physiology and psychology that are common to operational environments and closed-chamber tests. These common features can be more completely studied in controlled ground-based studies. Equally important is knowledge of the differences that may exist between the environments so that these differences may be considered in the interpretation of the results of all studies. Closed-chamber tests with humans in the loop lead to new technologies for monitoring and for countermeasures to untoward effects of isolation. Technologies that have the potential to enhance nonintrusive monitoring of individual and group performance may have a positive impact on enhancing crew performance (Chapter 3.6: Spaceflight Cognitive Assessment Tool for the Lunar-Mars Life Support Test Project Phase III Test). Thus, future research may place more emphasis on additional specific concerns.

Earth benefits from these types of tests (technology utilization for non-NASA applications)

Table 7.1-2 illustrates some of the Earth benefits from the test results reported in this book.

Table 7.1-2 *Earth benefits from recent advanced life support tests*

Area	Examples
Habitability	Designing living space for maximum human performance Tools for evaluation of safety of habitat
Psychological/Social	Noninvasive methods for measurement of circadian rhythms and sleep quality Tools for tracking crewmember's psychological health and team work Evaluation of psychological status in isolated environments Cognitive assessment tools
Engineering design	Tools for movement patterns of groups within controlled and limited environmental design
Air and water quality	Importance of trend analysis for air and water quality Water recycling methodologies including microbiological, metals, and organic compound measurement technologies Problems related to cleaning of surfaces when dependent on air recycling/indoor air quality Wearable detectors for air quality, e.g., formaldehyde sensors

Table 7.1-2 continued *Earth benefits from recent advanced life support tests*

Area	Examples
Food systems/nutrition	Food processing with water limitations and air recycling Utilization of food frequency questionnaires as a dietary assessment tool Palatable diet with vegetarian diet and/or limited variety of foods
Exercise	Effectiveness of aerobic exercise with resistive exercise; overtraining
Microbiology and medicine	Decreased immune responsiveness with latent viral reactivation under stressful and isolated conditions
Medicine	Utilization of telemedicine with untrained crewmembers in isolated conditions
Training/Education	Just-in-time learning and evaluation of different types of training: video, computer-based, virtual reality

The Future

There is no doubt that larger and more fully integrated tests are required to validate exploration-class and low Earth-orbit mission scenarios. The principle that guides these efforts is technology-based and is illustrated in Figure 7.1-4. The next generation of technologies draws from the evolving knowledge base in information technology. Advances in information technology will improve analysis and manipulation of data and will provide biocomputations for image analysis and essential training simulation efforts utilized for medical support through telemedicine. Information technology research may elicit understanding of the control systems for the large variations in human activities, yet at the same time minimize the hardware, energy, and resupply needs. The ongoing modeling efforts to determine the best combination of systems must be evaluated in an integrated test bed before they can be utilized in Earth-orbital or exploration-class missions. Advances in biotechnology will result in improved sensors, and new developments in micro- and nanotechnology will provide the basis for design and construction of better hardware for maintaining a closed life support system (Figure 7.1-5). With highly reliable autonomous life support systems, the spacecraft can ensure a breathable atmosphere, potable water, food production, solid/water processing, and thermal control. Through the automatic detection and remediation systems, microbial and chemical contaminations due to humans, food processing, and waste management can

be controlled (Figure 7.1-6). Future research in microbiology and immunology (Chapter 4.3: Microbiology; Chapter 5.3: Reactivation of Latent Viruses; and Chapter 5.4: The Influence of Environmental Stress on Cell-Mediated Immune Function) is needed, as this is an important area given the experimental nature of the air revitalization, water recycling, waste management, and food processing activities that will occur within these small, enclosed systems. Figures 7.1-4 and 7.1-5 illustrate some examples of the requirements for ongoing efforts in micro- and nanotechnology development. Critical research areas include development of adaptive user interfaces and displays, onboard systems for refresher training and skills monitoring, continuous assessment of mental status and, of course, personal communications and recreation through integrated systems (Figure 7.1-7). As seen from Figure 7.1-4, the goal is to move from a strong human interface with the life support system not just to an automated system requiring little direct human intervention, but rather to a self-analysis system, and then finally to a self-repairing system. This will lead to decreased hardware mass requirements that reduce launch mass, a critical concern for efficient achievement of low Earth orbit. Finally, a major benefit is that crew time can be used for exploration and scientific missions rather than for repair, maintenance activities, training, and health monitoring. Critical areas for future research include habitation systems, such as advanced life support, environmental health, food and nutrition, and human behavior and performance. However, besides these basic areas for research, ground-based closed-system test beds are excellent analogs for improving and verifying clinical care capabilities and multisystem integration (Table 7.1-3).

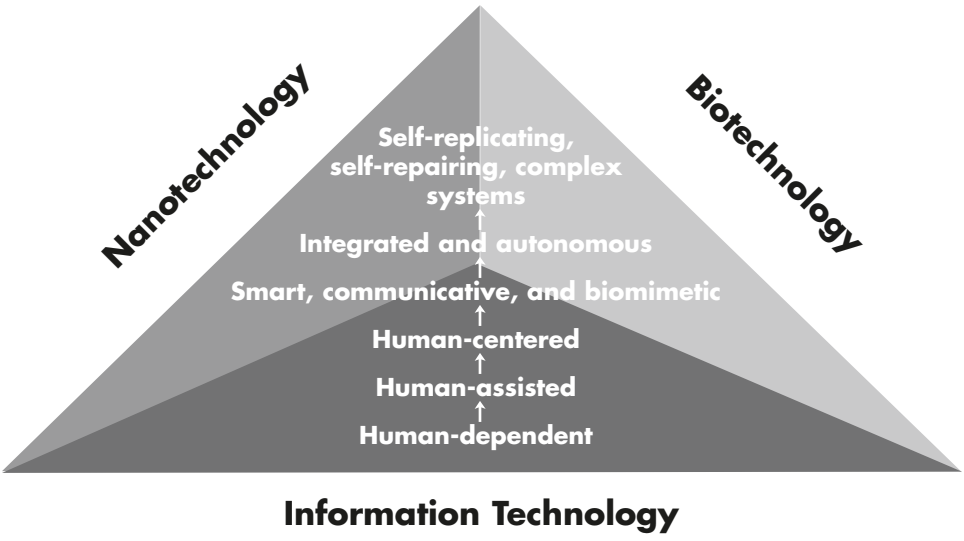

Figure 7.1-4 *Technologies of the future*

Life support
- Sensors and effectors
- Bioregeneration
- "Human-on-a-chip"
- Biological niches

Medical care
- Diagnostic probes
- Treatment and delivery systems
- "Keyhole" surgery
- Tissue replacement

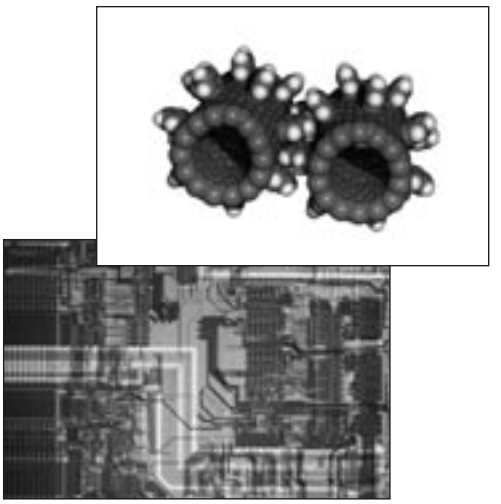

Figure 7.1-5 *Nanotechnology: research and design at the molecular level*

Highly reliable, autonomous life support systems to provide:
- Breathable atmosphere
- Potable water
- Crop production and processing
- Solid waste processing
- Thermal control

Automatic detection and remediation of environmental factors
- Microbial and chemical contamination in air, water, and food
- Radiation events or cumulative exposure

Figure 7.1-6 *Critical area: advanced life support.*
Image courtesy of Bob Sauls of John Frassanito and Associates

- Nonintrusive monitoring of individual/group performance
- Adaptive user interfaces and displays
- Onboard systems for refresher training and skill monitoring
- Continuous assessment of mental status
- Personal communications and recreation through integrated systems

Figure 7.1-7 *Critical area: crew performance. Computer-generated image courtesy of Bob Sauls of John Frassanito and Associates*

Table 7.1-3 *Critical areas for research*

Function	Discipline Risk Areas
Habitation systems	Advanced life support Environmental health Food and nutrition
Adaptation/Countermeasure systems	Bone loss Cardiovascular alterations Human behavior and performance Immunology, infection, and hematology Muscle alterations and atrophy Neurovestibular adaptation Radiation effects
Medical care systems	Clinical capabilities Multisystem (cross-risk) alterations

NASA's future plans include a much larger closed life support system potentially composed of six chambers – 15 ft in diameter by 37 ft in length with a 12 ft diameter, 63 ft long tunnel, and a 1.5 ft long node 12 ft in diameter (Figure 7.1-8). The six chambers are interconnected yielding an internal volume of approximately 44,000 ft^3.

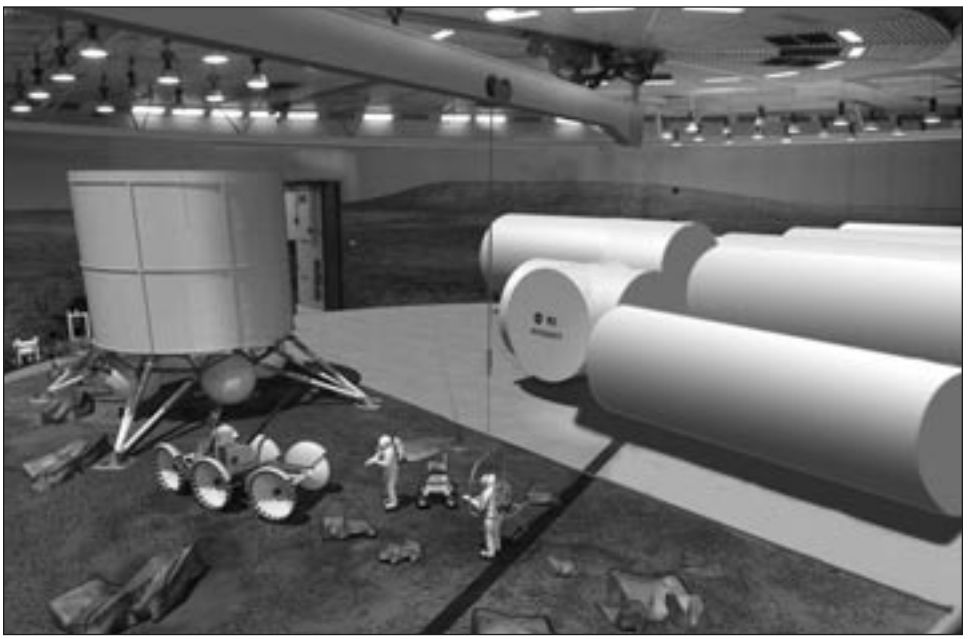

Figure 7.1-8 *An artist's rendering of a future closed life support systems test bed. Image courtesy of Bob Sauls of John Frassanito and Associates*

The goal is for all systems (i.e., air, water, power, thermal control, waste management, plant production, food processing, and human habitat) to be contained within the chambers. This will provide the capability for monitoring mass balance and for acquisition of the data necessary to enhance modeling of life support systems for long-duration space flight. A laboratory chamber is proposed that will provide chamber air, water, and other essential monitoring and analysis. This effort will require development of micro- and nanotechnologies since inadequate space exists for traditional analytical instrumentation. The integrated test system will have a control room adjacent to it for monitoring and control of the series of planned tests. The major driver for development of this large ground-based closed life support test bed is to provide capabilities for integrated tests of advanced life support engineering: water recycling, air revitalization, waste management, crop production, food processing, and thermal management within a closed system. Advanced sensors and new types of information technologies along with modeling technologies will be routinely tested and verified. Beyond these activities, additional studies are planned that will include human factors research.

Confinement is an analog for several avenues of research including psychological, immunologic, and training studies. Additionally, confinement can provide a good model for research into the effects of light on human subjects. Historically, little attention has been given to mimicking the intensity and spectral output of sunlight, despite our knowledge that intensity and duration of light can have

significant effects on circadian rhythms and that ultraviolet B radiation is required for vitamin D biosynthesis. Light conditions similar to those found on the Martian and lunar surfaces can be simulated in these test chambers. Furthermore, the limited and delayed communications expected with exploration-type missions can be mimicked to provide improved communication methodologies necessary for effective psychological and medical support.

Conclusions

Each chapter of this book reflects the types of investigative activities that can benefit from closed life support chamber studies. As advances in space flight-related sciences, technologies, and engineering approaches occur, these can be evaluated in a long-duration integrated test in order to more clearly define the interactions between systems. Critical areas of life sciences research are listed in Table 7.1-3. It is important to continue ground-based systems testing within these critical areas if the reality of human exploration of our solar system – and ultimately beyond – is to be achieved.

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