NASA/TM–2005–213162

KC-135 and Other Microgravity Simulations

Summary Report

Space and Life Sciences Directorate
Human Adaptation and Countermeasures Office
Johnson Space Center, Houston, Texas

August 2005

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National Aeronautics and
Space Administration

Lyndon B. Johnson Space Center
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PREFACE

This document represents a summary of medical and scientific evaluations conducted aboard the KC-135 from June 28, 2004 to June 27, 2005. Included is a general overview of KC-135 activities manifested and coordinated by the Human Adaptation and Countermeasures Office. A collection of brief reports that describe tests conducted aboard the KC-135 follows the overview. Principal investigators and test engineers contributed significantly to the content of the report describing their particular experiment or hardware evaluation. Although this document follows general guidelines, each report format may vary to accommodate differences in experiment design and procedures. This document concludes with an appendix that provides background information concerning the KC-135 and the Reduced-Gravity Program.

CONTENTS

1 Overview of KC-135 Flight Activities sponsored by the Human Adaptation and Countermeasures Office

From June 28, 2004 to May 31, 2005, five weeks were specifically reserved for flights sponsored by the Human Adaptation and Countermeasures Office (HACO). Seats were obtained aboard flights during two additional weeks for HACO customers with other organizations sponsoring the flights. A total of 25 flights with approximately 40 parabolas per flight were completed. The average duration of each flight was two hours. The KC-135 coordinator assisted principal investigators and test engineers of 29 different experiments and hardware evaluations in meeting the necessary requirements for flying aboard the KC-135 and in obtaining the required seating and floor space. HACO customers purchased a total of 256 seats. The number of seats supported and number of different tests flown by flight week are provided below:

Flight Week	Seats	# Tests Flown	Sponsor
June 29 – July 2, 2004	68	6	HACO
July 13 – 16	30	6	Undergraduate Program
July 27 – 30	20	4	Undergraduate Program
August 10 – 13	32	3	HACO
August 31- Sept. 2	51	5	HACO
Sept. 9	13	2	HACO
Sept. 28 – Oct. 1	42	3	HACO

Support was provided to the undergraduate program during July 2004. Local and major network radio, television, and newspaper journalists accompanied the students on some of these flights. A large ground crew from the respective academic institution supported the in-flight experiments.

In October 2004, the Aircraft Operations Division (AOD) at the Johnson Space Center retired the KC-135 931 aircraft used for parabolic flights since September 1995. The resumption of parabolic flight opportunities by life science investigators is being planned for the summer of 2005 when the replacement C-9 aircraft becomes available.

2 Medical and Scientific Evaluations aboard the KC-135

2.1 Microgravity-Compatible Flow Cytometer

FLIGHT DATES:
June 29, 2004
August 10 – 11, 2004
September 2 and 9, 2004

PRINCIPAL INVESTIGATOR:
Brian Crucian, Wyle Life Sciences
Mayra Nelman-Gonzalez, Wyle Life Sciences
Clarence Sams, NASA/Johnson Space Center

GOAL:
To validate a prototype spaceflight-compatible flow cytometer and an associated microgravity-compatible cell staining device for medical support during long-duration space missions. Five KC-135 evaluations of the developed hardware were performed in FY04.

OBJECTIVES:
A spaceflight-compatible flow cytometer would be useful for the diagnosis of astronaut illness during long duration spaceflight and for conducting in-flight research to evaluate the effects of microgravity on human physiology. Until recently, the primary limitations preventing the development of a spaceflight compatible flow cytometer have been largely mechanical. Standard commercially available flow cytometers are large, complex instruments that use high-energy lasers and require significant training to operate. Standard flow cytometers function by suspending the particles to be analyzed inside a ‘sheath’ fluid for analysis. This requires the presence of several liters of sheath fluid for operation, and generates a corresponding amount of

liquid hazardous waste. The particles are then passed through a flow cell which uses the fluid mechanical property of 'hydrodynamic focusing' to place the cells in single-file (laminar flow) as they pass through a laser beam for scanning and evaluation. Many spaceflight experiments have demonstrated that fluid physics is dramatically altered in microgravity (MSF [Manned Space Flight] Fluid Physics Data Sheet-August 1997) and previous studies have shown that sheath-fluid based hydrodynamic focusing may also be altered during microgravity (Crucian et al, 2000). For these reasons it is likely that any spaceflight compatible design for a flow cytometer would abandon the sheath fluid requirement. The elimination of sheath fluid would remove both the problems of weight associated with large volumes of liquids as well as the large volume of liquid waste generated. It would also create the need for a method to create laminar particle flow distinct from the standard sheath-fluid based method.

The spaceflight prototype instrument is based on a recently developed commercial flow cytometer possessing a novel flow cell design that creates single-particle laser scanning and evaluation without the need for sheath-fluid based hydrodynamic focusing. This instrument also possesses a number of design advances that make it conditionally microgravity compatible: it is highly miniaturized and lightweight, uses a low energy diode laser, has a small number of moving parts, does not use sheath fluid and does not generate significant liquid waste. Although possessing certain limitations, the commercial cytometer functions operationally like a standard bench top laboratory flow cytometer, aspirating liquid particle samples and generating histogram or dot-plot data in standard 'FCS' file format. In its current configuration however, the cytometer is limited to three parameter/two-color capability (two color PMTs + forward scatter), does not allow compensation between colors, does not allow linear analysis and is operated by rather inflexible software with limited capabilities. This is due to the fact that the cytometer has been designed and marketed as an instrument specific to a few particular assays, not as a multi-purpose cytometer.

The NASA-JSC Center Directors Discretionary Fund has funded the Cell & Molecular Research Laboratory to: (1) construct a prototype flight instrument based on the framework of the commercial cytometer; (2) perform ground-based and microgravity validation of the instrument; (3) design and validate a set of medical assays compatible with the prototype instrument; (4) design and validate a microgravity compatible cell staining device for sample processing that can interface with the instrument. In FY04 the initial stages of instrument design and validation were successfully completed, as well as the development of the cell staining unit and medical assays. The FY04 KC-135 evaluations that took place were as follows:

Flight #1	6-29-2004	Validation of fluidics/function, standard sample acquisition
Flight #2	8-10-2004	Std. Data acquisition, evaluation of precision sample delivery pipettes
Flight #3	8-11-2004	Initial evaluation of new cell staining unit and associated instrument port
Flight #4	9-2-2004	Complete blood-to-data system evaluation part 1
Flight #5	9-7-2004	Complete blood-to-data system evaluation part 2

METHODS AND MATERIALS

Blood donors. Whole blood samples were obtained from adult donors into ACD anticoagulant vacutainers. This includes the KC-135 flight experiment and all developmental work performed

in preparation for the experiment. The subjects had been screened by the NASA-JSC Human Test Subject Facility for most major infectious diseases and were found to be in good health. Institutional Review Board (NASA-JSC) approval was obtained for this study and written informed consent was obtained from all subjects.

Cell staining. A complete 2-color antibody panel was formulated to resolve most major leukocyte subsets yet remain within the limitations of the instrument. The cell populations resolved included: leukocyte subsets; T cells; B cells; NK cells; T cell subsets and activated T cells. Cell surface markers were stained prior to flight. For the bead-based cytometry samples either fluorescent calibration microspheres or linearity fluorescent microspheres were used.

Flow cytometry analysis. For ground-based analysis, a Beckman-Coulter XL flow cytometer was configured as a reference cytometer for 3 parameter/2 color analysis so that it would mimic the function of the cytometer. Analysis was performed using the XL as a reference cytometer, ground-based control data was generated using the prototype flight cytometer. For data collection during microgravity the sampling apparatus of the cytometer was altered for ease of operation during parabolic flight. To ensure data collection occurred during microgravity only, samples were mixed and affixed to the sampling probe and the instrument was primed prior to the initiation of the microgravity phase. Priming took place during the 2g phase between parabolas. Data collection was initiated as the parabola was initiated and ended as the aircraft exited the parabola.

RESULTS:

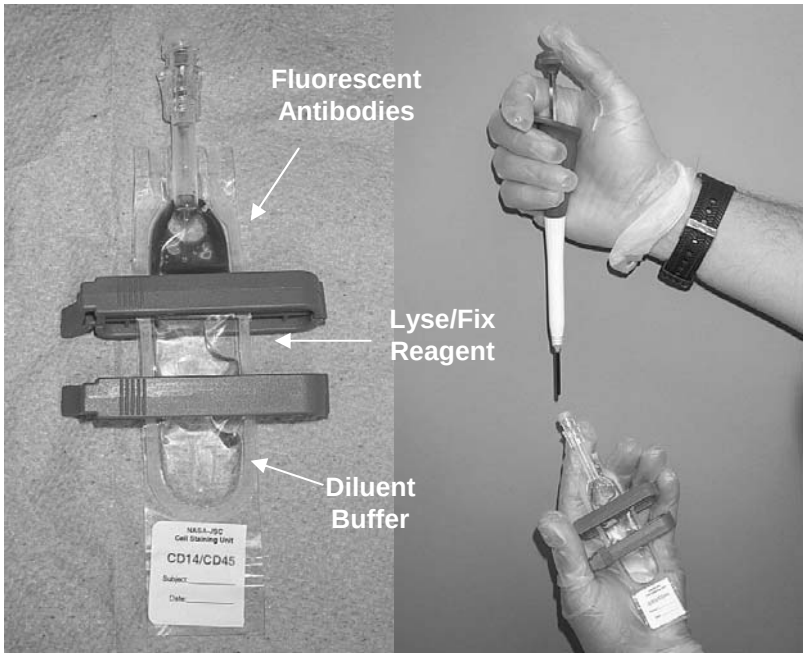
In FY04 the basic configuration of the prototype flight cytometer was prepared, the associated cell-staining unit (interfaces with instrument) was developed and validated, the medical assay panel was developed and validated and microgravity evaluation of all hardware was successfully performed.

Development of prototype instrument: The internal components of a novel commercial cytometer were used as a basis for development of the basic prototype flight cytometer. The basic prototype flight cytometer consists of the unit with the minimal alterations required for microgravity evaluation and further development. (An advanced prototype flight cytometer will be completed in year two of this project that will be completely re-engineered for optimum compatibility with on-board flight operations) The areas of the commercial cytometer that were deemed not microgravity compatible were modified. These included: (1) replacing the waste collection system with an inflatable Teflon bag fixed to a one-way check valve and (2) modifying the sample uptake port with 10cm of silicone peak tubing and an injection port. The newly developed injection port was designed to be compatible low-flow fluidics and compatible with the injection port of the cell staining unit.

Development of cell staining unit/sample processing procedure: A novel cell staining unit was developed by the CMR Laboratory in 1996, and evaluated onboard the Shuttle on STS-71. This unit, the Whole Blood Staining Device (WBSD), consists of tubing separated into chambers with clips with an injection port at one end. By filling the chambers with staining reagents, a blood sample may be injected and processed during microgravity. The WBSD was initially considered and evaluated with the prototype flight cytometer. It was found, however that the hard tygon tubing unit body created a negative pressure that hindered the instrument from withdrawing sample. This effect was found to dramatically alter the capacity of the instrument to provide

absolute cell counts. A novel second generation unit was designed that used a sterile low-adherence collapsible Teflon bag, a Baxter Interlink Injection Site and Weland clips for chamber separation. This unit, (the WBSD2) interfaced perfectly with the instrument, provided reliable absolute counts and was found to be robust and reliable.

To generate absolute counts it is necessary to deliver a precise volume of blood sample into the staining device. For this purpose a 100ul positive displacement pipette was used. Several types of blood collection tubes were evaluated, and a 4.0 ml EDTA Starsted monovette was found to have several distinct advantages: (1) they are plastic; (2) the top screws into place and is not held by vacuum; (3) they are without vacuum during storage, the user creates the vacuum at the time of sample collection; (4) they make excellent storage vessels if there is an unexpected delay in processing; (5) the port is thinner than a vacutainer, meaning that the plastic pipette tip may pass through and sharp needles are not required.



Development of medical assay panel: A panel of clinical laboratory hematology and immunology assays, important in infection and immunity, was developed to be compatible with the limitations of the Prototype Flight Cytometer. This assay panel was achievable within the two-color cytometry limit of the instrument and would provide critical medical information currently not available on-orbit. The developed and evaluated assays were as follows: white blood cell count, leukocyte differential, lymphocyte subsets, T cell subsets, activated T cell levels and DNA content. The two-color antibody matrix developed for these assays was as follows:

PE	PC5	
CD14	CD45	WBC Differential: granulocytes/lymphocytes/monocytes.
CD3	CD45	T cell percentage
CD19	CD45	B cell percentage
CD16	CD45	NK cell percentage
CD3	CD4	Helper inducer T cell subsets
CD3	CD8	Cytotoxic/suppressor t cell subsets
CD45RO	CD4	T cell subsets; memory/naïve T cell subsets
CD45R0	CD8	T cell subsets; memory/naïve T cell subsets
CD69	CD3	Activated T cell subsets.

Microgravity evaluation of instrument and associated hardware: The instrument, all associated hardware, and the panel of assays were all evaluated real-time in microgravity conditions onboard the KC-135 aircraft. The sample delivery/processing hardware was found to function well during microgravity conditions, in a manner comparable to ground operation. The operators noted that care needed to be exercised to prevent air bubbles in the liquid samples from skewing the volume of sample primed in the pipette for delivery. However, the clear-bore design of the precision 100ul positive-displacement pipette allowed for easy inspection of the sample. The WBSD2 units functioned extremely well during microgravity and the interface with the instrument was found to be simple and reliable.

The Prototype Flight Cytometer bolted to the floor of the KC-135 aircraft for microgravity evaluation (left) and evaluation of the precision sample delivery and cell staining hardware

The cytometer itself was found to function well in microgravity condition. Due to the sample priming delay caused by the temporary addition of the exterior peak tubing to which the WBSD sample port was attached, the operators had to begin priming the samples during the 2g phase of flight; however data were only collected during the microgravity phase. All peripheral blood leukocyte subset data collected during microgravity were found to be essentially identical to ground data and reference cytometer control data.

DISCUSSION:

The flow cytometer is an extremely versatile laboratory instrument with a broad-spectrum of uses in both clinical medicine and basic science research. It is therefore highly desirable to develop a spaceflight compatible cytometer for use on the International Space Station. While currently limited in ability, the cytometer represents an attractive design option for the design of a spaceflight compatible flow cytometer. It has the potential to be made completely microgravity compatible and serve as a prototype spaceflight instrument with relatively minimal alterations in design specifications.

The cytometer's level of miniaturization, use of a low energy diode laser, and elimination of the sheath fluid requirement all uniquely meet the existing prerequisites for use during spaceflight. It is likely that the current limitations of this instrument could be overcome by modifying the software, adding additional lasers, color PMTs, side scatter, color compensation ability and further miniaturization. The sample delivery apparatus of the cytometer is not microgravity compatible and would require significant modifications. These limitations notwithstanding, the cytometer may be well suited to be the prototype from which a spaceflight compatible flow cytometer is designed.

The successful microgravity evaluation of the cytometer and the need to collect real-time experimental data onboard ISS for both research and clinical diagnosis purposes warrant the continued development of a spaceflight prototype flow cytometer. The versatility of the flow cytometer for general research (biological, microbial, environmental and physiological studies) and diagnostic medicine would be a major asset to the ISS/lunar/mars programs.

CONCLUSION:

A basic evaluation version of a prototype spaceflight-compatible flow cytometer was successfully created and evaluated. An associated sample processing system that interfaces with the instrument was also successfully developed.

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Crucian, B., Norman, J., Brentz, J., Pietrzyk, R. and Sams, C. Laboratory outreach: student assessment of flow cytometer fluidics in 0-gravity. *Laboratory Medicine* 31(10):569-572, 2000.

NASA-Marshall Space Flight Center (1997). Microgravity Research Division: Microgravity Fluid Physics Discipline Brochure. Available online at *http://mgnews.msfc.nasa.gov/db/fluid-phys.pdf* (PDF document) or *http://mgnews.msfc.nasa.gov/db/fluids/fluids.html* (HTML document).

PHOTOGRAPHS:

JSC2004E27340
JSC2004E27342
JSC2004E27347 to JSC2004E27349
JSC2004E27372 to JSC2004E27375
JSC2004E35188
JSC2004E35197 to JSC2004E35203
JSC2004E35218 to JSC2004E35227
JSC2004E35238
JSC2004E35293
JSC2004E35652 to JSC2004E35673
JSC2004E40306 to JSC2004E40320
JSC2004E40641 to JSC2004E40659

VIDEO:

- Zero-g June 29 – July 2, 2004, Reference Master: 718394
- Zero-g August 9 – 13, 2004, Reference Master: 718620
- Zero-g August 30 – September 9, 2004, Reference Master: 718586

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.2 Experimental Microfluidic System

FLIGHT DATES:

June 28 - July 2, 2004

PRINCIPAL INVESTIGATORS:

Christopher Culbertson, Kansas State University
Steve Gonda, NASA/Johnson Space Center
John Michael Ramsey, University of North Carolina

GOAL:

The ultimate goal of this project is to integrate microfluidic devices with NASA's space bioreactor systems. In such a system, the microfluidic device would provide realtime feedback control of the bioreactor by monitoring pH, glucose, and lactate levels in the cell media; and would provide an analytical capability to the bioreactor in exterrestrial environments for monitoring bioengineered cell products and health changes in cells due to environmental stressors. Such integrated systems could be used as biosentinels both in space and on planet surfaces.

OBJECTIVE:

To demonstrate the ability of microfabricated devices to repeatedly and reproducibly perform bead cytometry experiments in μ, lunar, martian, and hypergravity (1.8g).

BACKGROUND:

Microfluidic or Lab-on-a-Chip devices are small platforms upon which a complete chemical analysis can be performed. These devices consist of a series of small interconnecting channels (10µm deep and 40 to 200mm wide) etched in glass, or molded in polymers, through which fluids can be moved. The fluids can either be controlled with electric potentials generating electric fields within the channels that move fluid electrokinetically, or by generating pressure differentials using a syringe pump or peristaltic pump that moves the fluid hydrodynamically. Using these two methods of controlling fluid flow it is possible to generate devices that have multiple uses. For example it is possible to use hydrodynamic flow to rapidly move large numbers of cells or large particles, such as beads, through a focusing intersection much faster than if one were to use electrokinetic flow. On the other hand electric fields allow for the separation of differentially charged analytes using capillary electrophoresis, something hydrodynamic flow alone can not accomplish. These devices provide many advantages over conventional bench top scale instrumentation as a result of their ability to integrate sample handling and sample processing operations with analyte detection on a single, monolithic substrate. Such integration allows for the efficient automation of chemical analyses. In addition to automation and integration, microchips have several other inherent advantages over conventional chemical analysis instrumentation. These advantages include (1) the ability to perform faster separations with no loss in separation efficiency, (2) lower reagent and sample consumption (< 1mL/year), (3) less waste production, and (4) the ability to fabricate many parallel systems on the same device. Thus far, their performance has been either equivalent to or better than conventional laboratory devices in all cases investigated. They appear to offer the rare combination of better-faster-cheaper simultaneously, and their ability to manipulate reagents and reaction products “on-chip” suggests the potential to perform virtually any type of “wet-chemical” bench procedure on a microfabricated device.

The advantages described above make these devices especially interesting for use in extraterrestrial environments where small, portable, rugged, and reliable devices capable of sustained remote automated operation will be required.

METHODS AND MATERIALS:

Microfluidic Experiment Description

The portable microfluidic device developed for these tests contained in a Bud box enclosure (NBA10148) which had exterior dimension of approximately 30 cm wide x 18 cm deep x 40 cm high. The microchips, in their custom machined 2-part PMMA holder, were attached to an x-y positioning plate (ST1XY-S; Thor Labs Inc.; Newton, NJ) and positioned above a microscope objective (CD-240-M40X; creative devices, Neshanic Station, NJ). This objective was used to focus the excitation light of a green laser pointer (The Laser Guy, Seabrook, TX). The laser was modified to have a remote switch and power supply (2 D cell batteries). The laser module consisted of an 808 nm laser diode which was shifted to 1064 nm and then frequency doubled to provide a continuous output of 5 mW at 532 nm. The laser beam was reflected off a dichroic mirror (560 DRLP: Omega Optical, Brattleboro, VT) prior to being focused into the microchip channel by the microscope objective. The fluorescence from the labeled amino acids was collected by the same microscope objective, passed through the dichroic mirror, a 1.0 mm pinhole, and a 565 nm longpass filter (565ALP, Omega Optical) prior to being detected at a channel photomultiplier tube (MD972; Perkin Elmer; Fremont, CA). The PMT was powered by a 5 volt power supply. The gain was manually controlled by a potentiometer which had a locking mechanism to prevent accidental change.

The high voltages used for making injections and performing the electrophoretic separations on the microchip were provided by two independent high voltage power supplies cabable of 125 ma outputs at up to 8 kv (C80; Emco High Voltage Corp.). Each high voltage power supply was powered by a 15 VDC source. The HV output was determined by a 0-5 VDC control signal provided by a National Instruments AO card (DAQCard AO-2DC). These power supplies each occupied only 19 cm^3 and weighed 51 g, making them very suitable for portable applications.

A syringe with a locking stop was used to provide pressure to drive the hydrodynamic flow for the bead and cell focusing experiments.

The entire instrument was controlled and data were acquired using in-house written LabVIEW software run off a laptop computer.

Protocol

Orange fluorescent polystyrene microspheres that have a 10µm diameter were diluted into the running buffer to make a bead concentration of $6.5x10^6$ beads/ ml (Molecular Probes, Eugene, OR). The run buffer was composed of 25 mm NaBorate and 0.01% (v/v) Silwet. The Silwet helped prevent aggregation of the particles and particles adsorption to the capillary sidewalls. Sonication of this solution was performed prior to focusing experiments to further reduce bead aggregation.

To perform the bead focusing experiments a 25 mm NaBorate and 0.01% Silwet solution were placed in channels B and C (Figure 1), while the bead solution was placed in channel A. A low pressure was applied to channel D using a syringe pump. This caused fluids from A, B, and C to converge at the cross intersection. The beads were consequently focused and detected using the LIF system described above.

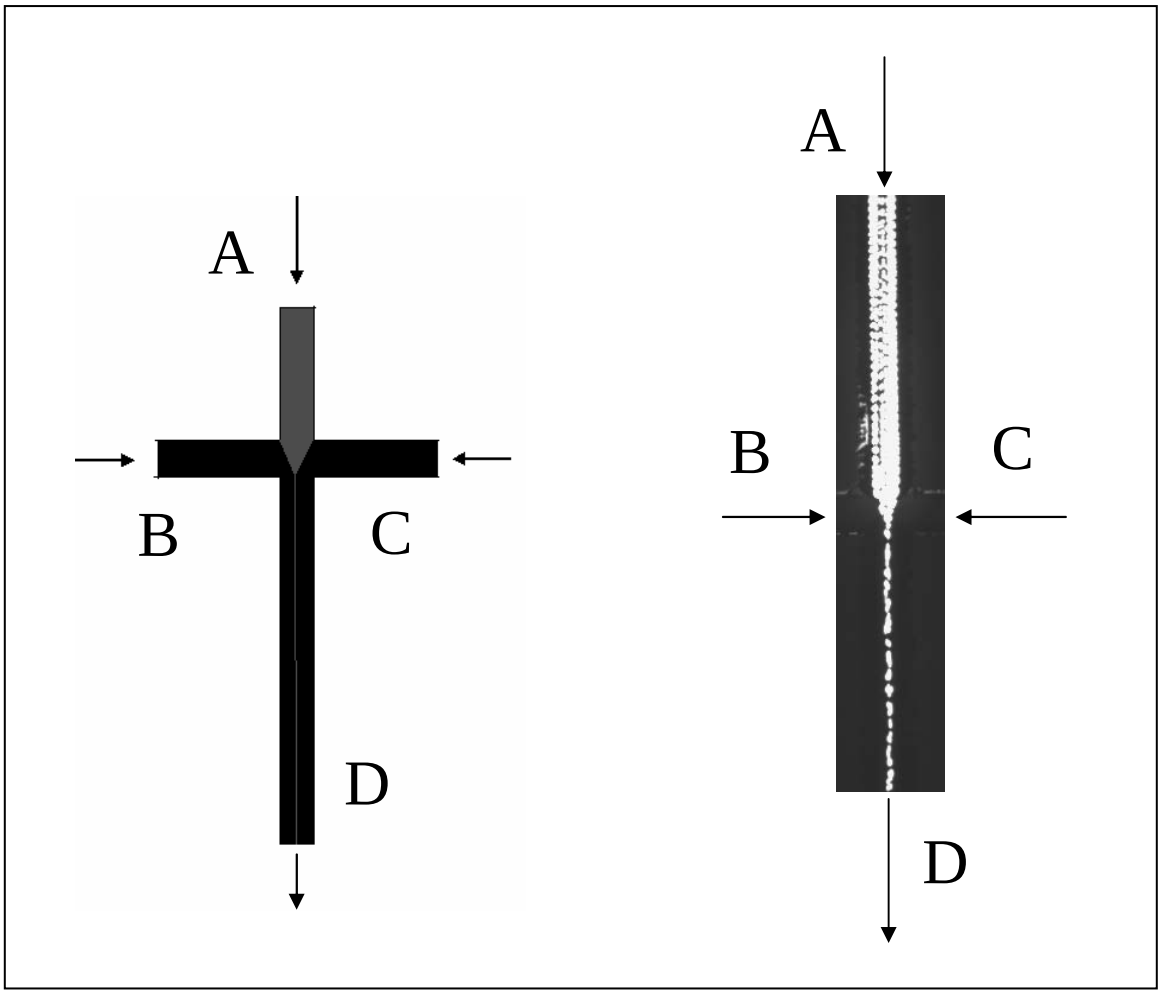


Figure 1: Schematic of bead cytometry experiment, and CCD imaging of 10 micron spheres being focused at the cross intersection.

RESULTS:

Bead cytometry was performed on two of the three flights, June 30th and July 1st. Figure 2 below shows an example of the bead cytometry experiment at 0.026 g. There was no significant difference between the cytometry experiment at 0.026 g, and 1.768 g. The particle velocity was 2.3 meters per second and the volumetric flow rate was 0.036ml/s. To properly sample the rapidly flowing beads, the data acquisition rate used was 20 kHz. The maximum particle count rate at this data acquisition rate is about 3,000/s. Our experiment had a count rate of 200/s with an average intensity of 0.95±0.4. The velocity of the beads, maximum count rate, average and standard deviation of the intensity and flow rate can be seen in Table 1.

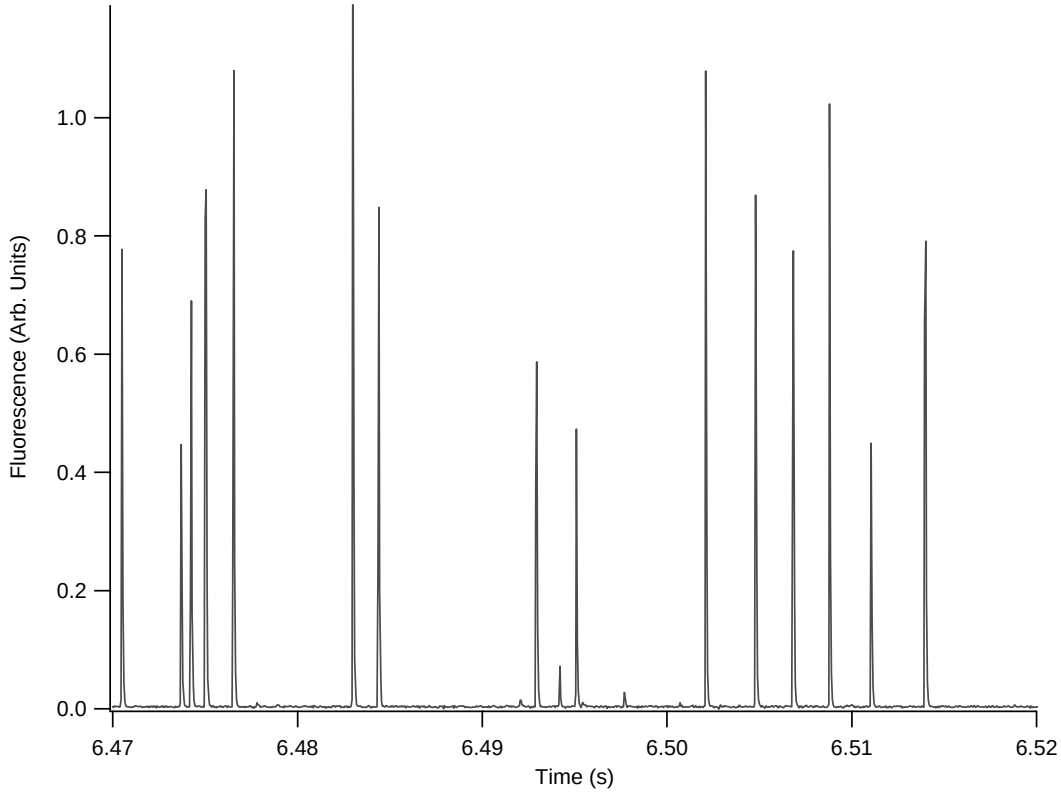
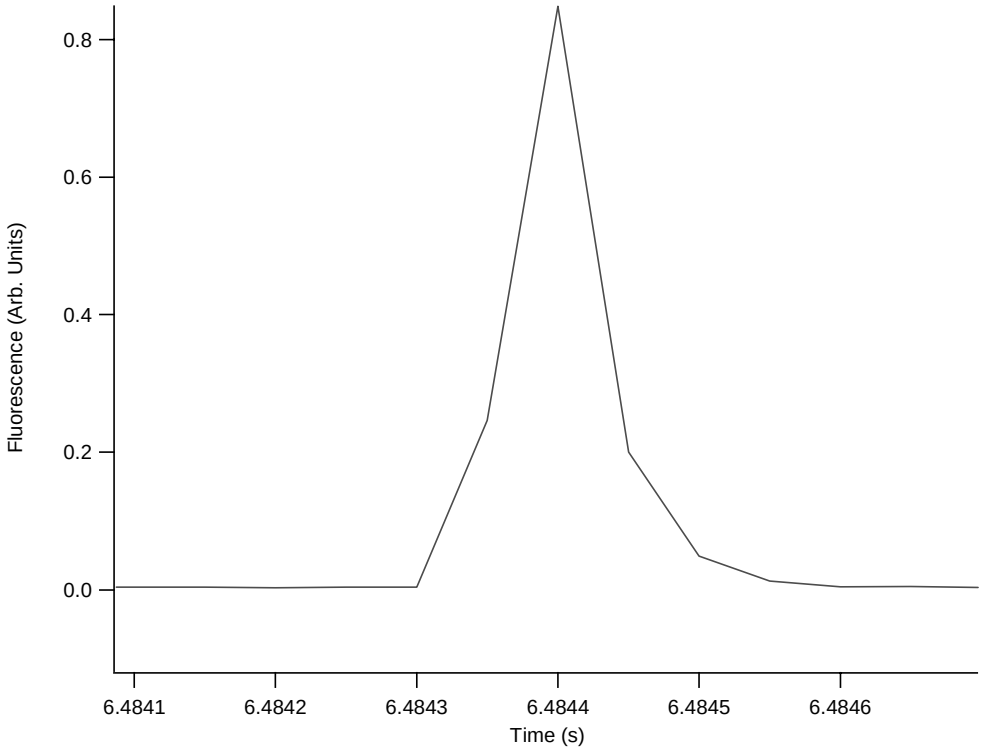


Figure 2: 10 seconds of bead cytometry at 0.026 g



The final flight on July 2nd was used to attempt to complete an experiment integrating a bioreactor with a microfluidic device to perform an on-line separation of amino acids within the bioreactor. The separation was working on the ground prior to the flight, but unfortunately an electrical failure with the laser catastrophically caused the experiment to fail. This problem was solved by integrating a new 30 mW laser that has a longer lifetime for future flights.

DISCUSSION:

This data shows that it is possible to perform bead cytometry in reduced gravity environments. The throughput of the device is absolutely outstanding with respect to the number of beads it can count per second, although the standard deviation of the mean amplitude of the fluorescent signal is relatively high. This suggests that the focusing at the intersection needs to be tighter to assure that the particles pass through the interrogation beam single file.

Table 1: Summary of Results for Bead Cytometry Experiments

Bead Velocity	2.3 m/s
Max. Count Rate	3,000 bead/s
Fluidic Flow Rate	0.036 mL/s
Average Peak Amplitude	0.9+/- 0.4

PHOTOGRAPHS:

JSC2004E28082 to JSC2004E28085
JSC2004E28254
JSC2004E28286
JSC2004E28399 to JSC2004E28401

VIDEO:

- Zero g June 29 – July 2, 2004, Reference Master: 718394

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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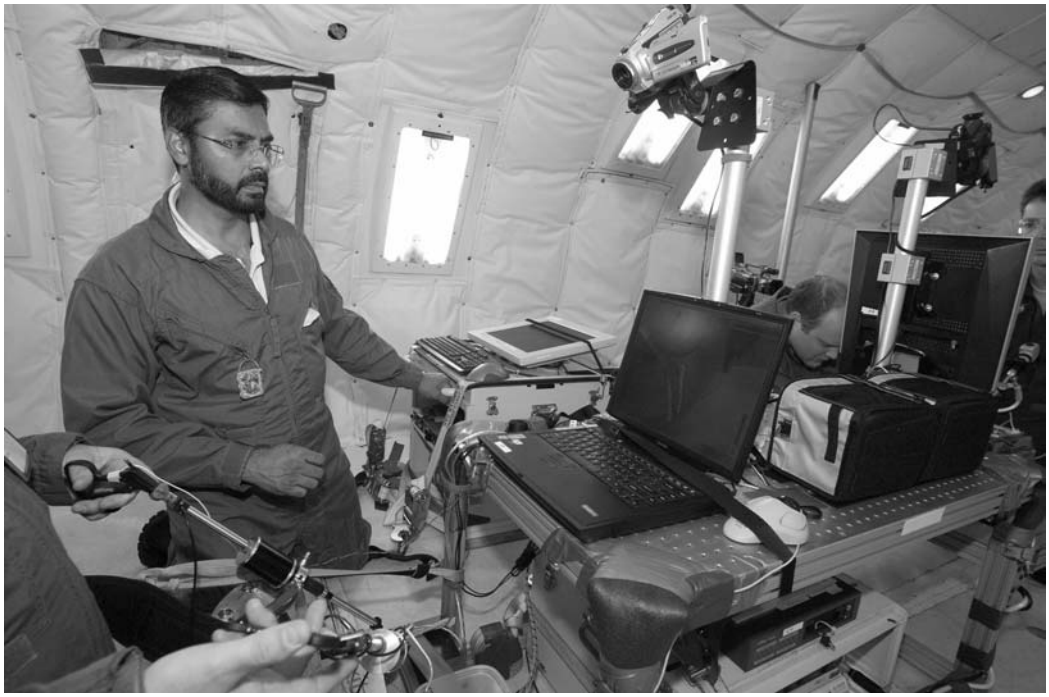
2.3 Surgical Task Execution in Microgravity: Ergonomic and Logistical Features Measured with Advanced Informatics

FLIGHT DATES:
June 29 – July 2, 2004

PRINCIPAL INVESTIGATOR:
Ronald C. Merrell, Virginia Commonwealth University

CO-INVESTIGATOR:
Azhar Rafiq, Virginia Commonwealth University



INTRODUCTION:

In this experiment, virtual reality (VR) and inanimate simulators were evaluated with regards to the assessment and training of basic surgical skills. Each participant performed the selected skill on the simulator as efficiently and effectively as possible within 25 seconds. Twenty five seconds was chosen so that evaluation would be completed during the 30 seconds of microgravity that occurs at the top each parabola. As detailed below, these experiments required 32 of the 40 available parabolas during each of 5 KC-135 flights. The basic premise of this research was to evaluate computer-based VR surgical skill assessment and training in the microgravity environment.

The **hypothesis** was that distinct differences in task strategy and task performance in microgravity compared to Earth gravity could be analyzed and adjusted to conform to clinical needs for surgical skills during flight missions. Additionally, we hypothesized that with a

structured simulation-based training program medical and non-medical flight crew members can be trained to carry out autonomous medical care during flight.

BACKGROUND:

Clinical care for flight crew in practice currently is based on incomplete knowledge of many facets such as pharmacodynamics, pharmacokinetics, decompression illness or gastrointestinal complications. The practice of on-board medical care is targeted towards the most common medical problems based on risk estimates. Delivery of effective and standard health care during spaceflight requires development and implementation of clinical capabilities in microgravity. To implement standards of care all crew members will require extensive training with microgravity simulation scenarios of basic surgical skills to provide autonomous therapeutic care.

Training for the capability to carryout autonomous clinical care will require integration of parameters that reflect the logistics, operability and reliability of performing clinical protocols during flight. The performance of therapeutic procedures in microgravity is one of the more difficult issues in designing in-flight clinical capabilities. The inclusion of basic surgical skills in training protocols will allow the crew to manage medical emergencies effectively. In the event that the crew medical officer may become incapacitated cross training of other flight crew members becomes paramount.

Logistical concerns to perform even minor surgical procedures by flight crew in weightlessness include technique, instrument deployment and operator/patient restraint. To fully evaluate the requirements for developing effective training protocols and simulation interfaces we used an inanimate workstation replicating laparoscopic surgical practice inclusive of tool placement with direct line of sight of the monitor displaying the digitized image of the operative field. The performance of four basic surgical skills in microgravity during parabolic flight was compared to conducting the identical tasks in standard gravitational environment on the ground. Microgravity environment was established with the use of the NASA KC-135 aircraft undergoing parabolic flight. The flight pattern provided repetitive 25-second windows of weightlessness, preceded and followed by periods of 1.8g’s as the plane regained altitude for the next parabola.

Tasks in space are not informed by gravity cues and are generally accomplished with more difficulty and very much more slowly than on Earth. This is certainly the case of surgical skills as demonstrated in the Medical Informatics and Technology Applications Consortium’s earlier work. However, the factors that erode performance should be identifiable and circumvented by well-designed ergonomic solutions.

METHODS AND MATERIALS:

In this experiment, VR and inanimate simulators were evaluated with regards to the assessment and training of four basic surgical skills. Each participant on the KC-135 flight was asked to perform the selected skill of either knot tying on the inanimate station or dissection on the VR workstation as completely as possible within 25 seconds. The task evaluation required a total of 32 of the 40 available parabolas during each of 5 KC-135 flights. The experimental method is presented below.

During day 2-5 the eight participants were paired in order to undergo microgravity skill assessment on the KC-135. Initially, all study participants underwent baseline surgical skill assessment at JSC using an inanimate simulator and the LapSim surgical dissection simulator

prior to their flight. The assessment of each participant on both simulators was in random order. Evaluation on the inanimate and VR simulators included the degree of task completion with each of the surgical skill performed in 25 second intervals. Following baseline skill assessment, participants were tested that same day on the KC-135 flight for skill performance. Thus two participants were evaluated for microgravity skill assessment on each of 4 separate flights spread out over four days. During these flights, the participants conducted skills via an inanimate simulator and LapSim surgical simulator. As both simulators were used concurrently, a total of 32 parabolas were required per flight to complete skill assessment of the 2 participants. Finally, post flight, all participants underwent final skill assessment at JSC via the inanimate simulator, and LapSim simulator.

RESULTS:

We implemented all methods specified above.

All 8 participants were able to perform assigned surgical skills and evaluate the logistics of performing minimal invasive surgical skills during microgravity. We were able to obtain analyzable data from all subjects in the study.

The quantitative analysis of the result indicates that performance of knot tying skills on the inanimate station and gall bladder dissection on the VR simulator was degraded to the extent that time required for task accomplishment was extended beyond expected limits. In analyzing the performance at the inanimate station the skill of knot tying was achieved with great effort due to a lack of sufficient pre-flight training. This was reflected in a greater amount of time required to complete the task and the lack of managing laparoscopic tools with ease of motion. In surveying the participants it was noted that the lack of force feedback for the laparoscopic tools was a hindrance in effectively tying the knots. There was no difficulty in viewing the flat screen monitor and capability to translate the two dimensional image into a three dimensional motion.

In comparison, performance in the virtual reality software for surgical task performance was more difficult for the participants. Considering that the performance was based on the ability of the software to perform adequately it was noted that the image refresh rate was not realistic. Additionally, the software did not allow for equal movements of the tools. This translates to the fact that a millimeter of hand motion did not translate to a millimeter of tool movement in the simulation software.

Additional data analysis is continuing to precisely identify the constraints in the workstations and the psychomotor variables influencing task performance in microgravity.

DISCUSSION:

This study revealed that the level of accurate (on target) success for each of the surgical tasks was reduced when performed in flight. This reduction in performance between pre-flight and flight noted in this report is in agreement with earlier studies during the Neurolab Space Shuttle Mission indicating there is a decline in sensorimotor coordination resulting in decreased level of performance. During this mission, astronauts were required to perform a tracking task performance where their hand was to move along a prescribed trajectory while viewing a video monitor. The conclusion was that motor responses become more variable and slowed down during spaceflight. The magnitude of slowing in performance is consistent regardless if the

movements involve whole-arm or fine finger movements. Task performance during parabolic flight confirmed that single task performance was substantially poorer in microgravity.

It should be noted that the task performance in this study required accurate translation of visual cues within a two-dimensional interface in order to maneuver in a three-dimensional environment. In experiments evaluating the capability to objectively evaluate laparoscopic surgical skills and suturing performances following identical training regimen indicated that basic skills could be acquired effectively in a brief course with relation to prior surgical experience. The conclusion is that implementation of simulation interfaces is a viable mechanism for training of surgical task performance in microgravity. With continued training using surgical simulators, spaceflight crews can be trained for basic surgical care and thereby provide effective, autonomous in-flight medical care. In a structured experimental environment, individuals without a medical background can also use simulation to learn basic surgical skills and these skills can be successfully performed in microgravity.

PHOTOGRAPHS:

JSC2004E27345 to JSC2004E27350
JSC2004E27377 to JSC2004E27383
JSC2004E28089 to JSC2004E28092
JSC2004E28098 to JSC2004E28099
JSC2004E28106
JSC2004E28128 to JSC2004E28133
JSC2004E28249 to JSC2004E28253
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JSC2004E28279 to JSC2004E28283
JSC2004E28287 to JSC2004E28290
JSC2004E28366 to JSC2004E28371
JSC2004E28402 to JSC2004E28405
JSC2004E28442 to JSC2004E28435
JSC2004E28447 to JSC2004E28448

VIDEO:

- Zero g June 29 – July 2, 2004, Reference Master: 718394

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.4 Evaluation of Life Sciences Glovebox (LSG) and Multi-Purpose Crew Restraint Concepts

FLIGHT DATES:
June 28-30, July 1, 2004

PRINCIPAL INVESTIGATOR:
Mihriban Whitmore, NASA/Johnson Space Center

GOAL:

The goal of this investigation was the assessment of the adequacy of different restraint types for various onboard configurations.

OBJECTIVE:

The primary objective was to evaluate the usability of multiple crew restraints for use with the Life Sciences Glovebox (LSG) and for performing general purpose tasks. Since this flight was follow-on to the March 2004 flight evaluation, the primary objective included the testing of refined designs from the March flight, as well as some new design concepts for LSG and general purpose use. Secondary objectives included: (1) the evaluation of target sizes for a tablet computer, (2) usability of a speech-based procedure navigation tool, and (3) audio recording of typical voice commands for post-processing by a voice recognition system under development. These tasks were used as representative onboard tasks to be performed during evaluation of the general purpose restraints.

INTRODUCTION:

Within the scope of the "Multi-purpose Crew Restraints for Long Duration Spaceflights" project, funded by Code U, it was proposed to conduct a series of evaluations on the ground and on the KC-135 to investigate the human factors issues concerning confined/unique workstations, such as the design of crew restraints. The usability of multiple crew restraints was evaluated for use with the Life Sciences Glovebox (LSG) and for performing general purpose tasks. The purpose of the KC-135 microgravity evaluation was to: (1) to investigate the usability and effectiveness of the concepts developed, (2) to gather recommendations for further development of the concepts, and (3) to verify the validity of the existing requirements. Some designs had already been tested during a March KC-135 evaluation, and testing revealed the need for modifications/enhancements. This flight was designed to test the new iterations, as well as some new concepts. This flight also involved higher fidelity tasks in the LSG, and the addition of load cells on the gloveports.

METHODS AND MATERIALS:

Test Conductors

A group of 4 test conductors (evaluators) per day conducted the KC-135 usability evaluations, which occurred over 4 days. Each evaluator was certified to fly onboard the KC-135 by passing a Class III physical and completing the physiological training course. The evaluators were recruited from the NASA Johnson Space Center, and included two crewmembers.

Apparatus

KC-135 test articles, including the crew restraints, Life Sciences Glovebox (LSG) mock-up, and support hardware, were built per the KC-135 User's Guide. Four camcorders were flown to record video/audio data from the entire evaluation for postflight analysis. The following items were also used during the test:

- Laptop computers
- Tablet PC
- Audio tape recorder

In addition to restraint design changes, the fidelity of the LSG tasks was also increased. Actual LSG equipment (i.e., Data Input Device, Integrated Control Panel, OptiCells, syringes) was borrowed from the NASA Ames Research Center to use in the evaluation.

Restraints: Six restraint concepts were evaluated. One LSG restraint concept had multiple configurations to allow for adjustability and comfort. Components of the LSG restraint that were evaluated included: foot plates, thigh bar and lumbar support (see tripod structure in Figure 3). An additional LSG restraint concept [Texas A&M University (TAMU) Roller bar] had roller bars for the feet and waist. Three multi-purpose restraint concepts were also evaluated - an augmented handrail, padded socks, and toe loops. A bungee cord was also provided for the evaluators to use on the last flight day (per crewmember suggestion). On this day, participants used the foot plates and wrapped the bungee cord around their back and through the handle, routing the cord in front of them and attaching to the other handle (see bungee cord in Figure 3). While it was recognized that this violated one of the initial LSG requirements (no attachment to the LSG), the investigators thought it was worth testing the basic concept of an elastic "belt" support.

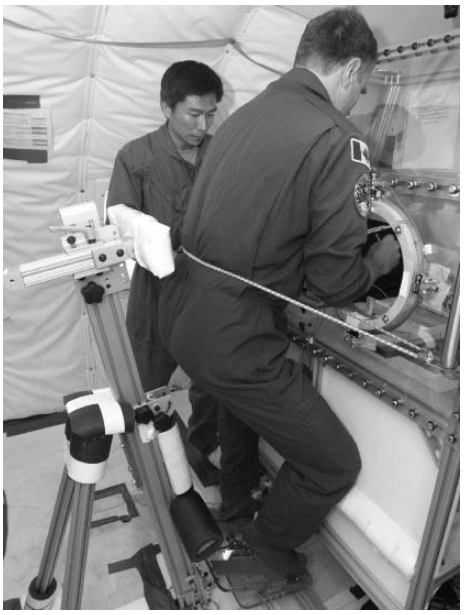

Figure 3. Evaluator using bungee cord and foot plates.

LSG Mock-up: The same mock-up used on the KC-135 flights in March was used during this evaluation, except that load cells were added to the gloveports for these flights. This was done in an attempt to quantify forces placed by crewmembers' arms on the gloveports while working in the LSG. These data will help determine how well a restraint supports the crewmember during glovebox tasks.

A scale-based questionnaire was developed for postflight administration. This questionnaire addressed the user interface issues and comfort of the crew restraint concepts.

Procedure

The restraints were evaluated in one of three different work areas on the workstation as shown in Figure 4: primary LSG (front), secondary LSG (side) and multi-purpose (back). A minimum of 30 parabolas per flight were dedicated for data collection. The remaining 10 parabolas were reserved for inadvertent disruptions such as turbulence or hardware problems. In-flight video (full body and close up views) of the tasks was recorded for postflight analysis. The questionnaire was administered post flight.

Tasks: The evaluators at the primary work area were performing simple representative glovebox tasks while in the LSG restraint. These tasks included: simulated filter change out by removing a foam core square from the back of the LSG work volume, and operations with the Data Input Device (DID), Internal Control Panel (ICP) and Opticells. The DID involved the use of a glide point computer input device; the ICP involved physical switch operations and the OptiCell injecting fluid into and from an OptiCell container.

The evaluators at the multi-purpose restraint site (back) were performing one of a number of technology evaluations while using a number of different restraints. These included evaluating pointing target sizes for a tablet computer, evaluating a speech-based procedure navigation tool, and making a voice recording.

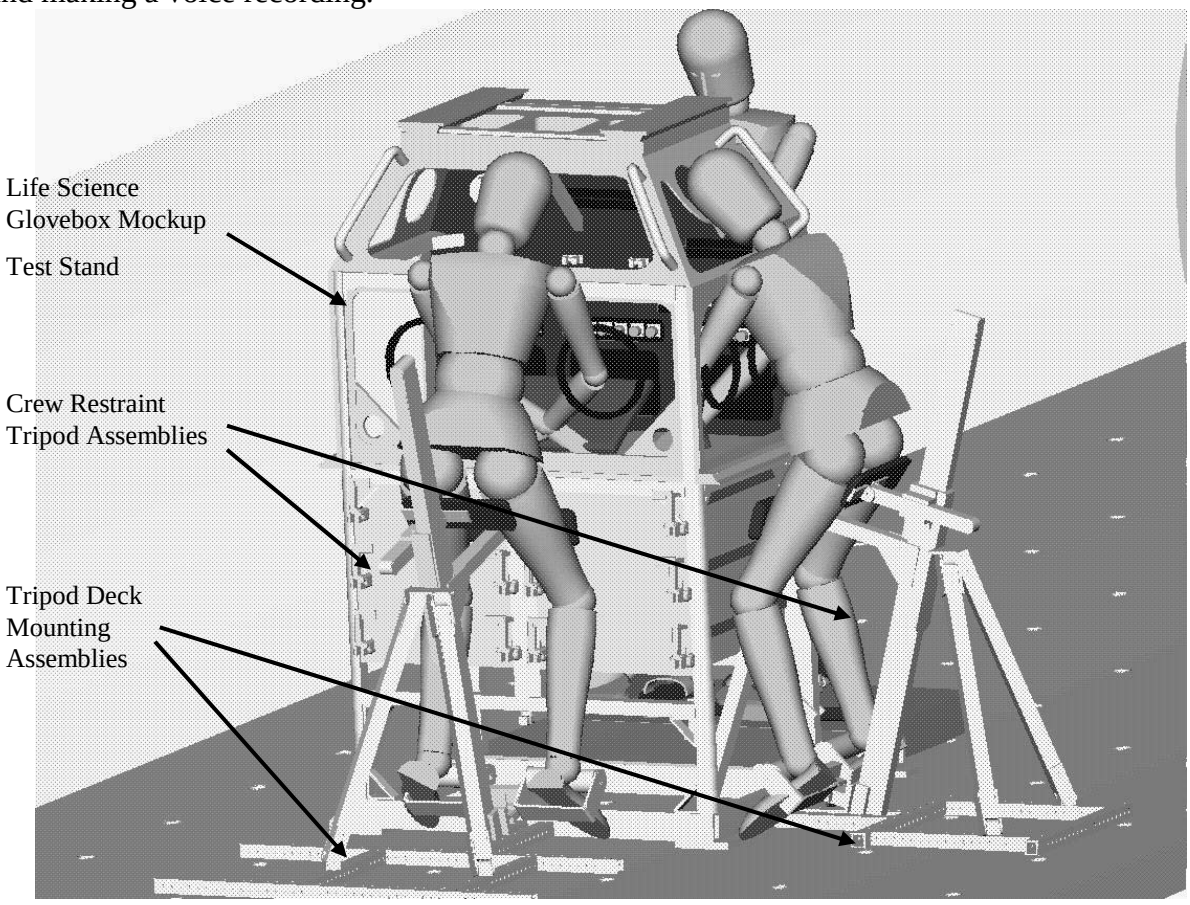

Figure 4: Test Configuration – View from Forward / Port Life Sciences Glovebox Test Stand

RESULTS:

Sixteen of the ratings gathered on the questionnaire were organized into two categories: Restraint Usability and Comfort. Ratings within each of the categories were very similar, and so were collapsed to produce two key metrics. Table 2 shows the two key metrics averaged over all participants.

Table 2. Ratings for Average Usability and Comfort.

Average Restraint Usability (ease of ingress/egress, stability, ease of performing operations, etc.)	**3.3**
Average Comfort Rating (comfort ratings for all parts of the body)	**3.5**

(1 = Needs Improvement, 5 = Excellent)

Summary of key comments from the evaluators:

- Restraint placed tall evaluators too high to perform glovebox operations comfortably.
- Thigh restraint hindered ability of shorter evaluators to reach to the back of the glovebox work volume.
- Thigh restraint prevented evaluators from floating into the LSG mockup.
- The foot plate strap should be wider and elastic.
- The bungee/foot plate combination worked well.
- The TAMU roller bar restraint had fabrication difficulties and did not function as intended

An in-depth analysis of the load cell data is currently in progress. Preliminary results indicate that whenever reaching is involved, there is an increase of force on the gloveports. This data will be beneficial to the LSG team in that it will provide some indication of the forces likely to be put on the glovebox when in use on ISS. An example graph of the load cell data is presented in Figure 5.

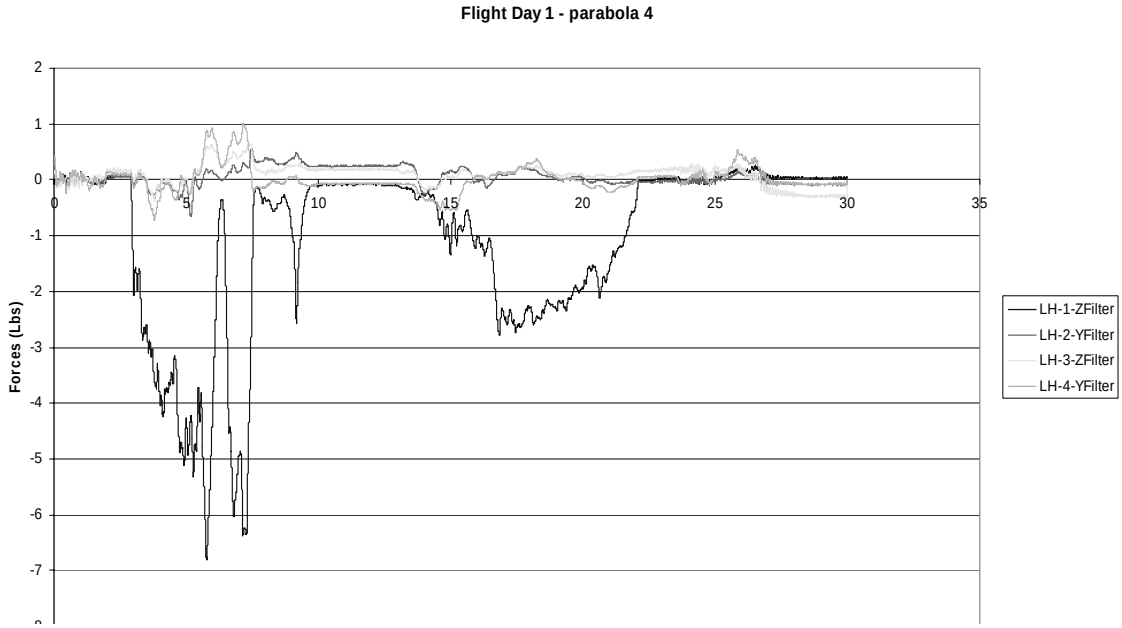

Figure 5. Graph of load cell data obtained from the left hand gloveport during parabola 4 on flight day 1.

The graph shows two periods of negative forces indicating times when contact was made with the left gloveport. The negative forces mean the load cell was in compression, so the evaluator during this parabola was pressing on the gloveport for stability.

Preliminary general purpose restraints results were as follows:

- The padded socks got mixed reviews. They were liked by some evaluators, but not others.
- The padded handrail performed well. Evaluators stated that it provided good stability and was very comfortable. It was also very easy to ingress and egress.
- The foot loops performed well.

Secondary Objectives

Target Size

Results showed that the smallest size targets (12 x 12 pixels) produced unacceptably high error rates. Further analyses are underway, and the data are being compared to ground-based pointing data before final conclusions are drawn.

Speech-based Procedure Navigation Tool

- It was difficult to hear the computer voice.
- Computer did not respond to some voice commands.
- Evaluators had to repeat themselves to get computer to respond.

The data and recordings were turned over to the principal investigators who developed the system.

Audio Recording of Voice Commands

- The recordings were turned over to the principal investigator who is developing the system.

CONCLUSIONS:

One of the problems seen to a greater degree in this evaluation was that very tall participants were unable to adequately adjust the restraint height (This flight had generally taller flyers). They had to crouch in order to place their hands/arms inside the glove ports. While the height of the footplates was adjustable, the limited height of the KC-135 cabin prevented adjustment beyond a certain point. The thigh restraint worked well for some and not as well for others; thus it was advantageous that it was optional (could be folded down). One of the comments captured on the questionnaire stated that the more experience you have in microgravity, the less restraint is required. The evaluators stated that the combination of the bungee cord and foot plates allowed for better access to areas within the LSG work volume. The bungee cord was stated to be very comfortable and provided the best posture for the evaluators. The implications of these comments need to be studied further, since attachment to the LSG is currently considered undesirable. However, the design concept of a flexible/elastic back support should be investigated further.

The padded handrail solution was probably the best of all the general purpose restraint concepts. It is a simple design that utilizes a hardware component already onboard ISS (handrail). It just involves the addition of padding. The rigid bar also appeared to result in less toe flexing than the foot loops, and therefore, probably longer term comfort.

PHOTOGRAPHS:

JSC2004E27355 to JSC2004E27357
JSC2004E27360
JSC2004E27384 to JSC2004E27385
JSC2004E27392 to JSC2004E27393
JSC2004E27395
JSC2004E28086
JSC2004E28093 to JSC2004E28095
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JSC2004E28104 to JSC2004E28105
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JSC2004E28113
JSC2004E28116
JSC2004E28121 to JSC2004E28127
JSC2004E28658 to JSC2004E28665
JSC2004E28278
JSC2004E28294 to JSC2004E28297
JSC2004E28406 to JSC2004E28415
JSC2004E28424 to JSC2004E28434
JSC2004E28446
JSC2004E28443 to JSC2004E28444

VIDEO:

- Zero-g June 29 – July 2, 2004, Reference Master: 718394

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.5 Microgravity Effects on Plant Boundary Layers

FLIGHT DATES:
June 29 – July 2, 2004

PRINCIPAL INVESTIGATOR:
Gary Stutte, Dynamac Corporation, Kennedy Space Center, FL

CO-INVESTIGATOR:
Oscar Monje, Dynamac Corporation, Kennedy Space Center, FL

INTRODUCTION:

The goal of these series of experiment was to determine the effects of microgravity conditions on the developmental boundary layers in roots and leaves and to determine the effects of air flow on boundary layer development. It is hypothesized that microgravity induces larger boundary layers around plant organs because of the absence of buoyancy-driven convection. These larger boundary layers may affect normal metabolic function because they may reduce the fluxes of heat and metabolically active gases (e.g., oxygen, water vapor, and carbon dioxide. These experiments are to test whether there is a change in boundary layer associated with microgravity, quantify the change if it exists, and determine influence of air velocity on boundary layer thickness under different gravity conditions.

METHODS AND MATERIALS:

Experiment Description

The following experiments were performed as part of the Microgravity Effects on Plant Boundary Layers project on the KC-135:

Rates of temperature change in microgravity from wet and dry wicks at various wind speeds were measured.

Rates of temperature change in microgravity from monocot (wheat) and dicot (radish) plant leaves with and without forced convection were measured.

Oxygen consumption rates in stagnant and stirred water containers in microgravity were measured. Tthese rates were compared to O_2 consumption rates in a moist substrate in microgravity

The results from this experiment provided insight into how microgravity-induced changes in boundary layers affect mass and heat transport from bulk air to plant organs (leaves and roots). Similar experiments have flown twice aboard the KC-135 aircraft, first as a part of the ISS PESTO experiment in 2000, and again as a Fluid Physics-Plant Growth Experiment on a combined flight of Advanced Life Sciences and Fundamental Biology experiments in early 2004.

Hardware Description

Oscar Monje, Ph.D., was the technical lead for these experiments and designed the payload hardware package and has been responsible for post-flight analysis of data.

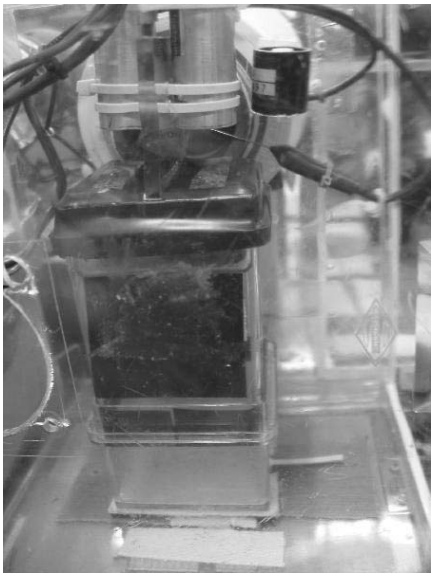
The experiment hardware consists of an aluminum base plate with a small rack that contains the plant chambers, fan, accelerometer, data logger, light and video equipment. The base plate was bolted to the floor prior to take-off and has the accelerometer, data logger, fan, and light attached. The video equipment and plant chambers were set up upon reaching level flight and were re-stowed prior to landing. All tools were located in a tool kit contained in the Reduced Gravity Program Offices' yellow container during takeoff, parabolas and landing. Access to the tool kit was not required during parabolas. Operators used straps for restraint during the parabolas.

Figure 6. The experimental configuration on the aluminum base plate.

The aluminum base plate provides structural support for crash loading and has flown numerous times aboard the KC-135. Figure 1 shows the experiment configuration on the aluminum base plate.

RESULTS

Experiment One: Microgravity effects on boundary layer development on wet/dry filter paper.



Objective

Changes in boundary layer resistance will affect heat and mass transfer rates of surfaces to air flowing above them. Quantifying heating rates of these cellulose surfaces (filter paper) in differential g's (1g, 0g, 2g) allows the hypothesis that boundary layers increase under microgravity conditions. The effects on boundary layer development were determined. Infrared thermometers measured the effects of changing gravity conditions (1g, 0g, 2g) on filter paper temperature at different levels of forced convection (wind tunnel) during the KC-135 parabolic flight. These measurements will allow the effects of gravitational forces to be quantified, and to establish whether gravity alters the impact of air velocity on boundary layer disruption.

Data collection

The *g* force was measured with an accelerometer, the air-flow with an anemometer, air temperature with thermocouples, and filter paper temperature with infrared thermometers.

Observations

The experimental hardware performed well during the parabolic flights. Temperature difference ($T_{surface}$-T_{air}) data was recorded during the 0g, 1g, and 2g at 0, 0.2, 0.4, 0.6, 0.8, and 1.0 m/s wind

speeds. The convective heat transfer for the dry surface increased as wind speed increased. However, for a wet surface the convective heat transfer increased only until the wind speed reached 0.4 m/s. At wind speed greater than 0.4 m/s, the convection decreases, probably because less heat eas transferred through convection, but rather through evaporation.

Conclusion

The boundary layer, as measured by temperature difference, was greater under 0g conditions than either 1g or 2g conditions. The effects of increasing air velocity on boundary layer were less in microgravity than 1g for both wet and dry surfaces. These data are consistent with the hypothesis that boundary layers in microgravity are greater than they are under 1g. The effects of microgravity on boundary layer increases could be mitigated by increasing air velocity to 0.4 m/s over the surface.

Experiment Two: Effects of microgravity on boundary layer development of monocot and dicot leaves.

Objective

Changes in boundary layer resistance surrounding plant organs will affect leaf transpiration and the resulting evaporative cooling of the leaf surface. Measuring the heating rates of plant canopies under differential g conditions (1g, 0g, and 2g) should allow changes in boundary layer resistance to be estimated. Hypothesized increases in boundary layer resistance under reduced gravity conditions should disappear as air velocities increase. These assumptions were tested by measuring changes in leaf temperature at different levels of forced convection (wind speed) and thermal load (light intensity) during parabolic flights on wheat (monocot) and radish (dicot) leaves.

Data collection

The g force was measured with an accelerometer, the air-flow with an anemometer, air temperature with thermocouples, and canopy temperature with infrared thermometers. Canopy temperature difference ($T_{surface}$-T_{air}) data was recorded during the 0g and 1g at various wind speeds over wheat and radish canopies. Light levels were measured with a light meter.

Observations

The experimental hardware performed well during the parabolic flights. Preliminary results indicate that it took about 3-4 parabolas for canopy temperature to reach equilibrium following a change in light level at constant wind speed. Generally, the plant canopies became hotter as the wind speed flowing above the leaves was reduced. The ground study was conducted in an environment (air temperature and relative humidity) simulating those in the plane using the

Orbital Environment Simulator chamber at KSC. Data analysis comparing the 0g and 1g data is underway.

Experiment 3: Determine the effects of microgravity on boundary layer development in rooting media.

Objective

Changes in boundary layer resistance may affect the supply and removal of metabolic gases (O_2, CO_2) to roots. It is hypothesized that a larger boundary layer in microgravity results in hypoxia in the root zone, which can become limiting to plant growth. If the boundary layer increases during microgravity, then the rate of O2 diffusion to the O2 sensor should decrease. To test this hypothesis, O2 concentrations in 1 mm glass bead media were measured during parabolic flight with and without forced convection above the root zone. It was hypothesized that forced air would reduce the boundary layer, and increase rate of diffusion, and that this change would be proportional to distance from the surface.

Data collection

The *g* force was measured with an accelerometer, the air-flow with an anemometer, and air temperature with thermocouples. Oxygen concentration was measured using two different types of sensors: galvanic oxygen sensors and Root Oxygen Bioavailability (ROB) sensors embedded in 1 mm glass beads.

Observations

The experimental hardware generally performed well during the parabolic flights. Unfortunately the ROB sensor output was outside the range of the data logger and valid data was not obtained. The galvanic O_2 sensors performed well. It was observed that it required 5-6 parabolas for the galvanic sensors to equilibrate following a change in wind speed. The Galvanic sensors indicated that the aeration in the root zone decreases only slightly (~0.3%), however, their output appears to be influenced by the atmospheric pressure changes in the plane, and changes in cabin air temperature. The methods for correcting for these changes are being formulated. The Galvanic sensors also appear to respond to air movement near the air intake of the sensors and dead air volume may confound data analysis.

Conclusion

The temperature and pressure dependence, as well as the response to air currents near the intake of the galvanic sensors needs to be better characterized in order to interpret the behavior of these sensors in 0g. Once the sensor performance is characterized then they can be used to test the hypothesis that boundary layers are increasing in the root zone.

Fractional g testing

During the 4^{th} day of the campaign, a request for two 0.16 (lunar) and 0.33 (mars) g parabolas to be performed was approved and fractional g data for Experiments 2 and 3 was obtained.

During these parabolas, the data suggests that the differential g forces result in a proportional effect on boundary layer development around the leaf and in the root zone. However, these data are very preliminary, and interpretation difficult due to lack of O_2 sensor stability after only 2 parabolas. However, these results are intriguing and proposed for future parabolic flight testing will be proposed under fractional *g* conditions.

ACKNOWLEDGEMENT:

Funding for this work was provided through an NRA grant (NNK04EB08A) from NASA's Office of Biological and Physical Research (OBPR) Fundamental Biology Program (FBP).

PHOTOGRAPHS:

JSC2004E27361
JSC2004E282118 to JSC2004E28120
JSC2004E28256 to JSC2004E28257
JSC2004E28298 to JSC2004E28299
JSC2004E282416
JSC2004E28423
JSC2004E2845

VIDEO:

- Zero-g June 29 – July 2, 2004, Reference Master: 718394

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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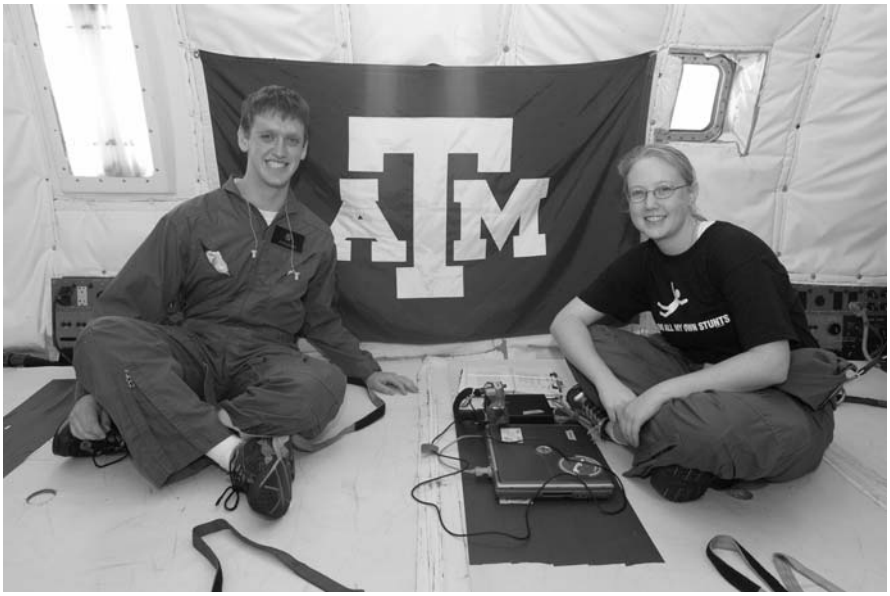
2.6 Undergraduate Program Flights – The Effects of Microgravity on Ocular Movement

FLIGHT DATES:
July 13 -14, 2004

PRINCIPAL INESTIGATOR:
Alicia M. Rutledge, Texas A &M University

CO-INVESTIGATOR:
Allison L. Barnard, Texas A &M University
Jesse A. Bowes, Texas A &M University
Chelsey A. Dankenbring, Texas A &M University



GOAL:

The primary objective of this experiment is to learn how well the human eye can track words while reading in microgravity. Spaceflight observations have shown that during microgravity, fluids relocate to the body's extremities: the arms, legs, and head. The hypothesis of this experiment is that the swelling of ocular blood vessels during periods of microgravity will reduce the subjects' eye motion.

OBJECTIVES:

This experiment will quantify what effects, if any, microgravity will have on subjects' ocular movements, specifically changes in their reading abilities. Determining these effects will allow greater insight into the capabilities of the human body to adapt to extreme environments and process visual signals. It will serve as the basis for future studies of the effects of prolonged periods of microgravity on skills such as reading, and aid in the development of programs to more fully prepare astronauts for the many difficulties they will encounter in microgravity. Test results might also prove useful in the creation of more user-friendly space mission products, such as larger print manuals or changes in control panels, to ease the negative effects of the microgravity environment.

METHODS AND MATERIALS:

Protocol Design

The Visagraph II consists of a pair of goggles (see Figure 7) attached to a computer. The goggles use infrared to track the subject's eye movement while reading.

Figure 7: Visagraph II Goggles[1]

Prior to arriving at Ellington Field, each test subject was required to read passages from the Visagraph II text booklet while wearing the Visagraph II goggles in a 1g environment. The goggles track the subject's eye movement, and the data was recorded on a laptop computer.

During take-off, the flyers stowed all Visagraph II equipment in the provided padded storage locker. This equipment was not removed from stowage until the test director said it was safe to do so.

While sitting on the floor of the KC-135 test section, using straps to restrain their legs, the team then conducted the experiment during each microgravity segment of the flight, silently reading the same passages from the Visagraph II text booklet as read during in the 1g environment. The subjects continued to read until the passage was finished or until the plane finished the microgravity segment whichever came first. No experiments were conducted during the 2g portion since the team was not seeking to gather data during this segment and also because

attempting to work during the 2g portion of the flight could have induced motion sickness. The test subjects continued the experiment by reading a different passage during each microgravity segment until twenty parabolas were flown or until physically incapacitated by motion sickness. At the completion of the flight, the data recorded by the laptop from this experiment was to be analyzed by the team and compared with the results from the 1g environment.

Equipment

The equipment used in this experiment is comprised of a pair of Visagraph II goggles, assembled by the manufacturer, a Visagraph II text booklet, and a Dell Inspiron 5100 laptop computer to acquire data. The goggles can be seen in Figure 7.

The goggles use infrared sensors to track the subject's eyes while reading. A subject slips them on and they are adjusted to his/her inter-pupillary distance. No further calibration or adjustment is needed.

The Visagraph text booklet is a pamphlet containing various passages suitable for readers of all ages and levels. Subjects will read from this booklet during the experiment.

The laptop computer, assembled by the manufacturer, will be provided by a team member. It will be used to collect and record the test data.

Pre-Flight Training and Baseline Data Collection

One month before the actual flight, the team will begin baseline data collection. This normal gravity data will be needed for comparison with that taken in microgravity. Twenty to thirty preflight experiments will be conducted in order to reduce error and familiarize the team with the equipment and procedure. Baseline data collection will be performed at the Texas A&M University Spacecraft Technology Center.

Procedure:

1. Attach Visagraph II goggles (see Figure 7) to laptop computer
2. Open Visagraph II software, configure for subject, turn video camera on
3. Subject slips on goggles, adjust to his/her inter-pupillary distance
4. Tester starts software
5. Subject silently reads passage from text booklet until finished
6. Tester saves data
7. Repeat 15-20 times

In-Flight Activities

Procedures:

1. Remain secured in passenger seats for two parabolas to adjust to new sensation
2. Before parabolas begin, remove equipment from cases and secure to users
3. During first two parabolas, allow time to adjust to sensation of microgravity
4. At start of third parabola begin testing
5. Subject begins reading from booklet at beginning of each microgravity parabola, continues until he finishes passage or when plane finishes parabola
6. Tester takes readings from software and saves to a specified file
7. Check subject's comfort level

8. Steps 4-6 are repeated for twenty parabolas or until subject is incapacitated by motion sickness

Equipment used:

1. Visagraph II goggles
2. Visagraph II text booklet attached to clipboard
3. Laptop computer

Approximate duration of testing:

Testing will consist of one session of twenty parabolas; one to two hours total.

Number of subjects, number of experiment tests: 4,1

The experiment will be run once each on two subjects, on two separate flights.

Post-Flight Activities

This experiment did not require any post flight testing. The team will spent post flight time engaged in saving data from each flight, recalibrating the goggles for the next subject, and recharging all necessary batteries.

RESULTS:

Visagraph readings for two subjects, referred to as A and B were compiled into the following table:

Table 1: One-g readings vs. Microgravity readings

Item of Interest	Subject A			Subject B		
	one-g	µg	error	one-g	µg	error
Average Fixations/100 words	62.50	60.00	4.00%	52.64	83.17	57.98%
Average Regressions/100 words	5.33	6.75	26.56%	4.79	21.83	356.22%
Average Span of Recognition (words)	1.86	1.76	5.43%	2.31	1.26	45.35%
Average Duration of Fixation (sec)	0.21	0.19	8.80%	0.24	0.18	25.69%
Cross Correlation	0.97	0.83	13.82%	0.99	0.63	36.13%

These results are discussed in the following section.

DISCUSSION:

In order to explain this experiment in full, a brief discussion of the parameters used to evaluate the subjects is required. Five parameters of interest were observed and tracked using the Visagraph II: (1) average fixations per 100 words; (2) average regressions per 100 words; (3) average span of recognition; (4) average duration of fixation; and (5) cross-correlation. *Fixation* refers to the number of times (for every 100 words) the subject's eyes stop for a split second and fixate on a word or words. *Regression* means the number of times a subject's eyes traveled from right to left instead of the usual reading pattern of left to right. *Span of recognition* is simply the number of words – one word is defined here as five letters – the subject recognizes in one fixation, and the *duration of fixation* is the time a subject's eyes linger during a fixation. Finally, *cross-correlation* refers to how well the subject's eyes move in synch.

Readings of all five parameters were taken in both normal gravity (on the ground) and during the microgravity segments of flight aboard the KC-135 aircraft. Both subjects were tested repeatedly and the results averaged. Table 1 contains the mean average of each data set.

As one can readily observe from Table 1, Subject A had less than ten percent error on three parameters: average regressions, average span of recognition, and average duration of fixation. Subject B, however, had over twenty percent error on all five counts. This discrepancy could be attributed to many factors: for instance, Subject A is female and Subject B is male. Perhaps women's eyes function more clearly in microgravity. Also, Subject A neglected to take the offered anti-nausea medicine while Subject B took it before the flight. Perhaps something in the medication influenced his performance. Subject B's extremely large percent error for regressions suggest that he had difficulty concentrating. Perhaps the flight was such an intense experience that he simply could not focus on the reading material, or was distracted by what seemed to be a kind of euphoria among the student researchers.

Though all of these explanations are somewhat plausible, the simplest and therefore most likely explanation is that both the subjects were not necessarily responding to microgravity itself, but rather to the sudden change in their perception of gravity. To attempt reading soon after one has made the transition from 2g to microgravity is no easy feat. In fact, Subject A was incapacitated by motion sickness after approximately fifteen parabolas. Her test readings for the parabolas that occurred at the time she was ill may be suspect for errors because of her lack of concentration.

During post-flight video review, subjects were observed to be moving their heads while reading. As the Visagraph II is meant to be used while the subject is reading using *only* his/her eyes, subject head movement probably contributed greatly to the data error.

Also contributing to the data errors is the fact that the Visagraph II equipment did not always function reliably. The cables were not secured as tightly as they should have been, and the connections came loose on several occasions. This fact alone makes some of the data suspect.

CONCLUSION:

Though this experiment did provide useful information about the effects of gravity changes and motion sickness on human ocular movement while reading, it did not entirely succeed in its goal to examine human ocular movement in microgravity. The results indicate that we need to further standardize future experiments; for instance, an implement similar to a vise could be constructed to restrict head movement. Also, the Visagraph equipment itself needs to be carefully examined for the best possible arrangement in microgravity, hopefully preventing future equipment errors. We hope to fly subsequent derivatives of this experiment and look forward to gaining a better understanding of ocular movement in microgravity.

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JSC2004E30015 to JSC2004E30016
JSC2004E30047

VIDEO:

- KC Undergraduate Flights, Group A, July 13 -14, 2004, Reference Master: 718428

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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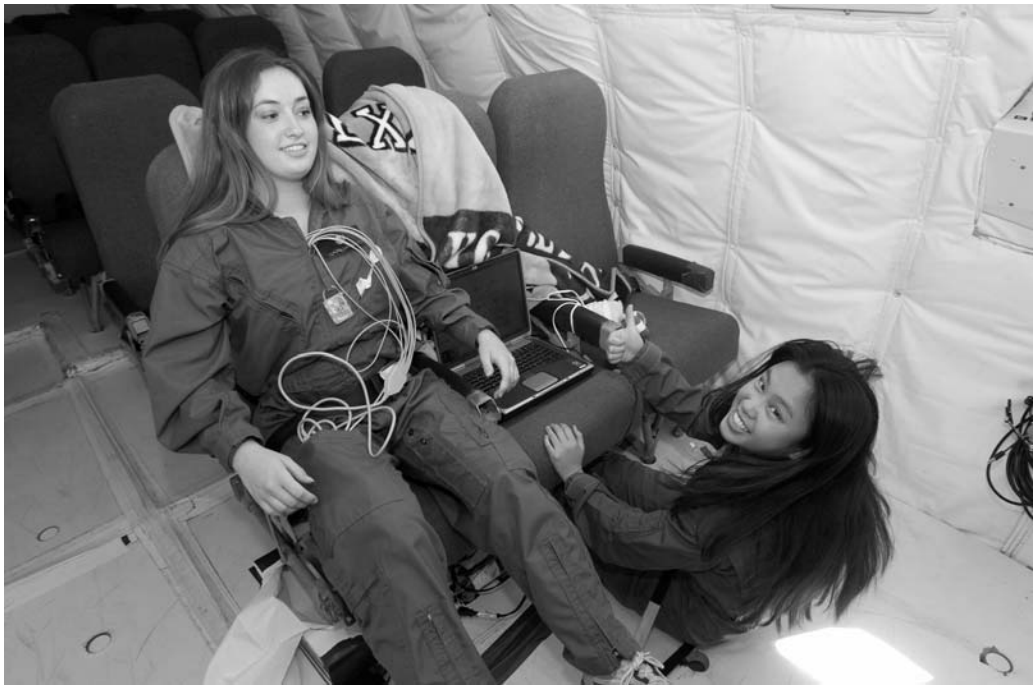
2.7 Undergraduate Program Flights – Effect of Acute Changes in Gravity on 12-lead Electrocardiography

FLIGHT DATES:
July 13 -14, 2004

PRINCIPAL INVESTIGATOR:
Madhurita Sengupta, University of Texas - Austin

CO-INVESTIGATORS:
James Rexroth, University of Texas - Austin
Laura Sarmiento, University of Texas - Austin
Waralee Sattam, University of Texas - Austin



GOAL:

Our main goal in conducting this experiment is to gather and analyze data pertaining to the effects of gravitational change on the human heart. Once data acquisition has been performed, we intend to analyze each test subject's recordings in order to differentiate the effects of acute microgravity and hypergravity on the electrocardiograph (ECG) recordings.

OBJECTIVES:

Parabolic flight offers the unique opportunity to experience actual micro- and hypergravity. The analysis of the acquired data will contribute to the understanding of how 12-lead ECG recordings reflect the effects of these environments. Although the changes occurring in the 12-lead ECG

during parabolic flight may resemble those occurring during postural changes in 1g, it would be premature to state that the 1g-related postural changes can "precisely" predict the gravity-related changes. Our data should aid aerospace medicine professionals in recognizing whether astronauts and cosmonauts are responding normally to the acute (early) portions of spaceflight in the future.

Several investigators have previously used 3-lead electrocardiography to describe changes in heart rate observed during parabolic flight. For example, it is well known that heart rate decreases upon entrance into parabolic microgravity and increases upon entrance into parabolic hypergravity [1-3]. On the other hand, to our knowledge, there are no published reports regarding changes in the full 12-lead ECGs of parabolic flyers. For instance, it seems very possible, based upon changes in cardiac electrical axes that occur during postural changes in 1g[4], that acute shifts in electrocardiographic axes will also occur during parabolic micro- and hypergravity. However, the determination of any such axis-related shifts during parabolic flight would, of course, be best performed using an electrocardiograph with more than two channels. In addition, although changes in QT intervals have recently been described in astronauts returning from long duration spaceflights[5], changes in QT and other electrocardiographic intervals during parabolic flight have not been described, in detail, in the past. In this study, we continuously collected 12-lead electrocardiographic data before, during, and after parabolic flight, in order to better define the changes that occur in the 12-lead electrocardiogram in response to acute changes in gravity.

Pre-flight, each team member participated in continuous, five minute, 12-lead ECG recordings in both the supine and seated positions to provide reference data for the in-flight ECG recordings. Post-flight, all pre- and in-flight data acquired by the ECG software were analyzed and compared.

One of our working hypotheses is that the QRS frontal plane axis will shift relatively toward the left during parabolic microgravity and then relatively toward the right during parabolic hypergravity. This hypothesis is based upon known shifts that occur in the QRS axis after transitions between the supine and the upright positions in 1g[4]. Moreover, in healthy individuals, an increase in relative sympathetic nervous system activity, such as might be expected during hypergravity[1], postural change, a submaximal bicycle exercise stress test[6], or a cold pressor test[7] on the ground, usually decreases not only the RR interval, but also the QT interval and the T-wave amplitude[8]. Another working hypothesis is therefore that the QRS and QT intervals and T-wave amplitudes will increase during parabolic microgravity, although not necessarily to clinically pathologic levels, and then decrease again during parabolic hypergravity.

Statistically speaking, as an *n* (number of subjects) of 4 provides too little data for drawing firm conclusions, a follow-up flight that would ensure an *n* of at least 6 is desired.

METHODS AND MATERIALS:

Prior to flight, in the spring of 2004, all team members participated in continuous 12-lead ECG recordings before, during and after a supine-to-seated change in posture. Each subject's ECG recording began with the subject in a supine position for five minutes, after which the subject slowly transitioned into a seated position (analogous to the transition between micro- and hypergravity). The subject was then seated for five minutes (analogous to the effects of relative hypergravity), before transitioning back into a supine position for one minute (analogous to the transition between hyper- and microgravity). Monitoring the subjects' ECG continuously

throughout all pre-flight postural changes in 1g has allowed for reference data for later comparisons to the in-flight data.

During the flight, both the initial test subject (test subject #1) and the initial operator (test subject #2) were pre-instrumented with stress-test-style adhesive electrodes and a set of standard 12-lead ECG lead wires. To reduce potential noise from the limb leads, the Mason-Likar lead configuration was used[7]. Considering the flight program consists of two sets of ten parabolas, a set of five or seven parabolas, and then another set of either five or seven parabolas (all sets with a 1g turn-around period in between), we collected continuous 12-lead ECG data on each team member (in the seated position) for at least one set of ten parabolas. At the second turn-around, the lead wire hub from test subject #1 was disconnected from the CARDIAX recorder, the initial operator's (test subject #2) lead wire hub was connected, and ECG data were collected continuously for at least one set of ten parabolas. As previously mentioned, these data were compared to terrestrial ECG data collected using the same hardware and software that was used during flight. Data collection continued during the period of straight and level flight, as well. Digital photography was used in order capture enough still and video data to ensure that experimental procedure was not compromised.

Since we were allowed to occupy the first row of seats aboard the aircraft, the laptop computer and the small, lightweight ECG hardware (about the size of a typical Holter recorder) was secured to the floor of the aircraft or to one of the seats using Velcro. All wires were carefully duct-taped to the aircraft floor to prevent any compromising safety situations.

The operator was responsible for aiding the subject in any way necessary and for ensuring that the data were appropriately collected. At the onset of the second turn-around period, when the team members "switch out," the previous test subject assumed responsibility for saving the data and starting another set of data for the new subject.

At the end of the last 20 parabolas, team members disconnected and powered down all equipment and were ready for return to Ellington Field.

PRELIMINARY RESULTS:

All subjects displayed similar patterns regarding heart rate. As predicted, the heart rate of each individual decreased in response to microgravity and increased in response to hypergravity. P and T axes shifted acutely during parabolic microgravity and hypergravity, and all subjects displayed consistent patterns with respect to Pd and PQ throughout the flight. In addition, all subjects' QT intervals elongated, within non-pathological levels, during parabolic microgravity, and shortened during parabolic hypergravity. However, discrepancies occurred with respect to changes in the QRS intervals of the subjects, as shown in figure 8; data from three out of four subjects supported the hypothesis that the QRS interval would lengthen during microgravity and shorten during hypergravity. Furthermore, some subjects' QRS axes did not follow the changes as outlined in the hypothesis. Two out of four subjects exhibited a cardiac frontal plane shift relative to the right during microgravity, rather than a shift to the left as hypothesized. An example of this shift to the right is shown in figure 9. Similarly, in these same two subjects, the QRS axes shifted to the left in the hypergravity environment. Additional data are presently being analyzed.

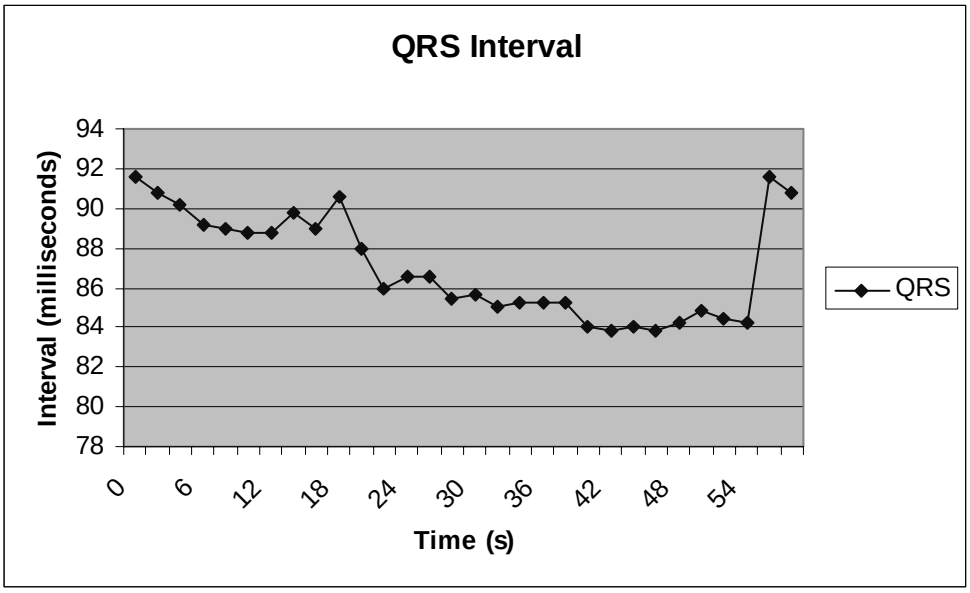


Figure 8. QRS interval during microgravity (10 – 18 seconds) and hypergravity (24 – 56 seconds).

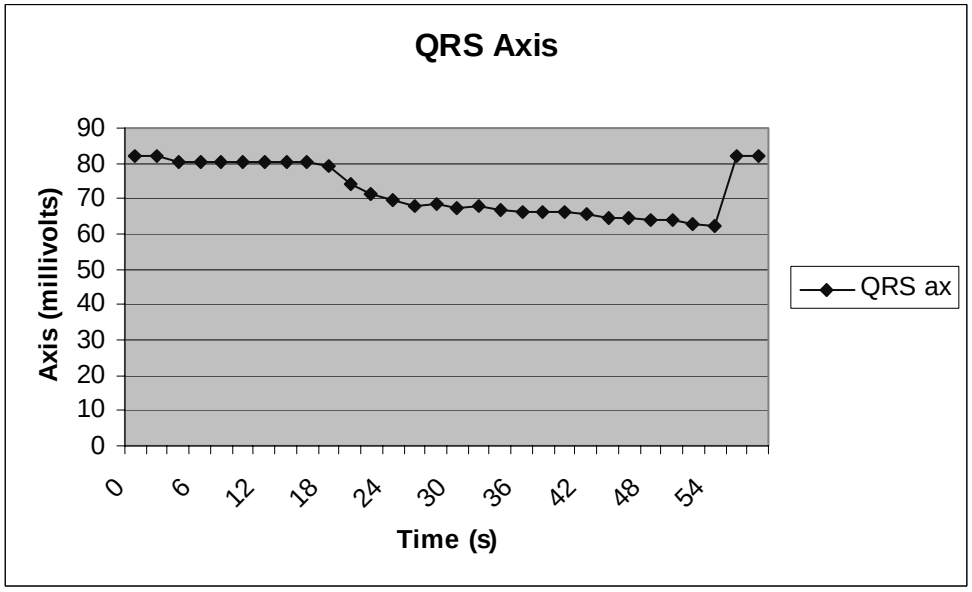


Figure 9. QRS axis during microgravity (10 – 18 seconds) and hypergravity (24 – 56 seconds).

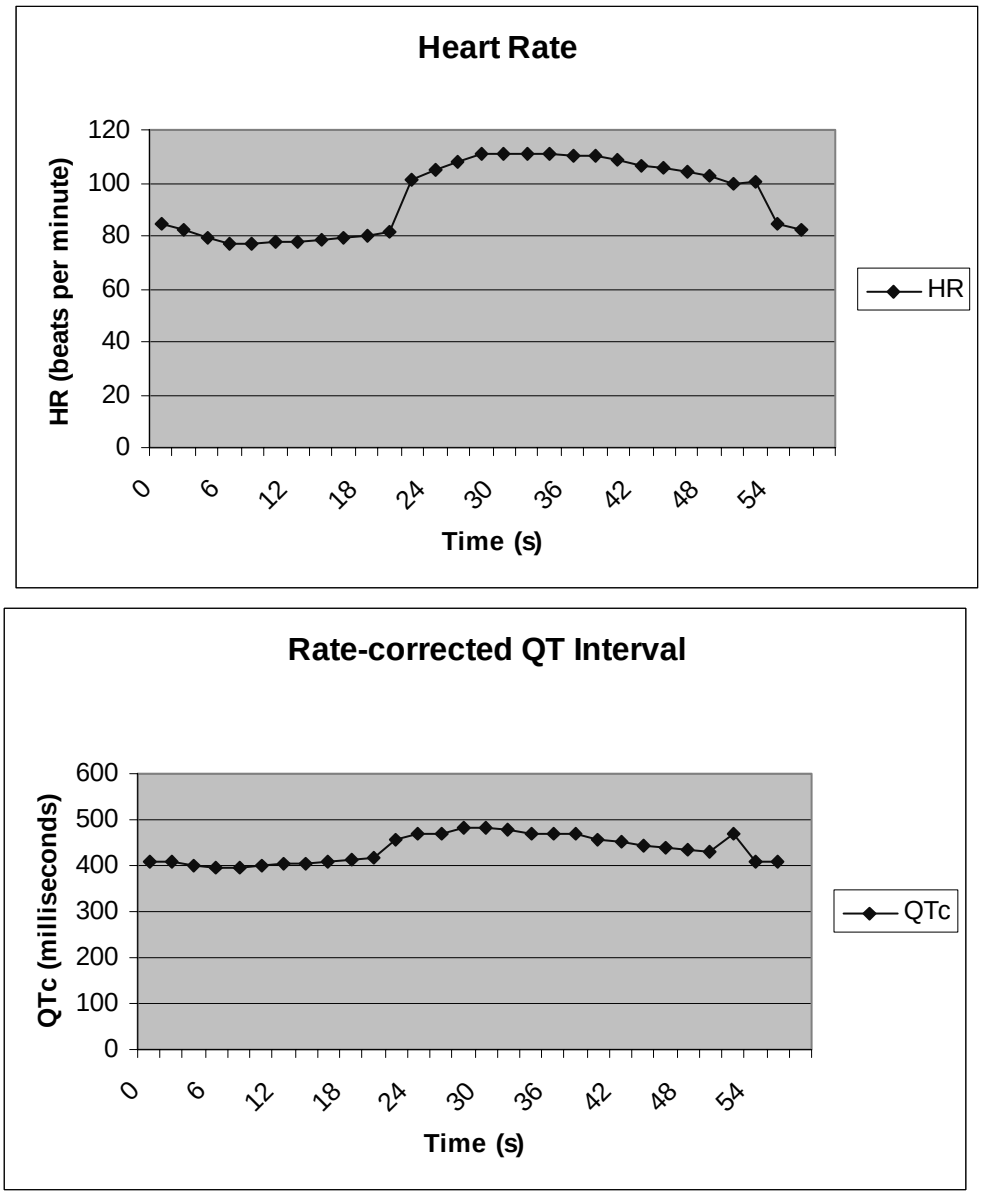


Figure 10 Comparison between heart rate and rate-corrected QT interval during microgravity (10 – 18 seconds) and hypergravity (24 – 56 seconds).

DISCUSSION:

The most interesting finding from this study thus far is that directional shifts in the frontal plane QRS axis did not always occur as predicted. QRS axis shifts have been observed after transitions between supine and upright positions in $1g$[4]. Based on these observations, we had hypothesized that the frontal plane QRS axis would shift relatively to the right during parabolic microgravity and relatively to the left during parabolic hypergravity. Although this pattern was seen in two of the four subjects, the other two subjects did not have such a pattern, and in fact had the opposite pattern.

As expected, the heart rate of each individual decreased in response to microgravity, and increased in response to hypergravity. These changes in heart rate in likely reflect baroreflex-mediated cardiovascular control mechanisms that serve to maintain circulatory homeostasis during changes in venous return due to changes in ambient gravity[2].

Our hypothesis that the QRS and QT intervals would lengthen during parabolic microgravity (although not to clinically pathologic levels), and then shorten during parabolic hypergravity, was generally verified. However, the changes in the QT interval may simply reflect those simultaneous changes in the RR interval. In addition, changes in the QRS interval in one of the four subjects were not consistent with the hypothesis, and thus the data at this point provide insufficient evidence to draw any firm conclusions. Further data collection, using a greater number of test subjects, may yield more conclusive results with respect to changes in the QRS interval and frontal plane axis.

At the present time, we are also analyzing those changes that occurred in heart rate-corrected QT (QTc) intervals during this study (e.g., using Bazzett's formula). However, it should be kept in mind that Bazzett's formula (or any other "generic" correction formula for that matter) is itself problematic due to individual differences in actual QT-RR relationships. It is well known for example that Bazzett's formula often tends to overcorrect the QT interval for any given change in the RR interval. QT intervals during microgravity and hypergravity in this experiment have not yet been corrected for RR interval influences, as exhibited by the similarities between the graphs of heart rate and rate-uncorrected QT interval in one of the subjects (figure 10).

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JSC2004E30023
JSC2004E30025
JSC2004E30047

VIDEO:

- KC Undergraduate Flights, Group A, July 13 -14, 2004, Reference Master: 718428

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

2.8 Undergraduate Program Flights – Soybean Sugar Extraction through Innovative Blending Design

FLIGHT DATES:
July 13 -14, 2004

PRINCIPAL INVESTIGATOR:
David Chipman, Iowa State University

CO-INVESTIGATOR
Jonathan Gettler, Iowa State University
Clayton Neumann, Iowa State University
Dustin Lunde, Iowa State University
Kevin Schroeder, Iowa State University
Russ Uthe, Iowa State University
Cheryll Reitmeier, Iowa State University,

GOAL:

The goal of this experiment is to test the blending efficiency of the proposed blender in microgravity and compare the results with ground based experiments.

OBJECTIVES:

The objectives of this project were to design, build, and test a blender that will function in a microgravity environment.

INTRODUCTION:

The purpose of this experiment is to test the function of a specially designed blender in a microgravity environment. The objective of this research is to explore the effects of microgravity on blending and food processing through the use of an innovative blending technique. The device will blend soybeans in microgravity over varied number of passes under a blending wheel during flight. Soybeans were chosen as our test specimen for their ability to be made into a wide variety of products (i.e., tofu, soymilk, etc.) and because their growth has been extensively studied in space (Watkins 2003). Post flight, the success of each test was evaluated by measuring the concentration of sugar content of each test trial and compared the results to those obtained from ground experiments of the same protocol. It is hypothesized that blending during flight will be equivalent to mirrored ground tests. Data collected from this experiment should provide information about the effects of gravity on the dynamics of the transformation of solid particles (soybeans) to a slurry mash (ground soybeans) and basic food processing techniques.

METHODS AND MATERIALS:

All methods and procedures were kept consistent between ground and flight tests. CyMix blending steps detail the process utilized to operate the "space blender" and mash the soybeans. The data collection procedure outlines the steps required in achieving accurate results. The following guidelines were used throughout the entire experiment, including ground, flight, and post-flight operations. Vinton 81 soybeans were used for this project.

In-Flight / Ground Test Procedures

1. Control Man: Open top compartment hatch and secure it in the open position.
   Bucket Man: Grab the next bag of soybeans to be blended.
2. Both Men: Unclamp the blended bag from the plate.
3. Control Man: Raise the blending wheel using the ratchet strap lift. Position the next bag appropriately.
   Bucket Man: Place the used bag into the cooler container.
4. Both Men: Clamp the new bag to the plate.
5. Bucket Man: Lower the blending wheel using the ratchet strap lift.
6. Control Man: Close and secure the top compartment hatch.
7. Control Man:
   a. Toggle Motor switch to ON.
   b. Turn the Controller switch to ON.
   c. Flip Clutch switch to ON.
   d. Select the appropriate revolution setting (5, 10, 15, 20).
   e. Depress the Start button
8. Control Man:
   a. Disable all revolution setting switches.
   b. Flip Clutch switch to OFF.
   c. Turn OFF the Controller switch.

d. Toggle OFF the motor switch.

Blended Soybean Data Collection Procedure

1. Beans transported and stored in cooler for approximately six hours after flight.
2. Photographs were taken of each tested bag of soybeans.
3. Open the test bag of soybeans.
4. Add 90 mL of room temperature tap water to the test bag to achieve a concentration of 170.1g of blended soybeans per 5 L of water.
5. Reseal bag of soybeans.
6. Gently turn bag for 5 minutes.
7. Filter slurry mash using Kim Wipes as filter paper.
8. Dab filtered solution on to the refraction surface of the refractometer and record the measured value.
9. Clean the refraction surface with tap water and a Kim Wipe.
10. Repeat steps 8 and 9 until 3 measurements per bag have been recorded.

Statistical Analysis

The data was analyzed using a standard test of equivalence. The equivalence region was defined to be 0.80 and the reciprocal of 0.8 (1.25) multiplied by the mean of the values at each pass setting.

RESULTS:

Table 3. Means for the sugar concentration values for each flight day, the combined flight results, and the ground results at each pass setting.

	Flight Day 1 (%Brix)	Flight Day2 (%Brix)	Combined Flight Tests (%Brix)	Ground Tests (%Brix)
0 pass	1.7	2.0	1.8	1.7
10 pass	2.6	3.0	2.8	3.5
20 pass	3.2	4.0	3.6	3.5
30 pass	3.2	3.3	3.2	3.3
40 pass	3.7	3.4	3.5	3.5

Table 3 shows the mean sugar concentrations (%Brix) for each pass setting and gravity environment. The mean sugar concentrations for the combined flight results and ground tests for 0 pass, 20 pass, 30 pass, and 40 pass are equivalent for each pass setting. The mean sugar concentrations for the combined flight results and ground tests for 10 pass are not equivalent.

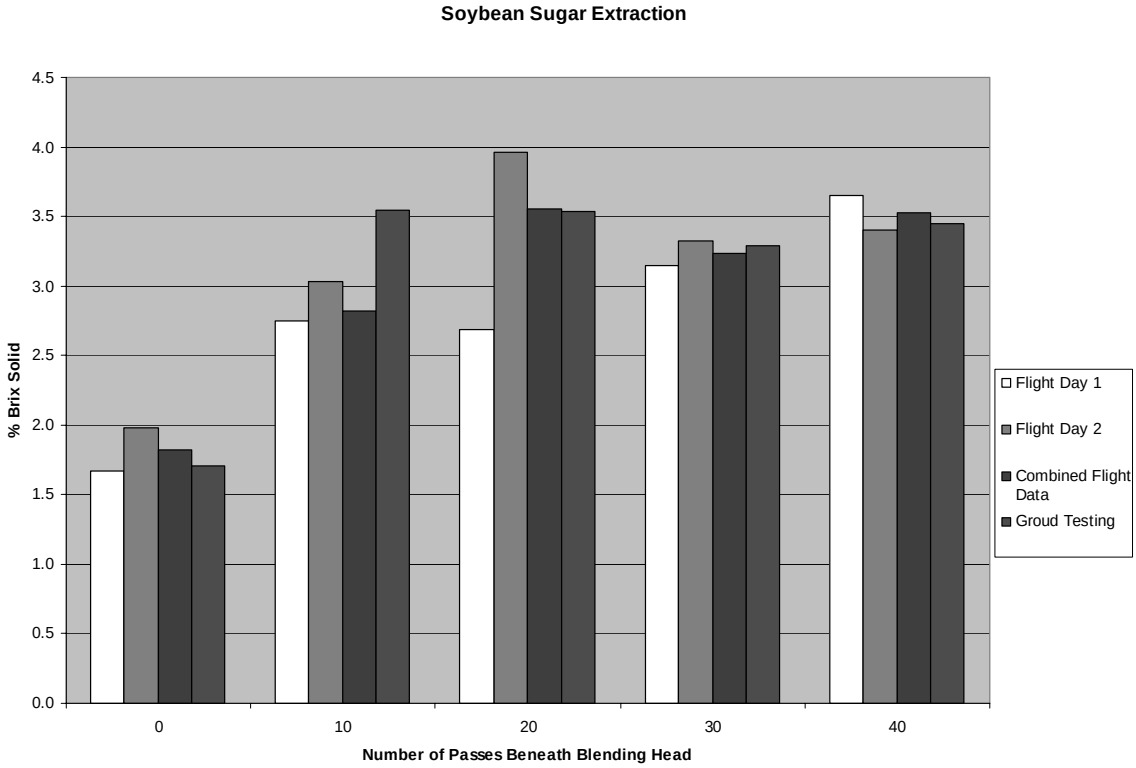


Figure 11. Graph of the mean values for sugar concentration (%Brix) for flight day 1, flight day 2, combined flight data, and ground testing for each pass setting

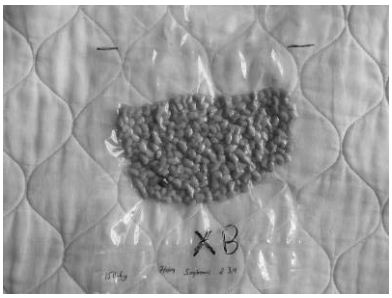
Figure 11 compares the mean sugar concentration between pass settings and test environments. The white and gray columns represent the mean sugar concentrations for flight day 1 and flight day 2 respectively. The blue and red columns represent the mean sugar concentrations for the combined flight data and ground results respectively.

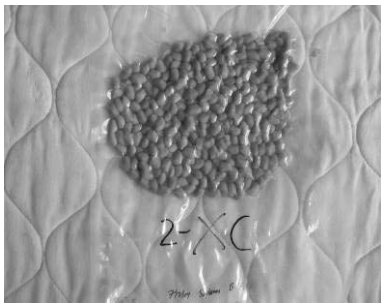
Figure 12 shows are examples of blended soybean bags. The sections are labeled according to the number of passes that the bags were run and the columns represent ground testing, flight day one, and flight day two trials respectively.

Visual Bean Comparison

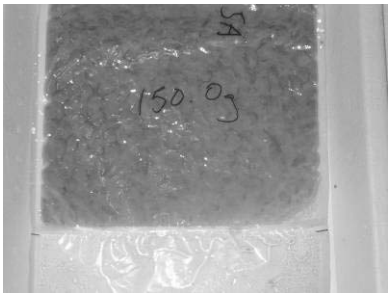
Ground Testing | **Flight Day 1** | **Flight Day 2**

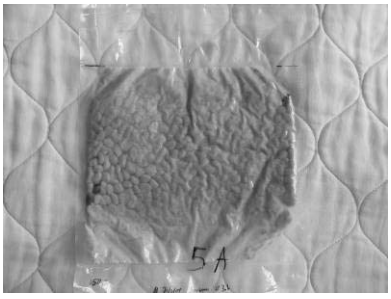
Control Group (Zero Passes)

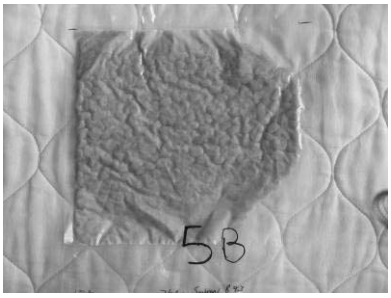


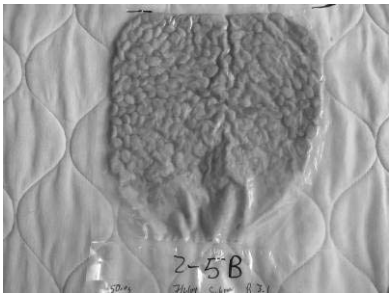


10 Passes

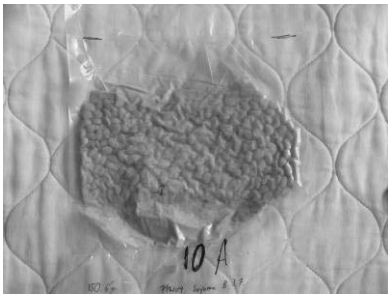


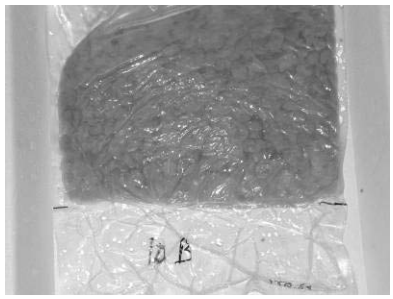






20 Passes

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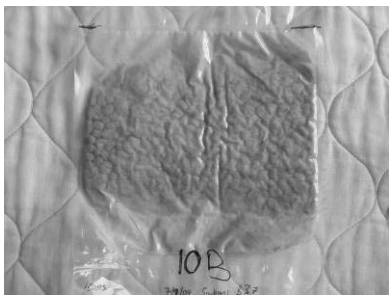
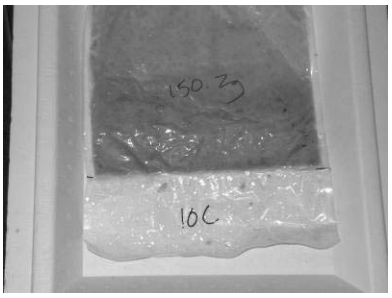
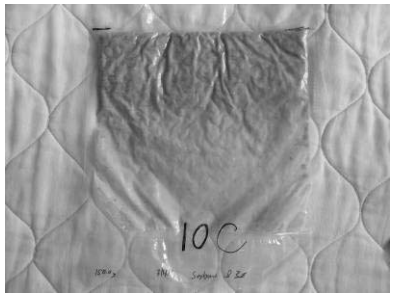

10B

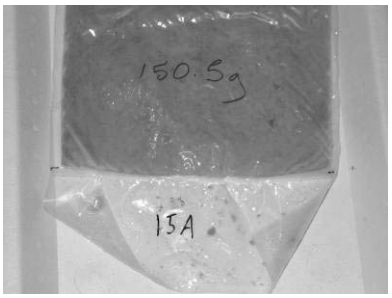

2-10B


150.2g
10C


10C

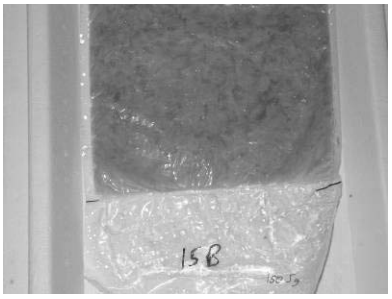

2-10C

30 Passes

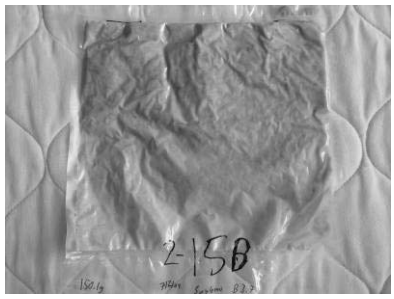

150.5g
15A

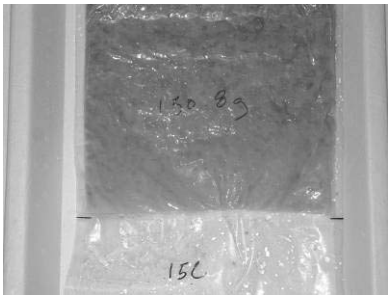

15A


2-15A


15B


15B


2-15B


150.8g
15C


15C


2-15C

40 Passes

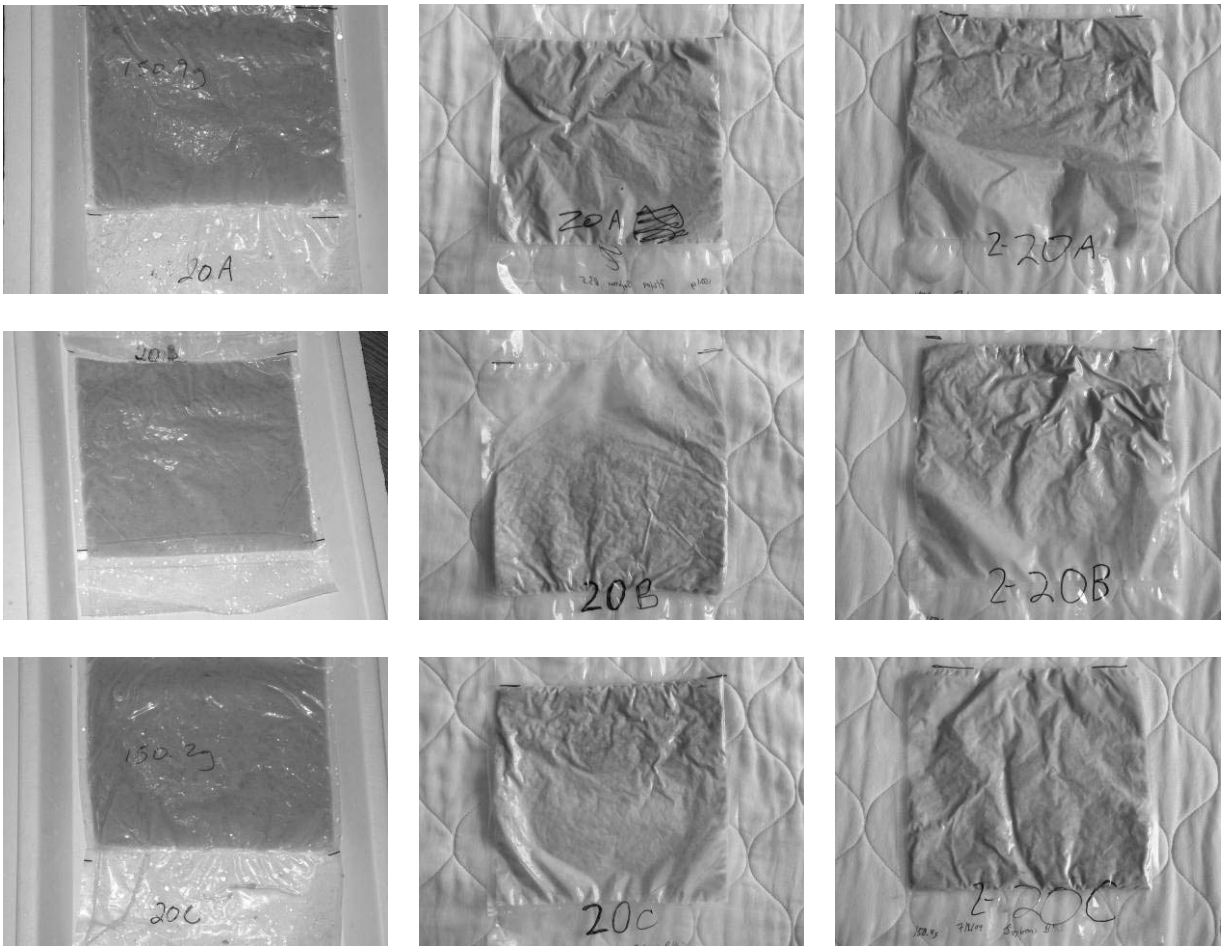


Figure 12 shows selected pictures from each test and pass setting. Visually, one can see the soybeans increase in blending as the number of passes increases.

DISCUSSION:

Analysis of Results

Statistically, the microgravity blender performs the same in microgravity as it did on the ground for all cases but one. All sugar concentration means but the 10 pass setting are statistically equivalent (Table 3). Several factors affecting the blending, including bag misalignment, could be the cause of the difference for the 10 pass settings. These factors are discussed in more detail below.

The 0 pass settings means for both ground tests and combined flight tests were 1.7 and 1.8 % Brix respectively (Table 3). This indicates that the 2g portions of the flight had little or no effect on the packaged soybeans. More broadly, this indicates that the aircraft environment did not affect the packaged soybeans. There is little to no visual difference between the groups either (Figure 11).

The mean sugar concentrations for the 20, 30, and 40 pass settings appear to be similar for both the combined flight tests and ground tests (Figure 11). For the combined flight tests, the 20, 30,

and 40 pass settings average a difference of 1.63 Brix percentage points greater than the control. For the 20, 30, and 40 pass settings for the ground tests, the average difference is 1.73 Brix percentage points greater than the control.

To make soymilk, the desired sugar concentration is about 5 % Brix (Reitmeier). Our lower results are likely due to the limited amount of blending time (30 seconds maximum) and the amount of water used to dissolve the sugar in the soybean mash. We are confident that if allowed to run for longer than the 30 second intervals constrained by the flight and with a proper water mixture, the blender could produce sugar concentration levels close to 5 % Brix.

Quality of Results

Several factors may have influenced the results of the tests and are discussed below.

The soybean bags were frozen for transportation to Houston because the proper equipment for soybean bag preparation was not available to the team in Texas. Before flight, the frozen bags were thawed in a two part process. The beans were moved from the freezer and placed in the refrigerator 15 hours prior to the flight. The beans were then transferred to a cooler for the trip to Ellington Field. The team underestimated the time required for the beans to thaw completely and did not identify that some of the samples were partially frozen when loaded onto the plane. This may have affected the soybean texture causing difficulty during processing, thus possibly resulting in decreased sugar concentrations.

Calibrating the tension for the blending wheel was difficult on the first flight day. It was hard to anticipate the performance of the blender during flight. The blending wheel was adjusted for the second flight day.

Aligning the soybean bags on the blending plate was important for proper blending. Misalignment could cause the beans towards the end of the bag to not be blended. Due to the short time frame allowed for the soybean bag change-out the experimenters were rushed and chances for bag misalignment increased. Bag misalignment may have contributed to lower sugar concentration results.

Performing the experiment within the period of 0g was difficult for the 40 pass settings. The operator was required to begin the experiment in the 0g portion of the parabola; however, sometimes the process may have started before true 0g was reached. While true 0g was not required to obtain results, the inconsistency could have varied the results.

The safety mechanism worked properly when a containment breach occurred, halting the experiment in place. The problem was that a safety breach could occur if the lid was not properly secured, which reset the microcontroller to the default start configuration. On a couple occasions the lid floated and the microcontroller was reset, causing the controller to lose count. Experimenters had to estimate how many passes occurred and manually finish the blending pass.

All of the soybeans used for experimentation could not be cooked in one batch, allowing the possibility for variability between batches. In an attempt to control this variable, each batch was cooked and timed in a consistent repeatable process.

On flight day one, one member of the team suffered from motion sickness after the third parabola, and the other experimenter was required to do both jobs listed in the methods section. The process is difficult to fully complete in the allotted time of the parabolas for two people, so

the loss of the other team member reduced the amount of time to ensure an accurate and consistent process.

The precision of the refractometer may also have affected the results.

Future Considerations

For future experimentation several changes are considered.

A different method of evaluating the effectiveness of the blender should be considered. While measuring % Brix solids provided a means of quantifying the effectiveness of the blender, a more precise way of measuring blending effectiveness is needed.

Manually changing the samples allowed for inconsistencies and wasted time. Automating sample changing would alleviate these problems and reduce human error. This would also allow for one team member to run the experiment should the other team member become incapacitated.

The samples were mixed with water after blending. In future studies, it may be useful to combine the blending of the sample with the mixing of water.

Adding textures to the blending head may allow for different blend "settings." These textures could allow for finer or courser blending, or could emulate dicing or chopping.

Using a blending bag with a textured inner lining may improve the blending quality of the samples and could act as a means of filtration.

CONCLUSION:

Overall the results support the hypothesis that blending during flight is equivalent to mirrored ground tests. Furthermore, the principals behind this basic concept may be useful in future food processing devices designed to work in environments ranging from microgravity to gravity environments found on planetary outposts.

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ACKNOWLEDGEMENTS:

Team CyMix would like to thank the following people for their efforts in advice, guidance, and contributions to the project: Dr. Cheryll Reitmeier, Dr. J Adin Mann, Ralph Patterson III, Dr. Charles Bourland, Dr. Roger Smith, Dave Larson, Ryan Richter, Tom Elliot, Regina Hendrickson, Dr. Anthony Pometto III, Dr. Lester Wilson, Ginger Gross, Jason Boyd, Scott Raymond, Jim Howe, Clyde Leonard, the Iowa State University Department of Aerospace

Engineering office, the Iowa State University Department of Mechanical Engineering, the Iowa State University College of Liberal Arts and Sciences, the Iowa State University Foundation, the Iowa Space Grant Consortium, and the NASA Food Technology Commercial Space Center.

PHOTOGRAPHS:

JSC2004E30047
JSC2004E30050
JSC2004E30057
JSC2004E30060 to JSC2004E30061
JSC2004E30063 to JSC2004E30064
JSC2004E30072 to JSC2004E30075

VIDEO:

- KC Undergraduate Flights, Group A, July 13 -14, 2004, Reference Master: 718428

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.9 Undergraduate Program Flights – Hemodynamics and Free Radical Production in High Gravity Shifts in the Human Cardiovascular System

FLIGHT DATES:
July 13 -14, 2004

PRINCIPAL INVESTIGATORS:
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Hanjoong Jo, Emory University/Georgia Institute of Technology)

CO-INVESTIGATORS
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Jessica O'Neal, Georgia Institute of Technology
Richard Moffitt, Georgia Institute of Technology
Mike Dorman, Georgia Institute of Technology
Kelly Griendling, Georgia Institute of Technology

GOAL:

To determine the effects of frequent high gravity shifts on the human cardiovascular system

OBJECTIVES:

In the human body, endothelial cells lining blood vessels are constantly subjected to shear stresses, generated by the drag effects of blood flow (1). These stresses trigger various biochemical and physical changes in cell structure and function. *Laminar shear stress*, a characteristic of healthy blood flow, has been shown to inhibit apoptosis and promote

atheroprotective effects, such as regulation of vascular tone, diameter, and vessel wall remodeling. These are all factors involved in suppressing the development of atherosclerosis (hardening of blood vessels (2). Conversely, exposure of endothelial cells to *oscillatory shear stress*, such as those found in regions of branched and curved arteries, has been shown to induce several inflammatory responses (e.g., reactive oxygen species and pro-inflammatory gene products) that contribute to early forms of atherosclerotic lesions (3,4). The greater implication of these studies is that any change in the shear stress on endothelial cells could contribute to the development of atherosclerotic vessels. Similarly, changes in arterial wall intramural stress have been linked to the generation of reactive oxygen species and increased inflammatory gene expression. Thus, both alterations in blood flow and blood pressure have the potential to induce the production of reactive oxygen species by the arterial wall.

Acceleration fluctuations, such as those experienced in the hypergravity and microgravity environment of NASA's KC-135 aircraft, result in dramatic alterations in blood flow as measured by Doppler Ultrasound (5). Parabolic flights have also been shown to induce acute effects on blood pressure and heart rate (6). It has also been shown that such simulated microgravity affects the baroreflex control of leg venous compliance (7). The known effect that parabolic flight has on blood flow patterns thus shows the potential to cause atherogenesis by inducing inflammatory response and oxidative stress.

The study attempts to find patterns between ECG waveform patterns, heart rate, and blood flow velocities as a function of a body's position in space. By using an accelerometer in-flight, the acceleration environment can be deduced post-flight by noting that the body will be still in the 2g portion of the flight, and will be moving in the 0g portion of the flight. Thus, physiological data can either be correlated with both the high g and low g environments separately, or can be examined succinctly to determine an overall effect of the transient periods experienced in-flight. A three-lead ECG configuration was used in-flight to capture waveform images, from which heart rate was deduced. Blood flow characteristics were captured from the radial artery using a Doppler Ultrasound probe in-flight. All data was recorded using BIOPAC software and modified in MATLAB.

Through an examination of the physiological changes resulting from 2g acceleration shifts, it is evident that abnormal stresses were placed on the blood vessels in-flight. The effects of these acceleration shifts on chemical components of the blood such as oxidative free radicals and brachial artery reactivity remain to be seen. However, a preliminary examination of the physiological effects is useful in deducing that 2g acceleration shifts do result in changes in the human cardiovascular system that may place added stresses on vessel walls.

Once the relationship between parabolic flight and free radical production is established (to be reported in subsequent papers), it will be a small corollary to suggest that parabolic flight can increase the risk of atherosclerosis in pilots and astronauts. Insight gained from this study will elucidate the possible mechanism by which parabolic flight can contribute to the development of atherosclerosis or other cardiac stresses. The implications of such findings would be important as larger populations are subjected to the stresses of spaceflight and other variable acceleration environments. Knowledge of the cardiovascular reaction to such stresses would be invaluable to future medical enterprises in the prevention of hypertension and atherosclerosis, as well as an important consideration in any medical treatment conducted under hyper/microgravity conditions.

METHODS AND MATERIALS:

There were several pieces of equipment required for this project. Ground based equipment included Acuson ultrasound machine, syringes, blood vials, sterilized needles, a cooler with dry ice to store blood samples, and a container to dispose of sharp needles and any disposables that have been exposed to human body fluids. Flight equipment included a mounting board, the Biopac payload, a Doppler probe, ECG leads, an accelerometer, the outreach box, and a laptop. Some equipment was needed upon return from Houston to analyze the blood samples. This equipment included hardware to perform the ELISA assay and the glutathione assay. For the ELISA assay, equipment used included a microplate washer, a microplate reader, ELISA kit, pipettes and other standard lab equipment. This was provided for use by Emory University. For the glutathione assay, the only necessary piece of equipment was the High Performance Liquid Chromatography unit (HPLC) which was provided by Dr. Dean Jones at Emory University.

Baseline data collection

Immediately prior to the flight, blood samples (2 mL) of flight team members were taken by a qualified phlebotomist to determine the baseline glutathione and prostaglandin levels. A pre-flight ultrasound was performed to determine pre-flight brachial reactivity.

In-Flight Experimentation

One subject per flight was connected to a BIOPAC system that monitored the electrical activity of the heart via a three lead ECG configuration, blood flow characteristics via a Doppler probe, and the body's position in space via a 1 axis accelerometer. The subject maintained a similar resting position (sitting upright) in each of the hypergravity portions of the flight to eliminate variables related to changing stresses due to the body's position in space.

Post-Flight

Immediately after the flight, blood samples were taken by a qualified phlebotomist for post-flight analysis of glutathione and prostaglandin levels. A post-flight ultrasound was performed to determine changes in brachial artery reactivity as a result of the acceleration shifts.

Non-Flight Day Procedures

On non-flight days, normal brachial reactivity baseline data and blood samples were taken to serve as a control by which post-flight physiological data would be compared.

RESULTS:

A representative section of physiological data obtained from subject 'J59' in-flight for three 2g portions and two 0g environment portions is shown in Figure 13. Correlations between heart rate and Doppler flow, accelerometer data and Doppler flow, and heart rate and accelerometer data, as well as the average r^2 value for all 6 portions, are reported in Table 4. There is a high correlation ($r^2 = 0.763$) between the heart rate and accelerometer data, suggesting that heart rate is directly related to the gravity environment. Indeed, the high-g portions of the flight show a consistent heart rate increase of nearly 50%. We also observed that as gravity shifted from 2g to 0g, the arterial blood flow sharply decreased, which was rapidly recovered back to the 2g level. In some cases (marked by an arrow in the Doppler flow panel, Figure 13), this sharp drop appears to be followed by an increase in heart rate, suggesting a potential baroreceptor type of feedback control leading to the heart rate increase. The correlations comparing accelerometer data and Doppler

flow, and heart rate and Doppler flow, are low due to the inconsistency in the blood flow data collection from the Doppler probe.

Table 4. Correlation Trends between Physiological Data Sets

Comparisons	r ('J59')	r^2 ('J59')	mean r^2
Heart rate vs Doppler flow	-0.4449	0.3799	0.193
Accelerometer vs Doppler flow	0.6164	0.1979	0.1258
Heart rate vs Accelerometer	-0.8735	0.763	0.3603

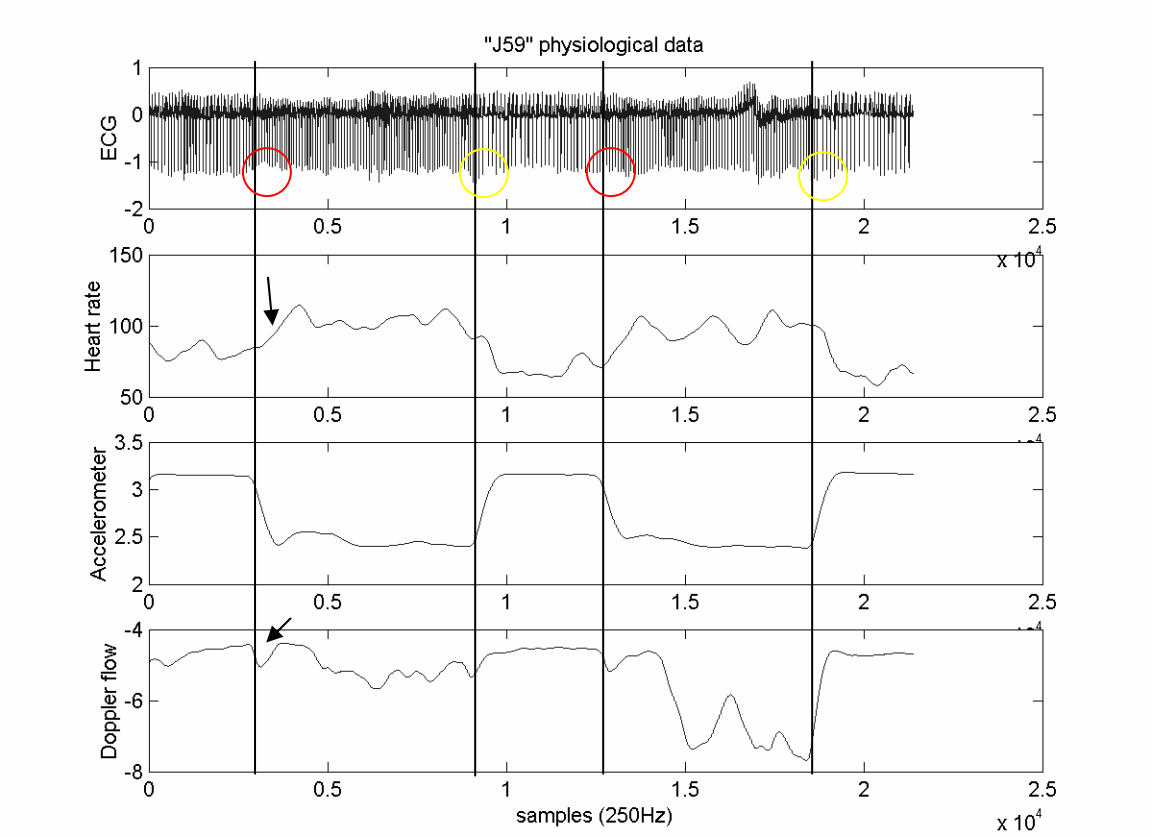

Figure 13. Representative Physiological Data resulting from Two Acceleration Shifts

From top to bottom: Raw ECG data, Heart rate, Accelerometer reading, and Doppler flow. General correlative trends are visible, especially between Accelerometer and Heart rate. Doppler flow is sometimes erratic due to motion of the probe and subject while attempting to maintain balance in-flight. Data shown (except ECG) is a result of a 0.1Hz Lowpass filter.

DISCUSSION:

The high correlation determined between heart rate and accelerometer data may be explained by considering that the classical baroreceptor feedback mechanisms (8) were triggered by the high-g. The increased g force during the high g will pull the blood towards the lower half of the body at two times the normal force that the body is acclimated to overcome. The blood pooling then decreases the amount of blood returning back to the heart (the right atrium), triggering a sequence of the baroreceptor feedback mechanism to ensure that blood circulates throughout the body including the brain. The decrease in the blood returning back to the heart decreases the

amount of blood ejected from it, decreasing the pressure in the arteries. This decrease in the pressure is then recognized by the baroreceptors located in the aortic and arterial sinuses and these send neural signals to the cardiac regulatory center in the brain. The brain then sends a variety of signals to the heart and the blood vessels to restore proper circulation levels. Some of these neural signals lead to an increase in the heart rate and contractility, increasing the cardiac output. Venous contraction is another target of the neural controls, forcing more blood to return to the right atrium. Our observation of the rapid and transient drop in the brachial artery blood flow, followed by a significant heart rate increase, provides support for this potential explanation.

One product of increased vascular mechanical stress is an increased production of oxidative free radicals that work to strengthen the walls of blood vessels. Such changes will protect blood vessels from potential rupture in light of increased stresses. However, prolonged hardening of the arteries increases the risk of developing atherosclerosis, which can result in heart disease and/or stroke. Subsequent papers will investigate the changes in oxidative free radical concentration in the blood and brachial artery reactivity to determine if such side effects are evident from the 2g acceleration shifts experienced on the KC-135.

We feel that our inaugural flight studies have successfully accomplished most of the specific aims and established a foundation that we can build upon in the future studies. Although studying the physiological changes occurring in the humans during the gravity shift conditions is important, this type of study is not easy to perform and has been significantly understudied. We have established a testable hypothesis through reviewing literature and have built and modified the device to measure physiological changed under gravity shift conditions. Using the device, we have been able to obtain useful data.

There were some aspects of this experiment that could be improved upon. An increased number of subjects would verify conclusions drawn in this paper and perhaps bring to light other patterns in physiological characteristics. Other things that could have been improved upon include a better way of attaching the Doppler probe to the arm so that it doesn't move in-flight and securing an isolated place to perform the brachial reactivity. We feel that repetition of this experiment with an increased number of subjects will help to verify our conclusions. We plan to submit a proposal to repeat this experiment during next year's flight campaign.

CONCLUSION:

The physiological data taken from the three parabolic flight paths demonstrated the high contrast between the functioning of the cardiovascular system in 0g and 2g environments. Though the increased stresses placed on the blood vessels may or may not have resulted in high levels of oxidative free radicals, the fact that high-g environments place increased stress on heart function cannot be disputed. The long-term effects of prolonged exposure to high-g environments have yet to be explored; however, results from this study have found evidence of the potential for the development of chronic cardiovascular problems, highlighting the need for follow-up studies.

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JSC2004E30031 to JSC2004E30038

VIDEO:

- KC Undergraduate Flights, Group A, July 13 -14, 2004, Reference Master: 718428

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.10 Undergraduate Program Flights – Sleep Hardware Concept Evaluation and Analysis

FLIGHT DATES:
July 15 -16, 2004

PRINCIPAL INVESTIGATOR:
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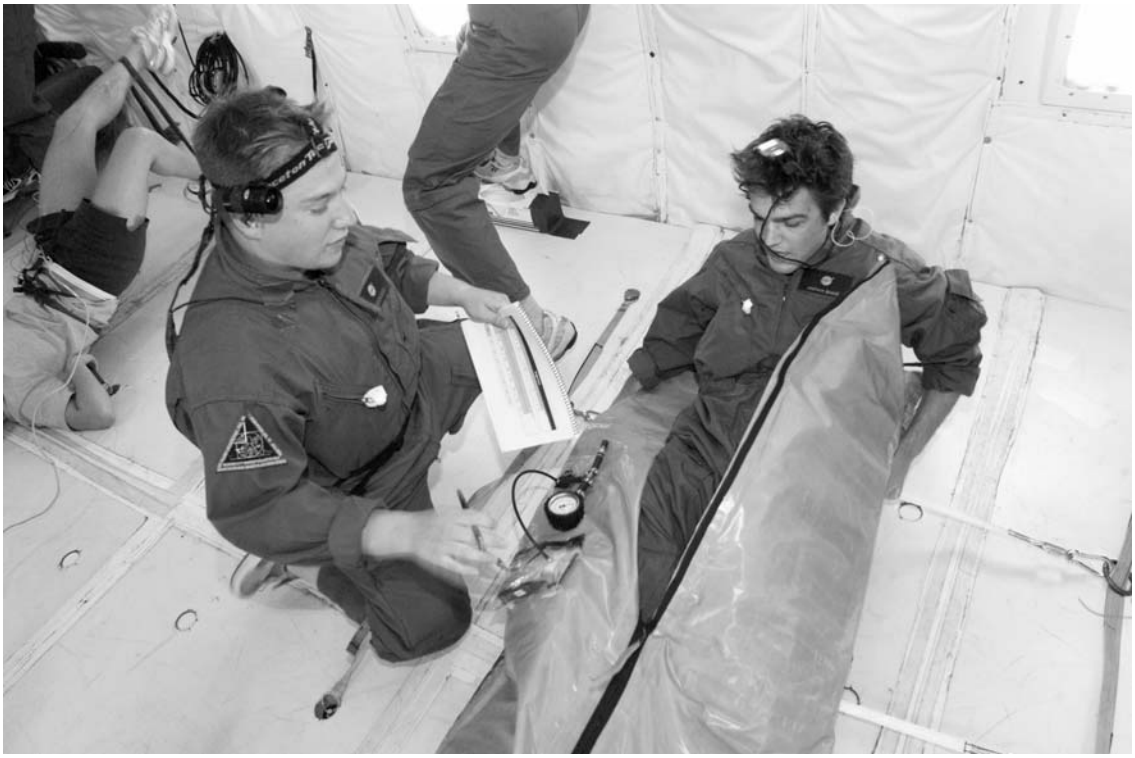
Brandon Dole, PSU
Zach Davis, PSU
Andrew Brahe, PSU
Amy Graeff , PSU
Angela Streich, PSU



EXPERIMENT BACKGROUND:

Our research into human elements that are lacking in 0g habitats led us to the sleep environment and the negative effects of sleep deprivation and astronauts. The results of these conditions are known as Space Adaptation Syndrome (SAS.) a NASA Astronaut responded to a student inquiry about sleeping in space. He stated in an email response, “I think the issue for sleep is more of adjusting to the 0g floating in your bed sensation rather than the 1g laying in a bed sensation. So

for me personally, I found it a little bit unsettling and difficult to sleep the first day or two primarily because I was floating." (1) This project was an architectural challenge to create, in a unique 0g environment, the sensation of weight, pressure, horizontality and other elements experienced while utilizing a bed/blanket in a 1g environment. The only way to know if our design was successful was to test it in a similar environment such as the one created by the KC-135. The device was tested on the KC-135 to collect a sufficient quantity of data of usable, measurable quality. It's our hope that a second-generation design will be tested on a follow-up flight in the 2005 Microgravity University program.

GOAL:

Sufficient, quality rest relates to comfort. We designed a sleep comfort device (SCD) that was to be experimented with for deployment, ingress, comfort, egress and stowage for hardware concept evaluation and analysis. The goal of the experience and experiment was to help us identify if a new design would rate better in the microgravity environment for astronauts' comfort as well as safety, deployment, accessibility, orientation, stabilization, isolation and confinement sensations. We had two hypothesizes. Hypothesis #1: By increasing pressure applied to the subject's surface-body area, via their sleep hardware, while in a microgravity environment, greater comfort associated with their usual 1g environment will be achieved. Null hypothesis #1 is that the increase of pressure on the body will not increase sleep hardware comfort in the microgravity environment. Hypothesis #2: By redesigning sleep hardware for greater comfort safety, ease and efficiency of ingress and egress can be maintained. Null hypothesis #2 is that the device will not maintain current standards of safety, ease and efficiency of ingress and egress of sleep comfort device.

OBJECTIVES:

It seemed to us that the sleep environment hasn't received as much attention as it should have in regards to living and working in space, that astronauts could be more productive, and healthier if more familiar conditions were present. As visits to outer space become longer and more frequent, a living environment must be considered as well as a working environment, therefore setting the stage of a great design opportunity for innovative architects.

We began this undertaking as one small piece in a broad scope of 0g habitat design. That first step we wanted to take was from the most intimate of spaces in the sleep environment in which we confine ourselves to on a daily basis, the bed. Creating the sensation of lying in a bed with the weight of blankets affecting the body similarly to a one gravity environment was the successful outcome we hoped to achieve from this experiment as well as the rate and ease of ingress and egress of the apparatus. To test our hypothesis, we planned on gathering qualitative as well as quantitative data by monitoring stopwatches and the SCD internal mbar pressure as it compared to the cabin pressure. In addition to these instrument recordings, the occupant was to answer questions from a survey to obtain results regarding their comfort during the various stages and cycles of occupying the device.

The objective was to collect enough numeric data to definitively state the actual amount of pressure the body must receive in 0g to experience the sense of weight, pressure, et cetera that would be associated with the familiar sensations we expect from a bed and blankets.

METHODS AND MATERIALS:

The device was pre-anchored to the floor of the KC-135 as the in-flight experiment began. Once the plane began its decent and 0g occurred the subject was to gain entry, interact and respond to the SCD's performance as it applied a controlled constraining pressure upon them to simulate 1g sensations of a bed comprised of a surface to sleep upon and blanket atop. These interactions with the device included lying on one's back and side as well as an attempt to curl up into the fetal position (commonly regarded by astronauts as the most comfortable way to rest in 0g). After the comfort experiment, the subject then exited the device and prepared to repeat the experiment.

The Sleep Comfort Device (SCD) was anchored and orientated perpendicular to the KC-135 fuselage deck. The SCD is an anchored cocoon-like sleeping bag with semi closed off sides. It is comprised of inflatable tubes that run parallel to one another and perpendicular to the occupant's vertical axis from their shoulders to their feet. The blanket was made of a manifold made from a several sheets of silicon coated airbag material and numerous I-beams (sewn of the same material), which were all laid out and adhered together. This process is similar to how an inflatable raft is constructed for white water. This method allowed the blanket flex and contour to match the occupant's form. The blanket was connected to a mattress, in parallel, sandwiching the occupant comfortably. The connecting system between the blanket and the mattress was a "Boa-lacing" system that permits even adjustment of the SCD's internal diameter size for individual comfort requirements. The lacing system applies constant pressure down left and right sides of the sleep device at all times. The lacing system thus bridges the air manifolds to a poly-batt filled polyurethane coated Cordura Nylon (2) pillow and mattress at the backside surface, which the occupant rests upon in the one- g environment or is received into in 0g. A non-locking quick release zipper (3) for ingress and egress was sewn into the blanket. The blanket is a closed circuit of air distribution manifolds that encircle 50% of the occupant's prone top surface area. A pneumatic blower (4) filled the manifolds with cabin air. As the tubes regulated pressure increased, sensation of comfort, based on ground tests of the device as it relates to the weight of blankets and sheet values (5), was to be achieved. The device was also fitted with a manual relief valve in case of cabin pressure loss.

Blanket air pressure, ingress and egress were monitored and recorded by the observing flyer. Sensations while occupying the SCD were assessed by the subject/occupant and verbally relayed to the observer. A numbered comfort scale of 1 to 5 (Likert scale, with the weight of a sheet (level 1) to a heavy blanket (level 5) was used to relay and record the sensations as they corresponded with mbar pressure (the survey was blind due to the subjects unawareness of the actual pressure at the time of the test).

RESULTS:

The short-term microgravity environment allowed us to analyze the device's 0g comfort in comparison to 1g sensations of a bed comprised of a mattress surface to sleep upon and a blanket that rests upon us.

The experiment was quite effective in generating the sensations associated with the weight of blankets. As the SCD blanket inflated the reduction of the interior area between the blanket and the mattress caused the occupant of the device to be contained and held in place while floating in 0g. The blanket performed and functioned as planned. The inflated SCD internal pressures associated with the comfort of a blanket, per human subject, ranged from 824 – 839.8 mbar (with

an external 824-826mbar barometric reading). The time of ingress was between 18 to 20 seconds and egress was 7 – 9.47seconds.

In addition to our own findings, the added evaluation of our design by a NASA astronaut and a NASA flight surgeon will allow us to further develop the device for a second trial run with the suggested improvements we gleaned from these professionals' constructive criticism.

DISCUSSION:

First off, we all had a great experience. The visits to the Johnson Space Center and pre-flight activities as well as the flights were fantastic. It was great fun and exciting to be hosted by the reduced Gravity Office at Ellington Field and to be apart of the NASA family of KC-135 0g Researchers. Their hospitality was exceptional at all levels of activity. Every member of the PSU Off Planet Architecture Team is grateful for the opportunity and will never forget it. We hope that others in the area of architectural study will take advantage of the program and share in this amazingly unique experience, which allows one to understand this strange environment and how to design for it.

The operation of our equipment and the gathering of our data were accomplished as planned. Both a NASA flight surgeon and an astronaut participated in the experiment on day two of our team's flights. They both thought that we were on the right track and that the device was functioning in a way that merited further research and design development. These NASA personnel are seasoned researchers; the four of us who flew on the mission were not. The flight manifest consisted of student flyers 1 and 2 on July 15th and flyers 3 and 4 on the 16th with the NASA personnel. It was the first time any of the flyers of the Portland State University Off Planet Architecture Team had ever flown on a 0g flight before. We were fresh to the 0g environment and the data we aimed to collect was a moderately difficult query for us.

When we began to interact with the device in 0g, the observing flyer was to record the time the occupant flyer took to enter and exit the device, inflate the device and account for the air pressure of the device as it was inflated and related to the occupant's sensational experience. The occupant of the SCD accounted for the experience of the devices sensations by using the pre-established Likert scale comfort rating system that we associated with the weight of a sheet (level 1) to a heavy blanket (level 5). This observation was then relayed to the observing flyer and allowed for quick and concise communication through out the experiment. This relationship of experienced and recorded internal barometric pressures of the device and the KC-135 cabin pressures, at different altitudes, allowed for the quantitative collection of data, but yielded potentially incongruent results from parabola to parabola since we hadn't incorporated a more accurate measuring instrument. The methods for gathering data were hard to manage by one observer (monitoring a stop watch and barometer as well as writing the data down and controlling the inflation of the device to maintain a level of blind surveying for the occupant to respond to).

Adjusting to the environment for the first time was a task in itself that was both exciting and eye opening. Even though we had thought the experiment through thoroughly, the gathering of the data was as difficult as managing oneself in the environment and none of us could have anticipated exactly how it would be like. Further data collection was cut short on the second day of flight due to a malfunctioning oil pump on one of the engines, thus causing us to end our

experiment early and return to Ellington Field. In retrospect, we did quite well, learned a lot and are ready to take the experiment further with an upgraded design influenced by the data we gathered. Over all, the experience of being able to relate to the environment will help the PSU Off Planet Architecture team to better design and prepare a plan for future evaluation with our next experiment with the SCD on a proposed follow up flight. The next generation of our experiment will be a SCD that incorporates tactile enhancements, an integrated dynamic array of low mbar pressure vessels and a more integrated quantitative measuring system that will allow us to more accurately monitor the correlation between the comfort of the weight/pressure of a blanket in 1g and 0g as it relates to the human condition and rituals of sleep.

The success of our project was due to many factors and people: the exceptionally creative design/build team of Off Planet Architecture, our helpful and encouraging advisors (Mark Weislogel, Jan Connolly, Cindy Hudy, Chuck Cooper, Dan Cox), and the generous material donors of Invista, Nautilus Sleep Systems, Ace Hardware, Sewing Center West, Rose City Textiles and Vans + the OR Space Grant Consortium and priceless field research input from NASA flight surgeons, astronauts and students Brahe, Davis, Dole, and Rudis. Our well-rounded experience of proposing a plan, implementing it, and following through to the completion of collecting data proves that we have successfully taken on a difficult experiment and design concept and gained real world results that are potentially waiting to be implemented into future use by our nations space program.

CONCLUSION:

In conclusion, we found that by using sleep hardware to increase pressure to the while in a microgravity environment, greater comfort associated with their usual 1g environment can be achieved. We also concluded that by redesigning the sleep hardware for greater comfort, safety, ease and efficiency of ingress and egress were maintained. The device worked well and was a good first step for a new sleep restraint device for astronauts to use in short duration flights onboard ISS and STS missions. The information we did gather was minimum, but did confirm the team's hypothesis that our design was capable of generating the sensation of the weight of a blanket in 0g and that we're "on the right track" (1).

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VIDEO:

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2.11 Undergraduate Program Flights – Free-Floating Resistance Exercises for Maintaining Postural Muscles in Weightlessness

FLIGHT DATES:
July 15 -16, 2004

PRINCIPAL INVESTIGATORS:
Stephanie Feldman, Dartmouth College

CO-INVESTIGATORS:
Chelsea Morgan, Dartmouth College
Lea Kiefer, Dartmouth College
Lauren Talbot, Dartmouth College
Jay Buckey, Faculty Advisor, Dartmouth College

INTRODUCTION:

Muscle atrophy is a serious problem for astronauts on long-term assignments in weightlessness. These astronauts particularly suffer upon return to Earth's gravity due to postural muscle atrophy. The current measures of prevention use heavy, bulky equipment and astronauts are scheduled for daily two-hour exercise periods to maintain muscle mass and aerobic fitness. Evidence exists showing that frequent, brief periods of intense exercise throughout the day may be as effective as or more effective than a single daily exercise session. If astronauts had a series of simple

exercises they could do anywhere aboard the ship with a straightforward resistance band, and without deploying cumbersome exercise equipment, they could realistically perform this series multiple times throughout the day. This may allow astronauts to maintain their muscles more efficiently in space.

The goal of this project, therefore, was to design a set of simple, free-floating exercises targeting five major postural muscles that astronauts can perform on their own throughout the day. This series of exercises is called Dartmouth Resistance Exercises for Antigravity Muscles (DREAM). The effectiveness of DREAM in weightlessness is determined by comparing the level of muscle activation in weightlessness during DREAM with the activation that occurs when postural muscles are activated in daily life on Earth (i.e., standing, sitting, climbing steps).

Muscle activation was measured using a surface electromyogram (EMG). Four subjects performed DREAM in weightlessness and DREAM again in Earth's gravity. These results were each compared to 5 normal daily activities on Earth to gauge the effectiveness of this exercise regime overall and particularly in weightlessness. We hypothesized that muscle activation in-flight using the DREAM resistance exercise program would be as great as or greater than muscle activation in daily activities. Weightless resistance exercises that produce substantial muscle activation can become a pilot suite of effective exercises that crewmembers can perform to maintain muscle mass while in space.

METHODS AND MATERIALS:

Equipment Description

40 Self-adhesive Electrodes; 4 Grounds
Portable EMG System (Base Unit, Laptop)
Digital Camcorder
Elastic Resistance Band: 4ft. x 4in.
Non-stretch Band: 4ft. x 4in.
Velcro Belt: 3ft. x 1in.

There is minimal equipment used in our experiment. We used two resistance bands – one elastic, and one rigid – as our exercise equipment. A surface electromyogram (SEMG) recorded muscle activity. The SEMG is comprised of a base unit and a laptop that sits on top of the base unit. Self-adhesive electrodes placed on each subject during preflight monitored individual muscle activation. The subject wore a Velcro belt that was attached to a foothold on the floor to keep the subject in front of the camera and in our experiment's area during the exercises. A standard handheld digital video camera monitored the subject.

Aboard the KC-135, the camcorder, EMG base unit and laptop were stored in their own carrying cases in 9g equipment boxes during take-off and landing. After takeoff, the researchers unloaded the EMG system and secured it to the cabin floor with pre-laid Velcro strips. The recording researcher sat with her back to the cabin's sidewall and secured herself with a foothold at her feet and one to her side. The EMG system was to the right and slightly in front of the recorder and angled to face her. The subject was lying perpendicular to the cabin wall with her head near the recording researcher's feet, giving her plenty of room to complete the primarily lower body exercises.

The digital video camera was mounted on a NASA provided camera pole a meter from the experimenting researcher's head and to the right side. The camera was at a height of five foot, five inches from the aircraft floor and facing the length of the experimenter's body.

Experimental Procedures and Protocol

One month prior to the flight all subjects began learning and practicing DREAM. Once each was comfortable with the exercises, electrode placement trials ensured that each studied muscle was being located and its activation recorded. Electrode placement referred to Dr. Aldo Perotto's referencing catalog and diagrams, *Anatomical Guide for the Electromyographer: The Limbs and Trunk.*

On the day of the flight, subjects arrived at Ellington two hours prior to flight time. Self-adhesive electrodes were placed on the two flying subjects over each muscle that was monitored. In-flight each subject completed the series of DREAM exercises. The subject performed each of the five exercises for fifteen seconds while the EMG recorded muscle activation – one exercise per period of zero gravity. The plane made thirty parabolic dives for each group of two student subjects. Each subject was given ten dives to complete her five exercises. The remaining ten dives were reserved in case specific exercises need to be repeated for any reason by either subject. The four elastic band exercises each targeted an individual postural muscle. The fifth exercise used the non-stretch band to stress five major postural muscles simultaneously. All subjects wore an adjustable Velcro belt while they complete their exercises. Prior to data collection, the subject loosely secured herself to a foothold in the floor of the KC-135 with this belt. The purpose of this system was not to stabilize the subject, but to prevent excessive movement around the cabin during weightlessness and to keep the subject positioned in front of the video camera. The investigator not performing the exercises was monitoring the EMG and recording the subject's data.

Post-flight, each subject again went through DREAM and then completed four daily motions (sitting up, standing up from a chair, toe raises, climbing stairs) that work the postural muscles. The immobile self-adhesive electrodes ensured consistent measurements, just as our same-day, within subject design negated any effect the motion sickness medication may have caused. This 1g DREAM data set is the primary baseline for each subject. The daily motions are a baseline for average postural muscle activation on Earth. Because all DREAM exercises are gravity independent, we expected the muscle activation in microgravity to equal the muscle activation in the post-flight baseline tests.

Description of DREAM:

Thera-Band (elastic resistance band) Exercises:
Hold each exercise for two seconds. Slowly return to starting position. Continue to repeat for 15 seconds/when your teammate tells you to stop.

Calf: Thera-band under ball of left foot. Toe slightly pointed then flexed, with the emphasis on the flex. Hold at peak flex, slowly return to starting position.

Quad: Thera-band double looped and place slightly above the ankle on the left foot and mid calf around the right leg. Both legs start even at a ninety-degree angle bent at the hip and knees. Feet are separated about a foot and a half. Without changing the angle of your hips and keeping the

right leg stable, straighten your left leg, hold, and slowly return to starting position. Keep knees separated by a foot.

Hamstring: Thera-band double looped and placed slightly above the ankle on the left foot and mid calf around the right leg. Both legs start even at a ninety-degree angle bent at the hip and knees. Feet are separated about a foot and a half. Without changing the angle of your hips and keeping the right leg stable, bend the left leg, moving the heel towards butt, hold, slowly return to starting position. Keep knees separated by a foot.

Iliopsoas (hip flexer): Thera-band double looped and place slightly above the ankle, even around both legs. Both legs are extended straight out. Keep feet separated by a foot. Keeping the right leg stable, using your hands for support if necessary, raise the left leg approximately one foot, hold, and slowly return to starting position.

Pull Squat: Place *non-elastic* band under the balls of feet, ankles slightly extended. Arms fully extended holding the band at knee-level, knees and hips at ninety-degree angles. Attempt to straighten legs. Arch back. Keep body tight. Hold continuously for 15 seconds.

There are five sets of electrodes for the five muscles we are targeting (Soleus, Quadriceps, Biceps Femoris, Iliopsoas, Erector Spinae). We recorded from all five muscles at all times.

DREAM was completed in this order:
• Calf-specific elastic band exercise
• Quad-specific elastic band exercise
• Hamstring-specific elastic band exercise
• Hip-specific elastic band exercise
• Pull squat non-stretch band exercise; targeting four major postural muscles (Soleus, Quadriceps, Biceps Femoris, Erector Spinae)

RESULTS/DISCUSSION:

All the subjects were able to perform the DREAM exercises easily in weightlessness. Subjectively, the degree of muscle activation achieved during weightlessness was similar to that achieved in 1g on the ground. The experiment demonstrated that astronauts could activate their postural muscles in space using simple exercises and equipment. Exercises like DREAM could be studied on long-duration missions to assess whether they could help to maintain postural muscle mass and function.

To provide an objective assessment of muscle activation, our team successfully recorded the electricity generated by each muscle with an EMG. The Nicolet Portable Endeavor System functioned very well throughout the flight between 0g and 2gs. The disposable electrodes, secured with Tegaderm, recorded EMG data with consistently low impedance for 3-5 hours. Unfortunately, EMGs are most often used as diagnostic tools and the software to provide the information we needed was not incorporated in the Nicolet system. To provide the analyses we need, we need to analyze the data using Matlab. Nicolet and Viasys have been very accommodating in helping us to convert the data into a form that can be read by Matlab. Viasys wrote new software to convert our data files into a Matlab format. This conversion program has some problems with data corruption and compatibility barriers, which we have been working on in collaboration with Viasys over the past few months. We anticipate being able to analyze our

data completely by this winter. These data should provide some objective measures of muscle activation.

Overall, we were able to complete our study successfully, which tells us that all five of the exercises in DREAM were doable in weightlessness, took up minimal space, and provided a strong muscle stimulus (subjectively). We were able to watch electrical output graphs on the laptop screen and noted that muscle activation was occurring for the experimenting subject in-flight, so we expect to see that these exercises were very successful.

Perhaps our biggest barrier in executing these exercises was the foreign nature of weightless movement. It took 3-7 parabolas to learn how to secure our bodies in a manner that allowed us to focus on the specific exercise at hand. Once comfortable with the environment, however, all of our exercises were very simple to complete and the bands were very easy to use in microgravity. One way to improve DREAM would be to eliminate the "stress and release" aspect of our exercises. A 15 second hold would work the muscle more efficiently. Also, the benefits of the counter stress created in "stress/ slow release" exercises in 1g are non-existent when exercises are completed in weightlessness. The only beneficial action in microgravity is working directly against the resistance band, so it would make sense to focus solely on that action in future exercise programs.

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VIDEO:

- KC Undergraduate Flights, Group A, July 13 -14, 2004, Reference Master: 718428

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.12 Undergraduate Program Flights – Change of Myoblastic and Osteoblastic Cellular Shapes due to Response of Nuclear Movement in Microgravity Conditions

FLIGHT DATES:
July 27 -28, 2004

PRINCIPAL INVESTIGATORS:
Isaac Chan, Duke University
Dan Choi, Duke University
John Fang, Duke University
Gary Sing, Duke University

GOAL:

The mechanisms of cellular responses directly due to microgravity conditions have not been concretely identified in previous research. Knowledge of such cellular responses to microgravity is crucial to the greater understanding of the present concern of muscle atrophy and loss of bone density during extended exposure to microgravity conditions. In our study aboard the KC-135 during the summer of 2004, our goal was to detect and observe a possible mechanism through which gravity might have a direct and relatively macroscopic effect on cellular morphology after minimal exposure to microgravity. This cell shape change potentially could come from two sources:

1) A loss of gravitational forces in the cell would cause a shift in the positions of the cellular organelles, including the nucleus, to a more energetically favorable state than with gravity.

2) The cell nucleus in myoblasts and osteoblasts would move in response to microgravity conditions and that this movement would change the overall shape of the cell via interconnections through the cytoskeleton.

Such changes in cell shape might have ramifications on the overall function or behavior of the cell, which is of immense interest to researchers examining the atrophy and bone loss issues.

OBJECTIVES:

Experimental evidence has shown that cell shape is responsible for influencing factors such as cell proliferation, gene expression (Vico *et al*, 96S), and general cellular metabolism (Tairbekov, 662), demonstrating the importance of such a direct effect of gravity on cell structure. Specifically, we predicted that a minimal time spent in microgravity conditions would directly cause noticeable movement and deformation of the cell nucleus and thus cause a change in overall cell shape through its connections with the cytoskeleton. We expected the general location of the nuclei within cells exposed to microgravity conditions to change in response to the loss of gravitational force. In Earth gravity (1g), since the density of the nucleus is much higher than the surrounding fluid, the nucleus would follow the gravity vector and settle in a convenient low energy configuration. However, without gravity, the nucleus would shift into another low energy position optimal for microgravity conditions. In addition to this shift in nuclear position, we expected the overall shape of the cell to change somewhat due to the interconnections of the cytoskeleton between the nucleus and the cell membrane. Cell shape is primarily dependant upon the organization of the cytoskeleton (Vico *et al*, 96S); thus, if the nucleus is interconnected with the cytoskeleton, movement of the nucleus would likely cause a change in cell shape. An example of such a theoretical shift in nuclear position is known as "focusing" where the gravitational response of the nuclear movement is amplified via the cytoskeleton and energy is transferred to a particular point or molecule on the cell membrane which can convert a mechanical signal to a chemical signal (Mesland, 17). To verify our predictions, we obtained cell samples fixed with formaldehyde while in microgravity, stained them with fluorescent dye, and analyzed the cell shape and nucleus position by examining the cells with a confocal microscope.

METHODS AND MATERIALS:

In order to fix the cells while aboard the KC-135, 10mL of Bouin's solution was added to slide flasks containing osteoblasts with serum free growth media and slide flasks containing myoblasts with serum free growth media. Once the cells were fixed, we stained them for examination under a confocal microscope.

In order to safely inject formaldehyde into the slide flasks containing the osteoblasts and myoblasts, the growth media required three levels of containment. The first level of containment was provided by the slide flask in conjunction with ½" foam weather stripping fastened by a rubber band around the mouth of the slide flask. The second level of containment was provided by an experiment box designed to allow injection of a fluid into the slide flask by a 10mL syringe via a 19 ½ gauge twist-on needle. The final level of containment was a large vacuum sealed bag that contained the experiment box while allowing operation of the syringes.

Once the box was placed inside the large vacuum sealed bag, the bag was placed on top of two file crates placed adjacent to each other. The box was secured to the file crates using adjustable

straps with ratchets. Under the file crates was an additional extra large plastic bag in case the first two levels of containment were breached.

In order to further prevent any leaking, all the corners of the box were padded with absorbent material to ensure the containment of each level.

Finally, in order to keep the cells incubated while inside the experiment box, we purchased two heating pads for babies to be placed between the box and the file crates. The heating pads were perfect because the temperature for the box is supposed to be 37° which the heating pads provided. Prior to the flight, the cells were transported from Johnson Space Center to Ellington Field in an insulated cooler with heating pads.

Once we arrived at Johnson Space Center (JSC), we unfroze the cells and began growing them in preparation to be put into the slide flasks. Between the first day at JSC and the flight days, we passaged the cells numerous times and eventually passaged them from the large flasks to the slide flasks to be used on the plane.

Culturing the cells involved changing the media daily, and when the cells became about 70% confluent, we passaged them into a larger flask until we had enough cells to use for the experiment on the KC-135. The cells had to be constantly incubated, which was accomplished using the cell incubator at JSC, a small incubated cooler and heating pad for the trip to Ellington field, and baby heating pads for the flight. This constant incubation was crucial to the survival of the cells.

Upon arriving at JSC, the box was not safe enough to contain the formaldehyde solution we planned on using. For several days, we modified the box to include a triple level of protection as described in the materials section. Once the box was complete, the experimental box was kept at JSC until the flight day.

Prior to the flight, the slide flasks were prepared by wrapping wire around the mouths of the slide flasks and sterilized using a UV light. Once sterilized, the cells were passaged into the slide flasks and the flasks were placed into an incubator for one night. The serum free media was also placed into the incubator to allow as much CO_2 to be absorbed into the media as possible. On the morning of the flight, the slide flask mouths were replaced with Parafilm and weatherproofing. The cells were then transported to Ellington field in an insulated cooler with heating pads to keep them warm.

At Ellington field, the slide flasks were placed into the experiment box with the myoblasts on the left side of the box and the osteoblasts on the right side of the box. The wire attached to the slide flasks were pushed through the foam surrounding the box so that they could be used for pulling the flasks into the needles for injection. Syringes filled with 10mL of formaldehyde were also attached to the box along with empty syringes. The whole box was then placed into the sealed bag and strapped to the file crates. A notebook was attached to the floor of the plane to write down which slides were successfully injected.

In the 2g pull, we punctured the slide flask mouth with the needle, and while in 0g we injected the formaldehyde while pulling air out to maintain the volume inside the flask. This process was repeated for each slide flask for approximately ten parabolas of the flight.

RESULTS:

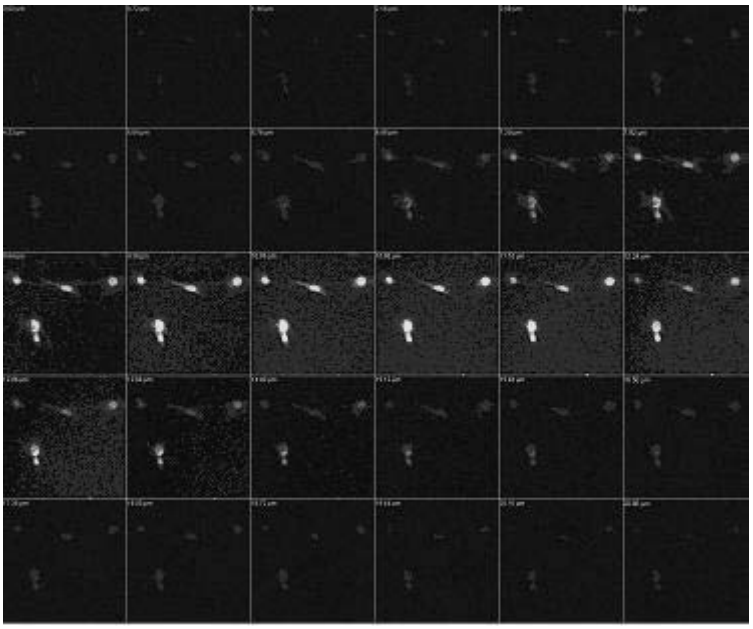

Figure 14: Control Myoblast Sample

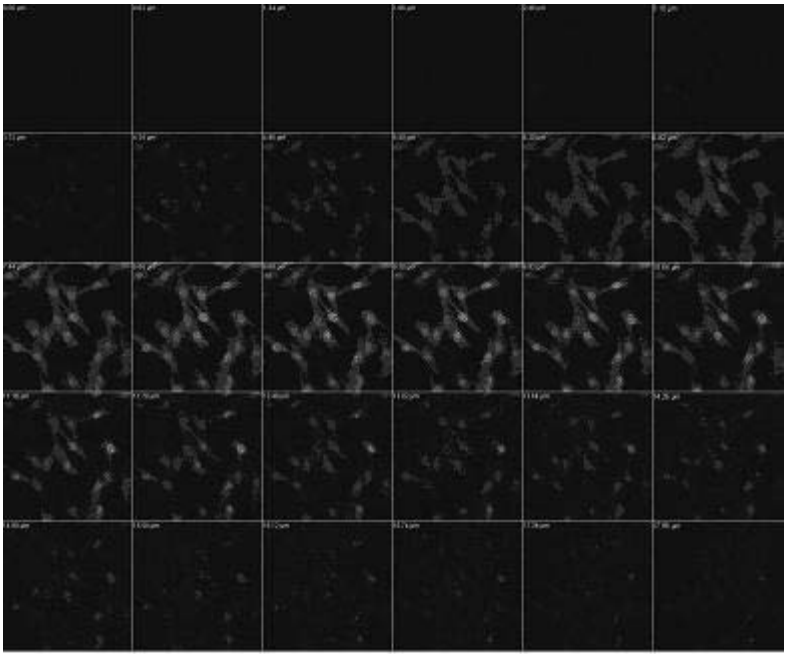

Figure 15: Myoblast cells fixed under microgravity conditions

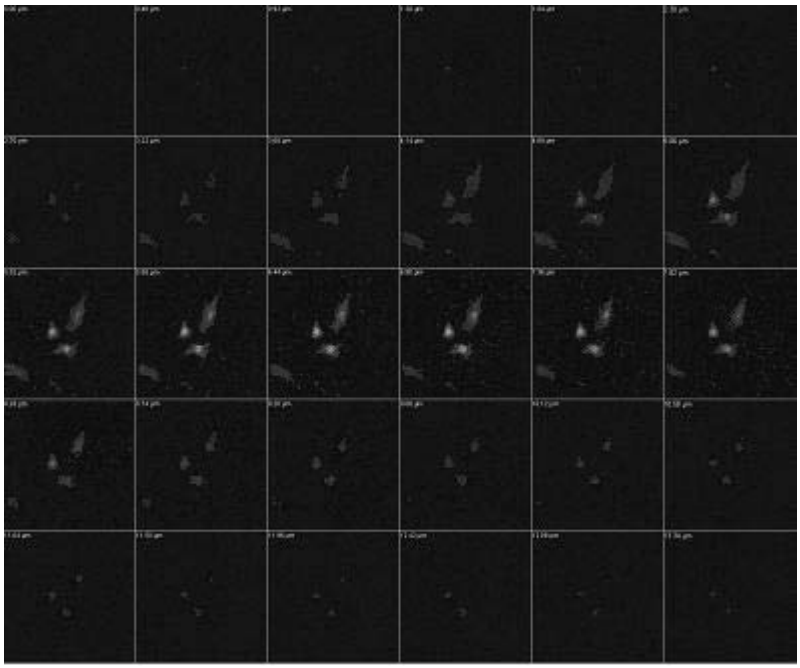

Figure 16: Myoblast cells fixed under 2G conditions

We successfully stained our cell samples with a dual-fluorescent stain. Our cell nuclei were stained with Sytox green and our actin cytoskeleton was stained with rhodamine phallodin. The stains were examined with a Zeiss LSM 510 confocal microscope. Using z-stack imaging, we were able to estimate by how many micrometers the cell nucleus moved. In most cases, microgravity actually caused the nucleus to move closer toward the bottom of the cell as can be seen by Figure 14 and Figure 13. Figure 14 represents our control myoblast sample; it was fixed on the ground while the flight was taking place. Figure 15 represents a myoblast sample fixed on the plane during the flight. We measured the slice at which the intensity of the nuclei was the greatest; in cells exposed to microgravity, this occurred when the slice of nuclei was closer to bottom cytoskeleton as compared to control cells. Osteoblast samples showed similar trends to myoblast samples. Our 2g results can be seen in Figure 16 which represents myoblasts fixed during the 2g acceleration experienced on the plane. There is no statistically significant data that indicates the cell nuclei moved more under a 2g influence than under a microgravity influence. Although we cannot provide quantitative data that microgravity had a direct effect on the cell nucleus, we can qualitatively say, through the images taken, that both the 2g pull and microgravity had an effect on the cell nuclei position.

DISCUSSION:

Our data was collected on the premise that the cytoskeleton would provide an adequate reference to the location of the cell nucleus. While qualitatively we saw that microgravity and hypergravity did have an effect on cell nuclei, we cannot infer, from the data we collected, any statistically significant patterns.

We have concluded that our images do not provide the precision necessary to enable us to discern the quantitative minute changes in nuclear position and cellular shape due to microgravity. Although the three-dimensional reconstruction of our confocal microscope images provided a general view of the cell profiles, measurements with respect to a definite plane of reference could

not be obtained due to the limitations of the microscope. More specifically, the delineation between the cell cytoskeleton and the microscope slide could not be easily distinguished because the interface between the cell and slide was extremely distorted due to the optical interference of the confocal microscope and leaky fluorescence from our stains. As well, because the scale of the required measurements was so small, the distortion of the image significantly hindered obtaining any meaningful data. This distortion leads to increased noise and blurriness of the image; therefore we are unable to conclude with certainty any movement of the nuclei.

Any optical microscope system, such as the Zeiss LSM 510, distorts the original object being viewed, and this distortion can be roughly approximated as a function of the optical properties of the microscope, such as the pinhole, numerical aperture, and laser intensities. This function, the point spread function, convolves the original object fluorescence into the more distorted final image we obtain from the microscope. In future experiments requiring image reconstruction and resolution of extremely precise features, such as the boundary between our cells and the slide surface, the images should be processed through a program to deconvolve the microscope images to obtain a truer image of the object. We attempted to process these images ourselves, but unfortunately we were unable to obtain the three-dimensional point spread function of our microscope necessary for our data analysis. We continue to investigate the reconstruction and deconvolution of our images in hopes that this will improve the analysis of future data obtained with the confocal microscope.

In addition to image noise, we also encountered other sources of error. Although we managed to collect the data, our stain intensity significantly faded while it was being analyzed. There are several theories as to why our stain faded. One could be attributed to a failure of the anti-fade agent we used to properly work. Another could be extended exposure of the samples to room light and the laser. The fading of the stain further complicated efforts for accurate measurement. The fading stain contributed to the blurring of the image and to the statistically insignificant data that we collected.

In hindsight, several alterations to our experimental apparatus also may improve the quality of the experiment and results. One significant improvement in our apparatus is in the extraction of media and injection of fixative. Our method does not allow us to know exactly when the cells are fixed in the period of microgravity since the fixative must mix with the existing media in the slide flasks before concentrations of fixative rise to a level capable of fixing the cells. Also, we do not know if the cells are fixed instantaneously or gradually over the period of microgravity (or beyond). One proposed alternative is the complete extraction of the media through a vacuum pump so that when fixative is injected, the cells are exposed to the full concentration of the fixative. Also, after observing the behavior of fluids in the slide flask in microgravity, the injection system must quickly and completely fill the chamber with fixative to ensure air bubbles do not prevent the fixative from reaching the cell surface. Also, although our containment system successfully kept the fixative from leaking, a rubber diaphragm and sealant should have been used to ensure that no fixative would leak from the slide flasks. Material limitations prevented us from finding the diaphragms which would fit our flasks.

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VIDEO:

- 2004 Student Campaign Group 5A & B, July 27 -30, 2004, Reference Master: 718435

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2.13 Undergraduate Program Flights – An H-reflex Investigation of Spinal Cord Excitability and the Effects of Microgravity

FLIGHT DATES:
July 29 – 30, 2004

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INTRODUCTION:

As technological advances lead to the progression of space exploration, human life in space becomes more of a reality. Although much scientific research has been conducted in microgravity, the effects of this new and unfamiliar environment on the human body is far from understood. Based on research conducted thus far, the effects of microgravity on the human body include cardiovascular de-conditioning (1), excess excretion of bodily fluids (2), alterations to the vestibular system affecting balance and coordination (3), changes to bone structure and growth (4), and muscular atrophy (5). As much as 20% of bone mass (6) is lost and 25%

muscular atrophy (7) occurs during prolonged space missions. These findings lead researchers to investigate the efficiency of astronaut exercise regimens as a preventative measure.

Exercise is the result of muscular contractions. The basic unit of contraction is the motor unit that is defined as the motor neuron and all the muscle fibers it innervates (8). Studies indicate that spinal cord excitability may be compromised during exposure to microgravity. If the excitability of the spinal cord decreases, a larger stimulus would be required to elicit the same muscular contraction. This would, in turn, make exercise more difficult and less effective for the astronaut. Despite the significance of such an occurrence, limited research has been conducted investigating the relationship between spinal cord excitability and microgravity. Results that have been gathered are variable and lack congruency.

Russian researchers were among the first to examine the effects of microgravity on muscular activity. Preliminary research focused on sensory system sensitivity assessed via T-reflex testing. Although the results documented by the Russians are ambiguous and lacking in details of experimental methods, decreased thresholds were reported and associated with decreases in T-reflex maximum amplitudes (9). Another Russian study utilized primates to investigate the effects of microgravity on the H-reflex. This time, H-reflex maximum amplitudes increased as a result of exposure to microgravity. It was concluded that there was a “marked increase in spinal mechanism excitability” (10).

Reschke, et al, also conducted research involving H-reflex testing. Results revealed initial potentiation of H-reflex amplitudes (after 24-hour space exposure) that were followed by a reduction of H-reflex amplitudes after seven days in-flight (11). These results have been disputed due to the variability of in-flight data for H-reflex testing (12).

Japanese researchers also have investigated the relationship between microgravity and the H-reflex. These studies revealed an increase in the H-reflex amplitudes during exposure to microgravity when compared to “normal gravity” H-reflex amplitudes. Based on these findings, it was concluded that spinal cord excitability was increased after exposure to microgravity (13 – 14).

Canadian Space Agency (CSA) scientists have conducted H-reflex testing research as well. On Spacelab IML-1, results revealed an increase in H-reflex threshold after five days in space, indicating a decrease in H-reflex loop excitability (15). In 2001, CSA research conducted aboard the ISS confirmed previous findings of decreased spinal cord excitability. These effects were examined via recruitment ratios (H max/M max) (16).

In this study, H-reflex testing was utilized to assess spinal cord excitability. The Hoffman reflex (H-reflex) is an electrical stimulation of a monosynaptic stretch reflex that bypasses the muscle spindle and instead directly stimulates the afferent nerve. For this investigation, H-reflex testing was conducted using the tibial nerve in the popliteal fossa. The stimulus travels along the Ia fibers, passing through the dorsal root ganglion, and travels across the central synapse to the anterior horn. At this point, a motor neuron is innervated and a muscular contraction of the soleus muscle results (17).

Research conducted last year aboard the KC-135 was aimed at assessing T and H-reflex thresholds and amplitudes through stimulation of the ulnar nerve. Data collection was limited for a number of reasons. T-reflex testing was determined to be unsuitable for the microgravity

environment; therefore no data was collected. H-reflex testing, however, was well suited to the microgravity environment and revealed an overall increase in H-reflex thresholds that were consistent with Watt's findings. H-reflex amplitudes were unable to be determined as the ulnar nerve loop was found lacking in length, thus making H and M wave discrimination difficult. It was determined that utilizing a nerve loop of longer length, such as the tibial nerve, would be more effective. Therefore, for this year's investigation, several alterations were made to the experimental design to further clarify and expand upon last year's results. Based on the results from last year's research, it was hypothesized that exposure to a microgravity environment would result in an increase of the minimum stimulus intensity (threshold) required to evoke a Hoffman reflex EMG response. Furthermore, it was hypothesized that the expected change in spinal cord excitability would become more pronounced with repeated exposures to microgravity. Increased thresholds served to indicate a decrease in spinal cord excitability.

The significance of this research is that it will expand the knowledge of the physiological effects of microgravity on the human body. Minimal testing has been conducted investigating the immediate effects of microgravity; most data on spinal cord excitability has been collected after twenty-four hours of microgravity exposure. The results of this study may be useful to researchers developing more efficient exercise regimens for astronauts.

METHODS AND MATERIALS:

Two subjects were used for this study. Ground tests, in-flight tests, and post-flight tests were recorded and analyzed. Hoffman reflex testing was utilized to assess spinal cord excitability.

H-reflex testing

The Hoffman reflex of the soleus muscle of each subject was tested. Each subject was connected to a series of three electrodes attached externally to the lower leg on the soleus muscle. One hand-held, safety-stimulating electrode was applied to the tibial nerve along the postero-lateral side of the knee. A 1-millisecond duration square wave pulse was used at a frequency of approximately 8 - 10 pulses per 30 seconds. The subject controlled the intensity of the stimulus by turning the voltage control dial located on the stimulator box. For stimulus presentation, the button on the safety-stimulator electrode was depressed. Once the subject was prepared for stimulus presentation, the subject gave verbal confirmation before testing began. The researcher then started computer-initiated stimulus presentation. H-reflex testing bypasses the muscle spindle and instead directly stimulates the Ia sensory neuron. In the spinal cord, the sensory neuron synapses on the primary motor neuron that then conducts the signal to the muscle. Subsequently, muscle contraction follows. Data regarding the stimulus intensity and the H-reflex response of the soleus muscle was recorded on a laptop computer. Six baseline in-flight tests were run. Data was then recorded in the first set of ten parabolas and the last five parabolas. Finally, seven post-flight tests were conducted on flight day.

RESULTS:

Threshold voltages required to evoke the H-reflex and H_{max}/M_{max} ratios were analyzed.

Subject 1(Threshold voltages and H_{max}/M_{max} ratios):

In-flight data was not obtained due to subject illness. Therefore, no data analysis could be completed.

Subject 2 (Threshold voltages):

Electrical stimulation of the tibial nerve consistently evoked motor responses in the soleus muscle and was recorded (Fig. 18). The stimulus threshold required to elicit the H-reflex was determined before, during, and after parabolic flight. Based on the measurements, it was found that 38.1% greater stimulus intensity was required to reach threshold during parabolic flight. The results obtained were as follows:

Pre-flight: Results of the six baseline tests revealed an average threshold of 29.3 volts. The determined standard deviation was 1.87. (Table 5 and Fig. 17)

First ten parabolas: Results of the ten tests revealed an average threshold of 29.8 volts. The determined standard deviation was 2.14. (Table 5 and Fig. 17)

Last five parabolas: Results of the last five tests revealed an average threshold of 40.5 volts. The determined standard deviation was 1.88. (Table 5 and Fig. 17)

Post-flight: Results of the seven tests revealed an average threshold of 45.7 volts. The determined standard deviation was 4.70. (Table 5 and Fig. 17)

Table 5 – Means and Standard Deviations of Threshold Comparisons (Subject 2)

	Mean	Standard Deviation
Pre-flight	29.3	1.87
Flight first 10	29.8	2.14
Flight last 5	40.5	1.88
Post-flight same day	45.7	4.70

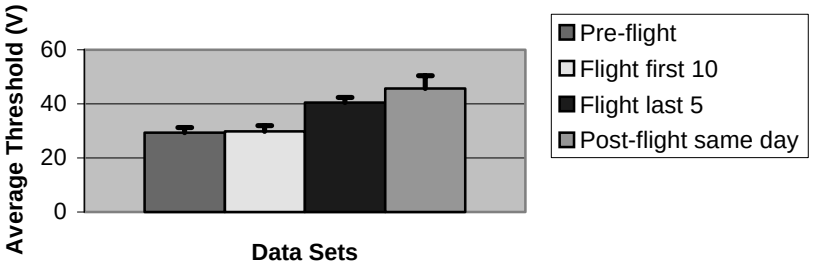
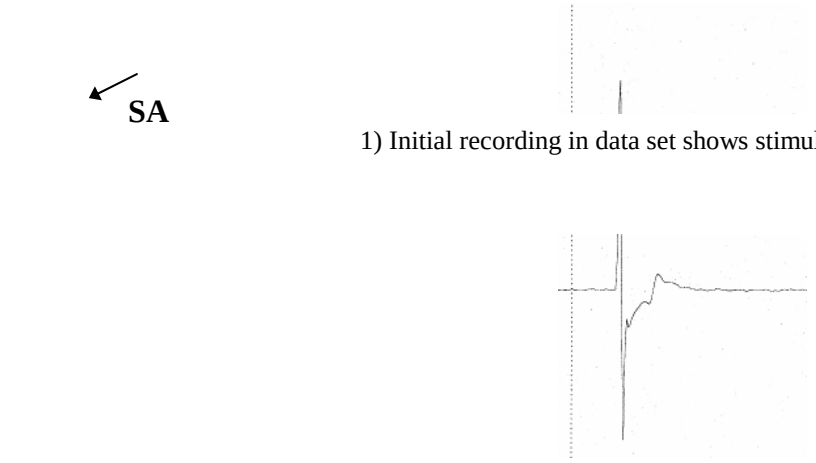


Figure 17. Threshold Comparisons (Subject 2)



1) Initial recording in data set shows stimulus artifact (SA) at 31.7 Volts.

2) Increasing the voltage incrementally (40.7 Volts) leads to the appearance of M & H-wave.

3) At 44.6 Volts, growth is noted in both the M & H-wave.

4) Maximum H-wave amplitude is reached at 50 Volts. Due to the intensity of the M-wave as voltage is increased; the H-wave is eventually overwhelmed and disappears from the recording.

Figure 18 – EMG Recordings as Voltage Stimulation is Increased

ANOVA analysis (Table 6) revealed that the observed differences between the following tests were statistically significant at $\alpha = 0.05$:

Table 6. ANOVA Analysis Results of Threshold Comparisons (Subject 2)

	p-value	Significant
Pre-flight v. overall flight	0.103	-
Pre-flight v. first 10	0.655	-
Pre-flight v. last 5	4.08×10^{-06}	*
First 10 v. last 5	3.50×10^{-07}	*
Overall flight v. post-flight	5.94×10^{-05}	*
Pre-flight v. post-flight	6.67×10^{-06}	*
Post-flight v. first 10	9.98×10^{-08}	*
Post-flight v. last 5	0.0418	*

Subject 2 (H_{max}/M_{max} ratios – Fig. 3)

The H_{max}/M_{max} ratios were determined via measurements of the largest H-wave recorded divided by the largest M-wave recorded in a single test. The results obtained analyzed via ANOVA testing (Table 7) revealed that statistically significant ($\alpha = 0.05$) differences existed between pre-flight v. last five parabolas and first ten parabolas v. last five parabolas.

Table 7 – ANOVA Analysis Results of H_{max}/M_{max} Ratio Comparisons (Subject 2)

	p-value	Significant
Pre-flight v. overall flight	0.611	-
Pre-flight v. first 10	0.563	-
Pre-flight v. last 5	0.00655	*
First 10 v. last 5	0.00347	*
Overall flight v. post-flight	0.865	-
Pre-flight v. post-flight	0.748	-
Post-flight v. first 10	0.378	-
Post-flight v. last 5	0.0420	*

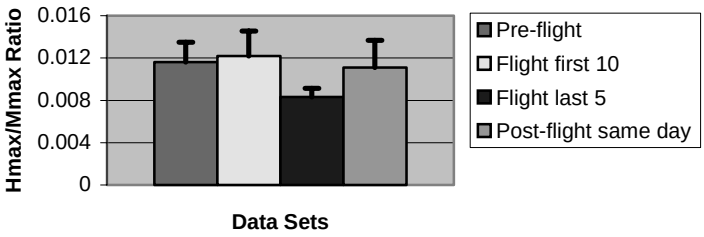


Figure 19. Hmax/Mmax Ratio Comparisons (Subject 2)

DISCUSSION:

Due to immediate illness upon entrance into parabolic flight, data was unable to be collected on Subject 1 during flight. This severely limited data reliability. Also, accidental power failure occurred during the last ten parabolas for Subject 2's flight, limiting data collection to the final five parabolas only. Despite these occurrences, the H-reflex testing methods and procedures utilized were found suitable for the microgravity environment.

Threshold data on Subject 2 was consistent with last year's research and research conducted by Watt via the Canadian Space Agency (CSA) aboard the International Space Station (ISS). As expected, results revealed decreased spinal cord excitability as evidenced by the increased threshold required to elicit the H-reflex (Figure 17). The decrease in excitability of the spinal cord was not immediately present upon entrance into microgravity; the decrease was noted during the last ten parabolas or after approximately one-hour exposure to microgravity. Watt's research indicates decreases were noted during the first in-flight test; however, the initial in-flight test was taken after a twenty-four hour exposure to microgravity. Therefore, based on the results found in this research, decreases may be taking place more quickly than originally thought. Still, spinal cord excitability does not have an immediate decline; instead it appears to occur gradually as exposure to microgravity continues.

In Figure 17, it appears that elevation of threshold continues to occur post-flight. Unfortunately, the medication factor cannot be ruled out as a possible cause for this occurrence. Scop-dex was administered to each subject prior to flight and information regarding its effects on spinal cord excitability is unavailable. Therefore, further research on the effects of Scop-dex on spinal cord excitability would be needed to clarify the results of the present study. In addition, further study to see if the elevated threshold levels paralleled the dose response curve of the medication would be beneficial.

Ratio comparisons of H and M wave maximum amplitudes (Figure 19) were consistent with those reported by Watt and the CSA. During the last five parabolas a significant decrease was noted. This again would serve to indicate a decline in spinal cord excitability as maximum wave amplitudes were reduced considerably. Still, the decrease does not occur immediately, but appears after continued exposure to microgravity.

Perhaps due to differences in methods, the results of this experiment differ starkly with those of the Japanese and the Russians. Each noted increases in spinal cord excitability. It is unclear if anti-motion sickness medications were utilized by the subjects of these studies. Therefore, this may contribute to the opposing results obtained.

SUMMARY:

Based on the results of the present study, it was concluded that spinal cord excitability decreased as a result of continued exposure to microgravity. Unfortunately, the role of the medication Scop-dex cannot be ruled out as an independent variable. Further research on the effects of this medication on spinal cord excitability is necessary. In addition, more subject testing is necessary to contribute confirmation and clarification of results.

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OUTREACH:

Website

The KC-135 UHCL Student Flight Team's website contained the abstract of the proposal and experiment title; team bios, pictures, and news; KC-135 information; and links to the team members' university and team e-mail address.
http://www.geocities.com/reflexions_2004/

Hitchcock ISD Outreach (Stewart Elementary):

The KC-135 UHCL Student Flight Team utilized the KC-135 Student Flight Program as an orientation program in which to educate and encourage underprivileged 4^{th}-6^{th} grade students to cultivate their dreams of a higher education in the field of mathematics and science. The UHCL team put together a Power Point presentation to promote NASA's KC-135 Student Flight Program, in addition to instilling the importance of teamwork and diligence in academic studies.

Pasadena ISD Outreach (Challenger Elementary):

The KC-135 UHCL Student Flight Team joined the teachers at this local alternative school to promote interest in mathematics and science. The team Power Point presentation was used to prepare the students for the ISS downlink scheduled by the Aerospace Academy. During the presentation, emphasis was placed on the importance of academic achievement and responsibility. An open forum took place at the end of the session to allow students the opportunity to ask questions about college life, microgravity, and future math/science careers.

Investigating Life Science in Space:

This was a teacher workshop for middle school science teachers offered through the Aerospace Academy. The workshop involved 30 to 40 teachers from 7 school districts located in close proximity to NASA JSC. The team served as workshop science assistants. In addition, the team presented their Power Point presentation to encourage teachers to promote interest in the fields of mathematics and science. The UHCL Student Flight team also attended the ISS downlink sponsored by the Aerospace Academy. Approximately 60 to 80 students participated in the downlink at Space Center Houston. Other students not in attendance watched via NASA television. It was projected that this activity reached a student audience of nearly 500 students depending on the availability of NASA TV. The event was featured in the SpaceTEC Quarterly Newsletter, *SpaceTEC Talk.*

UHCL Student Conference:
The UHCL Student Flight team presented their research at the National Student Research Forum which was held at UHCL in April. A poster session gave a brief overview of the research conducted. Team representatives were present to answer questions regarding their research.

Girl Power event:
After an overwhelming expression of interest in the team's adventure aboard the KC-135, Girl Scout involvement will be expanded this year to reach more troops. The KC-135 UHCL Student Flight Team is committed to interacting with area Girl Scout troops to further promote and educate students of the unique opportunities available in science and math. The team plans to show the girls a video of last year's flight and intends to interact on a personal basis by way of sharing with them personal experiences aboard the KC-135. Following the presentation, the team will be holding question/answer sessions for the girls to address any inquiries regarding the KC-135 Student Flight Program or to answer questions about space science in general. This Girl Power event is scheduled for mid-January 2005.

Expanding Your Horizons:
This event is scheduled to take place February 12, 2005. The focus of EYH is to encourage junior high girls to pursue careers in mathematics and science. Two workshop sessions will be hosted by the team detailing their research activities aboard NASA's KC-135.

ACKNOWLEDGEMENTS:

The UHCL Student Flight Team would like to thank Dean Charles McKay and the department of School of Science and Computer Engineering at the University of Houston – Clear Lake as well as the Texas Space Grant Consortium for their financial sponsorship of this research. We also would like to thank Dr. Rick Puzdrowski, our faculty advisor, for all of the time and support he provided us with during this project. His help and guidance made this project a reality for the team. In addition, the team would like to thank Dr. Ron Mills for all his help in securing funding for the research as well as lending support in data analysis. Finally, the team would also like to extend a thank you to the RGSFOP and RGO for all their assistance during this flight campaign.

PHOTOGRAPHS:

JSC2004E33225 to JSC2004E33227
JSC2004E33238 to JSC2004E33239
JSC2004E33244 to JSC2004E33245
JSC2004E33250 to JSC2004E33254

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2.14 Undergraduate Program Flights – S.E.C.U.R.E. II: Single-rescuer Emergency CPR while Unrestrained in Reduced gravity Experiment

FLIGHT DATES:
July 29-30, 2004

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Tina Tanhehco, Harvard University

FACULTY ADVISORS:
Nathan Shapiro, Harvard Medical School
Peter Lee, Harvard Medical School
Gregory Jay, Harvard Medical School

INTRODUCTION:

The growing presence of humans in space makes the need for effective medical emergency procedures critical. Asphyxiation, electric shock, and toxic inhalation are a few of the possible causes which can lead to cardiac arrest in orbit. To effectively treat cardiac arrest, a rescuer must perform cardiopulmonary resuscitation (CPR) to increase the victim's coronary and cerebral blood flow. Furthermore, commercial flights carrying civilian passengers may begin to take place in the near future. Since physical screening and training may not be as rigorous for civilians as it is for astronauts, the need for an effective CPR procedure is imperative.

Manual CPR suffers great limitations in microgravity, as well as on firm ground. Rescuer fatigue and inaccuracy in rate and depth of chest compressions are important factors that may limit blood flow to vital organs. To remedy these shortcomings in Earth-bound rescues, the Revivant Corporation designed the AutoPulse™ Resuscitation System (ARS), a non-invasive device that performs automated consistent chest compressions to improve coronary and cerebral blood flow during cardiac arrest. The ARS has the potential to improve a victim's chance of survival in microgravity, should the necessity for CPR arise.

OBJECTIVES:

Our experiment sought to determine (1) if the ARS would function properly in microgravity, and (2) if it would be an improvement upon manual CPR. The KC-135, administered by the Reduced Gravity Office, provided the microgravity environment that was central to this study.

Current NASA protocols for CPR in microgravity require manual compressions and ventilations with the victim and rescuer restrained using the Crew Management Restraint System (CMRS). We compared compressions using the ARS to manual compressions administered using the CMRS. We used the guidelines for CPR published by the American Heart Association (AHA) to compare the efficacy of both methods. Our test results indicated that the ARS can provide chest compressions that are consistent with AHA standards and superior to manual compressions in terms of the average depth of compression.

METHODS AND MATERIALS:

A standard half-body mannequin was pre-intubated with an intubating Laryngeal Mask Airway (iLMA). A bag valve mask (BVM) was connected to the iLMA. The depth of the chest compressions (anterior to posterior) was measured with a calibrated potentiometer fitted into the mannequin's thoracic cavity. The ventilations were measured with a piezoelectronic pressure sensor. An accelerometer was attached to the deck of the aircraft to certify that the rescue maneuvers were performed during the microgravity phase of the flight pattern. Signals from the three devices were recorded by a laptop computer using the BioPac MP100 hardware and AcqKnowledge Software. Additionally, a video camera was set up to record all of the procedures and rescuer comments.

For each flight, the ARS was tested during ten parabolas. At the start of each microgravity period one flyer initialized and monitored the ARS while administering ventilations using the BVM. The other flyer executed the data capture on the portable computer. The manual rescue maneuver using the CMRS was performed during another 10 parabolas, with the each flyer completing five parabolas as the rescuer.

The same in-flight protocol was performed in ground-based trials pre- and post-flight.

Tether

To insure that our experiment would not collide with other objects aboard the KC-135, our mannequin was secured by a tether wrapped around the torso of approximately 1.5 meter in length.

Crew Management Restraint System

For the purposes of this experiment, a replica was built due to the lack of availability of a bona fide CMRS. The main part of the restraint system was a plywood board (18 in. x 42 in.) bolted to the floor of the KC-135. A series of nylon straps formed a “v” restraint over the shoulders of the mannequin. The “v” restraint was fastened to an “o” ring under the abdomen of the mannequin.

Pre-intubated mannequin

A half-body adult mannequin, designed as a trainer for both airway management and CPR, was used. The mannequin was fitted with potentiometers that were calibrated to measure the depth of compression during CPR compressions and during CPR ventilations. These measurements were recorded by a laptop computer.

Intubating Laryngeal Mask Airway

The iLMA is a rigid, anatomically curved airway tube which accepts a standard 15 mm connector and an 8.0 mm cuffed endotracheal tube. The iLMA is lightweight and was pre-inserted before the flight and remained in the mannequin for the duration of the flight. Proper tracheal intubation was confirmed by bag valve mask ventilation of the mannequin prior to the flight.

Bag Valve Mask

The bag mask valve is a manual resuscitation device used to provide respiratory support to a respiratory distressed or non-breathing patient. Because the mannequin had the iLMA tube inserted into the trachea, the mask component of the bag valve mask was not used, and only the bag of the bag valve mask was attached to the iLMA tube. The bag was attached and secured to the mannequin prior to the flight.

Kistler Instruments Single-axis Capacitive Accelerometer

This accelerometer was attached to the floor of the plane and provided in-flight g-load data, which was recorded by a laptop computer. This accelerometer had been previously tested in reduced gravity flights.

Biopac MP100 Data Acquisition Unit

The MP 100 is a system designed to capture analog and/or digital data signals. This unit converted the analog signals given by the mannequin and accelerometer into a stream of digital information recorded by the laptop computer.

IBM T30 Portable Computer

The PC was equipped with Pentium 4 processor at 1.6 Ghz and 512 MB’s of RAM, running Microsoft Windows 2000 with Service Pack 4.

Biopac AcqKnowledge® Software v. 3.7.3

AcqKnowledge® is a comprehensive data capture and analysis suite that collects data from the MP100 acquisition unit and stores it on the hard drive of the computer. This software has an extensive collection of data analysis sub-routines specifically designed for life-sciences research.

Furthermore, it has the capability to export the data files into standard tab-delineated columns which can be read by the majority of data analysis programs.

Revivant AutoPulse® Resuscitation System
The ARS is a automated CPR device, produced by the Revivant Corporation. It is capable of delivering compressions to a patient that result in a twenty percent anterior posterior displacement of the patient's chest cavity for at least 30 minutes per battery. It weighs 27 pounds (with two batteries) and measures 8.4 cm H by 46.2 cm W by 82.5cm L.

RESULTS:

Depth of compression, ventilations, and on-board g-forces were recorded simultaneously in digital format using the AcqKnowledge software. The raw data were analyzed to identify the peak depth of compression values for each compression and the length of time between compressions. The time to complete each 15:2 compression-ventilation cycle was also calculated. These data were compiled for each of the four rescuers and for each of the following two configurations: Manual (CMRS), Automated (ARS). For each group and rescuer, the mean and standard deviation of all measurements were calculated.

The mean ± SD depths of compression were: Manual: 1.30 ± .46", Automated: 1.70 ± .13" while the compression per minute (cpm) rates were: Manual: 126.11 ± 20.2, Automated: 83.36 ± 15.1. The lengths of time to complete a 15:2 cycle were Manual: 11.21 ± 1.84 s, Automated: 13.64 ± 1.3 s.

DISCUSSION:

The primary objective of this study was to compare the quality of CPR that can be provided by performing compressions manually and with the assistance of the ARS. The criteria by which success of CPR was measured included the AHA standard for depth of compression of 1.5 – 2.0 in (anterior to posterior displacement); a compression rate of approximately 100 compressions per minute and a 15:2 compression to ventilation cycle length of approximately 2-3 seconds.

When CPR was performed manually on the CMRS, the majority of rescuers were able to achieve compressions greater than 1.5 in. However, there were some instances where the rescuers had difficulty attaining that minimum depth, partly owing to either motion sickness or failure in the restraints of the CMRS. The alternating microgravity and 2g periods placed an undue physical stress on the flyers which could have contributed to their inability to perform CPR correctly. Some of the rescuers were also smaller and lighter than the others, pointing to the need to consider an individual's size and strength when determining his/her ability to perform adequate CPR.

When CPR was performed with the assistance of the ARS, all compressions attained the minimum depth of 1.7 in ± .13.

The AHA recommendations for compression rate are "approximately 100 cpm." The results indicate that a compression rate of 100 cpm was attainable for rescuers performing CPR manually.

Although the ARS performed at 83.36 cpm, the timing controls can be adjusted by the manufacturer in the software of the device.

It is also important to note that the ± 20.2 and ± 15.1 sec. standard deviatios for the cpm of manual and automated compressions, respectively, were due to (1) the 20-25 sec. microgravity intervals that didn't allow for the compression-ventilation cycles to be performed for a prolonged period of time, and, in the case of ARS-assisted rescue, (2) the difficulty in timing the initialization of the ARS with the actual moment when the aircraft enters a microgravity portion of the parabola.

All the rescuers were minimally experienced in performing CPR before the flights, showing that the protocol can be executed without extensive medical training. The requirement of a single rescuer to perform CPR with the aid of the ARS can increase the chance of survival for a victim of respiratory and/or cardiac arrest in microgravity. Despite the fact that some of the rescuers were not able to meet the minimal depth of compression due to problems specific to this experiment aboard the KC-135, the ARS has demonstrated that it can provide consistent effective compressions in microgravity and therefore can be presented as a viable alternative to current CPR protocols for orbit-bound missions.

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JSC2004E33246
JSC2004E33306 to JSC2004E33307
JSC2004E33312 to JSC2004E33316
JSC2004E33325 to JSC2004E33329

VIDEO:

- 2004 Student Campaign Group 5A and B, July 27 -30, 2004, Reference Master: 718435

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.15 Human Patient Simulator Evaluation Flights Evaluation of HPS Function

Evaluation of HPS as a Training Platform

FLIGHT DATES:
August 10-13, 2004

PRINCIPAL INVESTIGATOR:
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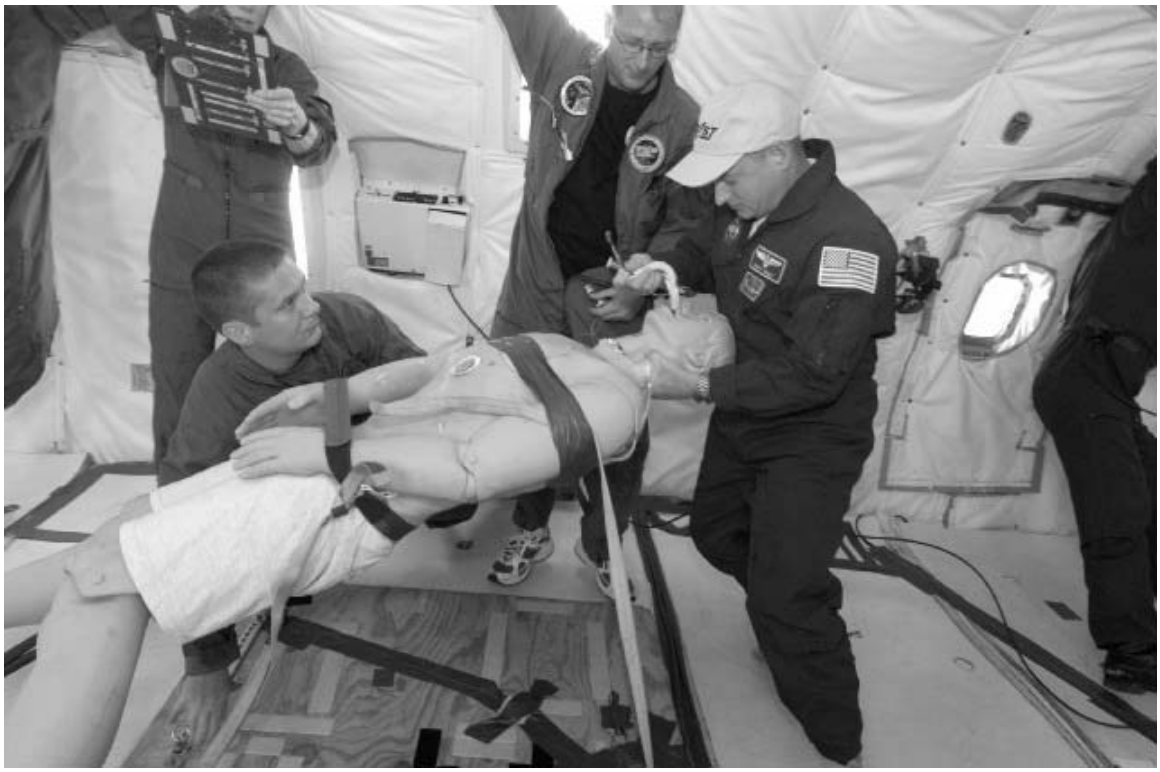
CO-INVESTIGATORS:
Kira Bacal, MD, PhD, MPH, NSBRI
Gabriel E. Mena, MD, Baylor College of Medicine



INTRODUCTION:

Human spaceflight has developed over more than 40 years in pursuit of knowledge and exploration of our universe. As space exploration continues to evolve, and mission lengths grow, we must continue to advance our efforts in securing the safety of our astronauts. To date we have been fortunate not to have experienced any major onboard medical contingencies. This is in great part due to NASA's rigorous screening of all astronaut candidates as well as the robust preventative medicine program instituted by NASA. As mission length increases and we prepare to extend these expeditions beyond low Earth orbit, we must prepare for the increasing possibility of a medical contingencies, requiring medical interventions. As the distances from Earth grow greater, the paradigm of "Scoop and Run," will no longer be possible and we will have to "Stand and Fight." In case of a medical emergency, the physician astronaut and CMO (Crew Medical Officer) will need to be capable of managing emergency medical events. Knowledge of basic skill sets such as insertion of a life saving airway device, like the Intubating Laryngeal Mask Airway (ILMA) will be necessary.

The CMO will need to be familiar with the performance of a basic physical exam such as the pupillary assessment and with the acute management of the airway in case of an emergency. Sustainment training of astronauts will be necessary to maintain emergency medical skills such as airway management and CPR on extended duration missions. The NASA reduced gravity testing program (KC-135) provides a high fidelity environment for this training.

Emergency medical procedures and vital medical equipment deployment can be accurately and efficiently evaluated and validated with the use of the METI Flight readied Human Patient Simulator (HPS). The HPS mimics real emergency scenarios and provides an excellent tool for astronaut and CMO training.

This experiment was designed to evaluate and determine the ability, time, efficiency and success of astronauts both with and without medical training and CMO analogs, while performing these clinical tasks. These experiments were also conducted to gather metrics on human performance in the microgravity environment. The following studies were performed: pupillary assessment, deployment and placement of the defibrillator, deployment and placement of the pulse oximeter, IV fluid set up, and ILMA insertion and ventilation in three scenarios.

Medical Operational Support Team (MOST) and the Human Patient Simulator (HPS)

The MOST was developed by the National Space Biomedical Research Institute (NSBRI) and was tasked by the JSC Space Medicine and Life Sciences Directorate (SLSD) to incorporate medical simulation into: medical training for astronaut-CMO and flight control teams and for evaluation of procedures and resources required for medical care aboard the International Space Station (ISS).

The MOST has incorporated medical simulation into Space Medicine Operations by using the HPS manufactured by Medical Education technologies, Inc. (METI, Sarasota, FL). The HPS has a very high fidelity and is an excellent tool for the training of CMO and astronauts in microgravity.

The efforts completed by the MOST in the past year have not been limited to curriculum development. The team has begun collaborating with an industrial leader in ventilation and a prominent military medical institution to conduct a study that would augment the standard of CPR care aboard the ISS. The MOST has also validated skill set training for surgical and non-surgical airway management as well as pneumothorax. The MOST has also developed a training regimen that allows NASA and Department of Defense (DOD) flight surgeons who are assigned for Russian Soyuz spacecraft recovery to administer anesthesia to a returning astronaut/cosmonaut “in the field” should it be necessary following the crew’s return from an ISS mission.

The establishment of the MOST by the NSBRI has enabled SLSD to begin augmenting the standard of medical training for medical flight control teams and expand other areas of medical training. In the coming year, the MOST will continue this effort by not only validating its training curriculums for the designated spaceflight disciplines but also by implementing a Continuing Medical Education credit program for flight surgeons as per the guidelines of the American College of Graduate medical Education (ACGME) and JSC Space Medical Operations.

MATERIAL AND METHODS:

All subjects first received a preflight safety briefing to comply with the reduced gravity program’s regulations. The participants were then briefed by the flight surgeon and were given an appropriate dose of both Scopolamine and Dexadrine. Prior to flight, a didactic training session was conducted by the investigators explaining in detail the medical procedures and specific tasks that would be required during the parabolic flight. Participants included astronauts, flight surgeons, CMO analogues, anesthesiologists, surgeons, and emergency medical specialists.The equipment and procedures were divided into five stations to be completed during the four day flight campaign. These stations included:

1. Intubating Laryngeal Mask Airway (ILMA)

2. Pupillary assessment
3. IV kit deployment and set up
4. Defibrillator deployment and set up
5. Pulse Oximeter deployment and set up.

The experiments were divided into four days. Each day consisted of 40 parabolas for a total of 160 parabolas available to complete the investigation.

Day #1 we conducted the pupillary assessment and IV kit deployment and set up study as well as simulator function.

Day #2 we conducted the pupillary assessment and defibrillator deployment and set up study. We also followed up on a few simulator function issues that needed repair.

Day #3 we conducted the ILMA and pulse oximeter deployment and set up study.

Day #4 we conducted only the ILMA study.

During the parabolic flights the participants performed the designated procedures. Not all participants were able to complete all of the procedures due to time constraints secondary to 0g periods. At the completion of each day, subjects were asked specific questions about each study and participated in a recorded debriefing session. The video was utilized for metrics. In addition the participants received a written questionnaire on each study to complete the data for those experiments. The small group debriefing post-flight was useful to obtain important data and offered a forum for the participants to share their ideas concerning the techniques demonstrated.

Intubating Laryngeal Mask Airway

During the pre flight experiment briefing (on ground), each of the astronauts and analogue personnel received a 20 minute class demonstrating proper techniques for ILMA insertion and mouth to ILMA ventilation using the HPS mannequin. The training session were conducted by the investigators (Anesthesiologist). Each subject was given “hands-on” time to practice their ILMA insertions. After familiarization with the procedure each subject was tested both pre-flight and at 0g.

Initially at 0g’s each subject was instructed again by the investigator, who showed them different approaches and positions for adequate ILMA insertion.
Subjects were timed from the moment they held the ILMA in their hands to the moment of positive mannequin chest rising after mouth to ILMA ventilation. During 0g’s each subject attempted different positions for ILMA insertion. Also the mannequin was available at different scenarios such as restrained to the floor, partially restrained and free-floating 3 feet off the ground.

Scenario #1	patient – restrained	//	caretaker -restrained
Scenario #2	patient - partial restraint	//	caretaker -restrained
Scenario #3	patient - free float	//	caretaker -free float

Pupillary Assessment

On the pupillary assessment station on ground each subject was given a pupillometer as a card to evaluate for pupillary shape, size and symmetry. Reaction of the pupils to penlight stimulation was also recorded. They were all trained by the investigator and timed pre-flight, 0g, 1g and post-flight by the test coordinator keeping track of the time. Subjects evaluated each other’s pupils on each different environment. Subjects were timed from the moment they hold the penlight in their hand until the moment they completed the pupillary assessment.

Intravenous Deployment/Set up
In this experiment we studied the length of time necessary for a subject to both deploy and set up an IV administration set. Each subject was timed from the moment that the IV checklist was read by another subject to the moment of completion of the task. During the flight the subjects would only work on their tasks in the 0g period of the flight. The experiment was conducted and timed pre-flight, 0g and post-flight in order to evaluate the effects of microgravity and possible effects of pre flight medication.

Defibrillator Deployment/Setup
During the experiment consisting of defibrillator deployment and set up on ground, each subject was timed from the moment that they deployed the defibrillator to the moment when the connected the paddles. The experiment was conducted and timed pre-flight, 0g, 1g and post-flight in order to evaluate the effects of microgravity and possible effects of pre flight medication.

Pulse oximeter Deployment /Set up
During the experiment consisting of pulse oximeter deployment and set up on ground, each subject was timed from the moment that they deployed the pulse oximeter to the moment when they connected the pulse oximeter probe on the mannequin's index finger. The experiment was conducted and timed pre-flight, 0g, 1g and post-flight in order to evaluate the effects of microgravity and possible effects of pre flight medication.

PRELIMINARY RESULTS:
Duration in seconds was recorded for each subject while performing each experiment pre-flight, 0g, 1g and post-flight. The raw data were exported from the time sheets to Microsoft Excel and mean and standard deviation was calculated for each study.

Pulse oximeter deployment and set up:
For the pre-flight time the mean ± standard deviation was 25.67±4.04, during 0g the mean ± standard deviation was 38.33+4.04, during 1g the mean ± standard deviation was 24.00±2.00 and for post-flight the mean ± standard deviation was 20.33±2.00 (figure1).

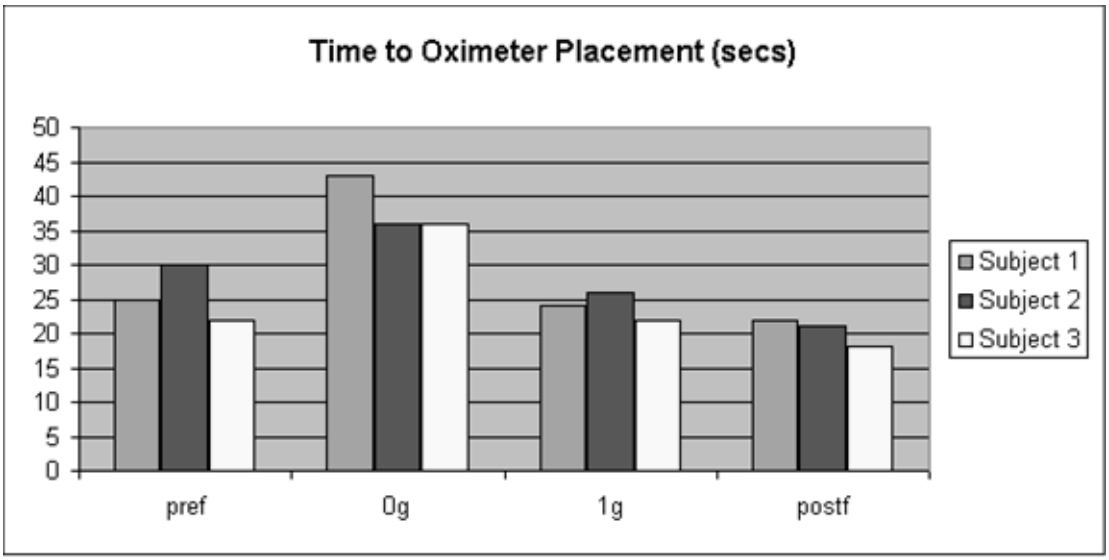


Figure 1 Pulse oximeter

Pupillary Assessment:
For the pre-flight time the mean ± SD was 21.57±4.58, during 0g the mean ± SD was 34.43±8.22, during 1g the mean ± SD was 24.14±4.88 and for post-flight the mean ± SD was 20.57±5.19 (figure2).

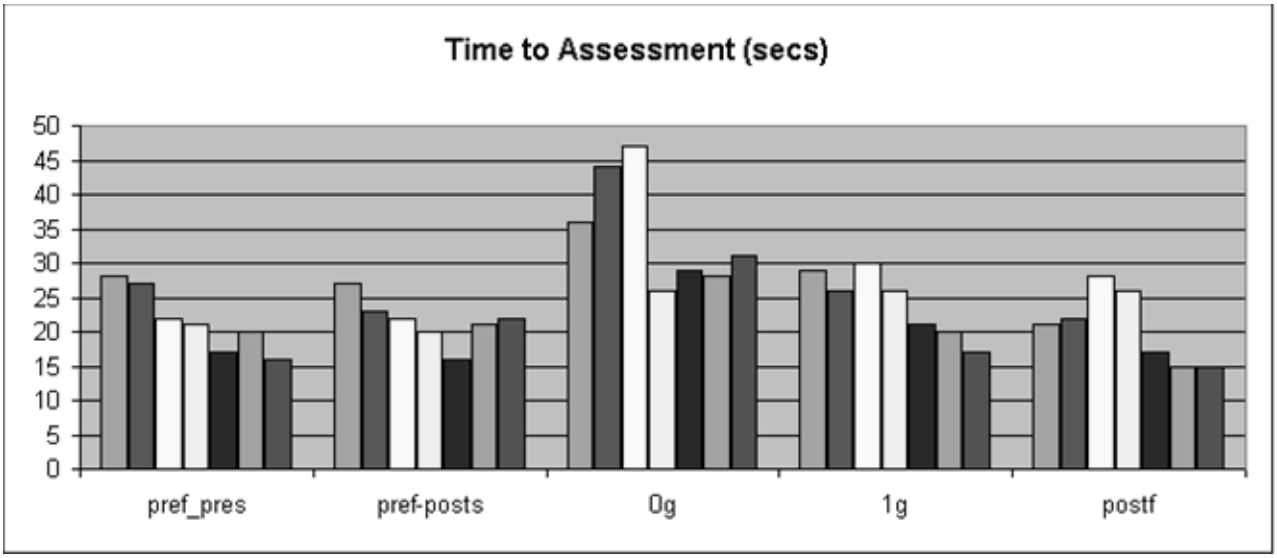


Figure 2: Pupillary assessment

IV kit deployment and set up:
For the pre-flight time the mean ± SD was 102.5±10.41, during 0g the mean ± SD was 105.25±11.38 and for post-flight the mean ± SD was 95±24.32 (figure3).

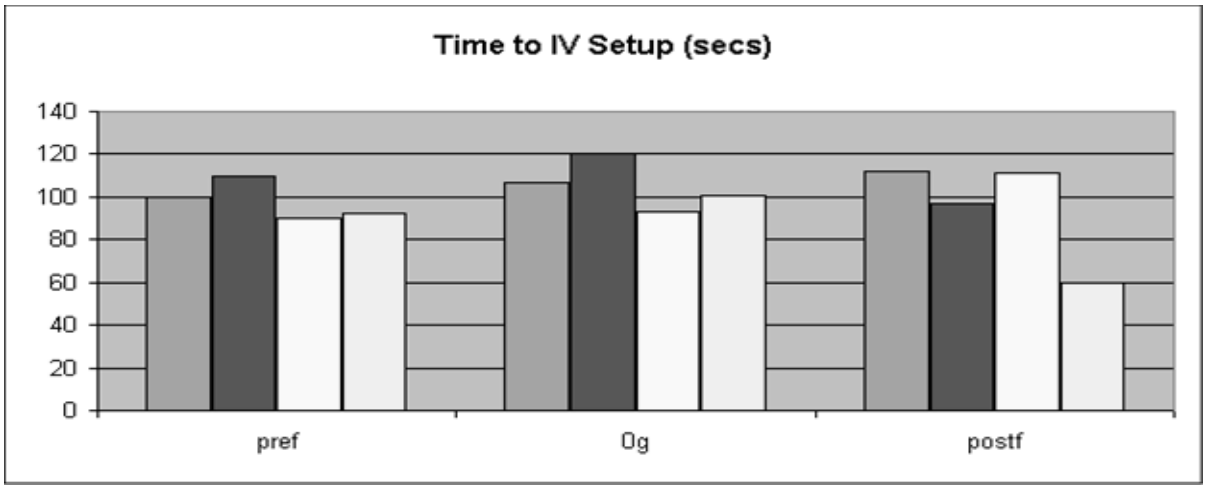


Figure 3 IV deployments and set up

INTUBATING LARYNGEAL MASK AIRWAY (ILMA):

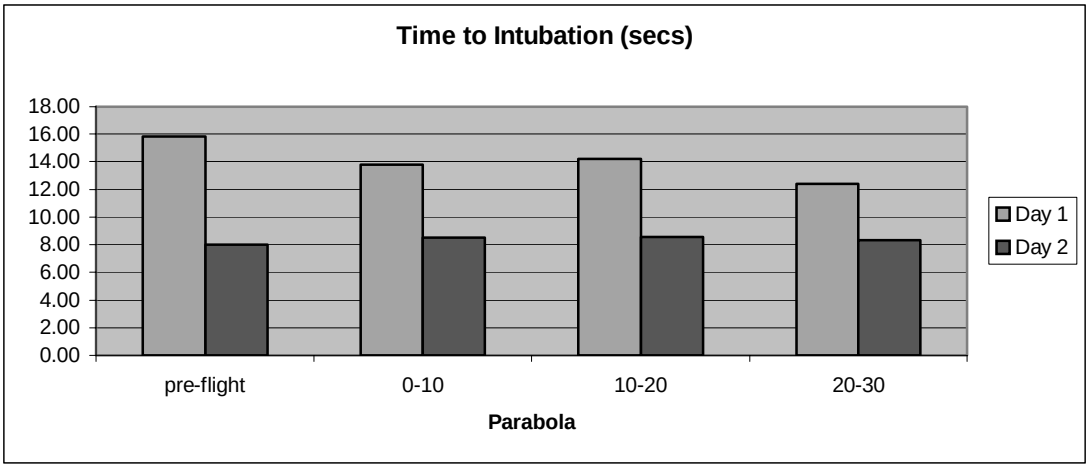


Figure 4 ILMA Insertion

DISCUSSION:

As expected, and has been reported in the aerospace literature, the time to successful completion of the tasks took longer when performed in microgravity.

There was a major delay on the IV kit deployment and set up study during microgravity. We attributed this finding to the fact that the checklist for the IV is long and the parts that match with the IV set are very small and difficult to assemble to each other in microgravity.

There was also time delay on the defibrillator deployment and set up study as well as the pupillary assessment and pulse oximeter deployment study in microgravity. The time delays on the experiments were mainly a result from the lack of gravity and the effects of microgravity on the neuro-vestibular system and orientation.

Interestingly enough, our data indicate that during ILMA insertion and ventilation in microgravity, the time in seconds was less when compared to the insertion and ventilation with the ILMA pre-flight at 1g. At the same time the success rate was very high (>95%).

We have shown that the ILMA can be satisfactorily inserted in a significant short period of time (<10 seconds) and used for mouth to ILMA ventilation by non-anesthesiologists with limited clinical experience in ILMA placement.

We concluded that the ILMA has a low failure rate in microgravity, with or without the use of restraints to patient or care giver. Insertion of this device does not require use of a laryngoscope; thus, one hand is free to stabilize the head and neck.

ACKNOWLEDGEMENTS:

We wish to thank the Reduced Gravity Flight Office, the individuals at WYLE Life Sciences, and NSBRI for their assistance on this project. We also want to thank METI for the HPS.

PHOTOGRAPHS:

JSC2004E35196
JSC2004E35204 to JSC2004E35217
JSC2004E35229 to JSC2004E35237
JSC2004E35240 to JSC2004E35276

JSC2004E35615 to JSC2004E35653
JSC2004E36240 to JSC2004E36265
JSC2004E36328 to JSC2004E36279

VIDEO:

- Zero-g August 9 – 13, 2004, Reference Master: 718620

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.16 Endosafe®-Portable Test System (PTS)

FLIGHT DATES:
August 31, 2004

PRINCIPAL INVESTIGATORS:
Jake Maule, Carnegie Institution of Washington
Norm Wainwright, MBL, Woods Hole

CO-INVESTIGATORS:
Dan Burbank, NASA/Johnson Space Center

OBJECTIVE:

Evaluate most effective fluid dispensing system for use on ISS.

INTRODUCTION:

The Portable Test System (PTS) is a hand-held device for monitoring the presence of potentially hazardous bacteria in the environment. It uses an immunological method derived from the horseshoe crab (*Limulus polyphemus*) to detect bacterial cell membranes and other molecular

components of a cell. Further modifications of the PTS will allow detection of individual hazardous species of bacteria.

This study was a follow-up of previous PTS and other immunological tests performed on the KC-135 during 2002-2003 (Maule *et al*., 2003, *J. Gravit. Physiol*.) and in the underwater habitat *Aquarius* during NEEMO 5 (Maule *et al*., 2005, *Appl. Environ. Microbiol* in prep.). The experiments described here were part of a final testing phase prior to use of the PTS on the International Space Station (ISS), scheduled for launch on 12A.1 on February 9th 2006. The specific aspects of PTS operation studied were those involving a fluid component: pumping, mixing, incubations and pipetting into the instrument. The PTS uses a stepper motor to move fluid along small channels, which may be affected by reduced gravity.

METHODS AND MATERIALS:

The dimensions and appearance of the PTS and cartridges are shown in figure 1 below. Figure 2 shows the layout of personnel and equipment in the aircraft. Three fluid dispensing techniques were tested and evaluated in l constant volume pipette and 3.µg: 1. Simple Pasteur pipette, 2. 25 Screw syringe pipette developed at NASA Marshall.

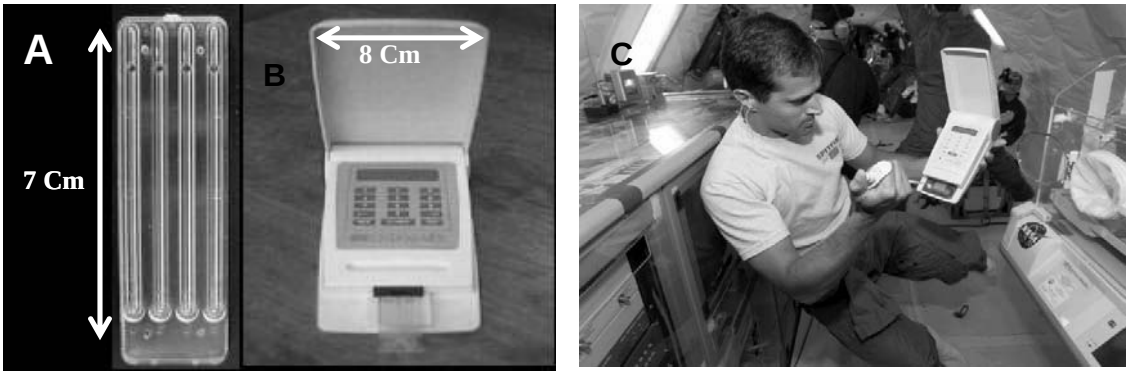

Figure 1. A, Portable Test System (PTS) cartridge. **B**, PTS (weight 2 lbs). **C**, Operator performing PTS operation in hand-held format in microgravity.

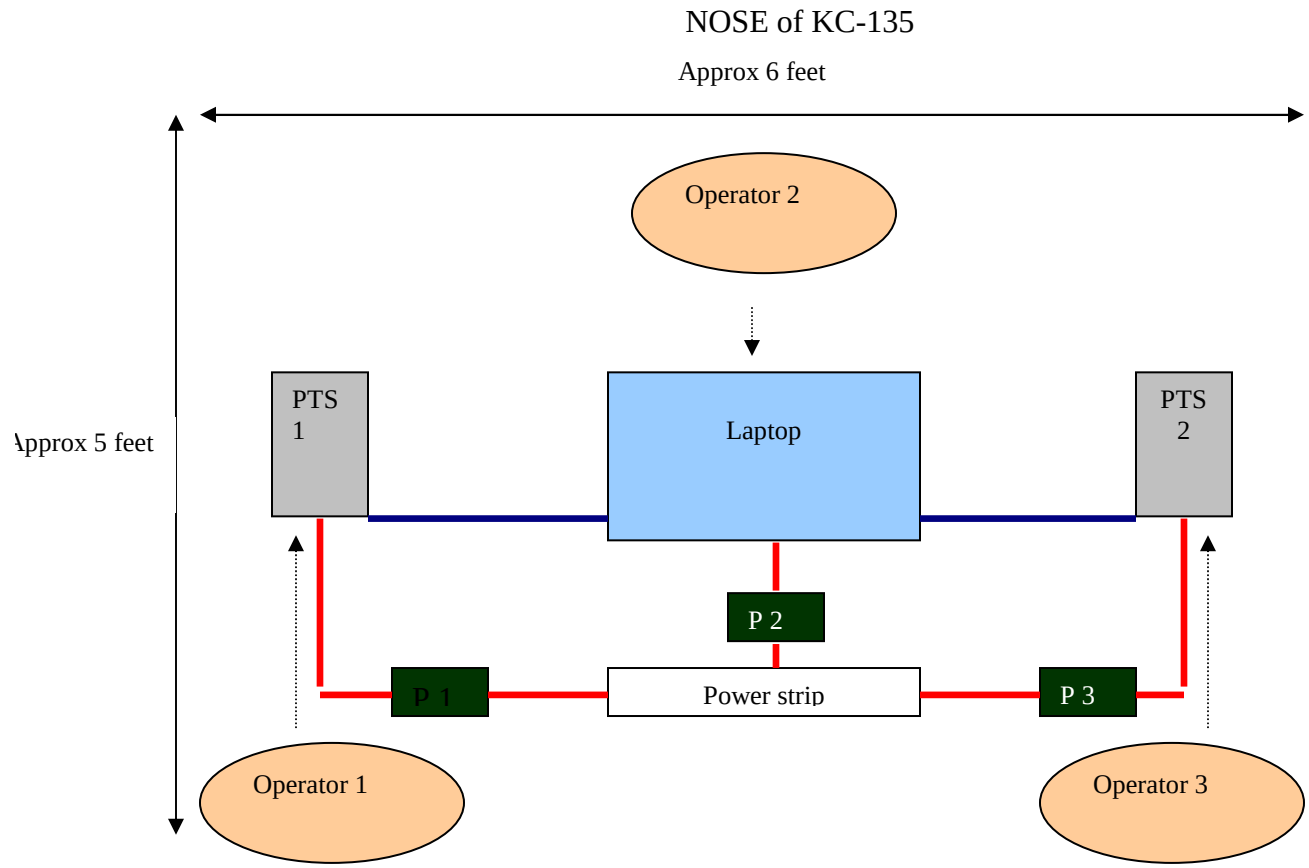

Figure 2. Layout of personnel and equipment on board the aircraft. P1-P3 = power supplies. Red line = power cord. Blue line = data connection.

RESULTS/DISCUSSION:

1. While the pasteur pipette dispensed fluid droplets of a uniform size and volume in 1g, this was extremely variable in microgravity: droplets would frequently expand and adhere to surfaces outside the well of the PTS cartridge.
2. Although the 25µl constant volume pipette took up a constant volume of fluid, this fluid often adhered to the upper section of the pipette tip. This prevented the expulsion of the complete 25µl volume into the PTS cartridge well.
3. The NASA Marshall pipette prototype delivered a constant volume, but following fluid adhesion to the surface of the PTS cartridge, further fluid volume was drawn out of the pipette. The pipette tip was too blunt to allow easy access to the PTS cartridge well; a narrowing/sharpening of this pipette would facilitate use with PTS cartridges.

CONCLUSION:

1. Sharpen tip to allow access to PTS cartridge wells
2. Enable thorough mixing in pipette

These modifications have been performed as of 3/25/2005 and require a final microgravity test before PTS phase 2 safety review on 5/8/2005 and launch on 12A.1 on 2/9/2006.

PHOTOGRAPHS:

JSC2004E39740 to JSC2004E39780

VIDEO:

- Zero-g August 30 – September 9, 2004, Reference Master: 718586

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.17 Biomechanical Evaluation of Russian BD-1 Treadmill during Non-Motorized Treadmill Locomotion in a Weightless Environment (KC-135)

FLIGHT DATES:
August 31 – September 2, 2004
September 9, 2004

PRINCIPAL INVESTIGATOR:
Grant Schaffner, Wyle Life Sciences

CO-INVESTIGATORS:
Inessa Kozlovskaya, Institute for Biomedical Problems, Moscow, Russia
Don Hagan, NASA/Johnson Space Center

GOAL:

The goal of this experiment was to evaluate the ability of subjects to walk and run under bungee loading on the Russian BD-1 treadmill during weightlessness provided by parabolic flight.

OBJECTIVES:

1) To measure the average speeds maintained by subjects and compare this with the corresponding target speeds.
2) To measure the external load (EL) provided by the bungee loading system and determine the load dependence on body size.
3) To measure subject ground reaction force (GRF) in weightlessness and compare this with ground reaction force in normal gravity.

METHODS AND MATERIALS:

Eight subjects (33.3 ± 6.0 years, 174.3 ± 8.6 cm; 75.1 ± 11.7 kg) ran at 5, 8, and 14 km/h (3.1, 5.0, and 8.7 mph) on the non-motorized Russian BD-1 treadmill during weightlessness (0g) onboard the KC-135 aircraft and on the ground (1g). Subjects were held down to the treadmill by means of an applied external load provided by the treadmill's bungee loading system connected to an upper body harness. Treadmill belt speed was measured and recorded by means of the speed sensor built into the treadmill interfaced with a LabView (National Instruments, Austin, TX) data acquisition system. Bungee loading was measured and recorded by means of load cells (Entran, Inc., Fairfield, NJ) placed between the bungee and harness attachment points and

connected to a second LabView data acquisition system. Ground reaction force was measured and recorded by a Tekscan pressure insole system (Tekscan, Inc., South Boston, MA). Subjects performed two trials for each set of conditions.

RESULTS:

Speed Maintenance

A chart showing the average and standard deviation of speed for each subject at each of the three target speeds is provided in Figure 1. The average speed for all subjects was within one standard deviation of the target speed for 5 km/h (walking) and 8 km/h (slow running). For the 14 km/h (fast running) condition, however, two subjects had average speeds that were more than one standard deviation below the target speed. Also, the plot trend suggests that small and large body size subjects had the most difficulty reaching and maintaining the 14 km/h target speed based on the deviation of their average speed from the target speed.

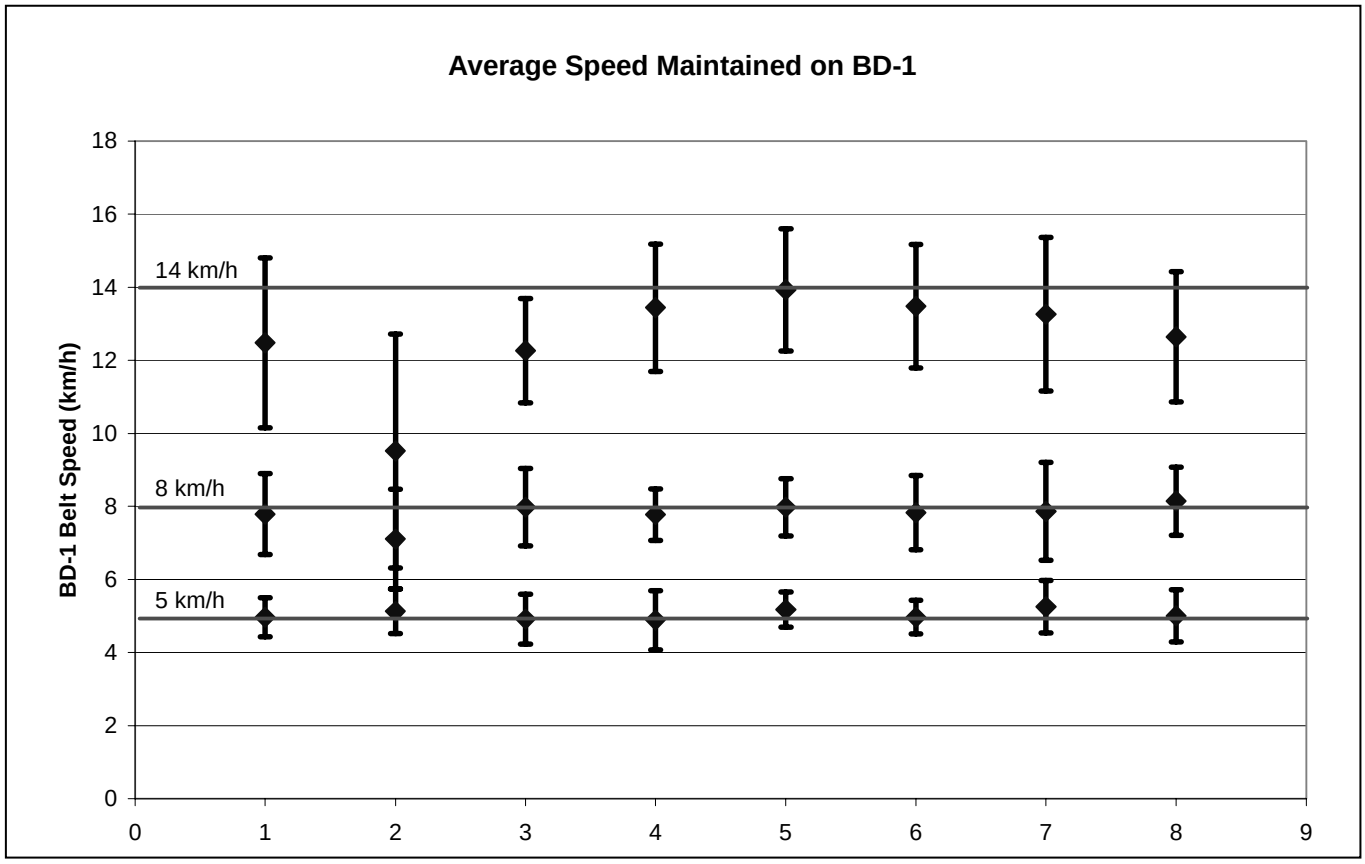

Figure 1. Ability of subjects to reach and maintain the three speed levels for walking and running in weightlessness.

External Load

The average external load provided during walking and running (all speeds) is presented according to body size in Figure 2. The average external load ranged from approximately 80 percent of body weight for the smallest subjects to approximately 60 percent of body weight for

the largest subjects. External load, as a percentage of body weight, showed a consistent decrease as body weight increased.

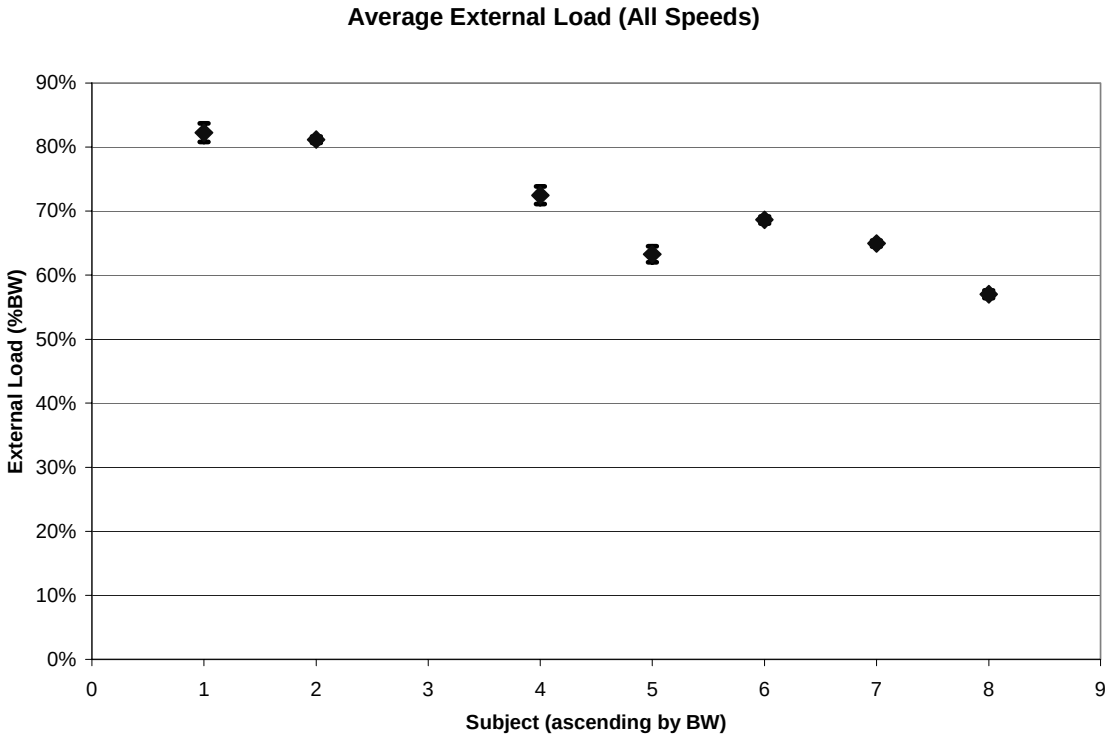

Figure 2. Average external load and standard deviation sorted according to subject size.

Ground Reaction Force

A typical ground reaction force plot for a single walking step at 5 km/h on the BD-1 treadmill in weightlessness is shown on the right side of Figure 3. For comparison, GRF plots obtained from a motorized treadmill in 1g and 0g at the same speed are shown on the left side of Figure 3. The shape of the motorized treadmill curves are similar in 1g and 0g. The BD-1 contact time tends to be less than with the motorized treadmill in 1g. The BD-1 impact GRF magnitude (first peak) is similar to the motorized treadmill, but the propulsive GRF magnitude (second peak) is greater for the BD-1.

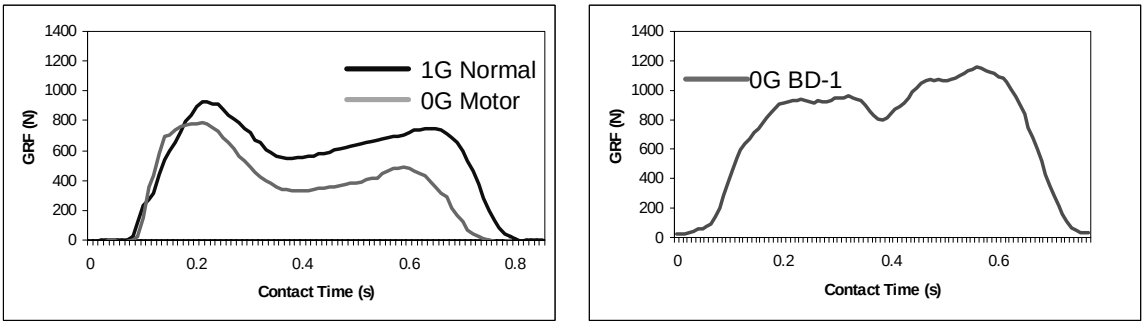

Figure 3. Typical GRF for walking at 5 km/h on a motorized treadmill in 1G and 0G (left) versus the BD-1 non-motorized treadmill in 0G (right).

A similar comparison of ground reaction force plots for a single running step at 8 km/h is shown in Figure 4. Again, the shape of the motorized treadmill curves are similar in 1g and 0g, but the impact peak is absent in the BD-1 GRF plot. The peak propulsive GRF on the BD-1 appears to be closer to the 1g peak for the motorized treadmill than the corresponding 0g peak for the motorized treadmill. The BD-1 contact time tends to be similar to that obtained on the motorized treadmill in 1g.

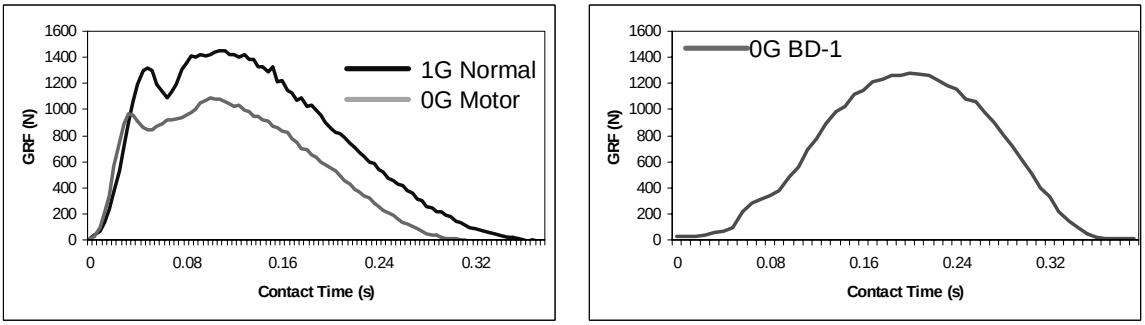

Figure 4. Typical GRF for running at 8 km/h on a motorized treadmill in 1G and 0G (left) versus the BD-1 non-motorized treadmill in 0G (right).

Ground reaction force plots for a single running step at 11 km/h on a motorized treadmill and at 14 km/h on the BD-1 are shown in Figure 5. The 11 km/h speed was the closest speed to 14 km/h available in the motorized treadmill data. As with the slower running step, the impact peak is absent in the BD-1 GRF plot. The peak propulsive GRF on the BD-1 appears to be lower than the 1g GRF peak for the motorized treadmill, but similar to the 0g peak for the motorized treadmill. The BD-1 contact time appears to be less than with the motorized treadmill in 1g.

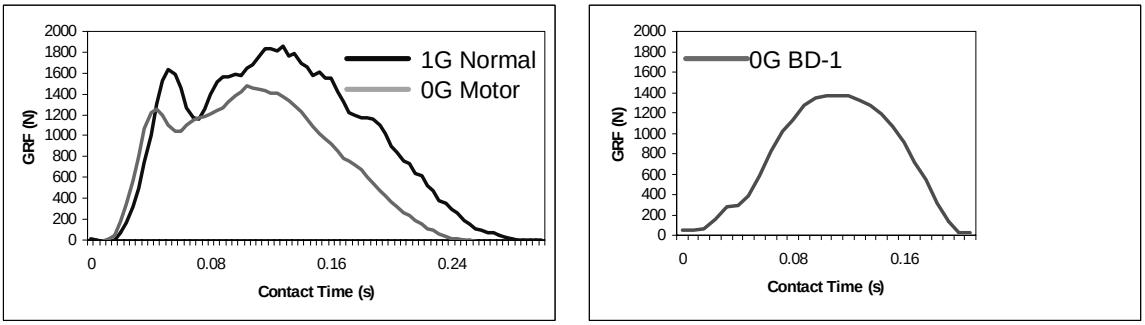

Figure 5. Typical GRF for running at 11 km/h on a motorized treadmill in 1G and 0G (left) versus 14 km/h on the BD-1 non-motorized treadmill in 0G (right).

DISCUSSION:

While subjects had no difficulty reaching and maintaining the walk (5 km/h) and slow run (8 km/h) speeds, it was clear that some subjects had difficulty reaching and maintaining the fast run (14 km/h) speed. This problem may be ameliorated with additional training and practice on the treadmill, but it is likely that some subjects may still have difficulty maintaining speeds in the range of 14 km/h for durations longer than about 20 seconds.
Due to the limited adjustability of the BD-1 bungee loading system, subject loading may be limited to a narrow range and depends on subject body size. Smaller subjects are limited to higher loadings closer to their full body weight while larger subjects are limited to load levels that may be as low as 60 percent of their body weight.

The ground reaction force obtained during BD-1 treadmill locomotion appears to have a heel strike peak during walking, but the heel strike peak appears to be absent during running. The absence of the heel strike peak may be due to the fact that the subjects held onto a handrail during locomotion on the BD-1 or it may be a natural occurrence in non-motorized treadmill running. Determining what factors contribute most to the absence of heel strike in the ground reaction force will require further study. The peak propulsive force appears to be similar to the 1g level, or at least equivalent to the 0g level, when using a motorized treadmill. The high rate of change of load associated with the heel strike peak along with the peak propulsive ground reaction force are both considered to be important factors in bone maintenance. Determining the extent to which these parameters are enhanced or diminished by non-motorized treadmill locomotion is an important topic for further investigation.

CONCLUSION:

Subjects are able to successfully perform locomotion exercise on the BD-1 treadmill in weightlessness. The BD-1 bungee system has limited adjustability and provides higher loading for small subjects and lower loading for larger subjects. Some subjects have difficulty reaching and maintaining higher speeds starting at around 14 km/h. The ground reaction force profile for BD-1 locomotion shows peak propulsive forces comparable to 1g, but the heel strike peak appears to be absent during running on the BD-1 in weightlessness. These observations have implications for exercise prescriptions for BD-1 treadmill use on-board ISS.

PHOTOGRAPHS:

JSC2004E39833 to JSC2004E39876
JSC2004E40321 to JSC2004E40346
JSC2004E40660 to JSC2004E40683
JSC2004E40904 to JSC2004E40964

VIDEO:

- Zero-g August 30 – September 9, 2004, Reference Master: 718586

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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2.18 The Effects of Microgravity on a Kinetic Reaction

FLIGHT DATES:
August 31 - September 2, 2004

PRINCIPAL INVESTIGATOR:
Vince LiCata, Louisiana State University (LSU)

CO-INVESTIGATORS:
Greg Thompson, LSU
Phil Speros, LSU
Jason Bell, LSU

GOAL:
To determine if microgravity alters protein kinetics and equilibria.

OBJECTIVES:
To standardize stopped flow equipment and control reactions under flight conditions.

METHODS AND MATERIALS:
Over the course of several parabolic microgravity flights, we measured the concentration dependence of ascorbic acid, on the microscopic pseudo-first order rate constant (k_{obs}) for the reduction of 2,6-Dichloroindophenol (DCIP), using a modified stopped-flow fluorometer. The reaction is one which has been used for many years as a "standard" to assure proper functionality

of stopped-flow apparatuses. (Tonomura et al., 1978) This reaction was chosen for a number of reasons. 1.) The reagents are relatively inexpensive, which allowed for exhaustive characterization in the laboratory. 2.) The reaction mechanism is well defined. It consists of a complete oxidation of the hydroxyl groups attached to the pyrene ring, with a subsequent reduction of DCIP, as shown in Figure 1. 3.) The reaction can be manipulated easily into pseudo-first order reaction conditions. Indeed, the reaction conditions used throughout the experiments are 200-1200 fold excess of Ascorbic acid to DCIP. 4.) The reaction is easily monitored. The reaction shows a dramatic loss of blue color on a millisecond timescale, which can be readily monitored with our fluorometer in a modified "absorbance" mode. 5.) The reaction is very pH sensitive. At low pH the reaction occurs very rapidly, while at higher pH the reaction occurs much more rapidly. Although the reaction rates seen with these reactions is much slower than those seen in diffusion-limited reactions, we needed a well behaved and well characterized reaction to determine proper functioning of the equipment in microgravity.

DCIP and Ascorbic Acid Reactions: DCIP was prepared to 140uM in water. In all cases the concentration of DCIP used was 140uM. Ascorbic acid was prepared in water and the pH was adjusted using concentrated NaOH or HCl. Final concentrations of Ascorbic acid were achieved by mixing (in the stopped-flow), using both low and high concentration solutions, mixed upstream in appropriate volumes before mixing with DCIP.

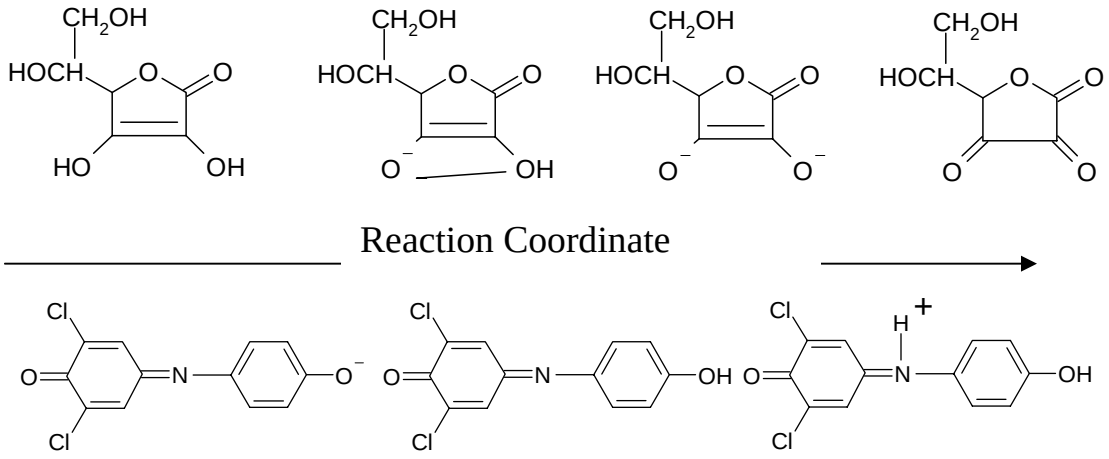

Figure 1: Ascorbic acid oxidation and subsequent reduction of DCIP. This reaction is monitored by a loss of absorbance at 420nm as the reaction proceeds.

Data Analysis: Kinetic traces were fit to single exponential equations. (Equation 1) In all cases tested, a double exponential fit did not improve the fit.

$$Y = Y_0 + A_1 e^{-(x - x_o)/\tau} \quad \text{(Equation 1)}$$

All reactions were performed under pseudo-first order conditions. The rate of a pseudo-first order reaction is determined by monitoring the k_{obs} or $1/\tau$ with respect to concentration of a variable reactant. (Cantor & Schimmel, 1980; Hammes, 2000) The first-order rate (K_1) is proportional to the slope of this relationship. (Equation 2)

$$k_{obs} = 1/\tau = K_1[A]_0 \quad \text{(Equation 2)}$$

A plot of k_{obs} vs. [ascorbic acid] yields a straight line, with a y-intercept of zero under perfect conditions. However, because small errors in k_{obs} can lead to very large errors in the y-intercept, the lines were fit in various ways. In addition to fitting the data to Equation 2, another fitting option was exercised. In some cases, as noted, data was fit to Equation 3, with a floating y-intercept term, B.

$$1/\tau = K_1[A]_0 + \mathrm{B} \quad \text{(Equation 3)}$$

RESULTS:

"In-flight" experiments, pH 6: This section describes the results measured onboard the KC-135 Reduced Gravity Laboratory. All reactions were measured on-board the aircraft either before microgravity parabolic maneuvers began, or under microgravity, as noted.

Given the extreme conditions of the KC-135 flights, it was necessary to determine whether data collected under microgravity conditions was as "good" as that measured under normal gravity conditions. Figure 2, Panel A shows the reaction measured at pH 6 under normal gravity, while Panel B shows the same reaction measured during a single period of microgravity. Each trace was generated from an average of 3 shots collected at common concentrations. It was determined that the quality of the data collected under microgravity was comparable to that of data collected during the onboard control.

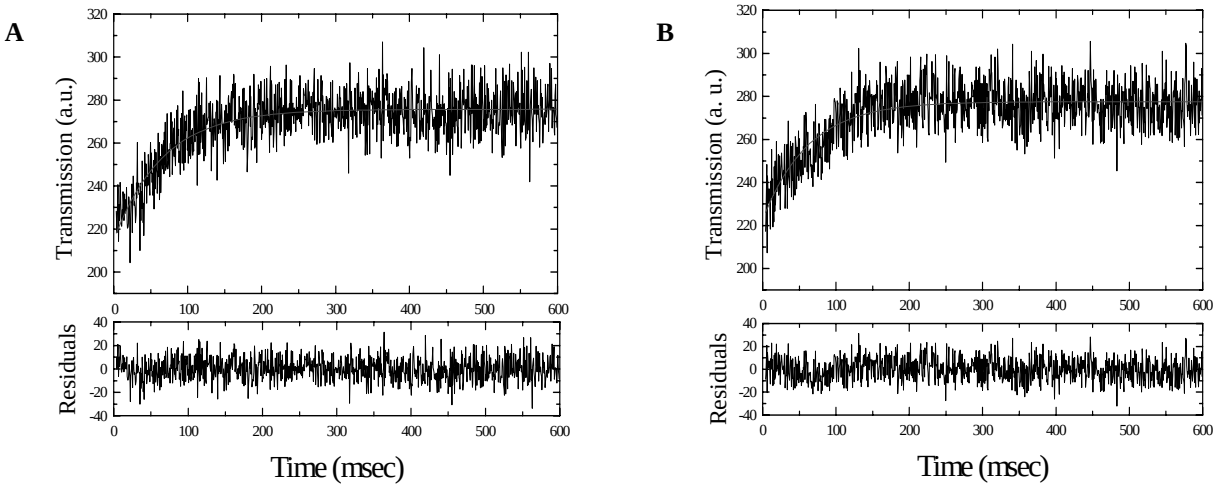

Figure 2: Representative curves collected while onboard KC-135 flights. An average of 3 independent shots of 21.42mM ascorbic acid (pH 6) into DCIP was used for each trace represented above. Panel A shows the shots collected during the on-board control. Panel B shows the data collected while under microgravity. Curves were fit to Equation 1, and the residuals to the fit are shown below each curve.

A full series of reactions was collected on-board at varying concentrations of ascorbic acid, under 1g gravity conditions and during periods of microgravity. At each concentration, the full series of data was collected and averaged, then fit to Equations 1, to determine the microscopic rate constant, k_{obs}. In all cases, at least 3 shots were collected and averaged. A plot of k_{obs} vs. [ascorbic acid] yields a linear relationship under all conditions measured. These data sets were fit in two ways. Initially, the data was fit to Equation 2 in order to determine the pseudo-first order rate (K_1) of 850.4 ± 42.8 $M^{-1}sec^{-1}$ under 1g onboard control conditions, and 851.9 ± 36.3 $M^{-1}sec^{-1}$ under microgravity. (Figure 3).

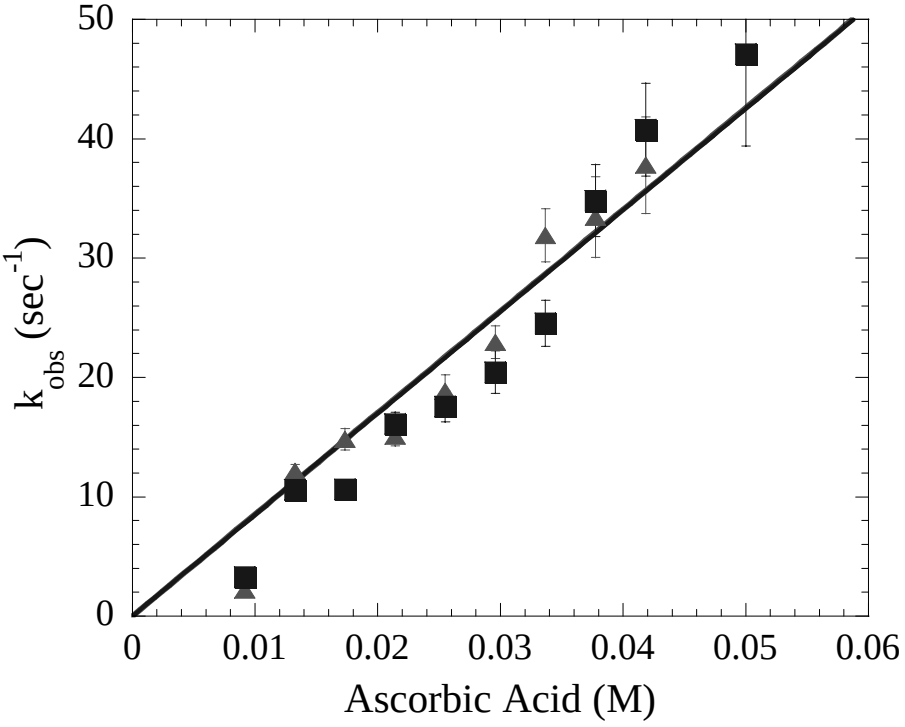

Figure 3: k_{obs} vs. [ascorbic acid] series measured under microgravity (red triangles) and under normal gravity (blue squares) collected onboard KC-135 flights. The lines represent best fits of data sets to Equation 2.

Additionally, the data sets were fit to Equation 3. This analysis was performed in order to obtain some understanding of the systematic error present in our data. Data collected under perfect lab conditions should have an invariant K_1 when fit to either Equation 2 or Equation 3, and each analysis should produce a y-intercept value of 0. Any deviation from that standard is probably due to systematic error in our onboard system. Fitting our data sets to Equation 3 (Figure 4) revealed a K_1 of 1065.8 ± 74.5 $M^{-1}sec^{-1}$ with an intercept of -7.2 sec^{-1} under normal gravity, and 1014.3 ± 76.8 $M^{-1}sec^{-1}$ with an intercept of -4.8 sec^{-1} under microgravity.

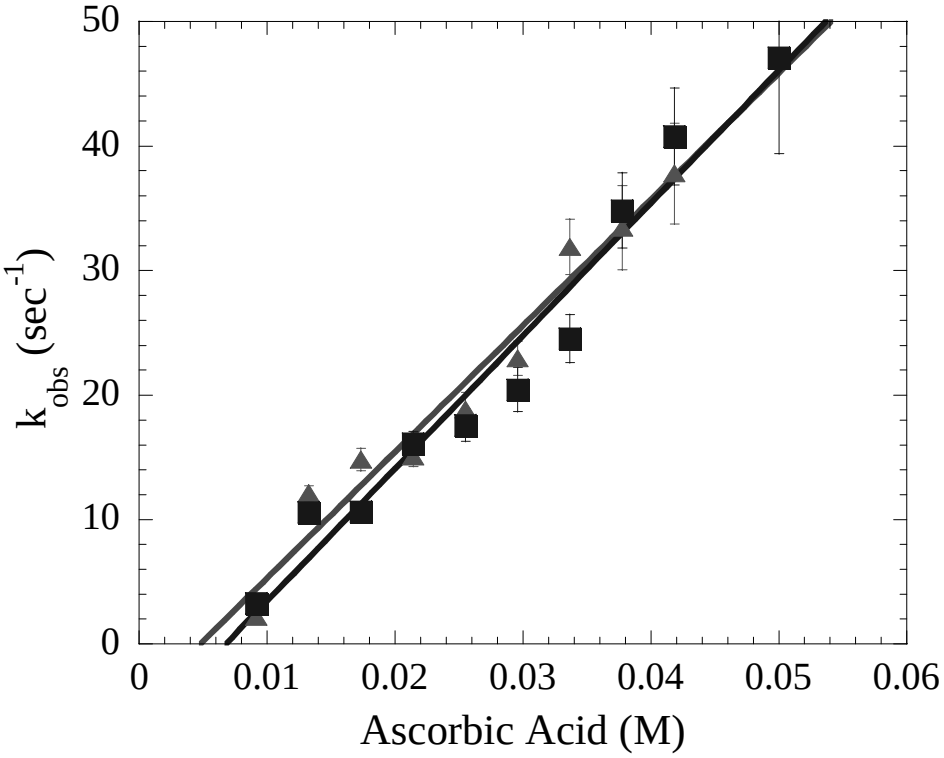

Figure 4: k_{obs} vs. [ascorbic acid] series measured under microgravity (red triangles) and under normal gravity (blue squares) collected onboard KC-135 flights. The lines represent best fits of data sets to Equation 3.

Despite the introduction of a systematic shift in the zero-concentration intercept, there does not seem to be any significant change in the reaction rate (K_1) from the control under microgravity. These are the expected results for this reaction system and confirm that the system is working adequately under microgravity conditions. In addition to the microgravity conditions, purposely introduced, there are a number of other significant deviations from "normal" lab conditions. Conditions beyond our control included; significant aircraft vibration, occasional loss of temperature control, and highly unregulated ambient temperatures, which fluctuated between 20^{o}C and 30^{o}C. Each of these conditions could contribute to the systematic deviation from zero-intercept. These results emphasize the need for onboard 1g control measurements for all future experiments, especially those where we hypothesize there should be a microgravitational effect.

CONCLUSION:

The goal of this flight series was to confirm the ability to measure microscopic rate constants in the millisecond time range in microgravity. After several iterative instrumental adjustments, corresponding 1g and micro-g data were collected over a wide range of reagent concentrations, which confirmed that rapid kinetics could be reproducibly measured in microgravity.

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Hammes, G. G. (2000) Thermodynamics and Kinetics for the Biological Sciences. A. J. Wiley and Sons. New York, NY. USA.

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PHOTOGRAPHS:

JSC2004E39824 to JSC2004E39832
JSC2004E40874 to JSC2004E40903

VIDEO:

- Zero-g August 30 – September 9, 2004, Reference Master: 718586

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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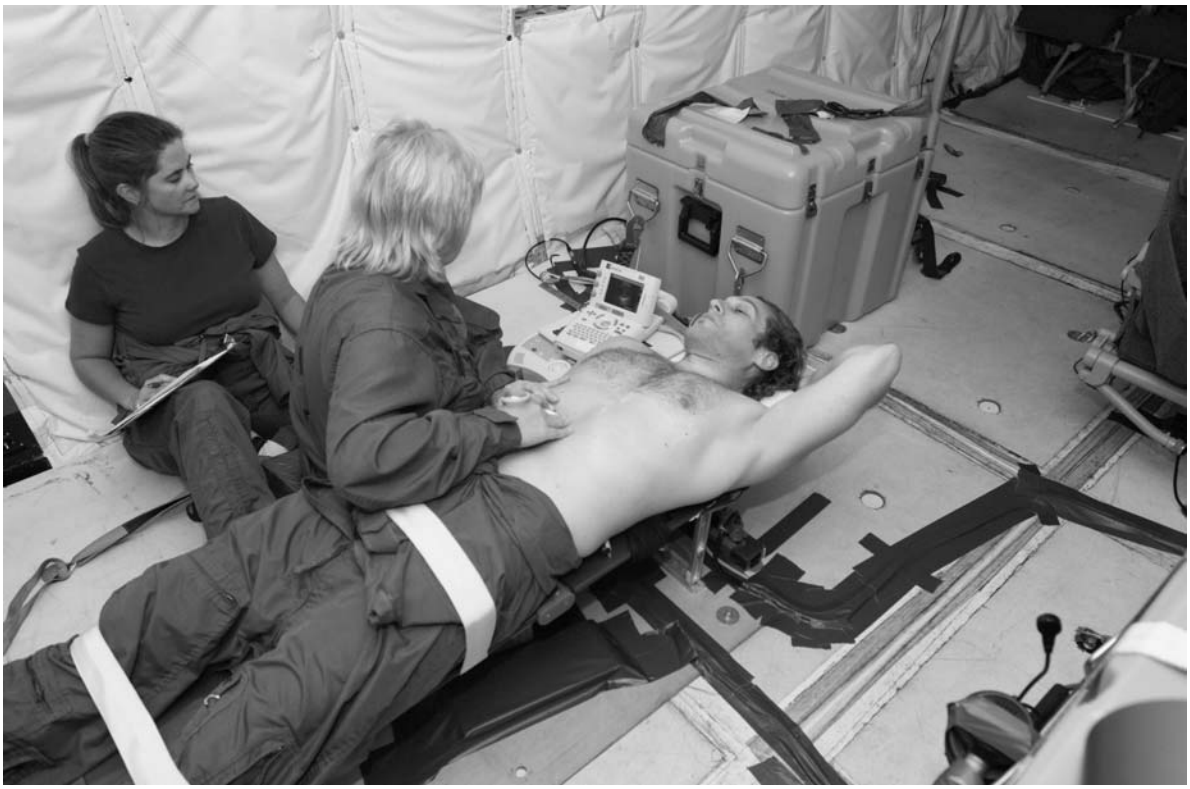
2.19 Minimally Invasive Diagnosis and Therapy of Microgravity Medical Contingencies

FLIGHT DATES:
August 31 – September 2, 2004

PRINCIPAL INVESTIGATOR:
Scott Dulchavsky, Henry Ford Health System

CO-INVESTIGATORS:
Shannon Melton, Wyle Life Sciences
Doug Hamilton, Wyle Life Sciences
Ashot Sargsyan, Wyle Life Sciences



GOALS/OBJECTIVES:

To evaluate, in a flight experiment on healthy volunteers, acute changes in human physiology and anatomy associated with changes in gravity, using diagnostic ultrasound imaging devices, and to demonstrate feasibility of performing similar imaging protocols for operational space medicine purposes.

- Test and validate the following experimental protocols (modified/designed by the Primary Personnel) in whole or in part, for use in microgravity.
    a. Thoracic Mapping for Lung Movement and Topography

b. Abdominal Renal/Pelvic Organ Morphometrics and Topography
c. Ophthalmic/Paranasal Sinus Protocol Evaluation
d. Cardiac Morphometrics, Physiology, and Topography
e. Internal Jugular Vein Hemodynamics

- Identify specific limitations and modifications, if any, in imaging windows, scanning sequence, and scanning techniques, and other aspects requiring attention in microgravity.
- Identify changes in organ shape, size and/or position related to gravity
- Identify vascular pressure /lumen / flow changes related to gravity
- Verify the applicability of basic terrestrial scanning protocols in 0g environment

INTRODUCTION:

The feasibility of ultrasonic imaging in human spaceflight has already been demonstrated. Hardware has been flown aboard both NASA and Russian spacecraft, and successfully operated by both physician and non-physician astronauts. Prior success and promising research opportunities have motivated the installation of a sophisticated ultrasound system aboard the International Space Station (ISS) as part of the Human Research Facility (HRF). The HRF ultrasound was successfully activated for autonomous use on the ISS on June 12, 2001, with no data transmission tested.

Ultrasound is the second most widely used clinical imaging modality in the United States. Its scope encompasses a large variety of medical and surgical conditions, many of which are considered possible in space. The diagnostic accuracy of this imaging modality is highly dependent on the professional knowledge, experience, and skills of the operator. Terrestrially, ultrasound data are acquired by a trained sonographer (technician or radiologist) and interpreted either during the process of examination (e.g., Europe, Russia, Japan), or through review of standardized sets of still images (North America). In the ISS setting, a trained sonographer is not available onboard; therefore, the essential expertise in both acquisition and interpretation is assumed to remain on the ground. Changes in anatomical norms and standard scanning "windows" can greatly affect the approach to diagnosis and treatment in a timely manner. This study attempts to identify some of these changes to better equip the experts on the ground for acquisition and interpretation. The acquired new knowledge is expected to be unique and valuable for future research programs concerned with human space physiology, space pharmacology, and other related disciplines. Certain products of these experiments may also be useful in the process of design and creation of the medical support systems for exploration-class missions.

METHODS AND MATERIALS:

Three KC-135 microgravity flights were conducted using healthy volunteer human subjects for all procedures. Ultrasound experts used multiple ultrasound devices to capture images of the heart, lungs, abdominal organs, eyes, and areas overlying paranasal sinuses.

Hardware

GE Logiqbook Ultrasound System (GE Medical Systems, Milwaukee, WI)

A portable ultrasound system approved for clinical use. Equipped with an abdominal (convex) and small parts (linear) probe.

Sonosite 180Plus Ultrasound System (Sonosite, Bothell, WA)
A portable ultrasound system approved for clinical use. Equipped with an abdominal (convex) and small parts (linear) probe.

Biosound MyLab30 (Biosound Esaote, Genoa, Italy)
A portable ultrasound system approved for clinical use. Equipped with a cardiac and vascular (linear) probe.

Crew Medical Restraint System (Wyle Laboratories, Houston, TX)
This device is currently flown on the ISS as part of the onboard medical support system for restraining an injured crewmember and the caregiver. It is also used in the conduct of ISS-based flight experiments (ADUM).

Airway Pressure Measurement and Biofeedback Regulation Device (Prototype, Wyle Laboratories, Houston, TX)
A breathing device that continuously measures and displays actual pressures within the breathing orifice, allowing the subject to maintain required pressure through biofeedback and adjustment of their respiratory effort.

Media Rack (Wyle Laboratories, Houston, TX)
A flight-rated rack that contains five video channels with recording systems, 3 audio channels and two real-time video monitors that can be switched between the multiple video sources. All video channels are mixed with overlays displaying flight time, g-levels, and parabola numbers. A remote viewing station was set up in a separate area of the plane with two-way audio loops for communication and video feeds from either one of the ultrasound systems.

Procedures

Multiple imaging protocols were performed on normal human subjects by expert sonographers. Two scanning stations were set up on each flight allowing for two subjects to be scanned simultaneously. In one station the subjects were restrained supine on the Crew Medical Restraint System. In the second station, the subjects were given reclined position in the aircraft seats, and restrained using the standard seatbelts.

Thoracic Mapping
Mapping of a standardized set of multiple lung fields along mid-clavicular, axillary, and mid-scapular lines for direction and amplitude of the respiratory movement of the pulmonary surface in normal respiration; this protocol was completed in 5 subjects in 1g, micro-g, martian-g, and lunar-g conditions.

Abdominal/Renal/Pelvic Organs
Imaging of the abdominal and retroperitoneal organs (Liver, Kidney, Pancreas, Spleen, and Gallbladder) was completed in four subjects through 2g-0g-2g cycles. Attention was primarily paid to shape, position, and linear dimensions of the organs, in order to detect any changes associated with the gravity level aboard the aircraft.

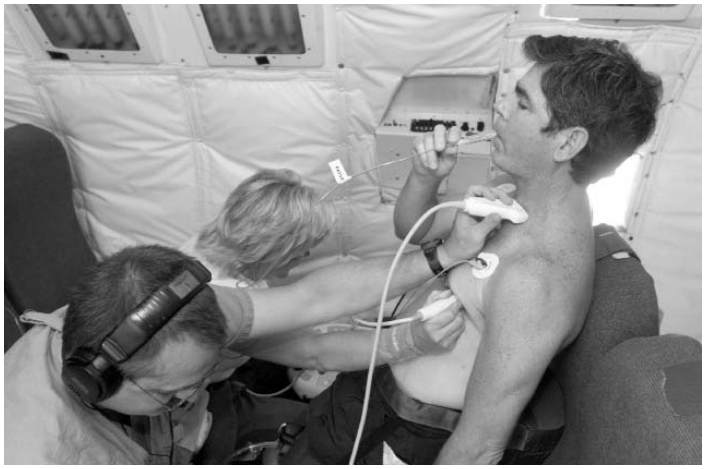
Ophthalmic Protocol

Imaging of the eye (closed eyelids) was completed in three subjects during 2g-0g-2g parabolic cycles focusing on the changes in imaging data, human factors, and scanning protocol associated with the gravity level aboard the aircraft.

Sinus
Imaging of the areas overlying the maxillary and frontal paranasal sinuses was completed in three subjects during 2g-0g-2g parabolic cycles focusing on the quality in imaging data, human factors, and scanning protocol associated with the gravity level aboard the aircraft.

Cardiac
Imaging of heart was completed on five subjects in supine position to view changes in sonographic "windows" and to record potential physiologic changes during 2g-0g-2g parabolic cycles.

Vascular
Simultaneous real-time imaging of the heart and the Internal Jugular Vein (IJV) was done in three reclined subjects in the following conditions:
2g-0g-2g transition in normal respiration, no thigh cuffs
2g-0g-2g transition in normal respiration, with thigh cuffs inflated
2g-0g-2g transition with subject inhaling at specific pressures at the breathing orifice
2g-0g-2g transition with subject exhaling at specific pressures at the breathing orifice



RESULTS/DISCUSSION:

Thoracic Mapping
Direction and amplitude of the respiratory movement of the pulmonary surface, as well as the imaging circumstances such as the chest wall thickness, were documented in all subjects in normal (shallow) respiration, for all lung fields per the designed protocol. The data were verified post experiment. The Primary Personnel will submit data for publication, to define the conditions for lung surface and potential pleural space imaging and to propose refined imaging protocols for terrestrial, microgravity, lunar, and martian conditions.

Abdominal/Renal/Pelvic Organs
Imaging of the abdominal and retroperitoneal organs (Liver, Kidney, Pancreas, Spleen, and Gallbladder) was completed in all subjects, as described in the Materials and Methods section

above. Data were verified post experiment. A comparison will be attempted between the data from this acute experiment and the information acquired in a similar fashion during long-term exposure to microgravity. Until such time, the data will be used to refine imaging protocols and measurement techniques, and to draw preliminary conclusions regarding the effects of microgravity on abdominal imaging data.

Ophthalmic Protocol

Imaging of the eye (closed eyelids) was completed in three subjects during 2g-0g-2g parabolic cycles focusing on the changes in imaging data, human factors, and scanning protocol associated with the gravity level aboard the aircraft. The most important results of this segment include observations and experience related to safety of ophthalmic imaging in variable and other unconventional gravity conditions. In view of the future ISS-based experiments involving eye imaging, these data are highly valued by the Primary personnel. Imaging data were verified post examination and are available for future analysis.

Sinus

Imaging of the areas overlying the maxillary and frontal paranasal sinuses was completed in three subjects during 2g-0g-2g parabolic cycles. Protocol development and validation were the most tangible results of this segment. Indeed, in normal subjects with air-containing paranasal sinuses devoid of fluid and/or other extraneous contents, imaging data cannot change in acute microgravity or hypergravity exposure. In long-term exposure of spaceflight, however, potential edema of the sinus mucosa, exudation, or infection may develop to become identifiable by ultrasound. This concept is confirmed by literature, as well as our own experience with animal sinusitis model.

Cardiac

Imaging of heart was completed on five subjects in supine position to view changes in sonographic "windows" and to record potential physiologic changes during 2g-0g-2g parabolic cycles. Both supine and Left Lateral Decubitus (LLD) positions were used. Data were verified post examination, and will be analyzed independently as well as in relation to other relevant data sets. The results will be used to refine the design and execution of operational echocardiography, prediction of ultrasound windows for specific crewmembers upon transition to 0g, and limited conclusions on physiological "norms" for acute microgravity and hypergravity.

Vascular

Real-time imaging of the heart and the Internal Jugular Vein (IJV) was done simultaneously. The protocol was repeated in three consecutive subjects. This hypothesis-driven experiment using a unique biofeedback-based device (D.H.) does not have precedents or analogues, and has created a new set of approaches to studying cardiovascular physiology in special environments. The data were verified post experiment, and will be analyzed and published at the earliest possibility

CONCLUSIONS:

The goals of the experiment were accomplished. New knowledge was acquired and documented. Data acquisition was designed to be compatible with the data from ground- and space-based experiments, allowing consideration of comparisons, correlations, and combined evaluation for protocol development purposes.

A new methodology of respiratory pressure biofeedback with complex imaging evaluation of cardiac and central vascular parameters was successfully tested and shown great promise. Further study in this direction seems to be warranted.

Secondary results include successful evaluation of the hardware used. The hardware and its setup, deployment, connectivity, and data acquisition did not exhibit any inadequacies, and fully supported the intended applications.

ACKNOWLEDGEMENTS:

The Primary Personnel highly values the essential contribution of the following individuals (equipment, experiment design, setup and support, logistics, serving as subjects, expertise):

- Ms. Kathleen Garcia (Wyle Laboratories, Houston, TX)
- Messrs. Richard Pettys, Terry Guess, and Ashkhan Moghaddam (Wyle Laboratories, Houston, TX)
- Mr. Grant Threatt (Lockheed Martin, Houston, TX)
- Dr. Kellie McFarlin, Mr. William Oddo (Henry Ford Hospital System, Detroit, MI)

PHOTOGRAPHS:

JSC2004E39877 to JSC2004E39926
JSC2004E40347 to JSC2004E40364
JSC2004E40827 to JSC2004E40873
JSC2004E41019 to JSC2004E41049

VIDEO:

- Zero-g August 30 – September 9, 2004, Reference Master: 718586

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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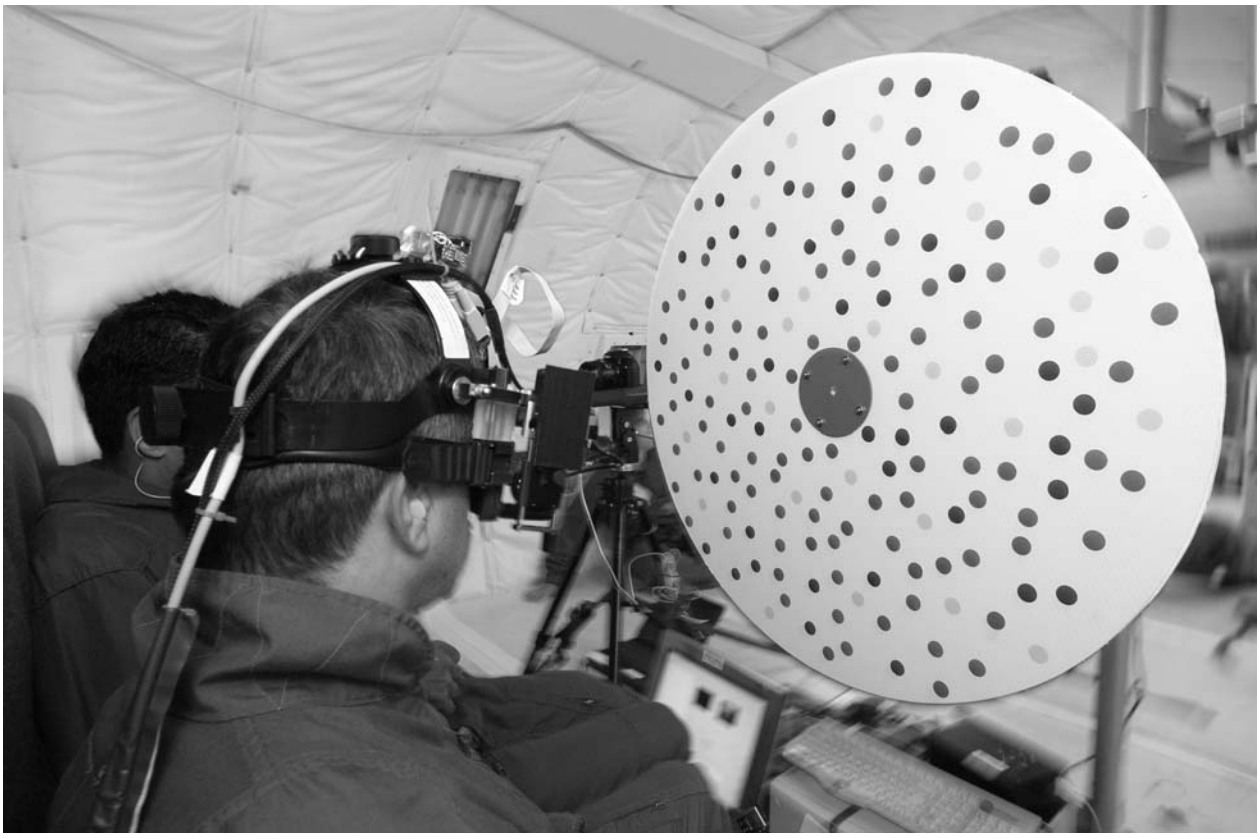
2.20 Vestibular Adaptation in Parabolic Flight

FLIGHT DATES:
September 28 – October 1, 2004

PRINCIPAL INVESTIGATOR:
Mark Shelhamer, Johns Hopkins University

CO-INVESTIGATORS:
Faisal Karmali, Johns Hopkins University
Ondrej Juhasz Johns Hopkins University



GOALS:

To determine the neural mechanisms of human adaptation to parabolic flight.

OBJECTIVE:

The purpose of this research study is to examine the ability of human subjects to adapt various behaviors (reflexive eye movements and orientation perception) to different conditions of gravitoinertial force (g level). The resulting information will be of value in determining how the

brain processes gravity information, in learning how humans can maintain different adapted states in different g levels simultaneously, and in aiding the design of future spaceflight programs.

INTRODUCTION:

On their first exposure to parabolic flight, many people experience motion sickness. Some subjects have been known to consider at this time not returning to fly the following day. Their experience the second day is, however, usually considerably better than that of the first day; motion sickness is much less prevalent. The adaptation process is dramatic and rapid, and some of it appears to occur during the intervening period while not flying. This phenomenon demonstrates aspects of adaptation and consolidation. One question which immediately arises is this: does adaptation to parabolic flight involve adaptation to each separate gravity level (context-specific) or is there a more generalized adaptation to the overall flight experience (implying for example a non-g-specific change in sensory weighting)? We are studying this with a series of measurements before, during, and after flight, on sets of first-time flyers and experienced flyers.

This study consists of multiple related experiments to learn more about how the human nervous system adapts to different gravity levels. We are particularly interested in adaptation of vestibular-mediated responses such as orientation perception and reflexive eye movements. These adaptive processes are important because of possible impairments in sensorimotor performance when astronauts undergo transitions between gravity levels. Some of these changes, and the adaptive processes that counteract them, may be similar to vestibular changes in ageing and ill people on earth. In order to investigate these changes, we measure oculomotor and perceptual responses in subjects exposed to various gravity levels, as provided by parabolic flight.

Our most prominent findings to date involve changes in torsional eye position. During the g-level changes of parabolic flight there are changes in torsional eye position (ocular counterrolling: OCR). These changes can be markedly asymmetric [Markham & Diamond 1993, Markham et al. 2000]. This change in torsional alignment may be due to a decompensation of otolith asymmetry in unusual g environments; on earth, the nervous system presumably compensates for natural asymmetries in otolith organ properties, but in hyper-g and hypo-g this compensation is inappropriate and produces torsional misalignment. A similar disconjugate change has been found during spaceflight [Diamond & Markham 1998].

METHODS AND MATERIALS:

We carry out a mix of sensorimotor and perceptual measures designed to examine a range of physiological responses, from low-level reflexive through high-order perceptual. Each test is carried out in level 1 g flight and in both g levels of parabolic flight, early and late in each flight. Experienced flyers are tested for one flight, since they are expected to exhibit almost immediate adaptation. New flyers are tested over the course of three consecutive flights in order to monitor adaptive changes. There are six main tests:

1. Ocular counterrolling (torsion) with the head upright and tilted. Torsional position of each eye is measured with a high-resolution digital camera.

2. Translational vestibulo-ocular reflex (TVOR) during transient lateral head motions. Lateral head motions are imposed by the experimenter, and the resulting reflexive eye movements are measured with a head-mounted video system.
3. Pitch angular vestibulo-ocular reflex (AVOR). The oculomotor response to pitch head movements at about 0.1 to 1.0 Hz is measured with a head-mounted video system.
4. Vertical alignment (skew). Vertical alignment of the eyes is assessed with a high-resolution digital camera.
5. Subjective vertical. The subject's sense of "down" (percept of vertical) is assessed in two ways. For subjective visual vertical, the subject sets a small line on a pair of goggles to the perceived vertical. For "postural" vertical, the subject sets a small indicator rod to the subjective vertical while seated.
6. Roll vection. The subject views a large rotating disk, which produces a sensation of self-rotation in a direction opposite to that of disk motion. The head-mounted video system measures torsion and the subject reports subjective sense of self-rotation (vection) with a joystick and a verbal rating.

RESULTS:

Early results center on two of the six measures in one subject who had not previously flown in parabolic flight.

The first result involves static torsional (roll) eye position in different g levels. Early in the first flight before any adaptation had occurred, there was disconjugate (unequal) torsional eye position with the head upright, in both g levels. The disconjugacy was smaller with the head tilted 45 deg to one shoulder. At the end of the first flight, the disconjugacy was reduced in both g levels with the head upright, but disconjugacy had developed with the head tilted. This suggests that a central compensation mechanism adaptively corrected for a pre-existing otolith asymmetry with the head upright, but this compensation was not appropriate for the case with the head tilted. By the third flight, the disconjugacy was greatly reduced in both g levels and with both head positions – the eyes remained aligned about the roll axis in all conditions. This indicates the development of appropriate g-specific adaptive mechanisms over the course of three flights.

The second result involves compensatory eye movements during pitching motions of the head. We found that the gain of the pitch AVOR appears to be slightly higher in 1.8 g than in 0 g. This gain difference is apparent at higher frequencies of head oscillation but not lower frequencies; this is surprising since the otolith contribution, which may be enhancing the gain in high g, is expected to be more prominent at lower frequencies. This result suggests that there is an imperfect integration of information from the otoliths and the semicircular canals in different g levels, which may take several days to adapt.

DISCUSSION:

This investigation is in its early stages, with one flight week completed out of a planned total of four. Our very preliminary analysis of the data suggests that, in accord with the time course of overall adaptation to parabolic flight, one of the most low-level of the neural responses that we measured – ocular counterrolling or torsion – shows adaptive changes very rapidly. One higher-level integrative response – pitch AVOR which involves interaction of both linear and angular vestibular sensors – shows clear differences between g levels both early and late in-flight. These

initial results indicate that low-level responses such as ocular alignment and compensation for otolith asymmetry occur very rapidly, while those responses that require integration of afferent information from several physiological sensors adapt more slowly. Further flights will be devoted to confirming these findings and more carefully characterizing the neural changes that occur upon repeated flights.

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JSC2004E43901 to JSC2004E43903
JSC2004E43905 to JSC2004E43910
JSC2004E43979

JSC2004E44008 to JSC2004E44046
JSC2004E44

VIDEO:

- Zero-g 09/28 to 10/01/04 , Reference Master: 718802, 806

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

ACKNOWLEDGEMENT:
This work was funded by NIH grant DC006090.

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2.21 Microfabricated Genomic Analysis System

FLIGHT DATES:
September 30 – October 1, 2004

PRINCIPAL INVESTIGATOR:
Steve Gonda, NASA/Johnson Space Center

CO-INVESTIGATOR:
René Elms, Texas A & M University

GOAL:

Assess the suitability of utilizing a microfabricated platform for gel electrophoresis of DNA for spaceflight applications.

OBJECTIVE:

Assess the integration of a microfabricated electrophoresis device with the microfluidics system, and the feasibility of a microscale electrophoretic approach for separation of DNA in low gravity.

INTRODUCTION:

Genetic sequencing and many genetic tests and assays require electrophoretic separation of DNA. In this technique, DNA fragments are separated by size as they migrate through a sieving gel under the influence of an applied electric field. In order to conduct these analyses on-orbit, it is essential to acquire the capability to efficiently perform electrophoresis in a microgravity environment. Conventional bench top electrophoresis equipment is large and cumbersome and does not lead itself to on-orbit utilization. Much of the previous research regarding on-orbit electrophoresis involved altering conventional electrophoresis equipment for bioprocessing, purification, and/or separation technology applications.

A new and more efficient approach to on-orbit electrophoresis is the use of a microfabricated electrophoresis platform. These platforms are much smaller, less expensive to produce and operate, use less power, require smaller sample sizes (nanoliters), and achieve separation in a much shorter distance (a few centimeters instead of 10's or 100's of centimeters.) In contrast to previous applications, this platform would be utilized as an analytical tool for life science/medical research, environmental monitoring, and medical diagnoses. Identification of infectious agents as well as radiation related damage are significant to NASA's efforts to maintain, study, and monitor crew health during and in support of near-Earth and interplanetary missions. The capability to perform genetic assays on-orbit is imperative to conduct relevant and insightful biological and medical research, as well as continuing NASA's search for life elsewhere. This technology would provide an essential analytical tool for research conducted in a microgravity environment (Shuttle, ISS, long duration/interplanetary missions.) In addition, this technology could serve as a critical and invaluable component of a biosentinel system to monitor space environment genotoxic insults to include radiation.

METHODS AND MATERIALS:

The Microfluidics system developed by Chris Culbertson of Kansas State University was utilized to accomplish DNA separations in a UV-polymerized sequencing quality acrylamide matrix using a microfabricated electrophoresis device. Detection was achieved by labeling the DNA with an intercalating dye (POPO-3) with an excitation/emission profile of 534nm/570nm. The DNA sample used was a 100 bp ladder. Separations were conducted by application of 30-60V across a distance of approximately 3 centimeters. A green laser was used for excitation of the DNA. A 100 μm pinhole was used for focusing of emitted light prior to entrance into the photomultiplier tube (PMT.) Also, prior to the PMT entrance is a 595 ± 30 nm filter. The Microfluidics system was controlled and data collected via LabVIEW. Following each set of parabolas, the system was opened and a new device placed into the system.

Device Fabrication and Assembly

The microfabricated electrophoresis device (microdevice) used during flight was designed by Burns and colleagues and measures 7 mm by 35 mm. Standard photolithographic techniques are used for fabrication of the device. The silicon substrate contains the electrodes, heaters, and temperature sensors, which are subsequently wire bonded to a PC board. The etched glass channel is bonded to the silicon substrate. In this research, it was necessary to use a glass substrate in order to facilitate alignment of the channel with the detection system. A photo-initiated polyacrylamide gel was used. This gel is a denaturing gel and is the same used for high resolution sequencing. Gels are cast by first filling the channel with the monomer/crosslinker mixture. Next, an opaque mask is placed on top of the loading ports and the gel polymerized by

UV illumination. After illumination, the mask is removed and the unpolymerized reagents are removed from the loading ports. The resulting gel interface is well defined, flat, and precisely positioned.

Microfluidics system

The Microfluidics system is housed in a metal enclosure. Fluorescently labeled test analytes are detected as they pass through a laser. The fluorescent emission from the analytes is collected using a microscope objective and detected with a channel photomultiplier (PMT). The signal from the PMT is sampled using a multiDAC card attached to a neighboring laptop computer. The current in each of the channels is monitored continuously and accelerations of ± 2g in the X, Y, and Z planes are recorded by an integrated accelerometer.

Device Preparation

The devices were attached to an interchangeable mount and the glass channels aligned prior to gel casting. A 5% Long Ranger acrylamide solution was used and crosslinking is UV-initiated, allowing for specific positioning of the gel interface.

RESULTS:

Three separations yielded usable data. This data from the flights did show separation of the DNA did occur throughout all gravity regimes. It is apparent not all 10 bands in the ladder were resolved. In all three separations, it appears that multiple bands were detected simultaneously. Similar results were achieved during ground studies.

DISCUSSION:

After further investigation, it seems that potentially the primary reason for the simultaneous detection of bands is a large plug width. Plug width is indirectly related to resolution. Plug width can be decreased and controlled on-chip by use of electrode-defined injection, increasing resolution. The Microfluidics system was not configured to allow for electrode-defined injection. Instead, the same pair of electrodes was used for injection and separation, resulting in a large plug width and loss of resolution.

CONCLUSION:

Separation of dsDNA was accomplished during all gravity regimes experienced onboard the KC-135. However, not all 10 bands in the 100 bp DNA ladder were resolved. It is believed this is a result of using a single pair of electrodes for both sample injection and separation, resulting in a large plug width. In order to increase the achievable separation resolution, it is suggested that electrode-defined injection and electric field directed DNA collection be integrated into the Microfluidics system for future microdevice DNA separation experiments. It appears from these experiments that the 1) microdevice integrated well with the Microfluidics system, and 2) a microscale electrophoretic approach for separation of DNA is feasible and functional in low gravity.

PHOTOGRAPHS:

JSC2004E43037 to JSC2004E43040
JSC2004E43978 to JSC2004E44046

VIDEO:

- Zero-g 09/28 to 10/01/04 , Reference Master: 718802, 806

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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Appendix

Background Information about the KC-135 and the Reduced-Gravity Program

The Reduced-Gravity Program, operated by the NASA/Johnson Space Center (JSC), provides engineers, scientists, and astronauts alike, a unique opportunity to perform testing and training in a weightless environment but without ever having to leave the confines of the Earth's orbit. Given the frequency of Space Shuttle missions and the construction and habitation of the New International Space Station, the Reduced-Gravity Program provides a truly ideal environment to test and evaluate space hardware and experimental procedures prior to launch.

The Reduced-Gravity Program was established in 1959 to investigate the reactions of humans and hardware during operations in a weightless environment. A specially modified KC-135 turbojet (KC-135A), flying parabolic arcs, produces periodic episodes of weightlessness lasting 20-25 seconds. The KC-135 is sometimes also flown to provide short periods of lunar (1/6) and martian (1/3) gravity. Over the last 35 years, approximately 100,000 parabolas have been flown in support of the Mercury, Gemini, Apollo, Skylab, Space Shuttle, and Space Station programs.

Excluding the KC-135 Flight Crew and the Reduced Gravity Program Test Directors, the KC-135 accommodates seating for a maximum of 21 other passengers. The KC-135's cargo bay provides a test area that is approximately 60 feet long, 10 feet wide and 7 feet high. The aircraft is equipped with electrical power, overboard venting system, and photographic lights. When requested and available, professional photography and video support can be scheduled to document activities in-flight.

A typical flight lasts 2 to 3 hours and consists of 30 to 40 parabolas. The parabolas are flown in succession or with short breaks between maneuvers to allow time for reconfiguring test equipment.

For additional information concerning flight weeks sponsored by the Johnson Space Center's Human Adaptation Countermeasures Office or other Reduced-Gravity Program opportunities, please contact:

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Explore the Zero Gravity Experiments and Aircraft Operations Web pages at:
http://zerog.jsc.nasa.gov/
http://jsc-aircraft-ops.jsc.nasa.gov

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave Blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2005	NASA Technical Memorandum

4. TITLE AND SUBTITLE
KC-135 and Other Microgravity Simulations, Summary Report.

5. FUNDING NUMBERS

6. AUTHOR(S)
Space and Life Sciences Directorate, Human Adaptation and Countrmeasures Office.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Lyndon B. Johnson Space Center
Houston, Texas 77058

8. PERFORMING ORGANIZATION REPORT NUMBERS
S-958

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

National Aeronautics and Space Administration
Washington, DC 20546-0001

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
TM-2004-213162

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Unclassified/Unlimited
Available from the NASA Center for AeroSpace Information (CASI)
7121 Standard
Hanover, MD 21076-1320 Subject code: 52

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

This document represents a summary of medical and scientific evaluations conducted aboard the KC-135 from June 23, 2004 to June 27, 2005. Included is a general overview of KC-135 activities manifested and coordinated by the Human Adaptation and Countermeasures Office. A collection of brief reports that describe tests conducted aboard the KC-135 follows the overview. Principal investigators and test engineers contributed significantly to the content of the report describing their particular experiment or hardware evaluation. This document concludes with an appendix that providesbackground information concerning the KC-135 and the Reduced-Gravity Program.

14. SUBJECT TERMS	15. NUMBER OF PAGES	16. PRICE CODE
Weightlessness, weightlessness simulation, parabolic flight, zero gravity, aerospace medicine, astronaut performance, bioprocessing, space manufacturing.	148	

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited