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Table of Contents

| | | Page |
|-----------------------|--|-------------|
| | Foreword | 1 |
| | Microgravity Sciences | 3 |
| | Life Science | 35 |
| Experiment | | |
| Identification | Experiment | |
| M-04 | Casting of Superconducting Composite Materials | 3 |
| M-05 | Formation Mechanism of Deoxidation Products in Iron Ingot Deoxidized with Two or Three Elements | 4 |
| M-06 | Preparation of Particle Dispersion Alloys | 5 |
| M-08 | High Temperature Behavior of Glass | 6 |
| M-17 | Preparation of Optical Materials Used in NonVisible Region | 7 |
| M-10 | Study on Solidification of Immiscible Alloy | 9 |
| M-11 | Fabrication of Ultra-Low Density, High Stiffness Carbon Fiber/Aluminum Alloy Composite | 10 |
| M-12 | Study on Liquid Phase Sintering | 12 |
| M-13 | Fabrication of Si-As-Te Semiconductor in Microgravity Environment | 13 |
| M-19 | Solidification of Eutectic System Alloys in Space | 15 |
| M-07 | Diffusion in Liquid State and Solidification of Binary System | 17 |
| M-14 | Gas Evaporation in Low Gravity | 18 |
| M-15 | Drop Dynamics in an Acoustic Resonant Chamber and Interference with the Acoustic Field | 20 |
| M-16 | Bubble Behavior in Thermal Gradient and Stationary Acoustic Wave | 22 |
| M-18 | Marangoni Effect Induced Convection in Material Processing under Microgravity | 23 |
| M-01 | Growth Experiment of Narrow Band-gap Semiconductor PbSnTe Single Crystal in Space | 25 |
| M-02 | Growth of PbSnTe Single Crystal by Traveling Zone Method | 27 |
| M-03 | Growth of Semiconducting Compound Single Crystal InSb by Floating Zone Method | 29 |
| M-09 | Growth of Spherical Silicon Crystals and the Surface Oxidation | 30 |
| M-22 | Crystal Growth of Compound Semiconductor in a Low Gravity Environment (InGaAs) | 32 |

| | | |
|-------|---|----|
| M-20 | Growth of Samarskite Crystal under Microgravity Conditions | 33 |
| M-21 | CrystalGrowth Experiment On Organic Metals in Low Gravity | 34 |
| PCG | Protein Crystal Growth Experiments on Spacelab J | 35 |
| L-05 | Crystal Growth of Enzymes in Low Gravity | 37 |
| L-06A | Studies on the Effects of Microgravity on the Ultrastructure and Function of Cultured Mammalian Cells | 39 |
| L-06B | Study the Effects of Microgravity on Cell Growth of Human Antibody Producing Cells and Their Secretions | 41 |
| L-06C | Organ Differentiation from Cultured Plant Cells Under Microgravity | 43 |
| L-07 | The Effect of Low Gravity on Calcium Metabolism and Bone Formation in Chick Embryo | 45 |
| L-12 | Circadian Rhythm of Conidiation in Neurospora Crassa | 48 |
| PCR | Plant Cell Research Experiment on Spacelab J Mission. Mitotic Disturbances in Daylily (<i>Heimerocallis</i>) Somatic Embryos after an 8-day Spaceflight | 49 |
| FEE | Amphibian Development in Microgravity: the STS-47 Frog Embryology Experiment | 51 |
| BCR | Bone Cell Research | 52 |
| FTS | In-Flight Demonstration of the Space Station Freedom Health Maintenance Facility Fluid Therapy System (E300/E05) | 54 |
| MRI | Magnetic Resonance Imaging After Exposure to Microgravity | 56 |
| L-01 | Endocrine and Metabolic Changes of Payload Specialist During Spacelab J | 57 |
| L-00 | Health Monitoring of Japanese Payload Specialist – Autonomic Nervous and Cardiovascular Responses under Reduced Gravity | 59 |
| L-02 | Neurophysiological Study on Visuo-vestibular Control of Posture and Movement in Fish during Adaptation to Weightlessness | 61 |
| L-04 | Comparative Measurement of Visual Stability in Earth and Cosmic Space | 63 |
| L-10 | Manual Control in Space Research on Perceptual Motor Functions under Microgravity Condition | 64 |
| LBNP | Countermeasure Against Orthostatic Intolerance After Spaceflight: The Combination of Oral Fluid Loading and Lower Body Negative Pressure | 65 |
| AFTE | Monitoring Astronauts' Functional State: Autonomic Responses to Microgravity | 66 |
| L-09 | Genetic Effects of HZE and Cosmic Radiation | 69 |
| L-11 | Studies on Biological Effects of Cosmic Radiation and Development of Radiation Protection Technology | 71 |
| L-03 | Separation of Biogenic Materials by Electrophoresis Under Zero Gravity | 75 |
| L-08 | Electrophoretic Separation of Cellular Materials under Microgravity | 77 |

Foreword

This report contains a brief summary of the mission science conducted aboard Spacelab J (SL-J), a joint venture between the National Aeronautics and Space Administration (NASA) and the National Space Development Agency (NASDA) of Japan. The SL-J mission was launched aboard Space Shuttle Endeavour (STS-47) on September 12, 1992, at 10:23 a.m., Eastern Daylight Time, from the John F. Kennedy Space Center (KSC). The crew comprised the following:

Capt. Robert L. (Hoot) Gibson, Commander
Maj. Curtis L. Brown, Jr., Pilot
Dr. Jay Apt, Flight Engineer
Lt. Col. Mark C. Lee, Payload Commander
Dr. Jan Davis, Mission Specialist
Dr. Mae Jemison, Science Mission Specialist
Dr. Mamoru Mohri, Payload Specialist

In addition, Dr. Takao Doi, Dr. Stan Koszelak, and Dr. Chiaki Mukai served as alternate Payload Specialists. The scientific objectives of the mission were to conduct a variety of material and life science experiments utilizing the weightless and radiation environment of an orbiting Spacelab. Spacelab ingress occurred at 00:03:21 Mission Elapsed Time (MET) followed by experiment operations until Spacelab deactivation at approximately 07:14:28 MET. Orbiter landing occurred on September 20, 1992, at 07:22:30 MET at the Kennedy Space Center.

The SL-J mission was successfully completed after eight days on orbit, which included an extra day of payload operations. All 43 experiments were activated—24 in microgravity sciences (material processing, crystal growth, fluid physics, and acceleration measurement) and 19 in life sciences (physiology, developmental biology, radiation effects, separation processes, and enzyme crystal growth). In addition, more than a dozen experiments benefited from the extra day through either additional experiment runs or extended growth time.

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Fred W. Leslie
SL-J Mission Scientist

MICROGRAVITY SCIENCES

Experiment M-04: Casting of Superconducting Composite Materials

Principal Investigator: Kazumasa Togano
National Research Institute for Metals

Coinvestigators: Hiroaki Kumakura
Hitoshi Kitaguchi
Hitoshi Wada
Hiroki Fujii
National Research Institute for Metals

The objective of this experiment is to investigate the influence of gravity on the formation of composite structure by monotectic solidification reaction. Such structural controls are expected to result in a great improvement of functional properties, such as superconducting properties. The ternary Al-Pb-Bi alloy system was selected for this flight experiment. This alloy separates into two liquid phases, that is, Al-rich and (Pb,Bi)-rich phases in the molten state. Under one gram, it is solidified not uniformly due to large differences in density, while under microgravity (Pb,Bi) particles are expected to be dispersed homogeneously in the Al matrix. It is also expected that we can fabricate a new type of Al-(Pb,Bi) composite superconducting wire by deforming the flight-processed Al-Pb-Bi alloy.

The experiment was done using the continuous heating furnace (CHF) of the First Materials Processing Test (FMPT). Three different compositions were chosen, which are Al-1at%Pb-1at%Bi, Al-2at%Pb-2at%Bi and Al-3at%Pb-3at%Bi. The alloy samples were heated in a cartridge to a temperature range of 1,577-1,580°K in ten minutes using the CHF, held for 34 minutes, and then cooled to 873°K in 70 seconds. For comparison, the same experiment was performed under one gram.

X-ray perspective views before cutting indicate the existence of some gas holes inside the flight-processed samples. The alloy samples were cut lengthwise and polished on the cross-section with alumina fine particles. The microstructure was observed by scanning electron microscopy. (Pb,Bi) particles were observed to be dispersed uniformly in the Al matrix for all the samples solidified under microgravity. The particle sizes range from a few to hundreds of micrometers in diameter, but more than 90 percent of the particles are below 50 nm. On the other hand, sedimentation of (Pb,Bi) particles was observed for gravitational direction for the samples processed under one gram.

The samples processed under microgravity were sheathed in a Cu tube and deformed into wires 0.35 mm in diameter. The wire shows the structure of the fine dispersion of (Pb,Bi) filaments elongated in the Al matrix and hence, zero resistance due to a proximity effect at low temperature. The critical temperature, upper critical field, and critical current density were 8.7°K, 1.9 T, and 5000 A/cm² (at 4.2°K, 0 T) for the Al-2at%Pb-2at%Bi wire.

We succeeded in preparing Al-Pb-Bi alloys with uniform structure using the microgravity environment and fabricating superconducting wire by deforming the flight-processed Al-Pb-Bi alloys. However, the problem of gas holes remained as a future subject. In this work, we also investigated the solidification of eutectic type Ag-Ln-Ba-Cu alloys and their superconducting properties after oxygenation.

Experiment M-06:**Preparation of Particle Dispersion Alloys****Principal Investigator:**

Yuji Muramatsu
National Research Institute for Metals

Coinvestigators:

Takehiro Dan
National Research Institute for Metals

Three Ni-TiC type dispersion alloys were prepared by powder metallurgical techniques in order to conduct melting and solidification in the microgravity environment. For this, a special furnace, referred to as Large Isothermal Furnace (LIF) was developed. The furnace was equipped with various systems and devices which accommodate the microgravity experiment. The microgravity experiment First Materials Processing Test (FMPT) was conducted on the SL-J mission. After the experiment, careful visual inspections on the cartridge and around the samples were done to confirm whether the experimental procedures were carried out normally and as scheduled. On the samples solidified, the state of melting and solidification were checked. Their microstructure and hardness were compared with those of samples melted and solidified on the ground in the same manner.

The inspections confirmed that all systems and devices worked correctly, and the melting and solidification was successfully achieved although a slight leak occurred in the FMPT. Experimental results showed that some definite differences in the micro structure took place in samples of the FMPT and terrestrial experiment. The samples processed in the FMPT showed uniform dispersion of TiC particles, while the samples processed on the ground illustrated macro- and microscopic segregations together with advanced grain growth of the TiC particles.

The reason why such segregations and grain growth take place is not clear up to now, but probably is due to thermal convection or bubble motion. FMPT samples had higher hardness values of which the deviation was smaller.

These facts demonstrate that the melting and solidification in the microgravity environment is favorable for uniform dispersion.

Experiment M-08: High Temperature Behavior of Glass

Principal Investigator: Naohiro Soga
Kyoto University

Coinvestigators: Kazuyuki Hirao
Katsuhisa Tanaka
Kazuki Nakanishi
Teiichi Hanada
Kyoto University

The melting experiment of a glass specimen floating under the microgravity environment has provided valuable information about the load-free thermal expansion, the internal flow and deformation, bubble formation and coarsening, and the surface crystallization; all of which are difficult to observe on Earth.

The experiment was performed at about 00:00:00 on the fourth day in orbit. Although the installation of the silica tube took a little bit more time, all the procedures including evacuation went on almost as scheduled. The heating started at about 01:00:00, and data were taken successfully by a video camera.

By analyzing the video images, the temperature-volume relation of a load-free glass sample was obtained. An abrupt volume increase was recognized around the glass transformation region from 400–500°C. However, when the sample was heated over 600°C, large bubbles appeared inside the specimen; additionally, the specimen was partially crystallized from the surface. Both of these phenomena had not been expected from the preliminary Earth bound experiments. Further analysis on these unexpected phenomena is required in order to improve the manufacturing and processing technique of glass materials in space.

The images at the highest heating temperature could not be obtained due to the second blackout. An inspection of the sample capsule after cooling revealed that the square shape of gold foils remained the same. The latter fact suggests the maximum targeted temperature 1,200°C, at which the gold foils melt and become droplets, was not attained.

Experiment M-17:**Preparation of Optical Materials Used in Non-Visible Region****Principal Investigator:**

Junji Hayakawa
Government Industrial Research Institute (GIRIO), Osaka

Coinvestigators:

Masaki Makihara
Kohei Fukumi
GIRIO

In microgravity, liquids do not require a container to hold them. Containerless melting eliminates the contamination from the container (crucible) and removes the chance of heterogeneous nucleation from the interface of the molten liquid and container. Using a containerless melting technique in microgravity, the glass forming tendency of a melt can be increased and new glasses with ultra-high chemical purity and interesting properties can be prepared.

We intend to establish the acoustic levitation technique, to study the chemical and physical phenomena of molten liquids, and to prepare new glasses with ultra-high chemical purity in the space experiment, SL-J M-17. The achievement of these objectives will show the advantage of microgravity for preparing glass will promote materials research in space, and will establish material manufacturing processes in space. That is, these results will lead to the preparation of new optical fibers for use in the non-visible region such as the infra-red.

Although the experiment was forced to stop due to a water leak in the Double Rack No.10, proper treatment by the Payload Specialist (PS) and the extension of the mission kept our three experiments on M-17. The PS started the experiment by setting the spherical raw materials in a platinum alloy cage which makes melting easy by the light conversion.

In the space experiments, we tried containerless glass forming by using an acoustic levitation furnace. The sample may drift irregularly due to the residual acceleration 10-4g. To avoid the drift, the sample is held at the focal position of the furnace by a standing acoustic wave in an acoustic levitation furnace which consists of a double ellipsoid reflector with two halogen lamps of 500W each. Since a gaseous medium is necessary for a sound wave to propagate, and the velocity of sound depends on the gas temperature, the frequency of the sound source must be adjusted continuously with increasing temperature of the gas so that a sound wave of constant wavelength is obtained. The operational performance of the acoustic levitation furnace was generally satisfactory for processing the samples.

In the first experiment, a $\text{CaO-PbO-B}_2\text{O}_3$ glass sphere made previously on Earth was levitated and remelted. We obtained many kinds of data for the acoustic levitation furnace, for the shaping of glass and for the flow of molten glass.

The second and third experiments used a spherical sample composed of sintered raw materials of $\text{CaO-Ga}_2\text{O}_3\text{-GeO}_2$ in the acoustic levitation furnace. Although the samples were not perfectly levitated due to the high temperature gradient from the melting temperature of $1,300^\circ\text{C}$, $\text{CaO-Ga}_2\text{O}_3\text{-GeO}_2$ samples were successfully melted and quenched to glass in space. In space, this is the first time that the clear glass was prepared without any crystallization.

In our space experiments, we tried to make the glass from sintered spherical raw materials in order to get the ultra-high purity glass. A few gas bubbles remained in the glass, but the amount was smaller than expected. The removal of bubbles from molten glass is one of the biggest problems of preparing glass in microgravity and this problem must be solved in fundamental space projects. Levitation techniques at high temperature must be improved in order to contribute to the development of new materials by containerless processing.

Experiment M-10:**Study on Solidification of Immiscible Alloy****Principal Investigator:**

Akihiko Kamio
Faculty of Engineering, Tokyo Institute of Technology

Coinvestigators:

Hiroyasu Tezuka
Faculty of Engineering, Tokyo Institute of Technology

Shinji Kumai
Precision and Intelligence Laboratory,
Tokyo Institute of Technology

Tsuneo Takahashi
Faculty of Engineering, Chiba Institute of Technology

Some alloys cannot mix on the Earth; just like oil and vinegar. The alloy, known as a monotectic alloy, generally consists of metallic components which are mutually immiscible and differ in specific gravity. The liquid alloy separates into two liquids in the hypermonotectic composition range above the solidification temperature. Therefore, sedimentation of the denser one (rich in the second component) takes place and that segregated alloy structure usually forms under gravity. In contrast, homogeneous structures exhibiting even dispersion of the second component particles in the matrix are expected in the alloy melted and solidified in space, where no gravitational effect operates. However, previous experiments in space have not shown this result.

The objective of the present study is to clarify the solidification mechanism of the monotectic alloy and to obtain fundamental information for solidification structure control. For this purpose, unidirectional solidification tests were performed in Al-In and Cu-Pb alloys of monotectic and hypermonotectic composition.

Flight records indicated that the experiment progressed as scheduled. In space, the samples were melted and subsequently, unidirectionally solidified using the GHF (Gradient Heating Furnace). When half of the sample solidified the residual melt was cooled rapidly in order to preserve the monotectic growth front morphology in the final solidification structure (interrupted quenching).

The monotectic Cu-Pb alloy solidified and interrupted quenching was successfully obtained. The microstructure exhibited alignment of irregular-shaped Pb rods in the Cu matrix. A lead condensed layer was observed ahead of the quenched monotectic growth front. General appearance of the monotectic solidification structure was similar to that grown at comparable growth conditions under gravity. The microstructure provides a lot of useful information concerning the effect of microgravity on the monotectic solidification mechanism. The effect of microgravity can be elucidated by means of further microstructural observation and quantitative measurement of the Pb phase distribution.

Effects of solidification conditions, such as growth rate and temperature gradient, should be examined in the future space experiments for further understanding of the solidification mechanism. Unfortunately, the sample cartridge was slightly damaged and the molten Al-In alloys leaked out of the sample container because of unknown reasons. This resulted in the lack of experimental data concerning the solidification structure of hypermonotectic composition. Therefore, additional tests are strongly required for Al-In alloys.

Experiment M-11: Fabrication of Ultra-Low Density, High Stiffness Carbon Fiber/Aluminum Alloy Composite

Principal Investigator: Tomoo Suzuki
Hokkaido University

Coinvestigators: Yoshinao Mishima
Seiji Miura
Tokyo Institute of Technology

The objective of the present experiment is to fabricate low-density, high stiffness carbon fiber/aluminum alloy composite to develop a new method of in-orbit fabrication of structural components in space. For this purpose, an aggregate of short carbon fibers coated with an aluminum alloy (Al-1 at%In) on the surface is encapsulated in a silica tube and is heated to above the melting point of the aluminum alloy. Six sample ampoules are prepared, heated at 700°C for ten minutes, and then cooled down to an ambient temperature using the Continuous Heating Furnace (CHF).

The aluminum alloy melts upon heating and acts as a bonding agent among short carbon fibers during cooling to consolidate the whole material. The composite thus made would be highly porous and, therefore, have a low density. High stiffness and resistance for buckling are expected from the structure having a large second moment of cross section and from the property of carbon fiber which is the major constituent. Such material would be suitable for structural components in space because the fabrication process, as an extrusion with heating at the gate of raw materials, is simple enough for in-orbit fabrication.

The experiment on the SL-J has been completed on schedule. By analyses on the flight data on temperature profiles of several different locations in the CHF, it turned out that the heat treatments on all the six samples were almost completely successful.

Analysis of the structure of the composite using a scanning electron microscope revealed that the bondings among fibers were generally achieved.

Consolidation of raw materials was successful and obtained a rigid composite material with densities below one tenth of that for aluminum. An unexpected observation was that there exist local coagulations of aluminum alloy. In the vicinity of such regions, it seems that surface of the short fibers is free from the aluminum alloy, and therefore the bondings among them are rarely observed. The reason for the occurrence of such local coagulations is unknown, but it could have been due to higher surface tension of the aluminum alloy melt than expected so that even a slight perturbation would have caused the phenomenon.

Mechanical properties of the composite were mainly examined by compression tests. It turned out that both elastic constant and compressive strength are, unfortunately, less than what were originally expected. It must be due to the presence of coagulated aluminum alloy, being distributed in the composites, which acts as a structural defect to substantially deteriorate the overall strength. The experiment on the SL-J has been, in many respects, successful and informative regarding the fabrication of such a composite material as we proposed. Establishing a method to avoid the local coagulation of aluminum alloy upon melting would be the next step toward developing further composite material of this kind with improved mechanical properties.

Table 1.**Summary of the flight data on the sample temperature during the heat treatment by the CHF**

| Sample No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|----------|----------|----------|----------|----------|----------|
| Max. Temp | 799°C | 785°C | 750°C | 763°C | 769°C | 765°C |
| Time to reach Max. Temp. | 00:04:03 | 00:03:13 | 00:03:33 | 00:03:20 | 00:03:36 | 00:03:36 |
| Holding Temp. | 718°C | 711°C | 700°C | 700°C | 706°C | 716°C |
| T for term 3 from melting point of Al | 58 | 51 | 40 | 40 | 46 | 56 |
| Isothermal holding time | 00:27:32 | 00:27:37 | 00:27:42 | 00:28:01 | 00:27:58 | 00:27:52 |
| Time held at above melting point of Al | 00:28:03 | 00:27:58 | 00:28:09 | 00:28:24 | 00:28:23 | 00:28:24 |

Experiment M-12:**Study on Liquid Phase Sintering****Principal Investigator:**

Shiro Kohara
The Science University of Tokyo

The objective of this experiment is to reveal the behavior of solid particles in a liquid matrix during liquid phase sintering.

Mixtures of tungsten and nickel powders with the compositions of 3.5, 7, 15, 20 and 30 mass% Ni were prepared and the mixtures were compacted into cylindrical specimens. Each specimen was inserted in an alumina receptacle, and five receptacles with different compositions were put in a boron nitride container. The container was enclosed in a tantalum capsule, evacuated, and filled with argon gas. The container was put in a tantalum cartridge. Two sets of cartridges were prepared for the spaceflight experiment.

The specimens were sintered in the Spacelab using a Large Isothermal Furnace (LIF).

Table 1. Conditions of sintering

| Experiment | Temperature | Time |
|-------------------|--------------------|-------------|
| M-12A | 1,500°C | 60 minutes |
| M-12B | 1,500°C | 300 minutes |

The schedule of the experiment was altered due to the trouble in Rack No. 10 during the space-flight. However, both experiments were finished as expected.

The specimens with 3.5 and 7% Ni kept the original cylindrical shape even after the sintering with liquid phase. However, the specimens with 15, 20 and 30% Ni changed to spheres after the sintering. The change in the shape of the specimens during liquid phase sintering depends on the quantity of liquid phase included in the specimens.

From the experimental results, it can be concluded that the mixture of liquid and small solid particles behaves like liquid under microgravity irrespective of density difference between constituent materials when a continuous liquid layer is formed at the surface. Further analyses of the data are still in progress.

Experiment M-13: Fabrication of Si-As-Te Semiconductor in Microgravity Environment

Principal Investigator: Yoshihiro Hamakawa
Osaka University

Coinvestigators: Hiroaki Okamoto
Kiminori Hattori
Chitose Sada
Osaka University

The objective of the flight experiment is to fabricate homogeneous, multicomponent, amorphous semiconductors in the microgravity environment in space, and to make a series of comparative characterizations of the amorphous structures, as well as their electronic properties for materials prepared in space and under terrestrial gravity environment.

Ternary chalcogenide Si-As-Te amorphous system involves various interests in view of both basic physics and technological applications. Since the Si-As-Te system consists of IV-III-II hedral bonding network, it has a wide glass forming region in which the physical constants can be controlled in appreciable ranges. For example, the electrical energy gap is designed from 0.7 eV to 1.8 eV by adjusting the atomic fraction x in $\text{Si}_x(\text{As}_2\text{Te}_{3-1-x})_{1-x}$ system. A systematic investigation on the compositional dependencies of physical constants offers an extended possibility to explore both the electronic and atomic properties of random network systems.

In the technological aspects, this material system could be applied to multi-layered, heterostructure devices and also optoelectronic functional elements in a wide spectral region from near-infrared to the visible light region. Moreover, due to the amorphous network, the material can be deposited on inexpensive substrates such as glass, ceramics and metals. These advantages meet with technological requirements for device fabrication processes in the future with good mass producibility and low cost.

Samples for the flight experiment were selected from the terrestrial experimental data to compositions $\text{Si}_x(\text{As}_2\text{Te}_{3-1-x})_{1-x}$ in which x is varied from 0.25 to 0.75. In order to examine the possibility of valency electron controllability, a trial is made on impurity doping with the Ni element of several atomic percent on the $\text{Si}_9\text{As}_{14}\text{Te}_{21}$ sample. The Si-As-Te samples already synthesized with Si, As and Te mixtures of designed atomic fractions (six kinds) are employed as the starting material of the experiments. They were grounded to 100 mesh, and sealed (total amount was one gram) three-fold with fused quartz ampoules, and encapsulated in the Ta universal cartridge for the CHF apparatus.

The material processing in the FMPT was carried out by using the CHF apparatus. All the scheduled material processings were completed safely and effectively during the allotted six hours; from 21:49 on the 13th to 03:45 on the 14th of September 1992. The processed samples, which reached us at the end of November 1992, have been subject to a series of systematic diagnoses. In contrast to the case of terrestrial processing in which the solidified Si-As-Te samples are formed as single ingots due to the presence of gravity, the FMPT specimens are separated into a few bulk ingots. This results from the trade-off between the saturation against fused quartz and the surface tension, as well as internal pressure during the quenching process.

The electrical and optical properties have been examined on both the FMPT and reference materials which were fabricated in the laboratory with atomic compositions and processing conditions identical to the FMPT materials. The control of material properties including optical energy gap is achieved equivalently for both the materials; however, some significant differences have been found between them in the quantitative aspects of material properties.

The FMPT materials exhibit wider optical band gaps with sharper absorption edge compared to terrestrial reference materials, clearly indicating that the FMPT materials involve structural disorder of a lesser degree. The reduced disorder implies longer carrier mean free path, which must have profound effects on transport property, that is, an enhancement of carrier mobility. Although any direct identification for these effects have not been achieved yet, a piece of evidence is provided from an increase in the conductivity prefactor which contains the carrier mobility.

The most striking effects of material processing in microgravity environment have been manifested in the valency controllability by Ni doping. The conductivity of $\text{Si}_9\text{As}_{14}\text{Te}_{21}$ increases by more than seven orders of magnitude, and the conduction type changes from p (undoped) to n type. So far, the valency controllability of such a large extent has never been achieved on a chalcogenide glass system. The result implies either a lower native defect density or a greater doping efficiency of doped Ni, while uniform distribution of Ni dopants would not be ruled out from the possible reasons.

All of the experimental results indicate the realization of homogeneous, amorphous network structure with a minimized disorder by the material processing in the microgravity environment. The material processing in space would contribute largely to the physics of disordered materials as well as to the development of new materials for semiconductor electronics.

Experiment M-19:**Solidification of Eutectic System Alloys in Space****Principal Investigator:**

Atsumi Ohno
Chiba Institute of Technology

Coinvestigators:

Tetsuichi Motegi
Chiba Institute of Technology

The objective of this experiment was to clarify the solidification mechanisms of the eutectic alloys under the microgravity conditions on board the Space Shuttle, Endeavour.

The authors have proposed the Crystal Separation Theory to explain the formation mechanism of the equiaxed crystals of isomorphous alloys and the equiaxed eutectic grains of eutectic system alloys. The primary crystals nucleate on the cooling site in the container, separate and precipitate forming the equiaxed zone. In eutectic system alloys, non-leading phase primary crystals do not have an effect on the formation of the eutectic grain zone, but leading phase primary crystals cause the formation of equiaxed eutectic grains. The thermal convection promotes the formation and separation of primary crystals from the cooling site in the container.

In general, no thermal convection occurs in the molten metal under the microgravity conditions. Therefore, the separation of primary crystals from the cooling site in the container cannot be expected to occur under the microgravity conditions.

To clarify the solidification phenomenon in the microgravity conditions, Al-Cu eutectic system alloys were melted and solidified on board. Six samples were used in space; four of them were the hypoeutectic Al-32.5%Cu alloy with and without the oxide film on their surface, and two were the hypereutectic Al-32.5%Cu alloy without oxide film. Oxide films were expected to prevent the Marangoni Convection under the microgravity conditions. The solidified structures of the pre-flight samples aligned straightly along the longitudinal direction. Consequently, it is very easy to understand the formation of the solidified structures. Each sample was contained in the graphite capsule and vacuum sealed in the double silica glass ampoule. They were heated at 700°C and held for five minutes. Next, they were cooled to 500°C in the furnace.

Experiments under microgravity were started as scheduled. After the flight, each sample was examined macro- and microscopically for their appearance and metallography. The silica glass ampoules and graphite capsules were not damaged. Each sample was contracted to the proportion of the last solidification. The diameter and length were a little bit larger before flight because of the melting and solidification. Hairline cracks on the oxide films appeared after the contractions.

Primary aluminum dendrite crystals in the Al-32.5%Cu alloy existed mainly near the container wall, but their distribution was independent of the longitudinal direction and oxide film on the sample surface. There was no difference between the samples with or without oxide film. The matrix of the hypoeutectic alloy showed columnar eutectic structures. On the other hand, few primary crystals, CuAl_2 , of Al-32.5%Cu alloy appeared near the container wall; however, no free crystal of CuAl_2 was observed. Small gas bubbles appeared near the capsule wall and large ones in the center of the sample. It is considered that the graphite capsule absorbed and then released the air. The air bubbles were difficult to move through the molten metal in space. On the ground, primary alpha aluminum dendrite and CuAl_2 crystals were located on the top or bottom of the samples

depending on the density difference of the liquid eutectic. Moreover, no bubbles appeared under these conditions.

Further study and more space experiments with larger samples will be necessary to ascertain the eutectic solidification under microgravity condition.

Experiment M-07:**Diffusion in Liquid State and Solidification of Binary System****Principal Investigator:**

Takehiro Dan
National Research Institute for Metals

Coinvestigators:

Yuji Muramatsu
Toshihiro Yamagata
Kennichi Hoshimoto
Takashi Kimura
National Research Institute for Metals

Under microgravity conditions, it is expected that the movement of liquid metal due to both density difference and thermal unevenness must be suppressed markedly. Such an environment must offer optimum conditions to liquid diffusion experiments. The objective of this research is to determine precise inter-diffusion coefficients in the liquid state of Au-Ag binary alloy system and to observe its solidification structure. The precise determination of diffusion coefficients is not only indispensable to the theoretical development on the diffusion mechanism and the structure of liquid metals, but also necessary for the optimization of almost all industrial manufacturing processes such as smelting, refining, welding, and soldering of metals.

The flight experiment was carried out well according to the predetermined schedule. After the flight specimens were observed on their outer shape and surface, they were cut and polished in order to investigate their solidified structure by means of optical and scanning electron microscopes and to determine the concentration distribution along the specimen axis by use of an electron probe microanalyser (EPMA).

It was apparent from these measurements that the diffusion in the flight specimens was faster than that in the ground ones, their concentration curves deviated from theoretical ones, and the reproducibility of their curves were not necessarily good. These results were ascribed to Marangoni convection flow, which became more dominant than the gravitational convection flow under the microgravity conditions.

It is indispensable that no free surface is formed on the specimens during the flight experiment. According to the strict safety guideline, specimens had to be enclosed with a three-fold container. As a result, the precise measurement of specimen temperature was very difficult. The compatibility of the flight safety with the experimental environment must be pursued.

The present research revealed some experimental difficulties which were peculiar to the microgravity environment. Nevertheless, the significance of it does not reduce in the least. It is expected that flight experiments will be undertaken without interruption.

Experiment M-14:**Gas Evaporation in Low Gravity****Principal Investigator:**

Nobuhiko Wada
Faculty of Science at Nagoya University

Coinvestigators:

Manabu Kato
Toshikazu Sato
Toshiaki Goto
Shinya Sawai
Masaya Sengoku
Masaaki Tani
Toshiaki Noda
Faculty of Science at Nagoya University

Minoru Dohi
Shizuoka Institute of Science and Technology

Gas evaporation is a technique for producing ultra-fine particles by evaporating material in a gas atmosphere[1] that has been used in various fields of science and industry. But the evaporation process of materials in a gas atmosphere has been investigated very little in contrast to that of evaporation in a vacuum. This is mainly due to the fact that every evaporation process in a gravity field is accompanied by the convection of gas—making analysis of the process difficult.

Fonda[2] has proposed an empirical evaporation formula of a tungsten filament in an incandescent lamp bulb, where he assumed a stagnation layer of flow on the evaporating surface (Langmir sheath) and that the evaporation rate is to be controlled by the diffusion velocity in this layer. This concept was originated by Langmuir and has been used for analysis of those kinds of problems up to the present [3,4]. The thickness of these layers are not deducible theoretically but they are estimated to be in the range of millimeters from the actual evaporation rate on the ground. For the gas evaporation in the low gravity, the thickness of the stagnation layer is to be as large as the distance from the evaporation source to the inner surface of the vessel. It is of the order of ten centimeters which is 102 times as thick as the one for the ground experiment. It means the evaporation rate will be suppressed too much to deposit smoke of fine particles in the low gravity, especially evaporation at a higher density of gas; the real situation will be known by the low gravity experiment.

Four experimental glass bulbs with a filament on the tip of which 50 mg of Ag is attached, are filled with Ar gas of 50 torr, 300 torr, and Xe gas of 5 torr. The filaments are lighted one by one in the low gravity field in space. The images of growing smoke are recorded on the VTR and brought back to the ground with the used experimental bulbs to the laboratory. The bulbs are cut and then deposited particles are examined by electron microscope.

The four bulbs worked and the heating temperature maintained the constant value of 1,110~1,150°C, which temperatures were too low to produce smoke on the ground. The heating temperature of bulb D was also kept at the same value for four seconds at the beginning of evaporation, but it rose up over 1,200°C and the filament was cut out in two minutes. The recorded images of smoke are very dark for bulb A and no smoke is observed for bulbs B, C, and D. Smoke balls around the filament appeared and the brightness increased in turn for B,C, and D. In the case of bulb D, a few branches of smoke gushed out in various directions at 1,110°C. It is the smoke burst that had been expected in this experiment. Fine particles of uniform size from 20–50 nm were deposited on the inside wall of the bulb

A, B, and C, but in the bulb D the particles of Ag and W were widely distributed in size from 20 nm to 10 μ m with clear habit.

The saturate vapor pressure of Ag at the evaporation is $\sim 10^{-3}$ Pa, while that of atmospheric Xe gas is 2 Pa. The burst of smokes may have proved the possibility of local accumulation of vapor atoms with pressure higher than that of the surrounding gas. It can not be interpreted in terms of a conventional diffusion model in the Langmire sheath. The phenomenon is a special one that appears only in the low gravity field and shows a possibility to analyze the fundamental molecular process of the gas reaction system, which has been difficult to carry out on the ground experiments.

The possibility of the local accumulation of high temperature material vapor in a gas system suggests the realization of nuclear fusion with high density fuel gas in space. It is also expected for new application to the material vapor processing with high quality and efficiency.

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Experiment M-15:**Drop Dynamics in an Acoustic Resonant Chamber and Interference with the Acoustic Field****Principal Investigator:**

Tatsuo Yamanaka
National Aerospace Laboratory

Coinvestigators:

Heihachiro Kamimura
National Aerospace Laboratory

Acoustic levitation technology, in which the molten material can be held in a position without contact against the walls of the crucible and be manipulated without a stirring apparatus, is useful for space materials processing. Acoustic standing waves excited in a resonant chamber, develop an acoustic radiation pressure gradient toward the loops of the velocity wave. Consequently, liquids and particles in the chamber are driven from the walls to the loops, collecting there and remaining until the excitation ceases. If the phase of the acoustic drivers between a couple of axes exists, a rotational torque is generated around the other axis, which could be used as acoustic stirrers. The authors have studied theoretical behaviors of the molten material drop in a tri-axial acoustic resonant chamber, ground based experiments, and aircraft ballistic flight experiments to develop a space experimental apparatus, which are compared with the STS experimental results.

In the theoretical studies, analytical solutions of perturbed surface tension waves are obtained for a rotating and a non-rotating liquid sphere in the loops of a tri-axial acoustic resonant chamber. When the strength of an acoustic wave is increased along one axis, the levitated liquid drop is deformed due to the axial acoustic radiation pressure. Assuming an oblate spheroid for the deformed liquid drop, we obtain an approximate solution to describe the oblateness with respect to the acoustic strength. A solitary non-linear wave equation is derived to predict a free liquid membrane, which is an ultimate shape of the deformed liquid drop due to a much stronger acoustic resonant wave.

In order to investigate the characteristics of a resonant chamber, as well as to compare the theoretical solutions with the experimental results, the authors developed a tri-axial acoustic resonant chamber. Experimental studies of stabilization and rotation of a styrofoam sphere were carried out by using the resonant chamber. Deformation of a small liquid drop due to the acoustic resonant wave was also measured, while attached to the injection syringe, in the chamber.

One of the major issues is the separation of a measured size of liquid drop for the successful space experiment. Such preparatory experiments cannot be performed in the ground based laboratory due to the Earth gravity. By using low gravity environments during ballistic flights of an aircraft (Mu-300), the authors investigated the following parameters: changes of resonant conditions between, before, and after the formation of a liquid drop in the center of the resonant chamber; the relevant drawing speed of the syringe from the resonant fields after formation of the drop; and the relevant acoustic strengths to conquer the sticking force to the syringe during separation.

In the space experiment [M-15], formation of a liquid drop and separation of the syringe were planned to be performed by manual operation of a Payload Specialist. Two events had caused the first drop to burst while attached to the syringe; (i) the timing of the separation was delayed a few seconds; and (ii) during the delay, the strengths of a couple of resonant acoustic waves increased higher than the preliminarily planned values. Some pieces of the

broken liquid adhered to an acoustic wave guide in the resonant chamber. Because the [M-15] did not provide a sweeping mechanism to eliminate such trouble, the space experiment did not perform the planned programs. During the first separation of a liquid drop from the syringe, however, images of the deformed drop were obtained, which were due to the strong acoustic resonant wave of the axis parallel to the movement of the syringe. The size of the liquid drop was fortunately large enough to allow a plot of the data which could not be obtained by the ground based experiment.

Experiment M-16:**Bubble Behavior in Thermal Gradient and Stationary Acoustic Wave****Principal Investigator:**

Hisao Azuma
National Aerospace Laboratory

Coinvestigators:

Shoichi Yoshihara
Sachio Ogihara
Mituru Ohnishi
National Aerospace Laboratory

In a microgravity environment, bubbles and droplets move not because of buoyancy as on the ground, but due to other factors. The most important one among these factors is thermal gradient which is always involved in material processing. Observation of bubble behavior in microgravity allows us to get data on bubble movement which cannot be obtained on the ground. A knowledge from these observations could be of use, for example, in the understanding of droplet behavior in the processing of particles dispersed alloy. Stationary acoustic wave is a strong means to move or remove bubbles positively. The objective of this experiment is to observe bubble behavior in a thermal gradient using stationary acoustic wave and to get data on migration velocity of bubbles and interaction between bubbles.

The experiment, which consists of three sub-experiments, was conducted as scheduled. From one sub-experiment, C, we obtained much data on bubble behavior in a thermal gradient.

In the sub-experiment C, an unexpected phenomena was observed: bubbles put in the silicon oil of a container moved towards the center of the container without a thermal gradient. This movement is inferred to be caused by electric charges on a bubble generated by forming the bubble through a syringe.

After injecting bubbles, a thermal gradient was applied and increased. During this process, bubble migration and interaction between the bubbles were observed. Due to a secondary flow caused by a thermocapillary flow around a bubble attached to the hot wall, bubbles far from the bubble attached to the hot wall were attracted to the top of the bubble and made a line along the thermal gradient. Moving velocities of bubbles due to a thermal gradient were measured and they were put in order by Marangoni number (a ratio of a force by surface tension to a force by viscosity).

From this we knew two features about the velocities. The first feature is the measured velocities of the bubbles were much smaller, theoretically and numerically, than that predicted for a bubble. This indicates the fact that when more than two bubbles move, the velocity becomes much smaller than in the case of a single bubble. The second feature is the measured bubble velocities near the hot wall exceeded the theoretically predicted values. This can be explained by the interaction mentioned above, i.e., bubbles are attracted by a bubble attached to the hot wall. Thus, the behavior of multi-bubbles in the container subject to a thermal gradient was made clear and the moving velocities of bubbles with large Marangoni number(s), where little data existed, were measured.

Experiment M-18:**Marangoni Effect Induced Convection in
Material Processing under Microgravity****Principal Investigator:**

Shintaro Enya
Ishikawajima-Harima Heavy Industries Co., Ltd.

Coinvestigators:

Keiichi Kuwahara
Hiroyuki Uchida
Jun-ichi Ochiai
Ryo Akiyoshi
Akiko Ohara
Ishikawajima-Harima Heavy Industries Co., Ltd.

For material processing in space using microgravity conditions, it is possible that the convection induced by surface tension difference (called Marangoni convection) occurs and strongly affects the quality of a grown crystal. Under microgravity, a visualization experiment has been conducted in order to study the characteristics of the convection in directional crystal growth (crystal is encapsulated in an ampoule and grown for axis-direction, which is called Bridgman crystal growth). By combining a numerical simulation with the experimental results, prediction of the flow field, the heat, and mass transfer will become easy. In addition, if the correlation between these phenomena and the crystal growth will become simpler. Furthermore, if the correlation between these phenomena and the crystal growth becomes clear, it is expected that the high quality crystals can be obtained by controlling these phenomena.

Our experimental model simulates a possible configuration of the melt state in Bridgman crystal growth. That is, the melt is not in contact with an ampoule and a cylindrical free surface is formed in uniform width at the grown crystal side. We used transparent paraffin as the sample liquid for flow visualization. Under microgravity, temperature gradients formed on the free surface after sample melting. Image data of the motion of tracer particles were recorded on video tapes.

Main objectives of this experiment are summarized:

- Stable liquid column formation with free surface
- Flow visualization of free surface and cross section of liquid column
- Observations of Marangoni convection and solidification phenomenon, which were carried out sequentially

Main results are as follows:

- Under microgravity, the liquid column (25 mm in diameter, 24 mm in length) with the free surface (6.5 mm in length) was formed and maintained stability.
- Visualization images (tracer particles and liquid column shape) were obtained. However, the clearness of the image was not very satisfactory.
- Marangoni convection did not occur in spite of the temperature gradient generation on the free surface and the suppression of buoyancy convection under microgravity. The sample, which was solidified under microgravity, was taken out from the experimental apparatus to analyze the change of composition or contamination. The examination results show high possibility of contamination by surfactant, including the free surface.
- Under no-flow condition, it was observed, because of the floating of a solid part of the sample, that the solidification front advanced—keeping the surface flat—and the melting speed became slower than on ground.

In the present experiment, Marangoni convection did not occur. Therefore, further experiments that must be done after procedures to prevent contamination are in place and improvements were made to the optical system and camera.

Experiment M-01:**Growth Experiment of Narrow Band-gap Semiconductor PbSnTe Single Crystal in Space****Principal Investigator:**

Tomoaki Yamada
NTT Basic Research Laboratories

Coinvestigator:

Kyoichi Kinoshita
NTT Basic Research Laboratories

$Pb_{1-x}Sn_xTe$ is a promising material for infrared laser diodes and photo detectors operating at wavelengths between 6-30 μ m. The electrical properties of $Pb_{1-x}Sn_xTe$ not only depend on the Pb/Sn ratio, but are also seriously affected by crystalline defects and impurities. Homogeneous, low-defect density, high-quality crystals are therefore required. In the gravitational field, however, thermal convection stirs the $Pb_{1-x}Sn_xTe$ melt contained in a crucible, causing unsteady crystal growth, and resulting in a heterogeneous crystal with a high defect density. The flight experiment attempts to produce a constant Pb/Sn ratio along the growth axis and a low dislocation density in the crystal by avoiding thermal convection in microgravity. Such high-quality crystals will be used for high-yield fabrication of high performance laser diodes and detectors.

$Pb_{1-x}Sn_xTe$ crystals were grown in a gradient heating furnace (GHF). By heating the furnace to about 1,000°C, $Pb_{1-x}Sn_xTe$ melt was formed in a boron nitride (BN) crucible doubly sealed by quartz ampoules and covered by a Ta cartridge. Part of a seed crystal remained unmelted. The melt was then solidified unidirectionally, from the seed crystal toward the other end of the melt. The temperature gradient at the solid-liquid interface was higher than 40°C/cm and the growth rate was set to be 5.5 mm/hr. Controllability of the growth rate by the movement of the heating elements of the furnace is a unique point of the present GHF.

In addition to a cylindrical crystal, 15 mm in diameter and 58 mm in length, 20-30 spherical crystals ranging from 0.5-11 mm in diameter were also obtained unintentionally. The overall cylindrical shape was the result of a graphite spring in the crucible pushing the melted material toward the seed, thus eliminating uncontrolled surface areas. This suppressed Marangoni convection, which develops on a free melt surface because of the surface tension difference in the melt.

The $Pb_{1-x}Sn_xTe$ melt seems to have escaped from the melt reservoir to the graphite spring enclosure through a small gap (about 0.1 mm) between the BN crucible and the BN plunger. The escaped melt then formed spherical melt drops in a hollow of the spring because of the surface tension of the melt and then solidified into spherical crystals. The graphite spring apparently facilitated spherical crystal growth by suspending melt drops and supplying open space and nucleation sites. The crystals reported here are the first spherical semiconductor crystals ever formed.

The cylindrical crystal grew nearly in the same direction as the seed crystal, but it contained many subgrains at the interface between the seed and at subgrain boundaries. It should be noted that the initial solid-liquid interface was shifted by about ten millimeters toward the higher temperature zone compared with terrestrial growth under similar heating conditions and the seed crystal (28 mm in length) was barely melted. The reason presented is that heat transportation by thermal convection is suppressed under microgravity. The short melt length of the seed and short soaking period may be the causes of the subgrains. In addition, the space-grown crystal had several large voids inside. Formation of these large voids might have been caused by Te vapor bubbles.

The void-free portion of the space-grown cylindrical crystal, however, shows improved compositional homogeneity, macroscopically and microscopically. A constant SnTe mole fraction of about 0.16 (i.e. a constant Pb/Sn ratio) is achieved from about 33–43 mm in the distance of about ten millimeters. A constant SnTe mole fraction is unattainable in terrestrial growth. The etch pit density (EPD) in the space-grown crystal is about one-tenth of a terrestrially grown crystal. The EPD of the seed crystal is between 2×10^6 and $3.5 \times 10^6 \text{ cm}^{-2}$ (average $3 \times 10^6 \text{ cm}^{-2}$), whereas that of the space-grown crystal is between 1×10^5 and $9.5 \times 10^5 \text{ cm}^{-2}$ (average $5 \times 10^5 \text{ cm}^{-2}$). The space-grown crystal also shows improved electrical properties: its intrinsic carrier density is lower and mobility is three times that of terrestrially grown crystals. The mobilities of the space-grown crystal are $1580 \text{ cm}^2/\text{Vs}$ at 77K and $2620 \text{ cm}^2/\text{Vs}$ at 4.2K, respectively. These prove effects of suppression of thermal convection under microgravity.

Spherical crystals might grow during the cooling process after directional solidification. It seems that the growth rate was too rapid, causing many Sn-rich inclusions in the largest crystal. Some small crystals, however, show low dislocation densities, on the order of $10^4/\text{cm}^2$, two orders smaller than the dislocation density of terrestrially grown crystals. The reduction of crystalline defects in such crystals might be due to the absence of mechanical stress, since the crystals do not touch the crucible wall.

The differences between space-grown and terrestrially-grown crystals were clarified in a $\text{Pb}_{1-x}\text{Sn}_x\text{Te}$ crystal growth experiment under microgravity, and valuable information on the improvement of crystal quality was obtained. Further investigation of the mechanism of spherical crystal growth is expected to lead to the growth of even larger and higher quality crystals.

Experiment M-02:**Growth of PbSnTe Single Crystal by Traveling Zone Method****Principal Investigator:**

Sohachi Iwai
The Institute of Physical and Chemical Research, RIKEN

Coinvestigators:

Yusaburo Segawa
The Institute of Physical and Chemical Research, RIKEN

The floating zone method is useful for growing a single crystal without impurity incorporation and crystal defect formation, because a molten zone is held by its own surface tension between two collinear solid rods without any container. On the Earth, this method has been practically applied to only a few materials with relatively large surface tension, e.g., silicone.

The objective of this experiment is to indicate the possibility to grow a PbSnTe single crystal, which is a promising material for opto-electronic devices in the infrared wavelength region. On the Earth, it is difficult to hold a molten zone with a diameter more than three millimeters. Under microgravity condition free from specific gravity, a large single crystal is expected to be grown by the floating zone method.

The vapor pressure of Te at the melting point of PbSnTe is around 1 atm. It was difficult to grow by the floating zone method using an image furnace (IMF), because of Te contamination on a quartz tube and mirrors in IMF. Therefore, the PbSnTe sample was enclosed in a quartz ampoule and the molten zone contacts the quartz wall of the ampoule during the crystal growth. Thus, the crystal was grown by a traveling zone method in a quartz tube.

A PbSnTe crystal of ten millimeters in diameter, which was grown by the Bridgman method, was used in the flight experiment. Because the vapor pressure of Te at the melting point of PbSnTe is approximately 1 atm, the source material of a PbSnTe crystal was enclosed in a quartz ampoule in a vacuum and was connected to a stainless steel holder with ceramic cement. The sample was set on a shaft in the IMF and was rotated at the rate of 3 rpm. A molten zone was formed in the center of the rod by focused light from two halogen lamps in the IMF. The molten zone was moved from a seed crystal to a feed crystal at the rate of 2 mm/hr. and a single crystal was grown on the seed crystal during the zone traveling for four hours.

In the molten zone, many bubbles of Te vapor appeared on the surface of the molten zone. During the experiment on the Earth, these bubbles moved upwards in the molten zone and a large void was formed in the upper part of the molten zone. During zone traveling, the molten zone became unstable and finally separated from the feed crystal. Therefore, it was difficult to grow a large single crystal even by traveling zone method.

In the flight experiment, many small bubbles were also observed. But these bubbles were distributed uniformly in the whole region of the molten zone because thermal convection did not occur. With the rotation of the molten zone at the rate of 3 rpm, the boundary between the molten zone and the solid could be observed more clearly than in the ground experiment. The molten zone length could be determined from the length of the bubble zone. After the flight experiment, the crystal rod was cut along the growing axis and the surface was polished. The shape of the molten zone was observed by chemical etching. The molten zone length was 16 mm, which was almost equal to the length obtained from the bubble zone. Inside the polycrystal of the molten zone, two voids with ball-like shape remained. But no void remained in the grown single crystal.

A single crystal with 7.5 mm length was obtained on the seed crystal by zone traveling for four hours. The composition of Sn in the grown crystal was measured by an x-ray micro-analyzer. At the beginning of the growth, Sn composition is 0.65 times as low as that of the seed crystal due to the segregation. With the distance from the seed crystal, Sn composition increased. Although the length of the grown crystal was small in comparison with the molten zone length, the measured Sn composition became almost constant in the grown region above six millimeters. This is in contrast to the result obtained from the ground experiment or the calculated value for the zone length of 16 mm.

Electric property was measured by van der Paw method. Carrier concentration in the crystal grown in space was about half of that in the crystal grown on the Earth. The carrier mobility was higher than that of the grown crystal on the Earth. These results indicate that the crystal grown in space has high quality in comparison with the crystal grown by the same method on the Earth. This high quality is considered to be due to weak contact of the molten zone with the quartz wall under microgravity.

The molten zone is stable during the zone traveling under microgravity for many hours. The result of the flight experiment indicates that the single crystal with large specific gravity can be grown under microgravity. Because the molten zone is stable for many hours, a large single crystal with uniform composition can be obtained by using the zone melting method. If the floating zone method is used, it is necessary to use a heating system regardless of Te contamination and to control the Te vapor pressure in order to get a high quality crystal.

Experiment M-03: Growth of Semiconducting Compound SingleCrystal InSb by Floating Zone Method

Principal Investigator: Isao Nakatani
National Research Institute for Metals

Coinvestigators: Satoshi Takahashi
Kiyoshi Ozawa
Isao A. Nishida
National Research Institute for Metals

Under microgravity, the floating zone method has great advantages for growing large-diameter single crystals and for growing high-quality single crystals. Previously, floating zone experiments have only been performed for Si single crystal and for Ge single crystal with respective diameters of ten and five millimeters. In this experiment, large-diameter single crystal of InSb was grown by the floating zone method using the image furnace aboard Spacelab J on STS-47. This was the first experiment that aimed at growing a single crystal of the compound semiconductor by the floating zone method.

The seed crystal was InSb single crystal rod with [111] orientation with 20 mm in diameter, and the rod stock was zone-refined polycrystalline InSb of the same size. Both the ends, separated with small gap, were heated by halogen lamps in the image furnace in the atmospheric pressure of Ar gas at the flow rate of 1,800 cc/minute. The electric power of the halogen lamps was carefully controlled by manual operations. Highly stable floating liquid zone of 45 mm in length and of 20 mm in diameter was formed, and traveled at the rate of 0.33 mm/minute.

The crystal of InSb processed in space has the size of 100 mm in length and 20–30 mm in diameter. The surface of the sample was chemically analyzed by x-ray probe microanalysis. The sample was cut into longitudinal sections with (211) surface orientation, polished and etched. Crystallographic characterization was made by an x-ray diffraction topography and optical microscope observations on the surface of the sections of the sample. Resistivity distribution were measured by 4-point probe method.

The surface of the molten zone was lightly contaminated by the oxide's thin film at the growth process in space. The floating liquid column was not sustained by its surface tension, but was sustained by the skin of the thin film of the oxide. The solidification process proceeded being confined in the skin over the surface. Some nucleations and some growth twins are generated from the oxide film on the surface. It appears from the x-ray topography that the crystal has high quality with low dislocation densities. Characteristic growth-striation patterns that arise from the convection flows were not observed on the x-ray topographies. The values of the electric resistivity rise up from 4×10^{-5} to 8×10^{-5} ohm-m along the growth axis, which indicates that an effective purification process proceeds in space. The thin film of oxide serves the growing system as a flexible container without stress that tends to cause crystal imperfections, e.g., dislocations, stacking faults, etc. Moreover, the oxide film is likely to suppress the Marangoni convection flow over the molten surface.

Experiment M-09:**Growth of Spherical Silicon Crystals and the Surface Oxidation****Principal Investigator:**

T. Nishinaga
University of Tokyo, Faculty of Engineering

Coinvestigators:

T. Sugano
Toyo University, Faculty of Engineering

O. Saitoh
Toyohashi University of Technology

T. Katoda
K. Asada
M. Kishi
University of Tokyo, Faculty of Engineering

Single Si crystals have been used for the fabrication of electronic devices such as integrated circuits, diodes, transistors, solar batteries, and so on. For the practical use of Si crystals, one should add some special impurity to get a certain conductivity type. Manufacturing devices with equal properties requires a uniform impurity distribution. However, when the crystal is grown on the ground from its melt, the crystal contains impurities with non-uniform distribution due to the presence of unsteady thermal convection.

The first experiment to grow Si in space was carried out by a German group in Spacelab 1 in 1983. When they investigated the impurity distribution in the space-grown crystals, they found strong non-uniformity, so called impurity striation, existing in the crystal. The presence of the impurity striation suggests that strong unsteady Marangoni convection existed in the melt under microgravity.

One of the reasons for the existence of such a strong Marangoni flow is the presence of strong temperature non-uniformity in the furnace. To improve the temperature non-uniformity, we employed a resistance furnace. Our objective was to grow Si from the melt under microgravity with much better temperature uniformity, thereby eliminating the unsteady Marangoni flow, as well as the unsteady thermal convection.

This experiment was composed of two parts. In the first part, a spherical single crystal of Si was used as a starting material. This crystal was melted in a furnace with almost uniform temperature distribution. The melting began from outside of the sphere and, after a certain time, growth was started by cooling the sample from the central crystal core kept unmelted. In the second part, the starting material was a Si rod of single crystal. The rod was heated in a temperature gradient to form a molten sphere at the high temperature end of the rod. The whole temperature was then decreased to start the growth from melt-solid interface using the unmelted rod as a seed crystal.

Heating and cooling processes for both samples were completed as programmed. After the furnaces were brought back to Japan, both samples were taken out of the cartridges which had been fixed in each furnace.

It was found that there was a loss of Si from the spherical melt in the first part of the experiment due to the eutectic reaction between the Si melt and Ta cartridge through a small hole in quartz crucible. The shape of the regrown crystal was a hemisphere and on

the surface of spherical part several facets were observed. This means that the growth occurred successfully outwards from spherical seed in the melt.

In the melting process of the Si rod, a molten sphere with a 19 mm diameter was first formed at the tip of the Si rod. However, this molten sphere moved from the tip to the side of the rod and the sphere touched with the quartz tube in the Ta cartridge. Due to this contact, the grown crystal was broken in pieces during the cooling period. However, it was possible to reproduce the original shape of the grown crystal near the unmelted rod. From this reproduced crystal, it turned out that growth was successful from the unmelted rod; however, the growth direction was perpendicular to its axis.

The hemispherical crystal grown in space was cut and polished. Then, the surface was chemically etched to see any impurity striation. Due to the loss of the Si melt, the grown part was not large. However, there were some parts where the growth did occur in space. The maximum thickness of the grown part was 1.2 mm. The layer grown in space contained no striations; while in the part grown on ground, there were many striations. In this experiment, this suggests that unsteady flow caused by both thermal and Marangoni convection has been suppressed. Hence, it can be concluded that by keeping the temperature uniform in the growth chamber, one can eliminate all impurity striations.

Experiment M-22:**Crystal Growth of Compound Semiconductor in a Low Gravity Environment (InGaAs)****Principal Investigator:**

Masani Tatsumi
Sumitomo Electric Industries, Ltd.

Coinvestigators:

Tsuguru Shirakawa
Shigeo Murai
Takashi Araki
Shinsuke Fujiwara
Sumitomo Electric Industries, Ltd.

The objective of our experiment was to grow a crystal of $\text{In}_{1-x}\text{Ga}_x\text{As}$ ($x=0.03$) having a homogeneous composition by the Bridgman method. That is use the crystal growth technique to solidify the melt in a crucible directionally from the edge of the melt in microgravity where thermal convection would not occur. Since ternary compound semiconductors, such as InGaAs could arbitrarily have a lattice parameter, which is spacing between atoms, by selecting the composition of the crystal, it is promising for the substrate of high speed transistors or laser diodes.

A sample was composed of $\text{In}_{1-x}\text{Ga}_x\text{As}$ ($x=0.03$) polycrystal measuring 12 mm in diameter and 26 mm in length with an InAs seed single crystal and charged in a crucible made of boron nitride. The sample was doubly sealed in two quartz tubes, which were sealed in a tantalum cartridge. We have equipped a plunger in the ampoule to press the melt during crystal growth in order to suppress the generation of a free surface on the melt. The free surface would generate the Marangoni convection, which is melt convection induced by surface tension. A crystal was grown at a rate of 4 mm/hr. using a gradient heating furnace (GHF) in microgravity. We also grew a crystal on Earth for reference. The experimental procedures in microgravity and on Earth advanced without any trouble.

The grown crystal in microgravity has a cylindrical shape measuring 12 mm in diameter and 23 mm in length. We have measured the longitudinal Ga concentration profile of the crystal and determined a segregation coefficient, which suggests a degree of homogeneity. A segregation coefficient closer to unity means that a concentration profile is more homogeneous. The segregation coefficient for the crystal grown in microgravity is 2.6. This value is closer to unity than the value of 3.2 for a reference crystal. This reduction of the segregation coefficient was achieved by suppressing thermal convection by means of carrying out crystal growth in microgravity and by suppressing Marangoni convection by means of preventing the generation of a free surface on the melt during crystal growth.

The result that the segregation coefficient is larger than unity means that weak melt convection was induced by residual gravity. Therefore, crystal growth in more reduced gravity environments is necessary to obtain a crystal having a completely homogeneous concentration profile.

Experiment M-20:**Growth of Samarskite Crystal under Microgravity Conditions****Principal Investigator:**

Shunji Takekawa
National Institute for Research in Inorganic Materials

Coinvestigators:

Isamu Shindo
ASGAL Co., Ltd.

Yoshinori Sugitani
Kanagawa University

The boule grown under microgravity conditions (Boule S) was 2.7 mm in diameter and eight mm in length. Boule S was composed of five phases: $(\text{Fe,Ca,Y})\text{Nb}_2\text{O}_6$, $(\text{Y,Ca,U})\text{NbO}_4$, $\text{Fe}_3\text{Nb}_2\text{O}_{16}$, Nb_2O_5 and $(\text{Y,Ca,Fe,U})_{1-x}\text{NbO}_3$; $x=0.04 - 0.1$. These phases were identical with those found in the boule grown on the Earth (Boule E). The distribution and the size of each phase in Boule S was different from those in Boule E. Nb_2O_5 grains were found everywhere in Boule E. In Boule S, Nb_2O_5 crystal lines were found only in the solidified molten zone, not in the boule. During the growth, the solidified body and the solution of the molten zone are well separated under microgravity conditions, but separation is poor on the Earth. In spite of this fact, samarskite with the identical composition of the feed rod was not obtained. It is necessary to examine the growth rate and the period of the single crystal growth.

On the Earth, during growth, there was a trapped liquid phase as an inclusion on the top of boule. These characteristics mean the sluggish diffusion of a solute from solution and a single crystal cannot be grown. The part disappears or is very small under microgravity conditions. This is consistent with the fact, that Nb_2O_5 crystal lines were precipitated only at the solidified molten zone in the case of the growth under microgravity conditions.

Due to the gravity on Earth, a high temperature solution of a molten zone migrates to a grown crystal. Accordingly, it is difficult to keep the volume of a molten zone constant on the Earth. On the other hand, under microgravity conditions, the volume of the molten zone can be easily kept constant and the boule with a uniform diameter was obtained. To keep a molten zone volume constant is essential for the growth of single crystals by the TSFZ method.

A large bubble was observed in Boule S. Its diameter was larger than a half of the diameter of Boule S. On the Earth, due to buoyancy, bubbles gather at the upper part of the molten zone. Accordingly, bubbles are seldom trapped in the crystals grown by the floating zone method. While under microgravity, bubbles are supplied with a flow in a molten zone and become large. Further study on the behavior of bubbles is necessary for growing single crystals of higher quality.

Experiment M-21:**Crystal-Growth Experiment on Organic Metals in Low Gravity****Principal Investigator:**

Hiroyuki Anzai
Electrotechnical Laboratory-Himeji Institute Of Technology

Organic metals have various interesting characteristics such as electrical anisotropies and metal-insulator phase transitions. In order to explore their intrinsic physical properties, large and good quality single crystals of organic metals must be obtained. The purpose of this work is to grow large, good quality single crystals of organic metals by an ideal diffusion method—without thermal fluctuation due to thermal convection and gravitational precipitation—for evaluation of those physical properties.

Tetramethyltetrathiafulvalene-Tetracyanoquinodimethane (TMTTF-TCNQ) composed of TMTTF as donor and TCNQ as acceptor was selected as the model of organic metal because the donor and acceptor are relatively more stable than others in the atmosphere. The two cells of “big” and “mini” sizes were used for the crystal growth. The crystal for evaluation of the physical properties was grown with the big size-cell, and the process of growing crystal was recorded by camera with the mini size-cell. Both cells are composed of three chambers (A, B, C) connected through cocks. The experimental flight period was prolonged for a day longer than the scheduled plan, and therefore, it was expected to obtain a bigger crystal.

Unfortunately, progress of crystal growth in the mini size-cell was not recorded on the film taken with the camera. The color of the solution in each chamber was the same or paler than the solutions put into each chamber on the Earth. Therefore, the seed crystals introduced into chamber C disappeared completely.

Also, there were no crystals obtained from the big size-cell. The color of the solution in each chamber strongly resembles each one of the solutions put into each chamber on the Earth. Seed crystals introduced into chamber C underground were confirmed.

Comparisons of the spectra of the solutions of the mini size cell with solutions of TMTTF, TCNQ and TMTTF-TCNQ suggest that TMTTF+ and TCNQ- probably decomposed with light for recording the crystal growth, and the seed crystals dissolved by decreasing the concentration of the solution, and finally disappeared. From comparisons with each spectrum of the solutions in each chamber of the big size-cell and noting no change of the shape of the seed crystals, I cannot help but conclude from analysis of the solution, that the cock was not opened under microgravity.

LIFE SCIENCES

Experiment PCG: **Protein Crystal Growth Experiments on Spacelab J**

Principal Investigator: Charles E. Bugg
University of Alabama at Birmingham

Coinvestigators: Christian Betzel
Shigeo Aibara
Wolfgang Weber
Daniel Yang

Protein crystal growth experiments aboard Spacelab J (SL-J) involved six proteins in a total of 60 vapor diffusion experiments carried out in one Refrigerator/Incubator Module (R/IM), at 22°C.

Two of the proteins, Factor D and peroxides, yielded minimal results producing no crystals or crystals too small for x-ray diffraction studies. The remaining four compounds, Trp-RS-tRNA, mouse monoclonal antibody, Lysozyme and epidermal growth factor receptor all yielded well-formed single crystals.

Trp-RS-tRNA produced many rectangular single crystals. Unfortunately, the crystals were too small for x-ray diffraction data collection, but they were clearly superior to Earth-grown crystals which adhere to each other during growth and become twined. Co-investigator, Dr. Daniel Yang feels that microgravity conditions combined with seeding may yield larger crystals.

Many well-formed crystals of mouse monoclonal antibody against dog lymphoma were grown on STS-47. However, these crystals were too small to produce usable x-ray diffraction data.

The largest crystal of epidermal growth factor receptor produced aboard SL-J was 0.6 x 0.43 x 0.2 mm, and it diffracted significantly better than any previous crystal. X-ray diffraction data was collected to 6 Å, which was 2–4 Å better than data collected from the best Earth-grown crystals. These data allowed, for the first time, the evaluation of the correct space group for the protein. The quality of the diffraction data collected from the space crystals indicates that the internal order of the crystal is significantly improved over the laboratory-grown crystals. Although the study of the structure of the epidermal growth factor receptor is in the early stages because Earth-grown crystals diffract poorly, the co-investigators, Dr. Christian Betzel and Dr. Wolfgang Weber, are confident that space-grown crystals will contribute greatly to the determination of this structure.

Lysozyme produced a few well-formed monoclinic crystals aboard SL-J. These crystals have different cell parameters than the Earth-grown crystals. Analysis of the x-ray diffraction data from these crystals is contributing to coinvestigator Dr. Shigeo Aibara's study of mechanism of protein nucleation both in micro gravity and in one gram environments.

In general, many diffraction-quality crystals were grown on SL-J. We believe that analysis of the data collected from these crystals will contribute significantly to determining the structures of these proteins and to understanding the mechanism of protein crystal growth.

Publications Citing Results of Protein Crystal Growth Experiments Aboard STS-47

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Weber, W., Betzel, C., Moore, K.M., DeLucas, L.D., and Bugg, C.E. Crystallization of the EGF Receptor Ectodomain on U.S. Space Mission STS-47, *Journal of Crystal Growth*, 1993 (in press)

Experiment L-05:**Crystal Growth of Enzymes in Low Gravity****Principal Investigator:**

Yuhei Morita
Fuji Oil Co. R&D Center

Coinvestigators:

Shigeo Aibara
Bunzo Mikami
Kyoto University Research Institute for Food Science

Charles E. Bugg
University of Alabama at Birmingham

Knowledge of the three dimensional structure of proteins determined by x-ray crystallographic analysis is essential for the elucidation of enzyme reaction mechanisms and for the development of drugs and food materials by protein engineering. Preparing protein single crystals is the first phase of determining the protein structure by the x-ray analysis. How the protein single crystals of good crystallinity can be obtained is the most important subject in this field of research. Lately, the microgravity environment in space has attracted considerable attention around the world as an appropriate environment for the production of such crystals.

This experiment was conducted using a microgravity environment in space to prepare single crystals of good crystallinity in which protein molecules are well arranged. On Earth, because of gravity, a mosaic structure (disorder of the crystal structure resulted from the uneven arrangement of protein molecules in the crystal) is large as a result of convection of the solution and sedimentation due to differences in specific gravity. It is therefore very difficult to build a precise model of the three dimensional structure of proteins using the crystals with large mosaic structures. If it becomes possible to obtain protein single crystals of good crystallinity using the microgravity environment in space, not only will it be possible to promote the development of drugs and food materials based on such a precise model of the three dimensional structure of proteins, but the dynamic crystallographic analysis of protein structure using the x-ray diffraction method can also become possible. Thus, the experiment of protein crystal growth, where a dynamic x-ray crystallographic analysis of proteins as an advanced technology could be materialized have a profound significance.

Six kinds of protein samples were used for crystallization in a space experiment which was conducted using the batch method and five of the samples produced crystals (Table 1). Two particularly noteworthy results were those of lysozyme (pH4.5) and w-amino acid:pyruvate aminotransferase. Single crystals of good crystallinity with little mosaic structure were obtained from these enzymes. Also, in this space experiment, since it is considered that the crystal nucleus would not sink or stick to the surface of the container due to the distinct characteristic of the microgravity environment, enough large and well-shaped single crystals of good crystallinity for the x-ray diffraction experiment would be expected to be obtained. However, compared with a control crystallization experiment on Earth, (1) crystal growth proceeded at a slower rate than expected; and (2) it was proved that depending on the protein sample and the crystallization conditions, the crystal nucleus grew in different shapes of crystals due to the influence of gravity; that is, the single crystals grown in space were not always of an optimal shape and size.

At present, x-ray crystallographic analysis is the only way to elucidate the structure (static, averaged structure) of the whole protein molecule at atomic level. Taking into account that it is one of the approaches to obtain the valuable knowledge about protein structures for the development of drugs effective in the intractable and the adult diseases, the space experiment is essential for preparing protein single crystals of good crystallinity and for improving the accuracy of protein structures. Also, dynamic x-ray crystallographic analysis should be done. However, for understanding the movement (dynamic structure) of the protein molecule at the same level, it is necessary to conduct a dynamic analysis using the Laue diffraction technique. For this purpose, single crystals of good crystallinity (with little mosaic structure) should be prepared although there still remain many problems to be solved. X-ray data collection of the order of milli-second should be attained. Consequently, the crystallization experiment using a space environment is indispensable for the elucidation of the dynamic structure of proteins.

The experiment was originally planned for seven days, and was extended one day. We were able to obtain a substantial crystal growth in seven days, but after the end of the space experiment, a new crystal growth was confirmed during transportation from United States. As a result, it is necessary to increase the amount of time of the space experiment. Also, protein single crystals grown in space are easily damaged by x-ray irradiation. Thus, the treatment of space-grown crystals after the space experiment remains a problem.

Table 1. Results of protein crystallization under the microgravity environment in space

| Sample protein | Space-grown | Crystals after space experiment | Shape compared with ground-grown crystals |
|--------------------|-------------|---------------------------------|---|
| Lysozyme, pH4.5 | Yes | | Different |
| Lysozyme, pH4.7 | Yes | | Different |
| Myoglobin | Yes | | — |
| w-Aminotransferase | Yes | Yes | Similar |
| Lipase | Yes | | Similar |
| Insulin | No | No | |

Experiment L-06A:**Studies on the Effects of Microgravity on the Ultrastructure and Function of Cultured Mammalian Cells****Principal Investigator:**

Atsushige Sato
Tokyo Medical and Dental University, Faculty of Dentistry

Coinvestigators:

Yasuhiro Kumei
Toshio Hongo
Kazuko Sato
Tatsuo Hamazaki
Ichiro Masuda
Tohru Nakajima
Takeo Ohmura
Tetsuji Sato
Kenjiro Wake
Tokyo Medical and Dental University, Faculty of Dentistry

Masayoshi Kaiho
Yamanouchi Pharmaceutical Co., Ltd., Safety Research Laboratory

Although microgravity affects development and function of bone, muscle, and other organs *in vivo*, it is not known whether microgravity exerts any direct or indirect effect on cells. Cytoskeletons are considered to have some important roles in the cell response to mechanical stimulation. It is important that by elucidating any direct effects of microgravity on the function of cell cytoskeletons, we have been able to identify the gravity sensing mechanism of cells, as well as, of organisms. Moreover, it is also important to analyze whether microgravity affects the biosynthetic processes of the cells as they relate to the possibility of progress in space biotechnology.

The aim of the present study is to elucidate by electron microscopy the direct influences of microgravity on the rearrangement of cellular cytoskeletons following isolation by trypsin treatment in space. The effects of microgravity on morphological cell changes, on cell proliferation, urokinase production, and glucose consumption were also examined. Another purpose of this study is to establish in space, a cell culturing system which is a fundamental technique in life science research.

In flight, the crews performed nominal microscopic observations, cultured medium renewing, trypsin-treatment, and cell fixation. In an extra-spaceflight day, additional observations were able to be performed. However, it seemed that the extension of the culture period in spaceflight without renewing the culture medium brought some damage to the cultured cells. The culture medium could not be completely exchanged, because the space shuttle was equipped with some different types of stems for syringes.

Four cultures (ACC1-ACC4) of monkey kidney cells, JTC-12 cells, were used in the flight experiments. The payload specialist (PS) carried out the experiments according to the set schedule. Two cultures (ACC1 and ACC2) were treated with trypsin/EDTA solution, to detach them from the substratum and dissociate their cytoskeletons. Then the cells continued to be cultured in new culture medium. Finally, they were fixed and stored in a refrigerator. The ACC3 cells were only used for the microphotography. One of the culture media of the chamber (ACC4) was exchanged for a serum-free culture medium. The

medium was collected after 24 hours of incubation and stored in a refrigerator. In an extra-flight day, the PS made additional observations and took microphotographs.

In general, there were no significant differences between the flight cultures and the ground control cultures at the initial time period with regard to the cell morphologies and proliferation rates. Analysis of the cultured cells of ACC1 and ACC2 revealed that there were few attached cells on the substratum in space flight after the dissociation by the trypsin treatment in comparison with the ground cultures.

After the flight, the cells were examined by scanning and transmission electron microscopy. For the flight cultures, a few cells attached onto the substratum but were slightly spread out after they had been treated by trypsin. In these cells, bundles of microfilaments could not be identified, although they reformed in the peripheral regions of the ground control cells. There were no differences between the flight cultures and the ground control cultures in the consumption of glucose and production of urokinase (Table 1).

The results of the present study revealed that a space flight of eight days has no essential effect on morphology, proliferation, and biosynthesis in mammalian culture cells. However, the lack of gravity induced the trypsin-treated dissociated cells to keep floating in the medium. Therefore, the attachment of the cells onto the substratum was delayed, and that caused difficulties in subculturing the cells. The present research also offers some important technical information on techniques for the establishment of cell cultures in space laboratories.

In the near future, there will be an increasing need to apply cell culture technologies in space life science. The present results showed that it was difficult for cells to reattach and adhere to the substratum after they had been separated by trypsin treatment during subculture. Thus, it will be necessary to develop some techniques for applying surface coating to the substratum in the future.

Table 1. Urokinase activity and glucose amount in the culture media by which JTC-12 cells were cultured 24 hours

| | Flight | Ground |
|--------------------|---------------|---------------|
| Urokinase activity | 0.038 IU/ml | 0.047 IU/ml |
| Glucose amount | 2.57 mg/ml | 2.61 mg/ml |

Experiment L-06B:**Study the Effects of Microgravity on Cell Growth of Human Antibody Producing Cells and their Secretions****Principal Investigator:**

Toshio Suganuma
Laboratory of Biological Science,
Mitsui Pharmaceuticals, Inc.

Coinvestigators:

Hisayoshi O'oka
Tamotsu Fukuda
Laboratory of Biological Science,
Mitsui Pharmaceuticals Inc.

Several attempts have been made to investigate the effects of microgravity on the growth and function of animal cells. Cellular activation of immune T lymphocytes is greatly affected by microgravity. On the other hand, little is known about the effects of microgravity on B lymphocytes, another major class of immune lymphocytes, growth or antibody production. Our approach to investigate the human B lymphocytes was to compare cell growth, nutrient consumption, and antibody secretion by a human B cell hybridoma between the cells cultured in space and the ground culture.

The cell line used in this study was a human-human hybridoma cell line designated JC-1, to produce an anti-sheep red blood cell IgM antibody. Cells were inoculated in cell culture chambers developed for this experiment and were cultured at 37.5°C for seven days. To observe the culture, the chambers were taken out of the incubator every day and microscopic photos were taken. The culture medium was changed with fresh medium on days 2, 4 and 6 after launch. The synchronous ground control experiment provided cell culture chambers for cultivation.

Human B lymphocyte hybridoma, JC-1, was grown in the space experiment. Table 1 shows the numbers of cells before and after the experiments. The cell proliferation was dependent upon the cell density at inoculation. The recovered cell numbers revealed no significant differences between the experiment done in space and the ground experiment. The viability of the cells cultured in the space experiment was slightly better than that in the ground experiment.

The IgM antibody secretion was increased two times or more during flight, although the number of recovered cells did not differ from that in the respective ground control culture.

The rates of glutamine consumption and ammonia production during the flight were slightly higher than those in the ground culture. The rates of glucose consumption and lactate production during the flight were significantly increased.

To examine the post-microgravity effect immediately after the experiment, frozen and preserved cells were thawed and cultured again on the ground. No significant differences in either antibody secretion or cell proliferation were observed between the cultures after the flight and the ground cultures. The increasing effect on the antibody production during the flight disappeared on the ground.

It is not clear by what mechanism microgravity causes enhanced antibody production and cellular metabolism. It may influence cellular metabolism and signal transduction by biological pathways. The present findings increase our knowledge of the effect of gravity on basic cellular mechanisms, and the effect of spaceflight on the immune system. Further, it may be applicable to develop useful biotechnological processes.

Table 1.**Numbers of cells before and after flight**

| Experiment | Cell Culture Chamber | Seeding Cell No. | Viability | Recovery Cell No. | Viability |
|-------------------|-----------------------------|-------------------------|------------------|--------------------------|------------------|
| Flight | BCC-1 | 5.0 x 10 ⁵ | 92% | 23.5 x 10 ⁵ | 48% |
| | BCC-2 | 2.0 x 10 ⁵ | 92% | 7.5 x 10 ⁵ | 58% |
| Ground | BCC-1 | 5.0 x 10 ⁵ | 92% | 23.3 x 10 ⁵ | 41% |
| | BCC-2 | 2.0 x 10 ⁵ | 92% | 8.0 x 10 ⁵ | 38% |

Acknowledgment:

We thank Dr. Y. Ono, Nihon University, for work and information on hybridoma establishment and Dr. A. Sato, Tokyo Medical and Dental University, for helpful advice on the space experiment.

Experiment L-06C:**Organ Differentiation from Cultured Plant Cells under Microgravity****Principal Investigator:**

Atsushige Sato
Tokyo Medical and Dental University

Coinvestigators:

Yasuyuki Yamada
Fumihiko Sato
Kyoto University

Hisashi Matsushima
Saitama University

Satomi Takeda
Osaka Women's University

Plant shoots develop against the Earth gravity, whereas roots develop toward the center of the Earth. These phenomena are known as a geotropism. However, while several attempts have been made so far, because of the relatively slow responses of plant cells to the environmental changes as well as the difficulties to set up the systems to grow intact plants under microgravity conditions, little is known about the long-term effects of microgravity on the growth and development of plant cells. Our research objective is to investigate the effect of microgravity on the organ differentiation and cell growth of tobacco cells using in-vitro cell culture systems. One of the advantages in using an in-vitro culture system is that we can dissect the differentiation processes and evaluate the effects of microgravity on each process.

For this purpose, tobacco stem tissues or calluses (dedifferentiated cells) were inoculated on the agar medium in a specially designed culture vessel. The culture medium contained the nutrients and plant hormones, i.e. auxin and cytokinin, to control the differentiation/dedifferentiation (cell proliferation) of plant cells. These inoculated tissues and cells are launched, and cultivated for eight days under microgravity conditions. After the return to the Earth, the differentiation and growth responses of flight samples were compared with those of the ground control. Because of the limitation of the growth period under microgravity conditions, the measurement of enzyme activities especially in lignin biosynthesis is planned to evaluate the level of differentiation and dedifferentiation (growth) of cells as well as the electron microscopic observation of ultrastructural changes. Furthermore, general gene expression pattern in flight and ground was compared using ³⁵S-methionine-labeling of proteins and their 2D-PAGE analysis.

The flight experiment showed that the culture vessel could be used even in microgravity condition without severe problems. While the culture period was limited to only eight days, flight experiments also showed that cultured plant cells or tissues could grow and regenerate shoots under microgravity condition. However, it was obvious that the growth of cultured stems grown under microgravity was more heterogeneous than those of the ground control and the increase in fresh weight of the flight samples was less than that of the ground control. Furthermore, the multiple shoot formation, which was often observed in ground controls, was not found in flight samples.

Microscopic observation showed that the meristem of regenerating shoot developed under microgravity was much smaller than that of the ground control. Electron microscope analysis showed that the development of chloroplasts was slightly more advanced in ground control

than in the flight samples. Furthermore, extensive arrays of microtubules were more clearly found in ground-control than in flight samples. On the other hand, the ultra-structure of callus cells of flight-grown and ground-control also showed some differences, in which the plastids were more dedifferentiated in the flight sample than those of the ground control.

Even though several ultrastructural differences were observed, two dimensional polyacrylamide-gel electrophoresis analysis of ³⁵S-methionine-labeled proteins suggested that the major gene expression in the flight samples would be similar to that of the ground control.

Flight experiments supported the previous observations that microgravity would not affect the initiation of the differentiation from cultured cells. However, the flight samples showed more heterogeneous growth and the total increase in fresh weight was slightly lower than the ground control. Less development of meristem of the shoot in the flight samples might reflect the little formation of multiple shoots in the flight samples. Those unexpected results indicated that further experiments are necessary for the characterization of the detailed effects of microgravity on plant development.

Experiment L-07:

The Effect of Low Gravity on Calcium Metabolism and Bone Formation in Chick Embryo

Principal Investigator:

Tatsuo Suda
Showa University, School of Dentistry

Coinvestigators:

Etsuko Abe, Toshimasa Shinki, Takenobu Katagiri,
Shusaku Yoshiki, Akira Yamaguchi, Satoshi Yokose,
Yoshiro Shibasaki, Kohtaro Maki, Masatoshi Mikawa, and
Yasuyo Hamazaki
Showa University, School of Dentistry

Hiroshi Horikawa
C. Itoh Feed Mills Co.

Yutaka Nagai
Tokyo Medical & Dental University, Medical Research
Institute

Katsuhiko Arai
Tokyo University of Agriculture and Technology

Fujio Suzuki, Tomomi Iwamoto, and Hiroyasu Iwamoto
Osaka University, Faculty of Dentistry

Hiroyoshi Endo and Kohtaro Kawashima
Teikyo University, Faculty of Pharmaceutical Science

Kousaku Maruyama
Chiba University, Faculty of Science

Shushichi Takahashi, Fumio Kita, Katsuya Koike, and
Koichi Metori
Nihon University, College of Pharmacy

Makoto Igarashi
Nihon University, University Research Center

Tsuneo Sato
Nihon University, College of Agriculture & Veterinary
Medicine

Teru Ishibashi and Seiji Kusuhara
Niigata University, Faculty of Agriculture

Emiko Watanabe
Tokyo Technical College

Sumiharu Noji
University of Tokushima, Faculty of Engineering

Isamu Kashima and Kousuke Nishimura
Kanagawa Dental College

Coinvestigators:
(Continued)

Sadao Yasugi
Tokyo Metropolitan University, Faculty of Science

Mitsuru Naito
National Institute of Animal Industry

G.M. Cohen
Florida Institute of Technology

G.W. Conrad
Kansas State University, and

R.S. Tuan
Thomas Jefferson University

The original aim of our space experiment was to examine morphologically and biochemically the mechanism of suppression of bone growth under microgravity using chick embryos flown in a space shuttle. After Vellinger performed a similar space experiment to ours using two and nine day old chick embryos in 1989, another aim was added to our experiment to confirm or disprove Vellinger's conclusion that the early stage of chick embryogenesis hardly occurs under microgravity.

The space shuttle Endeavour was launched on September 12, 1992 and landed on September 20, 1992. Thirty fertilized chicken eggs were preincubated for 0, 7 and 10 days (ten eggs each) on Earth before space flight and further incubated in the Spacelab module for seven days and three hours. After landing, survival of the chick embryo was examined by candling and half of the living embryos were immediately dissected. Their cartilage growth, bone formation and resorption, and muscle development were examined morphologically and biochemically. The rest of the embryos were further incubated until hatching at KSC and various parameters on bone and muscle growth were examined immediately after hatching. The same number of fertilized eggs was incubated for the ground control and the control groups under 1g gravity using an engineering model (EM) incubator and a commercially available conventional incubator, respectively, according to a simulated experimental protocol.

Table one shows the survival rate of chick embryos after landing. Twenty out of 30 eggs (9/10 in ten day old; 10/10 in seven day old; and 1/10 in zero day old) survived in the flight group. Out of ten 0-day-old eggs, two were nonfertilized eggs. Out of the remaining eight fertilized eggs, seven were recovered as dead embryos at KSC. In the seven dead embryos, two had survived for five days and the remaining five survived for one to three days. The only living embryo of the 0-day-old egg named "Space Embryo" finally died 24 days after launch. The dead embryo was 16-days old.

Table 1.**Survival rate of chick embryos after eight day flight**

| Group Total | 0-day old | 7-day old | 10-day old |
|-----------------------|------------------|------------------|-------------------|
| Flight: 20/30 | 1/10 (2)* | 10/10 | 9/10 |
| Ground Control: 27/30 | 7/10 (1) | 10/10 | 10/10 |
| Control: 26/30 | 10/10 | 10/10 | 6/10 |
| Total | 18/30 | 30/30 | 25/30 |

* The number in parenthesis means non-fertilized eggs.

The high mortality of the 0-day-old eggs appeared to be due to the specific inner structure of the eggs, in which specific gravity of the yolk (1.029) was significantly smaller than the albumen (1.040). Simulation experiments on Earth indicated that the yolk might not have floated in the albumen under microgravity. When the blastoderm (zero day old embryo) which was situated at the top of the yolk sank into the albumen for more than two days, most of the embryos died. The high survival rate of the seven and ten day old embryos may be due to the fact that these embryos had already been fixed in the correct position in the egg and angiogenesis had started prior to the time of launch.

Bone and cartilage formation was examined biochemically and morphologically in seven and ten day old embryos. No applicable changes were recognized in any of the tissues in the flight group compared to the ground control and the control groups, except that the bone mineral density of humerus and cervical vertebrae was slightly decreased in the seven day old embryos and that very little type XI collagen deposited in the territorial matrix of chondrocytes. In femoral cartilages, an irregular or abnormal pattern of type II collagen was also observed in the flight group.

The present study suggests that the high mortality of zero day old chick embryos under microgravity is due to the subtle difference of the specific gravity between the yolk and albumen. Contrary to our expectations, neither calcium metabolism nor bone formation of the seven and ten day old chick embryos were hampered significantly under microgravity. This may indicate that bone formation of rapidly growing embryos is rather insensitive to the presence or absence of gravity. Gravity may be critical for maintaining the balance of bone remodeling.

Three hours after the space shuttle landed, survival of the zero day old chick embryos was examined by candling. Candling (a) and morphology (b) of the dead embryo, was estimated to be five day old. Candling (c) and morphology (d) of the live embryo, indicated it died at the stage of 16-day-old.

Experiment L-12:**Circadian Rhythm of Conidiation in *Neurospora Crassa*****Principal Investigator:**

Yasuhiro Miyoshi
University of Shizuoka

Coinvestigators:

Yuji Moriyasu
Mineo Iseki
University of Shizuoka

To test whether a circadian rhythm can be observed in an environment without 24-hour periodicities arising from rotation of the Earth and in an environment of microgravity, an experiment using *Neurospora crassa* (band A strain) which shows an obvious circadian rhythm in its condition on the ground was conducted on the Space Shuttle flight STS-47.

The conidia were rhythmically formed in the flight experiment as well as in the ground control experiment. A reduction in the clarity of the rhythm and arrhythmicity which were shown by F. M. Sulzman, and others, were not observed in all six flight tubes. But there were some differences between flight tubes and ground control tubes, for example, the growth rate of the hyphae and the density of conidia were higher in flight tubes, and a morphological difference of the conidia was also observed.

Experiment PCR:**Plant Cell Research Experiment on Spacelab J Mission.
Mitotic Disturbance in Daylily (*Hemerocallis*) Somatic
Embryos after an 8-day Spaceflight****Coinvestigators:**

A.D. Krikorian
Stefania A. O'Connor
R.P. Kann
State University of New York at Stony Brook
Department of Biochemistry and Cell Biology

Embryogenic cells of carrot and daylily were exposed to space in metal Petri dish-type containers. The objectives were: (1) to evaluate whether spaceflight affected the developmental progression of embryogenically competent daylily and carrot cells from one well-defined stage to another; (2) to determine whether mitosis and chromosome behavior during cell division were modified by the space environment.

Embryogenically competent cells from a 200–400 fraction of daylily and carrot cell cultures were grown in plant cell culture chambers obtained from NASDA. Two plant cell culture dishes, one for each species, were used to grow cells on a semi-solid agar support. Progression to later embryogenic stages occurred in space when the pH-altering metabolic activity of the cells changed the environment to a permissive one as they grew. The timing of the set-up of the experiment was such that embryogenesis could proceed in space from poised cells. Although growth on the agar substrate, etc. that was used and the biology of these somatic embryogenic cell systems are readily amenable to fixation in space, available hardware did not allow this. However, the dishes with live cultures of somatic embryos were worked on within eight hours of recovery to the Stony Brook laboratory for post-flight evaluation of development and karyology. This was done after treatment with colchicine to arrest cells in metaphase, and by continued monitoring by “grow-out” procedures (initially aseptic and later non-aseptically in the greenhouse) of some of the rescued embryos.

Somatic embryogenesis occurred during the flight. Significant alterations in the karyology of somatic embryos developed in space were observed. Analysis has thus far concentrated on the daylily, but the carrot shows similar effects as well. The responses include in-flight samples—a substantial number of binucleate cells among those that are normally uninucleate. The ground control samples were uniformly uninucleate. Radiation levels during the flight were said to be in the expected range (personal communication from Dr. Nagaoka of NASDA); so, at this point, until we see detailed flight radiation data, attempts at interpretation will not focus on those potential effects.

Since our methodology for karyotype analysis involves treatment of cells and somatic embryos with colchicine, we have taken care to eliminate the possibility of an increased sensitivity of the flight-exposed cells to colchicine, leading to a doubling of the nuclei. Serial sampling and examination of flight samples after recovery, beyond what we refer to as sampling one (i.e., the first sampling after recovery) indicate that the number of binucleate cells diminish from some, but are not eliminated. Embryos reared into plantlets of both flight and controls as part of our follow-up studies indicate poor root formation potential and development in daylily plantlets in particular. In addition to the condition of double nuclei, aberrations in chromosome structure have been encountered as well. Reasons could include: 1) impairment of DNA-repair mechanisms due to the space environment; 2) effects of altered signalling at critical stages of cell division due to changed interfaces brought on by space effects, especially those affecting behavior of fluids and gases; 3) modification of

location and/or efficiency of energy-yielding or substrate availability-affecting organelles, especially at the putative cell plate; 4) the malfunctioning of spindles and phragmoplasts (future cell wall sites), which if so, would indicate perturbation in the interdependence of differentiated microtubule configurations in mitosis in space. It is perfectly conceivable that more than one of these factors is involved.

Whatever the cause(s), we conclude: 1) The PCR-type experiment, especially if carried out with fixation in space, offers promise in enabling us to understand atypical nuclear and chromosome behavior in space-grown plant materials; 2) We have demonstrated that cultured embryogenic cells can serve as models for the study of development in higher plants in space environments and in microgravity; 3) The experiment adds to the data base on chromosome effects on higher plants encountered in space by confirming that the level and fidelity of division achievable in higher plant cells on Earth, even when they are randomly oriented and the effects of gravity are neutralized by means of rotating clinostats, are not sustained in their counterparts during or immediately after space flight; 4) We have added embryogenic cells of daylily and carrot to those species that have shown change in earlier flights—namely, karyological changes in root cells of Shuttle-grown oats, mung bean and sunflower, daylily (*Hemerocallis*) and *Haplopappus gracilis* which were grown as seedlings and aseptic tissue cultured propagules; 5) The fact that significant chromosomal damage (between 3 and 30%) that altered subsequent cell division raises the question that specific species, the way they are grown, and the duration of flight can have an effect on the results. Less extreme karyotype changes (deletions and translocations) modify genetic make-up, but really adversely affect growth. In this context, continued development of the plant with changed genotype might be the result of a sort of space adaptation. On the other hand, declining cell division and chromosomal damage and mitotic disturbances in space-grown materials suggest that prolonged duration flights would present greater difficulties in achieving optimum growth of plants. This would mean that there is a “penalty to pay” for growth in space. On the other hand, it could also be that a better adaptation occurs after the initial stress and that with increasing duration of flight and once the adjustments to growing in space are made, plants will grow quite well.

An expanded version of the experiment is expected to be carried out on the International Microgravity Laboratory 2 mission (IML-2) scheduled for 1994. It is anticipated that both PCR and the IML-2 experiments will permit us to place the events of somatic embryogenesis and cell division in space on a firmer footing. This means it will be considerably easier to achieve the kind of control over the system necessary to separate direct from indirect effects and thus enable us to move towards resolving the still many outstanding questions. This work is supported by NASA.

Experiment FEE:**Amphibian Development in Microgravity: The STS-47 Frog Embryology Experiment****Coinvestigators:**

K. A. Souza
A. M. Ross
NASA Ames Research Center

S. D. Black
Biology Department, Reed College

R. J. Wassersug
Department of Anatomy and Neurobiology, Faculty of
Medicine, Dalhousie University

The amphibian egg is known to be particularly sensitive to gravity. On Earth, fertilized eggs rotate within the fertilization envelope so that the animal-vegetal axis is aligned with the gravity vector. Moreover, eggs experimentally inclined with respect to gravity form their dorsal structures on the side of the egg uppermost in the gravitational field. In view of these responses to gravity, we utilized the STS-47 flight opportunity to investigate whether gravity is required for normal development of *Xenopus laevis*, the African clawed frog.

Four female frogs were injected with chorionic gonadotropin which induced ovulation in microgravity. Eggs were fertilized with a sperm suspension and placed in 30 plastic chambers half of which were incubated at microgravity and the remainder incubated on a centrifuge which created a one gram condition during the flight. Fertilization efficiency was greater than 70% in both groups and morphology of embryos and larvae fixed at a variety of developmental stages was generally similar in the microgravity and one gram groups. Some differences in early embryonic structure and larval behavior were noted: a) there was a significant thickening of the blastocoel roof in the microgravity group ($p=0.0001$); b) the blastopores of the microgravity group were displaced toward the vegetal hemisphere ($p=0.02$); c) the lung volume of the microgravity group was significantly smaller than the centrifuge control group ($p=0.02$); d) the swimming behavior of tadpoles fertilized preflight and launched at the neurula stage developed upwardly bent tails and swam in backward somersaults both during flight and during the immediate postflight period; e) tadpoles fertilized and hatched in space did not exhibit looping behavior similar to the larvae fertilized preflight; f) tadpoles in the microgravity group tested within five hours postflight for their ability to follow a rotating pattern of vertical stripes exhibited a stronger response than did the centrifuge control group ($p=0.06$).

This experiment marks the first demonstration that vertebrates can ovulate, that the eggs produced can be fertilized and develop into a free-living stage in the virtual absence of gravity.

Experiment BCR:**Bone Cell Research****Principal Investigator:**

Nicola C. Partridge
University of St. Louis, School of Medicine

Our experience was that photomicroscopy and media exchanges generally went well. The major problem we encountered was the loss of dividing cells during the first media exchange due to misinterpretation by the crew of this procedure. The communication link was satisfactory. However, even though we were aware that the Japanese astronaut was performing the first media exchange incorrectly, my team was prevented from communicating with him to tell him to change what he was doing. This one step will affect all of our science since our cells never reached confluence.

We received detailed information about the cells' appearance. Extending the mission improved sample collection for the last media exchange. Samples were delivered to Hangar L within the scheduled timeframe and arrived in good condition, still frozen.

Downlink communication indicated sizable cell loss in flight chambers. We conducted parallel ground controls which did lose a majority of cells during the first media exchange due both to duplication of the crew's activities and inferior chambers in which cell adhesion was not good. Our best chambers were used for flight. One ground control chamber also incurred a fungal infection by the second media exchange which is probably indicative of a problem in doing these procedures out on the bench in Florida. The labs at Hangar L are not really an exact control for the Shuttle which is probably a cleaner, more controlled environment.

We repeated the ground control portion of the experiment protocol using the flight chambers and had better cell growth and no fungal contamination. We developed the flight and both sets of ground control films. The contact prints of the flight films look excellent. Repetition of the ground control experiment has helped to confirm slight differences in morphology of the flight cells.

We assayed the flight and ground control samples for collagenase by ELISA. Due to sizable loss of dividing cells during flight and in the parallel ground controls, the results were below the limit of detection for the standard curve. We have investigated means of concentrating the media before assaying and found spin columns which will concentrate the samples 50-fold. We are undertaking this now.

Bradford Bio-Rad Protein assays were performed on cell lysates and the values indicated low but significant levels of cell protein confirming the microscopic data. We will use these values to normalize the collagenase data. In addition, it is possible that we will be able to assay another osteoblastic marker, alkaline phosphatase, from these samples.

To measure rat tissue inhibitors of metalloproteinases (TIMPs), we had a peptide synthesized to generate antibodies for the rat TIMPs to use to establish either an ELISA or RIA for these proteins. The peptide was cross-linked to keyhole limpet hemocyanin and injected into rabbits repeatedly. This seemed to have been relatively successful and the rabbits produced antibodies to the synthetic peptide. However, the affinities of the antisera did not improve with further immunization. We have concluded that we are unable to obtain a high-titre antiserum using this peptide and would need to repeat the process with another peptide to be able to assay the TIMPs. However, this would not be possible with our current budget since peptide synthesis alone costs \$1,500. The alternative method

would be to measure the TIMPs by a functional assay, but this is also not possible since the BCR media samples contain 10% fetal bovine serum which is abundant in TIMPs. This problem would be exacerbated by concentration of the media 50-fold. So, with the budget cuts we have withstood, I do not envision us being able to finish this part of the project.

In conclusion, there appears to be some morphological changes in the cells under microgravity but as of this date we do not have any other scientific data to present.

Experiment FTS:**In-Flight Demonstration of the Space Station Freedom Health Maintenance Facility Fluid Therapy System (E300/E05)****Principal Investigator:**

Charles W. Lloyd
NASA, Johnson Space Center

Coinvestigators:

Gerry Creager
Terry Guess
Moureen Smith
KRUG Life Sciences, Inc.

Debra Giglio
McDonnell Douglas Space Systems Co.

BACKGROUND:

An integral part of medical care consists of providing intravenous (IV) infusion of fluids to sustain an ill or injured crew member. Due to the space station's weight and volume constraints, an adequate supply of the required solutions cannot be carried.

GOAL:

Assess the concept and performance of an integrated set of components designed to produce and deliver medically acceptable quality fluids for IV administration.

OBJECTIVES:

Formulate Sterile Water for Injection (SWI), Normal Saline (NS), and Dextrose 5% in Water (D5W) from potable water and concentrates. Compare samples manufactured in-flight to ground samples utilizing United States Pharmacopeia XXI standards. Verify the performance of a modified terrestrial IV pump at four flow rates (25, 80, 125, and 300 mL/hr.).

RESULTS:

Analysis of the baseline water taken from the Kennedy Space Center revealed that the tap water contained an endotoxin concentration of 43.2 EU/mL and common water born bacteria. Water samples assayed from the Flight SWC, taken directly from the tank into an IV bag, contained an endotoxin concentration of 124.8 EU/mL and a bacterial count of 4.6×10^6 per 100 mL. Water taken from another SWC used for the ground testing was found to have an endotoxin concentration of nearly twice that found in the flight tank of 286.5 EU/mL and a bacterial count of 5.9×10^8 per 100 mL. Only the ground samples were found to contain endotoxins (6/6 samples). After 21 days; 3/9 preflight, 0/8 in-flight, 0/8 ground, and 2/9 postflight, one liter IV bag samples produced by this process were identified as containing bacterial growth. Chemistry analysis of the samples produced indicated at the percent concentration of D5W ranged from 4.8% in 1,030 mL of fluid to 5.5% in 907 mL. For the NS samples produced sodium chloride, concentration ranged from 0.86% in 1,074 mL of solution to 1.04% in 925 mL. The concentration of trace metals, anions, and cations were within normal limits for all samples types based on USP XXI standards. Intravenous pump flow rate testing suggested that a zero-pressure head induces a 2-4% negative error from the set rates. When the in-line filter is added to the fluid administration set at a standard pressure head of 20 inches, the resistance associated with the in-line filter resulted in a 1-3% negative error from the set rate. When these two factors are combined, the total error ranges from 3-9%. In-flight results, the lowest error was noted on Channel B when running a set flow rate of 80 mL/hr. (5.3% error) and the highest was noted on Channel A when running at a set rate of 125 mL/hr. (9.9% error). In results from the testing being performed on the ground, the lowest error was noted on

Channel A when running at a set rate of 80 mL/hr. (5.8% error) and the highest was noted on Channel B when running at a set rate of 300 mL/hr. (7.8% error).

CONCLUSIONS:

The system was able to consistently produce the three types of solutions required for medical care. The hardware performed consistently within the experimental goal of < 10% error rate at all flow rates.

Experiment MRI:**Magnetic Resonance Imaging After Exposure to Microgravity****Principal Investigator:**

Adrian LeBlanc
Baylor College of Medicine

Coinvestigators:

Harlan Evans
Victor Schneider
Richard Wendt
Thomas Hedrick
Baylor College of Medicine

Magnetic resonance imaging (MRI) was performed on four members of the crew of the eight day Shuttle mission SL-J, on five occasions: L-90, L-60, L-30, R+1 and R+14 days. The pre- and postflight imaging were performed in the morning using two Siemens 1.5 Tesla magnets (Magnetom 635P). All images were acquired in a 256x256 matrix with oversampling. Using the whole body coil, spin echo acquisitions of the calf were performed with an aluminum shield over the lower torso. For these images, a single acquisition of 32 contiguous one centimeter slices was obtained using a $T_e=22$ msec, $T_r=1,500$ msec, and a 256x256 matrix. Following the calf images, the shield was moved to cover the lower legs and imaging of the thigh was performed using the same parameters as above. Following these images, a spine coil was used to obtain three separate sequences. A sagittal scout image was used to center the coil through L3. A coronal scout image was used to position a one centimeter region of interest through the center of the spinal column. A Carl-Purcell-Meiboom-Gill (CPMG) sequence with a TR of one second and spin echoes at 20,45,72, and 106 milliseconds and four acquisitions were used. This was followed by a multislice sagittal, a gradient echo sequence with a $T_e=7$ msec, $T_r=800$ msec, 24 contiguous slices of three millimeters each. Finally, a transverse spin echo sequence was obtained consisting of 20, 0.5 cm transverse slices centered on L3 and with a $T_e=20$ msec, $T_r=1$ sec, two acquisitions.

T2 calculations of the intervertebral discs and bone marrow of the lumbar spine showed no statistical change relative to preflight values. Similarly, the intervertebral disc areas and lumbar spine length were not significantly changed relative to preflight values indicating that 24 hours of normal ambulation following eight days of weightlessness is sufficient to return values to normal. The muscle volume changes found in the four crew of SL-J were: -3.9% from the anterior calf, -6.3% from the soleus + gastrocnemius, -6.0% from the quadriceps, -8.0% from the hamstrings, -10.3% from the intrinsic lower back, and -3.1 % from the psoas. Repeated measures ANOVA showed that these changes were significant except the psoas ($p=0.13$) and quadriceps ($p=0.06$). Two weeks following flight, recovery was evident in all areas but was incomplete in the hamstrings and intrinsic back muscles.

**Experiment L-01: Endocrine and Metabolic Changes of Payload Specialist
During Spacelab J**

Principal Investigator: Hisao Seo
Nagoya University Research Institute of Environmental
Medicine

Coinvestigators: Nobuo Matsui
Chukyo University, Faculty of Physical Education

Yoshiharu Murata
Norihiro Miyamoto
Sachiko Ohmori
Fukushi Kambe
Yoshitaka Hayashi
Nagoya University Research Institute of Environmental
Medicine

Yoshihiro Tamura
Nagoya University College Medical Technology

The objective of the FMPT/L-1 is to study endocrine and metabolic changes of a payload specialist during Spacelab J. Urine and blood samples were obtained at four different periods. The first data collection was urine and blood sampling from three Japanese payload specialists for three days from May 19–21, 1991. This data collection served as baseline control data. The urine samples were collected for three days immediately after waking, every three hours during the day and just before sleep. The blood samples were collected every three hours from 07:00–22:00 on May 20. The second data collection was urine sampling from the prime payload specialist, Dr. Mohri. The urine samples were collected from L-3 to L-0 as outlined above. The third set of data was in-flight urine sample collection. Urine samples were obtained by using the Urine Monitoring System (UMS). The last set of data was urine and blood sample collection during the postflight period. A blood sample was obtained two hours after landing (EDT 11:15) and two more samples were drawn on the same day. During the subsequent two days, blood samples were drawn at 07:00 and at the same time when the first sample was obtained after landing (CST 10:15). Urine samples were obtained in the same manner as the preflight sample collection. Urine and blood sample collection during pre- and postflight period progressed nicely as scheduled. As for the in-flight urine sampling, a UMS anomaly was reported. The anomaly was the connection of UMS flush line with waste water port. This might have caused contamination of Dr. Mohri's urine sample with waste water—creating difficulty in analyzing the data during mission days. Later analysis by NASA revealed that the maximum contamination in volume could be 20%. Thus, we believe the data during the mission days represent Dr. Mohri's. Also, alterations of the schedules for urine sampling and sleep hours during mission days were noted; especially the frequency of urine sampling was less than that in the original schedule during the initial period of space flight—creating a difficulty to analyze the alterations of fluid-electrolyte metabolism upon arrival at space and diurnal variation of the endocrine and metabolic systems.

The analysis of the samples obtained at L-1 year revealed that most of the parameters in blood and urine were within normal range. Several hormones such as cortisol, aldosterone exhibited typical diurnal variations. Excretions of sodium, potassium, calcium and

phosphorus also exhibited typical diurnal variations. Analysis of the data obtained pre-, in- and postflight periods revealed the results not expected from the ground-based experiments. For example, decreased excretion of antidiuretic hormone (ADH) upon arrival to space was not observed. A strong correlation between the excretion of ADH or aldosterone with that of cortisol was observed during the in-flight period, indicating the secretion of fluid and electrolyte-regulating hormones were strongly modified by stress reaction. Circadian rhythms of the excretory pattern of sodium and potassium were lost during the pre- and in-flight periods and restored promptly upon return to the Earth. Although it is difficult to understand the underlying mechanism, the disturbance in circadian rhythmicity of the metabolic system needs confirmation since it may create a serious problem during long stays in space.

Experiment L-00:**Health Monitoring of Japanese Payload Specialist
Autonomic Nervous and Cardiovascular Responses under
Reduced Gravity****Principal Investigator:**

Chiharu Sekiguchi
National Space Development Agency of Japan

Coinvestigators:

Tadashi Murai
National Space Development Agency of Japan

Masanori Ishii
Jikei University School of Medicine

Kazuyoshi Yajima
Nihon University School of Medicine

Kiyoshi Nakayama
Sophia University

The first and primary objective of this experiment is to monitor the health of the Japanese payload specialist during space flight. The second objective is to investigate the autonomic nervous system's response to space motion sickness. To achieve this, the function of the autonomic nervous system will be monitored using non-invasive techniques. We will compare autonomic function in gravity-free and 1g environments. Data obtained will be employed to evaluate the role of the autonomic nervous system in space motion sickness and to predict the susceptibility to space motion sickness. The third objective is to evaluate the adaptation process of the cardiovascular system to 0-gravity. The last objective is to create a database for use in the health care of Japanese astronauts by obtaining control data.

Flight duration was extended from seven days to eight days, so health monitoring by the physiological monitoring system (PMS) was conducted from launch to mission elapsed time (MET) day three before sleep, and from MET day five until landing. Cardiac hemodynamic measurements using echocardiography were not made because the crew members were too busy during flight.

Heart rate (HR) and blood pressure (BP) during flight increased on the first day of flight (MET day zero), recovered to approximately normal on MET day two, then remained approximately normal until the last day of flight. From the results of R-R variability analysis of HR, the coefficient of variance (CV) revealed very good correlation with the Graybiel's motion sickness (MS) score except the value on MET zero. The CV is thus considered to be a good objective index of space motion sickness (SMS). On the TV monitor downlinked on MET day zero, we observed that the J-PS's mobility was not smooth, that performance was degraded, that the J-PS's face was puffy, and that there was facial venous engorgement with several clinical symptoms (MS score 24–25) of SMS. On MET day two, BP and HR recovered to normal, significant symptoms of SMS were decreased, mobility was smooth, and performance became better. However, we noted the puffy face and facial venous engorgement until the last day of flight. The stand test after flight showed increased HR response compared to the preflight value. This suggests that one week of spaceflight slightly influenced the J-PS's cardiovascular system. However, other physiological parameters recovered to the preflight values three days after flight. These results suggest that health and performance of the Japanese astronaut were appropriately

maintained during flight except in the early stage. Moreover, an important database was created for healthcare of Japanese astronauts.

A bulky PMS significantly affected the J-PS mobility and performance. Moreover, the data recorder did not record the time code with data in tapes, and data in tapes had much noise. This noise contamination is considered to be due to bit errors caused by a mismatch of analog-digital data transformation. Therefore, we could not analyze all data which was recorded on tapes.

We have learned the following, which should be useful for a future space experiment from our experience:

- The physiological monitoring system (PMS) must be more portable.
- The PMS must record the time code with continuous data on tape.
- An infrared telemetry system is useful for data transmission in Spacelab because it does not limit the subject's mobility area.

Experiment L-02:**Neurophysiological Study on Visuo-vestibular Control of Posture and Movement in Fish During Adaptation to Weightlessness****Principal Investigator:**

Shigeo Mori
Nagoya University Research Institute
of Environmental Medicine

Coinvestigators:

Genyo Mitarai
Nagoya University

Akira Takabayashi
Fujita Health University

Sadaharu Takagi
Nagoya University Research Institute
of Environmental Medicine

Shiro Usui
Tetsuro Nakamura
Toyohashi University of Technology

Manabu Sakakibara
Tokai University

Makoto Nagatomo
Institute of Space & Astronautical Science

Rudolf J. von Baumgarten
Mainz University, Germany

In order to collect evidence for the sensory conflict hypothesis on space motion sickness (SMS), the investigators attempted to detect adaptive changes in the light-dependent response, dorsal light reaction (DLR), and cerebellar activity (EEG) of normal carp, and to compare them with those of an otolith-removed one. The experiment was conducted during the seven day flight mission of Spacelab J and also during a period of re-adaptation after the landing.

The DLR and EEG data were completely acquired on the twice-a-day, ten minutes each collection rule, as scheduled throughout the experimental period. An extra run was not made on the extended mission day, MET day seven.

Supporting the hypothesis for SMS origin in the normal carp, the DLR was unstable during the first three days of the mission and regained stability by the fifth day. Remarkable changes in the cerebellar EEG activity occurred in the second and fourth days. In the otolith-removed carp, tight twisting of the EEG cable immobilized the fish from the third in-flight test session until the end of the whole experiment. However, in the second in-flight test session (MET 1d+04h), the DLR became unstable without any twists of the cable, suggesting that the neural mechanism in the integration center, which had been rearranged for compensation for the otolith removal, was disrupted again. Both carp stood still on the bottom of the container most of the time after landing, implying that any light-dependent behavior could be hardly induced.

After completion of the experiment, both carp lost twice as much body weight as expected. In addition, findings from the water analysis made from water taken from the fish container suggested that the carp's metabolism was highly accelerated.

The present study will be extended further to the goldfish experiment in IML-2.

Experiment L-04:**Comparative Measurement of Visual Stability in Earth and Cosmic Space****Principal Investigator:**

Kazuo Koga
SMRC/RIEM, Nagoya University

Coinvestigators:

Tadaaki Mano
Yoshihiro Ohta
Keiichiro Tsuji
Takuo Goto
Nagoya University

Ryoji Osaka
Mitsuro Kida
Aichigakuin University, Department of Psychology

How humans obtain visual stability even with posture changes on the ground has been investigated. Visual stability can be categorized as static or dynamic. Static visual stability is concerned with orientation; and dynamic stability is concerned with motion perception. The perception of visual stability is modified by many other sensations, such as somatosensory, vestibular, and muscle tension. We will mainly focus on modifications by vestibular inputs to visual perception produced by eye movements in microgravity.

The Vestibular-Ocular Reflex (VOR) is a well-known characteristic which gives a tight relationship between eye mobility and vestibular afferent inputs. Eye movements also modify dynamic visual perception, such as perceived object motion velocity. The VOR is constantly stimulated under one gram conditions here on Earth. In fact, human beings have been habituated and "programmed" for orientation (visual stability) in their everyday, one gram environment. When humans are exposed to an environment with different gravity vectors, this programmed behavior must change, i.e., it is reprogrammed. This "reprogramming" is called habituation or familiarization. We hope to examine how object motion perception is perturbed in the microgravity environment.

The subject was Japanese payload specialist (JPS): Mamoru Mohri. The experiment was performed three times during the flight, once on mission day one (MD1), on MD2, and again on MD6. All three sessions followed identical protocols. The JPS with the help of MS1 was required for the experiment. The subject of the experiment was the JPS. In-flight data were distributed after the mission. Data analysis was completed and we have several new findings: (1) There are no differences in eye motility with and without gravity; (2) the antigravity muscle in the neck did not show muscle discharge during the task; (3) there is no cooperative behavior in the neck and eye; (4) adaptation processes for the low gravity were not observed; (5) subjective introspection from the JPS showed some disturbance for visual stability; and (6) there were differences between perception and behavior in postflight behavior.

Experiment L-10:**Manual Control in Space Research on Perceptual Motor Functions under Microgravity Condition****Principal Investigator:**

Akira Tada
National Aerospace Laboratory

Coinvestigators:

Masanori Okabe
National Aerospace Laboratory

Microgravity effects on human factors were observed through a series of manual control experiments in space. In space, the human perceptual motor functions including the vestibular, the oculo motor, and the hand movement control systems must be strongly affected by the microgravity condition. Therefore, the characteristics of the human operator may differ from that in the normal ground condition. The purpose of this research is to obtain basic data for designing man-machine systems in space, as well as to investigate human perceptual motor functions. The point of interest was whether there is any operator characteristics difference in space or on ground.

The Japanese Payload Specialist, Dr. Mamoru Mohri, tracked a displayed light spot that moved up and down by operating a joystick to control a double integral controlled element against a pseudo-random disturbance. Each experiment run is for 130 seconds. An entire session is composed of eight formal runs. The session was repeated on L+1, L+3, and L+6 days of the STS-47 Space Shuttle flight. The same sets of experimental sessions were also conducted for baseline data collection —immediate preflight and postflight as ground experiments.

All experiments were conducted in good experimental conditions and at scheduled times. There were slight data acquisition losses but without severe prejudice to analyses.

The most important newly found phenomenon is that the subject felt pain when tracking eye movement during orbital experiments. He was obliged to fix his line of sight at the center of a display and to watch the light spot movement using peripheral view. During these kinds of ground experiments, without exception, the subject's line of sight naturally tracks the light spot movement. Another phenomenon found is that the subject felt difficulty in supporting his body against the reaction force of his hand movement. Apart from a subjective feel of muscle workload, enough design care should be taken to support the operator's body during operations with fast or high frequency body part movements. After landing, a disturbance was observed in the subject's posture. His line of sight was not perpendicular to the display.

Further experiments, including clinical and physiological tests for these phenomena are suggested. A study should be planned and performed as soon as the next space experiment opportunity allows. For development and space use of man-machine systems, the relations between human operator characteristics and control conditions such as controlled element dynamics and forcing function frequency should be established through future space experiments.

Experiment LBNP:**Countermeasure Against Orthostatic Intolerance After Space Flight: The Combination of Oral Fluid Loading and Lower Body Negative Pressure****Principal Investigator:**

John B. Charles
NASA Johnson Space Center

The usefulness of a combined countermeasure against orthostatic intolerance after spaceflight was investigated on STS-47/Spacelab J (September 1992) and five other Space Shuttle missions in 1990-1992. This combined countermeasure includes ingestion of salt tablets (eight, one gram tablets) and water (32 oz.) during the first hour of a four hour decompression in a lower body negative pressure (LBNP) device. The treatment was applied after at least three days in flight (not on the last day in flight), and evaluated by brief LBNP tests one and two days after treatment, simulating re-entry one and two days after treatment. Four subjects (including both Spacelab J participants) completed the protocol as planned. Two subjects completed the four hour decompression, but did not ingest the full fluid load; one subject ingested the fluid but completed only 2.5 hours of the treatment due to timeline conflicts. Data on treatment effectiveness on the other five subjects were lost due to early mission termination (2), hardware difficulties (2), and unexplained factors (1).

Results include the following:

- (1) The treatment restores heart rate responses to simulated orthostatic stress (provided by the LBNP tests) one day after treatment toward preflight values; two individuals were able to complete the LBNP test one day after treatment who could not do so only a few days before.
- (2) Increase in leg volume after the four hour treatment was no greater than after the brief LBNP tests, indicating that fluid sequestration in the legs is probably not a major contributor to the treatment's effectiveness.
- (3) The treatment is at least as effective in women as in men (unlike a prediction based on bed-rest studies).
- (4) Partial protection is still present two days after the four hour treatment.
- (5) While time-consuming, the treatment does not prohibit the crew member from performing other useful tasks requiring mobility.
- (6) Incomplete treatments (LBNP without fluid loading, or fluid loading without four hours of LBNP) are not effective (confirming bed rest findings).
- (7) Baseline LBNP tests in flight confirm the time-course of the loss of orthostatic tolerance first determined during the Skylab missions.

The LBNP countermeasure is intended to provide maximum protection after just one application, on the day before landing. Future flight tests on Spacelabs IML-2 and USML-2, and other flights, will apply the treatment on the day before landing, and evaluate its protective effects during and after the actual stresses of re-entry and landing.

Experiment AFTE:**Monitoring Astronauts' Functional State:
Autonomic Responses to Microgravity****Principal Investigator:**

Patricia S. Cowings
NASA Ames Research Center

Coinvestigators:

William B. Toscano
University of California at Los Angeles

Neal E. Miller
Yale University

Shuttle Flight Experiment Design**In-flight Procedures:**

1. Continuous day-time monitoring: all subjects.
The AFS-2 biomedical monitoring instrument is worn during launch. It is donned during the post-sleep activity period and removed during the pre-sleep activity period of the first three mission days.
2. Time-lined and symptom-contingent diagnostic: all subjects.
Crew members are required to use the diagnostic log books to report symptoms at specified times during the mission day, (i.e., time-lined), and at any time that symptoms increase (i.e., symptom contingent).
3. Time lined and symptom-contingent Autogenic-Feedback Training (AFT) sessions: AFT subjects only.
Treatment crew members are required to perform a one five minute AFT practice session daily (i.e., time-lined) and at any time during the mission that symptoms increase (i.e., symptom contingent).

Postflight Procedures: All subjects

On the day of the landing, a ten minute debrief; Two weeks post-flight, two hour debrief.

The Autogenic-Feedback System-2 (AFS-2). An ambulatory monitoring system as worn by crew members.

AFS-2 Hardware Description**Data Recorded on Cassette Tape:**

- Electrocardiogram (ECG)
- Skin Conductance Level (SCL)
- Expansion of the Abdomen (from Strain Gauge)
- Triaxial Accelerations of the Head
- Skin Temperature
- Blood Volume Pulse (BVP)
- Event Signal (Initiated by Crewmember)
- Greenwich Mean Time (GMT)
- AFT Practice Session Time-Windows

- Hardware Malfunction Information
- Instrument Identification Code

Information Provided On Crew members' Wrist Display Unit:

- Heart Rate
- Respiration Rate
- Skin Conductance Level
- Blood Volume Pulse Temperature
- AFT Practice Session Time-Windows
- Hardware Malfunction Information
- Greenwich Mean Time

Table 1. Self Reports of Symptoms Using the Motion Sickness Diagnostic Scale

| Symptom Code | Description | Point Value | | |
|--------------|-----------------------|-------------|----------|--------|
| | | Mild | Moderate | Severe |
| EA | Epigastric Awareness | 1 | | |
| ED | Epigastric Discomfort | 2 | | |
| NSA | Nausea | 4 | 8 | 8 |
| SAL | Increased Salivation | 2 | 4 | 8 |
| PAL | Pallor | 2 | 4 | 8 |
| SWT | Sweating | 2 | 4 | 8 |
| DRZ | Drowsiness | 2 | 4 | 8 |
| HAC | Headache | 1 | 1 | |
| DIZ | Dizziness | 1 | 1 | |
| TMP | Increased Warmth | 1 | 1 | |
| VMT | Actual Vomiting | | | 16 |

Relevance to NASA's Goal:

Permanent human presence in space

1. To study physiological and behavioral indicators of human adaptation.
2. To use Autogenic-Feedback Training (AFT) to facilitate this adaptation and readaptation to Earth.

Two weeks following the mission, each crew member attends a private debriefing. Here the crew members are provided with graphs of their own physiological data obtained in-flight. Each response is plotted against Mission Elapsed Time (MET), here showing two hours, ten minutes before launch (-L/02:10) to three hours, 30 minutes into the first mission day zero (0/03:30). Physiological data may be related to specific payload activities. These graphs show that the most stressful part of launch is MECO. Data was interrupted as the crew members removed space suits.

Results to Date:

1. Two of the three crew members who received AFT experienced either no symptoms or only minor symptoms during the mission.
2. Two of the three control subjects who took antimotion sickness medication (i.e., received no AFT) experienced multiple vomiting episodes on the first few mission days.
3. Final verification of the efficacy of AFT requires data from space of 16 people (eight treatment and eight controls).
4. Preliminary findings suggest that AFT may be an effective alternative to antimotion sickness medication and produces no deleterious side-effects.

Experiment L-09:

Genetic Effects of HZE and Cosmic Radiation

Principal Investigator:

Mituo Ikenaga
Kyoto University, Radiation Biology Center

Coinvestigators:

Isao Yoshikawa
Moto Kojo
Toshikazu Ayaki
Nagasaki University School of Medicine

Haruko Ryo
Osaka University, Faculty of Medicine

Kanji Ishizaki
Tomohisa Kato
Hanako Yamamoto
Ryujiro Hara
Kyoto University, Radiation Biology Center

The purpose of our experiments is to examine possible effects, particularly the genetic effects, of space radiation on living organisms. For this, we have analyzed mutations induced in fruit flies of the species *Drosophila melanogaster* during the space flight.

Drosophila strains used were wild type strains and a radiation-sensitive strain *mei-41*. Two different developmental stages of samples were sent into space; young adult males to analyze sex-linked recessive lethal mutations and about 36 hour old larvae to detect somatic mutations in wing epidermal cells. For each strain, we have loaded 200 male flies and about 6,000 larvae. They were placed in two fly containers, installed in a low temperature incubator, and then exposed during the entire mission to space radiation.

To detect sex-linked recessive lethal mutations induced in X-chromosomes of male reproductive cells (sperm, spermatogonia, etc.), male flies returned from space were mated to virgin females of a tester strain, and the presence of lethal mutations was detected at the F2 generation.

Table one shows the results of such mutation studies. The frequencies of recessive lethal mutations in the flight groups were two and three times greater for Canton-S and radiation-sensitive *mei-41* strain, respectively, than those in the ground control groups.

Most larvae sent to space emerged as adult flies about ten days after landing. The presence of wing-hair somatic mutations, which give morphological changes in hairs growing on the surface of wing epidermal cells, was analyzed under microscope.

As shown in Table two, the frequencies of wing-hair mutant spots were similar between flight and control groups for Canton-S-derived strain and another wild type strain *Muller-5*. By contrast, for *mei-41* strain the mutation frequency was lower in the flight group than in the control group.

The observed higher frequency of lethal mutations in the flight group might be due to a possibility that radiation effects on reproductive cells could be greatly enhanced under microgravity. However, if this were the case, we do not have any appropriate explanation for the apparent absence of such synergistic effects on the somatic wing-hair mutation system.

In order to estimate human radiation risk during the future long-term space journey, it is urgently needed to prove or disprove possible synergistic effects of radiation and microgravity by carrying out more and more precise experiments in space.

Table 1. Frequency of sex-linked recessive lethal mutations

| Strain | Experiment Group | No. of X-Chromosomes Examined | No. of Lethal Chromosomes | Frequency (%) |
|--------------|------------------|-------------------------------|---------------------------|---------------|
| Canton S | Flight | 9,176 | 22 | 0.240* |
| | Ground | 9,177 | 11 | 0.120* |
| mei-41 | Flight | 9,355 | 37 | 0.396** |
| | Ground | 8,975 | 12 | 0.134** |
| * $P < 0.05$ | | ** $P > 0.01$ | | |

Table 2. Frequency of wing-hair somatic mutations

| Strain | Experiment Group | No. of X-Chromosomes Examined | No. of Lethal Chromosomes | Frequency (%) |
|--|------------------|-------------------------------|---------------------------|---------------|
| Wild Type Canton-S derived | Flight | 2,398 | 124 | 5.17 |
| | Ground | 2,384 | 132 | 5.54 |
| <i>Muller-5</i> | Flight | 2,226 | 78 | 3.50 |
| | Ground | 2,240 | 52 | 2.32 |
| Radiation- Sensitive <i>mei-41</i> | Flight | 1,188 | 69 | 5.81 |
| | Ground | 1,170 | 111 | 9.49 |

Experiment L-11:**Studies on Biological Effects of Cosmic Radiation and Development of Radiation Protection Technology****Principal Investigator:**

Shunji Nagaoka
NASDA, Space Experiment Group

Coinvestigators:

Tadayoshi Doke
Takao Hayashi
Waseda University

Koichi Ogura
Hiroaki Yamada
Nihon University

Tan Takahashi
Fumio Yatagai
RIKEN; Institute of Chemistry and Physics

Osamu Yato
Kagoshima Agricultural Experiment Station

Masanobu Ishikawa,
Fumio Takashima
Tokyo University of Fisheries

Hiroshi Tanooka
National Cancer Research Center

Kazuki Harada
PL Botanical Institute

Takeo Ohnishi
Nara Medical College

High energy proton and HZE are of great concern for space flight crews because they easily penetrate into the spacecraft and generate secondary radiation by interactions with inside materials. Unpredictable solar activity is also a remarkable and intense radiation source, sometimes 100 times greater than the average level. Although much data have been accumulated from the past manned and unmanned space flights, the detailed mechanism of the biological effects of such radiations is not yet resolved. In this experiment, a primary objective is to investigate the biological effect of cosmic radiations with various biological systems, in conjunction with the physical measurements and characterization of the intra-vehicle radiation environment during the STS-47 (Spacelab J) mission.

The equipment used is called "Radiation Monitoring Container and Dosimeter" which are passive dosimeter systems. Two Radiation Monitoring Containers included plastic nuclear track detectors called "Hartslus" and biological sample stacks. The Hartslus was an equivalent polymer to CR-39, but the sensitivity and surface property for chemical etching were improved by adding 0.01% of antioxidant, Naugard 445. Both containers were set at the aft-end cone of the Spacelab module approximately eight hours after the launch. The dosimeter was a flat aluminum package which included the track detectors and two types

of thermoluminescent dosimeters (TLD), LiF and MgSiO₄-Tb(MSO). Thirteen dosimeters were set at seven locations in the Spacelab and middeck area to evaluate low LET radiation environments.

Biological specimens used were two types of F1 plant seeds of heterozygotes at chlorophyll locus, maize seeds (yg^{2+}), and soybean seeds strain L65, and dry brine shrimp eggs (*Artemia salina*), 13 kinds of bacterial strains including *E. coli*, *B. subtilis* and *D. radiodurans* and shuttle vector plasmid DNA (pz189). Eleven *E. coli* mutants were used. The mutants lacked DNA repair systems in different enzymes and degree of the deficiency (Table two). The mission duration was approximately eight days, and all flight and ground control samples were transferred to Japan without additional x-ray exposure during the transportation.

LET Distribution of HZE in Spacelab

A typical LET spectrum obtained from the track detectors placed in *E. coli* stack provided 2.8 g/cm³ to 5.6 g/cm³ as local shielding. Additionally the calculated LET spectra behind shielding of 20 g/cm³, 30 g/cm³, 40 g/cm³ is based on the results of Spacelab D-1 mission in October 1985. The LET spectrum in the Spacelab was almost equivalent to the high LET radiation environment obtained from the past Spacelab missions of the same orbital inclination and altitude, D-1 and IML-1. Absorbed dose rates of the track detectors in the dosimeters at various locations were calculated from the LET distributions and listed in the Table one (Dc).

Low LET Radiation Environment in Spacelab

Of the two types of TLDs used, MSO provided consistent values with those measured during the past missions aforementioned, whereas LiF, however, gave unreliable data with very large variances. The absorbed dose rates obtained from MSO are listed in Table one (Dt). The values measured at different locations in the Space Shuttle or Spacelab varied over 30%; clearly reflecting the total shielding behind the dosimeter, where A-F were located at aft-end cone, G: rack number seven blank panel, H: in middeck locker, I-K: in incubator with fly container, L: fungi growth chamber, and R: in middeck Refrigerator/Freezer.

Absorbed Dose Rate and Dose Equivalent Rate

Absorbed dose and dose equivalent rates are summarized in Table one. The dose equivalent (Ht) values were calculated assuming the weights of LET dependent quality factors proposed by the ICRP(1971)².

Table 1.**Absorbed Dose Rate and Dose Equivalent Rate**

| TLD (MSO) | Dt (mrad/day) | Dc (mrad/day) | Hc (mrem/day) | Ht (mrem/day) | Ht/Dt |
|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------|
| A001 | 17.5 | 1.62 | 8.83 | 24.7 | 1.41 |
| B002 | 16.9 | 1.11 | 6.27 | 22.1 | 1.31 |
| C004 | 15.4 | 1.22 | 7.70 | 20.9 | 1.36 |
| D006 | 16.3 | 1.56 | 9.84 | 24.6 | 1.51 |
| E007 | 15.0 | 1.05 | 6.03 | 20.0 | 1.33 |
| F008 | 15.7 | 1.13 | 7.05 | 21.6 | 1.38 |
| G009 | 15.2 | 1.72 | 8.93 | 22.4 | 1.47 |
| G010 | 13.5 | 1.15 | 4.39 | 16.7 | 1.24 |
| I011 | 14.3 | 1.22 | 6.66 | 17.7 | 1.24 |
| J012 | 13.5 | 1.15 | 6.56 | 18.9 | 1.40 |
| K013 | 13.0 | 0.77 | 4.60 | 16.8 | 1.29 |
| L014 | 13.8 | 1.14 | 6.69 | 19.4 | 1.40 |
| R015 | 12.3 | 1.05 | 5.30 | 16.6 | 1.35 |
| Average | 14.8 | | | 20.2 | 1.36 |

Pycnosis in *Artemia* Embryo

Frequency of pycnosis which appeared in *Artemia* embryonic cells as well as the hatching rate was analyzed. The eggs, which were hit by HZE, were selected from the tracks analysis in the sample stack. The pycnosis in the embryo was found to be significantly increased in the flight specimens in which the eggs were hit by HZE.

Tetracycline Resistance in *B. Subtilis*

The spores of *B. subtilis* ISW1214 transformed with shuttle vector plasmid pHY300PLK were evaluated in the radiation effects by the efficiency of germination and the tetracycline (Tc) resistance. Both viability and Tc resistance were decreased in the flight sample, especially in the Tc resistance (88% : ground control/ 68% : flight sample). The result suggested that the plasmid DNA could not replicate due to damage on the plasmid DNA itself and/or vigorous damage of chromosomal DNA.

Table 2.**List of *E. coli* Mutants**

| | | | |
|---------|---|-------------------|---|
| H/r30R | (argF-amber) | Hs30R | (argF-amber, uvrA-) |
| NG30 | (argF-amber, recA-) | KMBL3835 | (trpE9777-) |
| KY383 | (trpE9777-, lexA3-) | KY385 | (trpE9777-, recA56) |
| KY386 | (trpE9777-, uvrA6-) | pol ^{ts} | (pol ^{ts} andexo ⁺ , recA ^{ts}) |
| polA107 | (pol+, 5'->3'exo-, 3'->5'exo+, recA ^{ts}) | | |
| polA1 | (pol-, 5'->3'exo+, 3'->5'exo-, recA ^{ts}) | | |
| resA1 | (pol-, 5'->3'exo-, 3'->5'exo-, recA ^{ts}) | | |

***E. coli* Mutants and Shuttle Vector Plasmid DNA**

Among those mutants, resA1 strain which lacks all enzyme activities of DNA polymerase and 5'->3' and 3'->5' exonucleases was found to be the most sensitive to the space environment. The averaged survivability of resA was decreased to 8%, whereas the ground control kept it more than 90%. The order of the sensitivities in lethality of these strains in space was resA1, polA1, polA107 (42°C) and polA107 (37°C), where recA gene is inhibited at 42°C and expressed at 37°C. The other strains such as NG30 or Hs30R were almost insensitive to the space environment. The results strongly suggested that DNA polymerase and 3'->5' exonuclease may be the highest enzymatic contribution to repair the cosmic radiation damage of DNA. In contrast, NG30 was known to be extremely sensitive to UV irradiation and Hs30R was hyper-mutable to UV irradiation³. This evidence also indicated that the UV endonuclease activity may not be effective to repair the DNA damages due to HZE. The shuttle vector plasmid DNA pz189 was originally employed to investigate specific mutation locus on the suppressor tRNA gene (supF). The agarose electrophoresis of the plasmid DNA, however, showed drastic degradation in all of the flight samples, whereas the ground control still formed clear bands. The results suggested that the unexpected heavy degradation might have happened during the space flight because none of the enzymatic repair mechanism exists in the plasmid itself.

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Experiment L-03:**Separation of Biogenic Materials by Electrophoresis under Zero Gravity****Principal Investigator:**

Masao Kuroda
Osaka University Medical School

Coinvestigators:

Tatsuya Tanaka
Hirosi Wada
Kunio Tagawa
Osaka University Medical School

Mitsuro Uozumi
Osaka Prefecture Public Health Institute

Among diverse methods for separation of biomolecules for analytical and preparative purposes, electrophoresis may be one of the most efficient methods.

At present, various types of apparatus for preparative (free-flow) electrophoresis are commercially available, but their separation efficacy is still unsatisfactory. The most serious problem that limits the resolving power of electrophoresis is a convective flow by joule-heat production occurring during electrophoresis. This leads to instrumental constraints including strict temperature control of a separation chamber, and reduction in the thickness of the chamber. The latter further decreases sample loading capacity, thereby restricting the application of the electrophoresis to preparative work.

Under the near zero gravity in space, the convection is negligible and hence the separation efficiency may be significantly improved.

The purpose of this research is to establish optimal conditions for the separation of biomaterials, such as proteins, peptides, and nucleic acids, by free-flow type electrophoresis under near zero gravity in space. We modified a conventional electrophoresis apparatus to be suitable for the use in space, and the separation conditions in space were compared with those on Earth using standard proteins.

Separation capability of a newly developed free-flow electrophoresis equipment was compared between in space and on Earth as regards the following experimental parameters:

- concentration of sample
- flow rate of solvent
- voltage of electrophoresis

The mixtures of cytochrom c (from a horse heart), conalbumin (from chicken white), and Bovine serum albumin (from bovine serum) were used as standard proteins.

Although our free-flow electrophoresis experiment was scheduled, the signals from a UV monitor could not be sent to us because of some trouble with the UV detector. Accordingly, the duration of the experiment was shortened.

We had intended to check the performance of the new instrument by preliminary experiments in our laboratory as regards the following points:

- sample injection position
- separation capability
- maximal resolution obtained at the highest voltage available

Unfortunately, the preliminary check on the instrument could not be performed because of a marked delay on constructing the new instrument.

The proteins separated by the electrophoresis were collected by a fraction collector, and then each fraction was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Free-flow electrophoresis experiments in space were performed for the first time. It is difficult to judge whether the separation efficiency of free-flow electrophoresis is higher in space than that on Earth since we unexpectedly spent a lot of time developing the new equipment. Thus, we have not yet evaluated the new instrument thoroughly in our laboratory.

However, electrophoresis in space theoretically has several advantages. Further improvement of the apparatus and the accumulation of data in space may provide as reliable results.

Experiment L-08:**Electrophoretic Separation of Cellular Materials under Microgravity****Principal Investigator:**

Teruhiko Akiba
The Institute of Physical and Chemical Research

The objective of the L-08 experiment was to investigate the separation of cellular materials using the vertical free flow electrophoresis unit (FFEU) and to evaluate its usefulness for purification of biochemical substances in space. Microgravity in space may eliminate two major gravity effects which adversely affect the resolution of samples in electrophoretic separation, i.e., sedimentation and thermal convection. Another objective was to determine if FFEU hardware functions in the orbiter as planned.

FFEU offers distinct advantage of continuous separation of charged molecules (enzymes, hormones) and cells (microorganisms, animals, plants, and organella). FFEU under microgravity is expected to give a better resolution of samples than under a terrestrial environment. Separation of bioactive cells is receiving strong interest from chemical and pharmaceutical research communities. Separation of denatured cells from active cells is also important in medical areas.

Several different types of cells and molecules have been examined using electrophoresis under microgravity. *Salmonella typhimurium* LT2 strains SL1027, SL3749, and SL1102 were used as cellular samples for the FFE experiment. These strains have different structures in the lipopolysaccharide layer of their cell envelope resulting in variant net surface charges to the cells, which also cause a difference in the sensitivity to antibiotics. The three strains were grown separately on 0.5% polypeptone medium at pH7.0 and 37°C for 16 hours. The cells were harvested, washed and suspended in 10 mM triethanolamine-acetate buffer (pH 7.5).

The experiment under microgravity was performed on schedule excluding a malfunction of real-time monitor of optical density (OD). All the preliminary experiments were, therefore, eliminated. An extra run was done on the extended mission day. All fractions collected in orbit were stowed in a refrigerator.

The sample fractions recovered were immediately analyzed for OD and cell numbers. No remarkable changes in OD measurements were detected in the run at 200V. The viable cell count of the separated fractions at 300V indicated that two strains SL1027 and SL3749 were separated into the two peaks. The strain SL1102 overlapped with the elution peak of SL1027. The migration distances of the two strains were consistent with predictions from the results of preliminary studies.

It was hard to get conclusive evidence that the electrophoretic separation under microgravity gave remarkably better results than that under ground conditions. Further investigation into FFE in space is necessary to ascertain effectiveness of microgravity on electrophoretic separation.