Paper Session II-B - Life Sciences Shuttle Flights-15 Years

B. P. Dalton
NASA-Ames Research Center, Moffett Field, CA

R. P. Hogan
NASA-Ames Research Center, Moffett Field, CA

G. Jahns

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Abstract

Fifteen years ago, the first Life Sciences Announcement of Opportunity offered the promises of "man-tended" microgravity flights. For the experiments involving "non-human" elements, i.e., plants, animals, tissues and cells, the Shuttle Transportation System (STS) flights posed both challenges and rewards. The transition from the 1-G laboratory bench to O-G environment has resulted in new information with each succeeding flight. These rewards are measured both in better understanding in methods and materials to conduct research within the microgravity milieu and interpretation of the data obtained.

The participatory record for Ames Research Center, the primary NASA Center responsible for implementation of nonhuman Life Sciences experiments, includes Spacelabs 1, 2, 3, Spacelab Life Sciences 1 (SLS-1), the International Microgravity Laboratory (IML-1), Spacelab J (SL-J), and an average of one mid-deck experiment every six months since "return to flight" in 1989. An inventory of proven equipment and the accompanying verification and operational procedures exist, which can benefit each succeeding flight. This paper will briefly describe the experiments and hardware operations performed during those missions.

Currently, experimenters on SLS-2 (launching 8/93) are using the Rodent Research Animal Holding Facility (RAHF) and General Purpose Work Station flown on SLS-1 and SL-J; experiments on Europe 1 (manifest 8/96) are utilizing hardware elements from IML-1 and SL-J. The Animal Enclosure Module, which houses 4-6 rats and had its origins with the Student Shuttle Involvement Program, has been flown on over ten flights and is being reconfigured to sustain the long duration missions such as SLS-3 (16 days). In addition, the SLS-3 mission is dependent on the environmental control system developed for the Rodent RAHF for their Rhesus Research Facility.

The engineering systems developed, operational knowledge gained over the past 15 years, and data base of experimental results being developed, can only enhance, support, and stimulate the scientific community's sights toward NASA's next direction - Space Station Freedom.
LIFE SCIENCES SHUTTLE FLIGHTS - 15 YEARS

In 1978 an Announcement of Opportunity (AO) for submission of experiments to fly aboard the Shuttle Transportation System (STS) Spacelab was released by the NASA Headquarters Life Sciences Division to the scientific community. From the original 400 proposals submitted, 24 human and non-human biological experiments were selected for flight. These investigations were to be part of Spacelab 4 (SL-4), a dedicated life sciences mission, scheduled to launch in 1981. Submission of these experiments was the beginning of life sciences research aboard the STS. Since that first AO, additional AOs (1984) have been released along with "Dear Colleague Letters," and NASA Research Announcements (NRAs) for participation in microgravity experiments, and have involved spacelab and mid-deck experiments, as well as sharing specimens post-flight. As a result of the various announcements, the Ames Research Center has supported experiments during three major spacelab missions and more than a half dozen secondary payload experiments within a two-year period.

The NASA Life Sciences Program has included investigations dealing with both human and non-human subjects. Non-human specimens have involved microbes (bacteria, yeast), cells, tissues, plants, amphibians (frogs, salamanders) and monkeys. Human studies, proposed for SL-4, involved investigations of cardiovascular function, lymphocyte proliferation, pulmonary function, protein metabolism, fluid-electrolyte regulation and vestibular function. The non-human investigations followed similar disciplines, but allowed in-flight and post-flight processing of the specimens at the tissue level.

Johnson Space Center (JSC) had earlier been identified as the developer of human experiments; Ames Research Center (ARC) was to develop all non-human experiments. As early as 1976, ARC had started studies toward release of an eventual Request for Proposal for design and fabrication of several major facilities including a Plant Growth Unit (PGU), Research Animal Holding Facility (RAHF), and General Purpose Work Station (GPWS).

The Spacelab Mission Development Test #3 (SMD-3) conducted at the JSC in 1977 was an exercise of conceptual spacelab hardware, including RAHF s for rodents, squirrel monkeys, and rhesus monkeys and a GPWS. The seven-day test, was the first union of human and non-human elements in a Spacelab type environment and was conducted at the JSC Spacelab mockup. The test verified the co-habitation of human and non-human experiments and specimens, i.e., 3 payload crewpersons, 48 rats, and 4 monkeys, and also logistically verified that, following development and testing, direct transfer of ARC hardware to the Kennedy Space Center (KSC) was more cost and time effective.

As planning continued toward the dedicated life sciences mission, Ames supported plant experiments in the mid-deck PGU, which contained its own lighting, thermal control, power, instrumentation, and terrarium-like chambers. Temperature was controlled by lamp heat, a strip heater, and two fans that circulated shuttle cabin air through the PGU. Mung beans, oats, and pine seedlings were grown in the PGU during STS-3. Without a gravity cue, some mung bean roots grew upward in space and also produced less lignin. During SL-2 in July, 1985, experimenters again grew mung beans, oats and pine seedlings with the objective of determining metabolic control of lignin formation under microgravity conditions. The hardware functioned well and has continued to be used in successive mid-deck "Chromex" flights managed by the KSC.

Rats were being flown in mid-deck units called Animal Enclosures Modules (AEMs) with potatoes as their source of water and RAHF food bars glued to the walls as a nutrient source. Waste management elements designed for the RAHF, specifically charcoal and treated absorbent materials, were incorporated into the AEMS, designed and fabricated by General Dynamics for the Student Involvement Program (SSIP). The program, managed by the JSC, with engineering and science support by ARC had lights on: STS-8, 10, and 29. Addition of a water box and an "in-flight" refill capability to the AEMs have made possible seven Secondary payload flights and the nine-day SLS-I flight.
Two versions of the RAHF were built to support the life sciences investigations, one to house 24 rodents and one to house four unrestrained squirrel monkeys and were delivered to SLSPO in 1981/1982 by the Lockheed Missiles and Space Company (LMSC). Elements of of the originally planned SL-4, were under development within Ames shops, i.e., the Frog Embryology Unit (FEU), or at investigator institutions, i.e., the Gravitational Plant Physiology Facility (GPPF) built at the University of Pennsylvania, Philadelphia. As planning toward SL-4 continued, it became, obvious that all originally proposed elements, by virtue of required rack space, power, and crew time, would not fit in one spacclab. In 1983 the "green things", represented by frogs and plants were shifted to other missions. The Frog Embryology Experiment was manifest on Spacelab Japan (SL-J) and the Gravity Threshold and Response to Light Stimulation: Phototropic Transients experiments were manifest on the International Microgravity Laboratory 1 (IML-1).

When Ames was offered the opportunity to fly the RAHF on SL-3, in 1985, it was recognized as a unique opportunity to verify the animal facilities, subsequent to the dedicated life sciences missions. Thus, SL-3 became the first Spacelab carrying a cargo of live animals, which included 24 rats and 2 squirrel monkeys. Hardware elements included the two versions of the RAHF, biotelemetry implants in 4 rats which measured heart rate and deep body temperature, and a Dynamic Environment Measurement System, which measured noise, vibration, and acceleration in the immediate vicinity of the RAHF during launch and reentry. The RAHF was designed to provide basic animal housekeeping functions of air, food, water, waste management, lighting, humidity removal and temperature control. Water was controlled via a set of lixits mounted just above the cage top in the cage module. Food was dispensed via a feeder cassette mounted on the side of the cage; waste management was controlled through the use of airflow to direct urine and feces into a waste tray at the bottom of the cage. Temperature and excess humidity removal were controlled via an environmental control system mounted on the rear of the cage module. A water separator system removed excess humidity and transferred liquid to a condensate collector bottle, changed out by the crew as required. Lighting was incorporated into the cage module. Activity of the rodent was monitored via an infrared beam. The hardware on SL-3 functioned as planned in terms of animal maintenance, but unplanned particulate release and animal odor, made rework of the RAHFs mandatory prior to their continued use in spacclab.

Meanwhile, SL-4 had been renamed Life Sciences 1 and eventually Spacelab Life Sciences 1 (SLS-1) and was scheduled to launch within two years of SL-3 (1987). The RAHFs which were to accommodate the 48 rats and 4 squirrel monkeys proposed in the experiments, could not be reworked and integrated into the flight processing flow in sufficient time. Containment in the GPWS, which had been planned for support of in-flight rodent dissections, was also questioned. In October, 1985, the SLS-1 Investigator Working Group proposed the use of the AEMs, in place of the RAHFs. There would be no in-flight processing, but the investigators would get immediate post-flight data.

Extensive post-SL-3 testing of the RAHF hardware, showed the cage module had leak paths preventing its operation as a negative pressure device. Air leaks were determined to be in an outward direction which accounted for the presence of odor in the cabin. The rodent cage top was 1/4" grid, with holes in the cage top for lixit access; waste trays were not sealed at cage front, and the foodbars crumbled. Airflow was erratic, turbulent, and non-existent within in some places in the cage.

RAHF Redesign 1985-1988: The RAHF was redesigned to prevent the recurrence of the particulate problems. Redesign included:

- Sealing the cage module to prevent odor escape and to insure inward airflow, along with improvements to produce linear airflow through the cages.
- Internalizing the rodent cage lixits, providing an improved waste tray and expanded food capacity and total interchangeability of all cage parts. Cages were sealed to prevent escape of all particles larger than 150 microns.
- Adding a Single Pass Auxiliary Fan (SPAF) to produce high inward airflow during cage
servicing operations such as feeder or waste tray replacement.

- Replacing all drinking water system parts with stainless steel to minimize corrosion.
- Upgrading components for reliability (water separator fan and other critical components).
- Sealing of cages to cage module to prevent escape of particles into the cabin. All exhaust air to the cabin to be filtered to 0.3 microns via the use of HEPA filters
- Providing modifications to address integration problems experienced at KSC.

New versions of the rodent RAHF were delivered to SLSPO in August 1988 and June 1989 for biocompatibility and system sensitivity testing. Members of the Astronaut Office and SLS-1 Payload Specialists participated extensively in the redesign activity including design of such items as cage latches, SPAF configuration, waste tray design, rodent viewing, etc.

A modified oil pipeline software program facilitated study of airflow within the RAHF. This program was used to reconstruct the SL-3 configuration and explain what had gone wrong and to determine the effect of various leak paths and sealing techniques. Extensive analysis and testing showed, with proper sealing, all airflow was inward (including leaks). Leak budget for the total system was ~10 CFM at one inch of water and indicated a leak of approximately 1 CFM at operating pressure of 0.25 inches of water. The actual measured leak rate was 2 CFM at one inch of water. Airflow was improved by placing a coarse mesh screen on the cage top which served as a turning vane for air coming from the inlet plenum of the ECS. Acetic acid smoke testing revealed linear airflow over the length of each cage and measured an average of 10 CFM through each cage. Change of waste tray packing material also improved the airflow through the cages. A separate layer of Filtrate™ on the bottom of the tray maintained the 150 micron retention requirement. Bondina™ was treated with phosphoric acid to prevent odor and eliminate microbial growth. Use of layers of fiber glass batting and loose charcoal resulted in inconsistent ΔP's across each cage.

Based on demonstrated test data and changes accomplished by the time of the Critical Design Review, the SLS-1 payload crew voted to include the Rodent RAHF, with live animals on the flight. The RAHF was manifested on SLS-1 in July 1987. Delivery of the new hardware from LMSC took place in August 1988. A new food bar was also designed, utilizing the binding characteristics of gluten, to minimize crumbing. Specifications on waste and odor control addressed efficiency of the particulate filters to insure minimum 99.95% removal efficiency for spherical particles of mean diameter >150 microns when challenged with rodent waste. During all phases of RAHF operation for 10 consecutive days, animal odor would not exceed a score of 1.5 based on the existing criteria. Escape of microorganisms into the spacetlab cabin would be minimized by providing all RAHF exhaust outlets with High Efficiency Particulate Apparatus (HEPA) filters having a minimum 99.97% removal efficiency for particles ≥ 0.3 microns.

**GPWS Redesign 1985-1988:** The GPWS was designed as a Class II workbench and was intended to be a sealed cabinet to be used for in-flight dissection and preparation of specimens in various toxic fixative solutions. In the event of a spill, the cabinet is closed off and operated in the recirculating mode which passes all air through a Trace Contaminant Control System, a multi layer charcoal bed designed to removes toxics (primarily aldehydes used in tissue fixation) which may be blown. As a result of SL-3, the following modifications were performed on the GPWS, upon delivery to ARC:

- The cabinet was sealed to NSF-49 Class II standards (contains particles ≤ 150μ).
- A side access window was added to allow entry of small items such as the rodent cage
- Gauntlet ports were added to the front and side doors to prevent particulate escape during operation and to keep the crew garments clean. These Tyvek™ gauntlets are standard clean room material and are designed for interchangeable gloves.
- Grill covers were added inside the cabinet to prevent particulates from entering the HEPA filter system.
- A General Purpose Transfer Unit (GPTU) was designed to accommodate transfer of rodent cages between the RAHF and GPWS.
The GPTU resembles a large wind sock (made from Tyvek™) and is attached to a lexan frame on the front of the RAHF. A cage is withdrawn into the GPTU and sealed by closing a door on the front of the unit. The GPTU is then transferred to the GPWS side access window and installed on the attachment frame, thus allowing exchange of cage location without exposure to the atmosphere. The GPWS has remained at KSC since its delivery for SLS-1 in 1988; immediately following SLS-1 it was integrated into and flew on SL-J and will be used in SLS-2.

**Spacelab Life Sciences 1 (SLS-1), June 1991**

A particulate containment demonstration test (PCDT) was conducted on SLS-1 to verify particulate containment capability of the RAHF and GPWS. Two of the cages were outfitted with particulate dispensers filled with simulated feces (black-eyed peas), food crumbs and rodent hair. Twenty rodents were designated to fly in the remaining ten double cages. A similar arrangement of particulate containers was designed for the GPWS along with a liquid container (crew drinking cup filled with colored liquid) to test the cleanup procedures needed for future flights. Per crew request and with the concurrence of NASA Headquarters, a successful completion of PCDT allowed transfer of a cage containing live animals to the GPWS in preparation for future mission operations on SLS-2.

Ten additional rodents were flown in the mid-deck in the Animal Enclosure Modules (AEM's). These animals were flown to increase the number of animals per experiment and to offer a direct comparison to rodents flown in the RAHF. Other major pieces of equipment flown on SLS-1 included the Small Mass Measuring Instrument (SMMI) and the Refrigerator Incubator Module (R/IM). The SMMI was calibrated several times during the mission as a measure of its capabilities and as a test for its use during the second dedicated Life Sciences mission, SLS-2 and performed flawlessly. An additional experiment involving >2,000 jellyfish flown in the R/IM provided interesting swimming patterns which are being reinvestigated on IML-2.

The most difficult aspect of the dedicated life sciences SLS-1 mission was the delayed launch attempts. Launch was scheduled for May 22, 1991 and was aborted just prior to installation of animals (~31 hours) due to computer problems. The second attempt was on June 1. The launch was within minutes of occurring when an IMU calibration failure was indicated. Animals were removed, cages refurbished and animals reinstalled at L-29 hours. On the third attempt, the Shuttle Transportation System (STS)-40 vehicle and SLS-1, the first spacelab post-Challenger, was launched from Kennedy Space Center (KSC) Pad 39A at 9:24:51 a.m. EDT, June 5 for a 9-day mission. STS 40 had a 150 nautical mile circular orbit with an inclination of 39.0 degrees. Landing was at Edwards Air Force Base, June 14, 1991 at 8:38 a.m. PDT, followed by turnover of mid-deck AEM animals 10:27 a.m. and turnover of Spacelab RAHF animals at 12:25 p.m. at the Ames Payload Receiving Facility.

In contrast to most spacetlans, SLS-1 in-flight payload operations were on a single 12-hour shift. In addition to the live cargo of 19 rats in the RAHF (1 cage could not be used due to inoperable lixit), 10 rats in the AEMs and congregations of jellyfish, crew members included the Commander, pilot, three mission specialists, and two civilian payload specialists. Hardware performed well; variations seen were accommodated. Three activity monitors failed but data was redundant with food and water counts. The failure of water pressure transducer on FD 3 was of major concern. Additionally, water count data was inaccurate due to experiment computer crashes of ~5 hours each. Because of the inability to accurately monitor water tank pressure, agar gel paks were added to all cages on FD 8. An in-flight pressure gauge has been provided for future flights (SLS-2) to circumvent the loss of volume data through the pressure transducer. A loose swage fitting was discovered during the AEM refill on FD 4. The crew initiated an Inflight Maintenance to tighten the swage fitting.

Post-flight turnover of specimens occurred as scheduled and science personnel were on hand to immediately process the animals. Between flight and ground samples, approximately 6,000 rat
tissue samples were provided to the scientific community under a Biospecimen Sharing Plan which involved American, Soviet, French, and Canadian investigators. The rats remained healthy during all phases of the flight, but on landing appeared flaccid in muscle tone, lethargic, and less inquisitive than real-time 1-g controls. Flight rats had reduced use of their tail as a stabilizing tool and displayed difficulty in balancing themselves on their hindlimbs in an upright posture. These changes were markedly reduced by the second post-flight day. Differences in behavior were indistinguishable between groups by the third post-flight day. Body weights were not different between flight or post-flight control groups at loading. Flight rats gained significantly less body weight during the flight period than post-flight control (4.2±0.2 vs. 6.0±0.2 g/day; p<0.0001; see Table 1). As a result, flight rats weighed significantly less at unloading. The flight rats lost 6.9±0.7 g/day for the first 2 days after landing compared to a net gain of 2.9±0.5 g/day in the controls. Though weight gain became comparable, body weights of flight animals remained significantly below those of the controls throughout recovery with no difference in body weights between flight RAHF and flight AEM rats.

### Table 1

<table>
<thead>
<tr>
<th>Food and water utilization/body weights: Flight rats vs Control rats</th>
<th>R+0</th>
<th>R+9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Body Weight</strong></td>
<td>Flight</td>
<td>CONTROL</td>
</tr>
<tr>
<td></td>
<td>300.2 ± 3.1 **</td>
<td>330.3 ± 5.1 ††</td>
</tr>
<tr>
<td></td>
<td>314.6 ± 3.1</td>
<td>374.3 ± 6.0</td>
</tr>
<tr>
<td><strong>Food</strong></td>
<td>Flight</td>
<td>CONTROL</td>
</tr>
<tr>
<td></td>
<td>28.0 ± .6</td>
<td>19.5 ± .5 ††</td>
</tr>
<tr>
<td></td>
<td>27.6 ± .4</td>
<td>27.3 ± .6</td>
</tr>
<tr>
<td><strong>Indexed Food</strong></td>
<td>Flight</td>
<td>CONTROL</td>
</tr>
<tr>
<td></td>
<td>9.4 ± .2 **</td>
<td>6.0 ± .1 ††</td>
</tr>
<tr>
<td></td>
<td>8.8 ± .1</td>
<td>7.2 ± .1</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td>Flight</td>
<td>CONTROL</td>
</tr>
<tr>
<td></td>
<td>30.3 ± 1.7</td>
<td>36.9 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>29.5 ± 1.8</td>
<td>30.1 ± 2.9</td>
</tr>
<tr>
<td><strong>Indexed Water</strong></td>
<td>Flight</td>
<td>CONTROL</td>
</tr>
<tr>
<td></td>
<td>9.8 ± .5</td>
<td>11.3 ± .7 **</td>
</tr>
<tr>
<td></td>
<td>9.1 ± .6</td>
<td>8.00 ± .8</td>
</tr>
</tbody>
</table>

Numbers are Mean ± SE. *p<0.05  **p<0.01  †p<0.001 ††p<0.0001
All weights are in grams. Indexed food weights are in grams/100 grams of body weight. Indexed water weights are in ml/100 grams of body weight.

Significant decreases in body weight without concomitant reduction in food consumption, results in greater caloric intake per body weight. Re-exposure to 1-G results in a reduction in body weight and in food intake. These changes appear reversed after 2 days of recovery, although stunted body weight and reduced appetite remain. Decrease in food consumption may be indicative of decreased caloric requirements, or possibly of dehydration. Though increase in water utilization by rats in microgravity (45 cc/day versus 30 cc/day) is indicated, it is not clear that all water is consumed.

The RAHF and AEM have both proved to be valuable habitats for the maintenance of rats during spaceflight. SLS-1 has shown that rats serves as a valuable models for basic research regarding the effects of spaceflight as well as a useful surrogates for certain human physiological studies.

**International Microgravity Laboratory (IML-1), January, 1992**

The launch of the Marshall Space Flight Center (MSFC) managed IML-1 Spacelab aboard STS Discovery occurred at 9:52 a.m. EST, January 22, 1992. Landing occurred at Edwards Air Force Base on January 30, at 7:06 a.m. PST. The GPPF and the Biorack US-1 experimenters, performed preliminary processing of their oat and wheat plantings and nematodes, respectively, at the Ames Dryden facility; the yeast from US-2 were transported by the investigator and co-investigator to Berkley for analyses; US-3 cells were transported by the European Space Agency (ESA) to the KSC Hangar 1 facility. Ames interfaced with the MSFC management team for integration of the GPPF and with the ESA for integration of the three Biorack experiments.
The GPPF, with its incorporated centrifuges, lights, videotape recorders, plant holding compartments, and temperature maintenance provided the tools necessary to study gravitropism and phototropism in oats and wheat, respectively. The GTHRES experiment studied the changes that occur when plants (540 oat seeds (180 planted)) are exposed to different levels and duration of gravity along with growing plants response to altered gravitational fields and the effects of microgravity on structure. The FOTRAN experiment investigated the response of plants (216 wheat seeds (96 planted)) to light in microgravity and the impact of microgravity on nutrition (the helical growth pattern of plant stems) and autotropism (the straightening observed in plants during gravitropic or nutational movement). The Ames hardware functioned, as planned, with only minor anomalies, specifically a ten-minute loss of GPPF taped video. Shadowgraphs of the growing seedlings were down-linked during the flight. Gas samples of CO₂ retrieved during the flight exhibited levels of 2,000 to 4,000 ppm. The significant finding was the precocious growth of the plants in both the FOTRAN and GTHRES experiments conducted in this hardware. Specific causes for the growth is still under investigation by the experimenters, who have been performing post-flight tests with the GPPF at their laboratories in Philadelphia, Pennsylvania.

The GPPF was designed and constructed at the investigator's laboratory. Components supporting the two experimental elements, GTHRES and FOTRAN, included: Control Unit, Test and Culture Rotor Assemblies, Recording and Stimulus Chamber unit, two redundant video tape recorders, Mesocotyl Suppression Box, and a stowage drawer called the Plant Holding Compartment. Due to the age of the equipment, lack of understanding of requirements during the design, and changing spacelab requirements post-Challenger, every unit had to be reworked or replaced after receipt at Ames. Problems existed with welds in the rotor housing structure resulting in insufficient safety margins, inadequate strength fasteners, lack of adequate EMI shielding in the Control Unit, safety problems with light bulb enclosure, and finally need for Finite Element Modeling and testing. In addition to verification testing, other activities conducted at Ames included development of a Failure Modes and Effects Analysis, update of all drawings and Materials Lists (often using spectral analyses for materials identification). The end result was full acceptance for integration into spacelab and the resultant sustained performance during flight.

Though the Ames supported experiments represented only 3 of the 17 Biorack experiments, the hardware development supporting Chondriogenesis in Micromass Cultures of Mouse Limb Mesenchyme Exposed to Microgravity (CELLS), Genetic and Molecular Dosimetry and HZE Radiation (RADIAT) and Microgravitational Effects on Chromosome Behavior (YEAST) was represented by >10,000 piece parts which required cleaning, inspection, and assembly in the last weeks prior to flight. Miniaturization is the essence of the Biorack hardware. All equipment must fit within the ESA provided Type I/O and Type II/O containers.

For the US-1 (RADIAT) experiment which examined the effects on soil nematodes (7.2 million microscopic round worms) exposed to atmospheric radiation, hardware was stacked radiation sensitive thermoplastic sheets (CR-39) along with Thermo-Luminescence Detectors and agarose gel on which the nematodes rested. Groups of lexan tubes also contained the nematodes and agar. The nematodes in their hardware, within the ESA containers were strategically placed around the Spacelab and tunnel to ascertain the areas of most exposure and potential mutation effects.

The US-2 (YEAST) experiment studied both microgravity and radiation effects by measuring genetic damage during mitosis and meiosis. Hardware consisted of a small chamber overlaying an agar covered plate on which the 3 billion brewer's yeast cells (Saccharomyces cerevisiae) rested. The chamber served as a vessel for addition of fixative solution during the mission. The fixative preserved the cells in their microgravity affected condition for analyses on return to 1-G. Pre-filled syringe assemblies were used to add the fixative.

US-3 (CELLS) used embryonic mouse cells which become cartilage, the precursor to bone formation. Study of gravity effects on cartilage differentiation provides greater insight to bone development on Earth. The US-3 hardware was similar to US-2, since cells were grown on a flat surface. The 32 million mouse limb-bud cells in the chamber vessel required changing the
overlying fluid several times during the mission. The US 3 experiment alone required >12 hours of in-flight crew time. The subtleties of tissue culture methodology, 11.5-12.5 timed pregnancies for appropriate gestational cells, along with miniaturization, proved a formidable task.

The IML-1 flight was an eight-day mission manned by a two shift operation and seven crew members. All hardware reportedly functioned as planned during the mission. The IML-1 experimenters have claimed the mission an outstanding success, especially with the extra day of data. Results of all post-flight analyses are provided by the experimenters, both GTHRES, FOTRAN, and US-1, 2, 3 in discipline publications.

**Spacelab Japan (SL-J), September 1992**

Endeavor was launched September 12, 1992 on its second flight carrying the Spacelab J mission and landed seven days later at Kennedy Space Center. Following, IML-1, SL-J was the second international spacelab flight in which Ames participated. Mission operations were jointly shared by the U.S. and Japan. The seven-person crew included one Japanese and 34 experiments sponsored by NASA; 9 sponsored by NASA. The two Ames associated experiments included the Frog Embryology Experiment (FEE) which investigated the effects of weightlessness on the development of amphibian eggs fertilized in space and the Autogenic Feedback Training Experiment (AFTE).

The mission provided the first flight for live frogs and joined the elements of Gemini experiments (conducted 20 years earlier by the investigator) and provided additional information to experiments flown aboard D-1 and IML-1. Hardly a home TV was without visions of frogs floating in space. The scientific interest was the role of gravity in the process of bilateral symmetry, dorsal/ventral axis formation, and the development of the inner ear and related gravity-sensitive components. Lack of gravity seemed not to affect normal formation of progeny nor of their swimming behavior. Fixed embryos and approximately 150 live tadpoles were returned to earth. They, along with their offspring are being studied in the investigator's laboratories at Ames.

The Frog Environmental Unit (FEU), the primary hardware used to support the experiment, contained four major systems: centrifuge, adult frog holding unit, O-G egg chamber storage, and electronics. The temperature controlled FEU maintained temperature at a set point in the range of 18-24°C. The adult frog chamber contained four female frogs in a sponge dampened environment. These frogs were removed, within the GPWS, and eggs obtained in-flight and examined microscopically. The egg chambers were small lexan structures configured with with valves for syringe access. The centrifuge provided an "in-flight" 1-G control.

The use of the AFTE on SL-J expanded the experiment sample size toward completing eventual data acquisition from 16 subjects. The hardware is designed to record ECG, respiration rate, skin conductance, blood volume pulse, triaxial accelerations, and temperature. Several of these parameters are displayed on a wrist display unit, which is used in-flight as a biofeedback aid. Final analysis of all data will allow AFTE training to be evaluated as a countermeasure for space motion sickness. This could lead to the ability to predict crewmember susceptibility to space motion sickness.

Both the FEE and the AFTE hardware were designed, fabricated, and tested within the Ames Research Center Code E Directorate support organizations. The other major elements of support to the FEE, the GPWS and Refrigerator/Incubator Module were refurbished from SLS-1 and integrated into the SL-J processing flow.

**Spacelab Life Sciences 2 (SLS-2), August, 1993**

SLS-2 is the implementation of SLS-1. SLS-2 is the second of the dedicated life sciences missions.
and will finally allow the "in-flight" manipulations proposed by investigators in their AO '78 proposals. Manipulations will include: rodent blood sampling, injections, and dissections. Biospecimens will include 48 rats housed within 2 rodent RAHF's. Other supporting hardware, the GPWS and SMMI, are units verified on SLS-1. The most intensive task in SLS-2 has been development of the Kits used by the crew for in-flight rodent operations. Currently >1,000 piece parts are contained in these Kits. The Biospecimen Sharing Plan being implemented for this flight will result in over 10,000 samples being distributed among U.S., French, Japanese, and Soviet scientists, many of whom will be present for the post-flight processing at the Dryden Flight Research Facility. In summary, SLS-2 will fulfill the AO presented 15 years earlier, but has expanded to provide a wealth of data to the scientific community.

**International Microgravity Laboratory 2 (IML-2)**

The IML-2 mission is scheduled to launch August, 1994. Ames supports two experiments, both studying gravity-receptor organs. The one uses newts and will be housed within Japanese provided equipment; the other involves jellyfish housed in German equipment.

**Spacelab Life Sciences 3 (SLS-3)**

The 16-day SLS-3 mission is scheduled to launch the first quarter of 1996. There will be 10 American and 11 French investigations using two rhesus monkeys as test subjects. No handling of the animals will occur in-flight, but the animals will be performing various computer games as a measure of their adaptation to the conditions of microgravity and will have telemetry implants. The results of the telemetry, games, and in-flight urine and fecal samples, along with similar data collected both pre and post-flight plus bone biopsies and blood samples, will provide a wealth of data on the effects of microgravity on primates.

Hardware to house the animals in Spacelab is being developed jointly by NASA-Ames Research Center and the Centre Nationale d'Etudes Spatiales (CNES) in France. The CNES is providing the unit housing the monkey termed the Experiment System for Orbiting Primate (ESOP), which contains the feeder, water lixit, urine collection system, fecal management system, and computer screen and joystick. The ESOP fits in the NASA provided Rhesus Research Facility, which is a version of the RAHF module with its environmental control system and water tank. NASA is also providing the Flight Data System which interfaces to the urine management software, primate games software, and relays the down-linked video and the Rhesus Measurement System data which consists of a telemetry system translating deep body temperature and electrocardiograph, and electromyogram measurements.

In addition to the primate experiments, two rodent experiments will be on-board housed in a futuristic version of the AEM called the Advanced Animal Habitat. The rodent experiments are assessing muscle activity and bone loss as a function of corticosteroids.

**Summary**

The Shuttle Transportation System has provided an unequalled in obtaining scientific data over the past 15 years. Our greatest challenge now is assimilating and analyzing that data along with the operational lessons learned to assure conclusive success in our Space Station efforts.