COMMERCIAL INVESTIGATION RESULTS FOR THE GENERIC BIOPROCESSING APPARATUS flown on UNITED STATES MICROGRAVITY LABORATORY - 1

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INTRODUCTION

The GBA payload was developed by BioServe to support the commercial flight development needs of our specialized consortia comprised of business, academic and government entities. The consortia have commitments to explore commercial opportunities in bioprocessing, biomedical models and closed agricultural systems. In addition, some members of BioServe have interests in the development and/or qualification of enabling flight hardware used in life sciences space flight testing. Some business and academic entities have interests in more than one of these consortia. To aid in payload development, flight, and analysis, each consortium member contributes resources ranging from proprietary expertise and materials, to hardware and cash. Professionals from business, academia and government often interact with each other via graduate research assistants who do much of the "hands-on" payload preparation and subsequent data analyses.

The GBA supported research, testing, and development activities for each different BioServe consortium. It produced an environment in which professionals from diverse backgrounds came together with a single focus. And, it provided a truly novel learning environment for a youthful new cadre of space professionals committed to the exploration of commercial opportunities presented by space.

Since the GBA supported a large number of different experiments, this paper will briefly describe the payload characteristics and the essential operations of the payload. Then a summary of the experiments will be presented. Finally, a few of the experiments will be described in detail highlighting some novel effects of space flight on life science systems. Portions of the reported work have or will appear in appropriate archival journals as cited in the bibliography. In several instances, data collected from USML-1 has been supplemented with related data collected on more recent STS missions.

I. PAYLOAD

The GBA was designed as a generic research tool which supports a wide variety of life sciences research. Using simple hardware, experiments can be supported in biochemistry, biophysics, microbiology, cellular biology, developmental biology and physiology. In any given mission, as many as 30 different experiments might be performed using in excess of 400 individual samples.

The GBA was designed from the beginning to support numerous bioprocessing investigations. To accomplish this goal, the hardware was designed based on relatively simple but common laboratory equipment. Similarly, procedures were written based on typical methods applied in a biology lab. This "generic" approach has led to a multiple use instrument that could support a new compliment of experiments with each flight (Luttges, 1992). The hardware has also been intentionally designed to ease the process of changing instrument configuration for subsequent flights so that specialized investigations can be accommodated.

Based on existing operational procedures, the experiments supported by the GBA can be subdivided into molecular, cellular and developmental categories. Molecular experiments are focused on biochemical or biophysical reactions that occur in a bulk fluid. Cellular experiments are those focused on living cultured cells whether they be microorganisms such as bacteria or algae or cells derived from mammalian tissues. Developmental experiments are those focused on whole intact organisms such as germinated seedlings, brine shrimp or miniature wasps.

Assembly of synthetic materials from collagen molecules is an example of molecular processing. Such materials, if formed under the right conditions to yield appropriate structures, could be used as artificial skin, blood vessels, cornea and other biomedical implants. In nominal gravity, formation of collagen materials is disturbed by convective mixing and sedimentation. Collagen materials formed under such conditions do not exhibit the strength and resilience of naturally produced materials. Reduced gravity provides a unique environment to assess the contribution of convective flows and sedimentation on collagen material characteristics. Space may provide conditions under which to manufacture improved biomedical implant materials.

Other molecular experiments include the assembly of liposomes and virus capsids, two different types of microscopic spherical structures that could be used to encapsulate pharmaceuticals. Both of these structures have the potential to be used as targeted drug delivery systems. Preliminary flight experiments have shown that these structures are produced with much greater efficiency in microgravity.

Protein crystals can also be grown using the GBA hardware. If grown to a large enough size and high quality, protein crystals can be analyzed to provide information on the structure of the specific protein. Such information could be used to understand the function of important proteins and design

drugs to inhibit or enhance the function depending on the desired result. The modified Fluid Processing Apparatus used to support protein crystal growth provides additional methods through which to achieve space flight crystal growth.

The GBA can also be used to study the effects of microgravity on cellular systems. For example, microorganism physiology can be studied. These experiments will help to lay the foundation for developing effective ecological waste treatment and water recovery systems for use in space and may lead to a greater understanding of gravitational effects at the cellular level. Such experiments may also shed insight into the alterations in bacteria and other microorganisms that might create health problems for crew members exposed to long duration space flight such as required for a Mars mission. At the same time these evaluations can determine whether space provides a new set of opportunities for achieving emerging novel biotechnological goals such as electroporation and cell fusion.

Experiments have been supported using immune cells such as lyphocytes and macrophages. Previous work has shown significant alterations to immune function in reduced gravity. Experiments can be done at the cellular level to better characterize the mechanism underlying the observed alterations. In addition, such information may shed greater insight into immune diseases that occur on Earth. Space flight immunological alterations may provide the basis for new immunological models in which to test therapies and to evaluate novel diagnostics.

In developing space flight biomedical models, cultured bone cells can be studied to learn more about the mechanism underlying bone mass loss observed in astronauts exposed to long duration space flight. Such information may lead to effective countermeasures that could be of tremendous benefit to crew health. Such countermeasures may also be effective in the treatment of osteoporosis and other bone diseases here on Earth.

The GBA also supports experiments on small organisms. For example, experiments are being done to evaluate seed germination and development in space. This work lays the foundation for developing the technology necessary to grow higher plants in space. It may provide knowledge for use in the closed agricultural industry where greenhouse crops will be more common in the fresh vegetable and flower markets as well as the plant-based pharmaceutical markets.

Other experiments focus on the development of small organisms during exposure to reduced gravity. In the brine shrimp, for example, starting from a cyst, this organism shows significant development during a typical space flight mission. Even though the brine shrimp is a simple organism compared with humans, such experiments may provide fundamental information on the importance of gravity in development and aging processes. Currently being used as a model for toxicological assessments in terrestrial laboratories, the brine shrimp can be used for toxicological assessments in flight, as well. Of similar importance are studies of miniature wasps. Again, these small organisms are

easily maintained in space, they are rapid to mature and they provide a test bed for chronic toxicological examinations. These organisms are also useful in assessing genetic materials inserted by a variety of parasitic mites.

II. Hardware Description

The GBA consists of three different hardware elements including the Generic Bioprocessing Apparatus (GBA), the Refrigerator/Incubator Module (R/IM) and a stowage locker. Each experimental sample is contained in multi-chambered device called a Fluids Processing Apparatus (FPA) (Fig. 1). FPAs were housed in sets of 12 (Fig. 2) in both the R/IM (Fig. 3) and the stowage locker.

FPAs are processed inside the GBA (Fig. 4). The GBA contains a temperature controlled Incubation Module that can hold twelve FPAs in individual incubator cylinders. Temperature is controlled from ambient up to 60°C through sensor feedback and heater control. Three independent electronic controls are used to virtually eliminate the possibility of overheating samples. Touch temperature indicators on the GBA front panel inform the crew if the FPAs are below 45°C and can be safely handled.

Optical sensors and light emitting diodes have been built into the incubation cylinders. The sensors generate signals based on turbidimetric properties of the samples contained in the FPAs. Such measurements relate to reaction kinetics, cell growth or organism behavior depending on the specific experiment. A Data Acquisition and Control Processor (DACP) controls data collection and storage. Each set of twelve FPAs can be treated in a different manner depending on the applicable crew procedure and specific software routine written for the DACP. The DACP is operated using a keypad and display located on the GBA front panel.

The crew can communicate with the DACP through a standard RS-232 interface. Using the Payload and General Support Computer (PGSC), software routines are uploaded into the DACP and data can be downloaded from the DACP. Downloaded data files were copied onto floppy disk for analysis following the mission and were downlinked to ground personnel for evaluation during the mission.

The GBA occupies the space of one middeck locker and attaches to a single payload mounting panel. The outer shell containment module is the only safety critical structure and encloses all other components. The GBA uses a maximum of 110 Watts of 28 volt D.C. electrical power.

The R/IM also occupies the space of a middeck locker and requires 90 Watts of 28 volt power (Fig. 3). The R/IM was operated at 22°C. The R/IM housed 8 sets of FPAs.

A vent port for cooling is located on the left side of the R/IM and must remain unobstructed for proper temperature control. The R/IM with contents was installed in the middeck approximately 24 hours before launch. This late access was necessary due to the relatively short viability of the biological samples. Power was maintained to the R/IM during both ascent and descent of the Shuttle.

Additional sets of samples were stowed in an ambient locker. Generally, these samples were not as temperature critical. These sets were also late loaded at approximately 24 hours prior to flight.

The Fluids Processing Apparatus consists of a glass barrel and four sliding seals that separate three chambers (Fig. 1). Generally, an experiment consisted of some type of process precursor, initiation and termination fluids contained in the three chambers. Up to 8 mls of fluid can be distributed amongst the FPA chambers. An outer Lexan sheath provides a second level of containment. For hazardous fluids, a third level of containment was in the form of a clear flexible bag.

Operation of the FPA is simple (Fig. 5). By pushing the plunger, the rubber seals and fluids can be moved forward in the glass barrel. As the column moves forward, one of the seals reaches a bypass formed into the glass barrel wall. Continued activation of the plunger transfers the fluid in chamber B via the bypass into chamber A. Following processing in the GBA for a specified period of time, the plunger can again be pushed to transfer fluid from chamber C into chamber A. This was typically done to terminate an experiment using an appropriate fixative or process inhibitor.

III. Payload Operations

Any of four different sample processing procedures were applied to a set of FPAs. These procedures were optimized to different classes of experiments as discussed above. These procedures are summarized as follows.

A. Sample Processing - Molecular. A set of FPAs is removed from stowage, activated by mixing the A and B chambers and inserted into the Incubator Module of the GBA. Temperature is maintained at the desired setpoint and turbidity measurements are obtained at regular intervals from a few seconds to a few minutes. Following incubation times up to 24 hours, the FPAs are removed from the GBA, terminated and returned to stowage.

The DACP data file is then downloaded to the PGSC for backup or downlinked to ground personnel. Additional sets of FPAs were processed sequentially using this procedure.

B. Sample Processing - Cellular. Similar to molecular processing, a set of FPAs is removed from stowage, activated and placed in the GBA. Under this procedure, however, samples are monitored only briefly and then removed from the GBA and stowed. Activation and monitoring is repeated for additional FPA sets processed under this procedure.

At approximately daily intervals throughout the mission, the cellular FPA sets are returned to the GBA and monitored. Depending on programmed information or on turbidimetric values, the DACP will inform the crew which, if any, FPAs are to be terminated on a given day. In this manner, individual FPAs in a set were terminated at different times on orbit.

Data files were downloaded to the PGSC on a periodic basis throughout the mission. This procedure was designed for cellular growth experiments where the expected growth occurs over a period of days.

C. Sample Processing - Developmental. Under this procedure, two FPAs from each developmental set are removed from stowage and activated. At periodic intervals during the mission, sequential pairs of FPAs are activated. By the end of the mission, all FPAs in the set have been activated but at different time points. Thus, an experimental time course is obtained for the biological system under study.

This procedure was designed to study developmental changes in whole organisms such as germinated seeds. The samples in each pair of FPAs were at different stages of development. After all FPAs had been activated, the crew recorded video images of the specimens and then terminated the FPAs.

D. Sample Processing - Undisturbed. Under this procedure, a set of FPAs is removed from stowage, activated and returned to stowage. Towards the end of the mission, the FPA set is removed from stowage, terminated and again returned to stowage.

This procedure was optimized for molecular reactions that require long reaction times and minimal disturbances. Protein crystal growth was an example of this type of experiment.

IV. Configuration and Operations on USML-1

The maiden flight of the Generic Bioprocessing Apparatus (GBA) took place June 25-July 8, 1992 aboard Space Shuttle Columbia as part of the first United States Microgravity Laboratory (USML-1). The record setting 14 day mission provided opportunities for a variety of investigations in biomaterials, biotechnology and life sciences. During the mission, more than 20 separate investigations were performed using 132 Fluids Processing Apparatuses (FPAs) (Table 1). An additional investigation was performed in the Glovebox facility using the Directed Polymerization Apparatus (DPA).

Some of the FPAs (36 total) were prepared for flight approximately 4 months prior to launch. These samples were stowed in the Spacelab Module under ambient temperature conditions. The remaining FPAs (96 total) were prepared, stowed in a Refrigerator/Incubator Module (R/IM) set at 22°C and turned over for integration into Columbia's middeck approximately 24 hours before launch. Preparation of late-access samples was done as planned without significant difficulty.

Following launch, payload operations were supported around the clock by BioServe personnel at the Payload Operations and Control Center (POCC) in Huntsville. Information and data obtained at the POCC were disseminated from there to appropriate investigators located around the country. Using such information, these investigators were able to make recommendations for changes in crew operations or timelines that might improve the science return from their investigations.

From both an operations and science return perspective, excellent payload success was achieved. All of the FPAs were activated and terminated according to the schedules laid out by the investigators and mission support personnel. Some anomalies did occur during the operation of a few of the FPAs. However, only two FPAs failed such that no data were obtained. The remaining 130 FPAs (98.5%) were operated successfully to yield data and samples for analysis.

During the mission, many of the FPAs were transferred from stowage to the GBA for on-orbit collection of optical density data. For many investigations, these data provided an indication of the rate at which a process occurred. Such rates were compared with the rates obtained from ground controls. Data collected in the GBA was transferred by the crew to the Shuttle Payload General Support Computer (laptop) and downlinked to the ground using the Orbiter data modem capability. Once received in the POCC, data was reviewed to determine experiment progress and success as well as to make decisions of when a certain reaction should be terminated.

Within three hours following landing at Kennedy Space Center, the R/IM containing the samples was removed from the Orbiter and transported to Hangar L for deintegration. Samples were photographed, packaged and shipped to each of the investigators. Since nearly all of the samples were preserved on-orbit prior to reentry, no specific processing or analysis was performed at Hangar L. For a few samples, high resolution photographs were obtained using microscopes with camera attachments. Approximately 40 hours after landing, the samples stowed in the Spacelab module were removed and transported to Hangar L. Again, FPAs were photographed, packaged and shipped to appropriate labs for analysis.

Results from some of the GBA investigations are presented in the remainder of this report.

V. Effects of Space flight on Tumor Necrosis Factor-alpha-Induced Cell Lysis

Dr. Keith Chapes

Tumor necrosis factor-alpha (TNF) is an important regulatory and immunological hormone. For example, TNF is involved in the direct rupture of virus-infected cells such as with pox-, adeno- and herpes simplex infected cells. Rupture of virus-infected cells involves the binding of TNF to a receptor on the surface of the cell. Subsequently, several biochemical second messenger systems are activated which ultimately induce rupture of the infected cell.

Previous investigations have suggested that clinorotation and reduced gravity can affect cellular and biochemical processes in the immune system. The present experiment was designed to determine whether TNF-mediated cell toxicity is different in microgravity than in a 1 x G environment (Chapes et al, 1992). This information should aid in learning more about the functional activity of TNF and about the signaling/receptor processes of space flight cells.

Space flight has been shown to affect the human immune system. Knowledge from this investigation should provide a better understanding of immunological changes in space flight and may lead to countermeasures that could be employed by astronauts and by people on Earth with compromised immune function. New models of TNF function are essential in expanding the medical potential of all tumorigenic pharmaceuticals.

A. Methods:

A common mammalian cell line (LM929) was used for this investigation. Cells were attached to Cytodex beads suspended in cell medium and loaded into the A chamber of six FPAs. Medium containing TNF was loaded into the B chamber of half of the FPAs. Medium alone was loaded into the remaining FPAs. Chamber B also contained ^{3}[H]-thymidine to measure cell proliferation. Fixative was loaded into the C chamber. FPAs were shipped to KSC for final integration into the payload.

Cells were exposed to TNF approximately 14 hours following launch by mixing the contents of the A and B chambers. The cells were fixed 29 hours later.

Following sample return, the number of cells remaining on the beads was determined. The amount of radioactive thymidine incorporation exhibited by the cells was also quantified.

B. Results:

Results clearly showed that TNF inhibited the incorporation of labeled thymidine into the LM929 cells by 22-59% in the simultaneous ground controls, as expected (Fig. 6). In contrast, essentially no inhibition was observed in the space flight cells. In addition, LM929 cells that were not exposed to TNF incorporated more labeled thymidine than equivalent ground controls.

C. Conclusions:

The incorporation of thymidine by the LM929 cells demonstrate that the cells were healthy at the time the experiment was conducted. The near total loss of inhibition by TNF during space flight was surprising. Two possible conclusions can be drawn from the data. Space flight inhibited the toxic activity of TNF or the samples were subjected to circumstances that did not allow for TNF-mediated toxicity. Additional flight experiments have been completed which confirm the results from USML-1 and show that inhibition of TNF toxicity is related to protein kinase C (Chapes et al, 1993).

VI. Viral Protein Assembly in Microgravity

Dr. Richard Consigli

The effects of convection-driven flows, sedimentation, buoyancy and other fluid phenomena are greatly reduced in microgravity. Thus, microgravity affords the opportunity to investigate molecular self-assembly processes in the absence of these disruptive forces.

This investigation focuses on the self-assembly of polyomavirus major capsid protein (VP1) (Fig. 7). From the BIMDA payload on STS-37 it has been shown that formation of virus capsomeres in space flight is more homogeneous and more efficient compared with ground control formation of these capsomeres (Chang et al, 1993) (Fig. 8). This investigation was to extend the results by focusing on the assembly of complete virus capsids, the outer protein shell of virus particles, beginning with the capsomere subunits.

Virus capsids have significant potential for use in targeted drug delivery. Under the right conditions, virus capsids can be formed to encapsulate a pharmaceutical or gene vector. These special capsids might then serve as carriers to deliver a drug to a specific tissue. As the virus capsids "infect" the tissue, the contents would be introduced directly into the interior of the cells. Thus, virus capsids may find application for the treatment of many diseases including cancer and genetic disorders.

A. Methods:

In one FPA, formed capsomeres were loaded into wells of a special low-volume insert in chamber A. Additional insert wells were loaded with a combination of formed capsids and capsomeres. A buffered solution containing calcium, designed to induce capsid formation, was loaded in chamber B. Two FPAs were used to study the effects of pH (5.0 and 7.0) on the capsid formation process.

The two FPAs were activated 12 hours after launch. Capsid formation was allowed to proceed through the full mission duration. Following sample return, the VP1 products were prepared for transmission electron microscopy. The number of capsomere and capsid units were determined and compared with equivalent samples processed on the ground.

B. Results:

Results from ground controls demonstrated that numerous capsids could be formed in the FPA hardware starting from either capsomer or combination capsomere/capsid samples. In sharp contrast, capsids were not observed in space flown samples. In fact, no capsids could be identified even from flight samples that initially contained capsid structures. Further, capsomeres exposed to extended reduced gravity were amorphous and swollen compared with ground samples.

C. Conclusions:

The results from this investigation are consistent with observations made following STS-37 (Chang et al, 1993). But, it appears that microgravity significantly interferes with virus capsid formation. The specific mechanism is unknown but may relate to effects of gravity on VP1 folding or availability of calcium, an essential component of capsid formation. Further investigation has been undertaken to understand these intriguing results.

VII. Brine Shrimp Development in Space

Dr. Brian Spooner

The brine shrimp is well suited to study the effects of space flight on embryonic and larval development. Shrimp development can be arrested in the cyst stage until activation with salt water. Then, significant development can occur during the typical Shuttle mission durations. This investigation is an extension from proof-of-concept experiments performed using the BIMDA payload flown on STS-37 and STS-43 (Spooner et al, 1993).

Brine shrimp are a major aquaculture food source, are used agriculturally as a quality protein source for livestock and can serve as a human food source. They may serve as a major component to a ecological life support system since they can graze on algae and provide animal protein to higher organisms. Before employed in this manner, however, the effects of microgravity on their development and growth must be understood. Such knowledge may also have application to the development and aging process of other animals, including humans. As noted above, the brine shrimp is currently a model for drug toxicological assessments; a use that can be extended to space flight evaluations.

A. Methods:

Dehydrated brine shrimp cysts along with a dried food source were loaded in the A chamber of eleven FPAs. Sufficient air space was provided as an oxygen source for the shrimp. Salt water was loaded into the B chambers and fixative into the C chambers.

Brine shrimp development was initiated at approximately 15 hours and 1 day 12 hours into the mission. Fixation was done at different time points in the mission. Thus, brine shrimp were obtained after embryonic, at larval and at pre-adult stages of development. Simultaneous ground controls were run for direct comparison with flight specimens.

Video recordings were obtained on-orbit using the Spacelab camcorder to provide data on shrimp swimming behavior.

Following sample return, brine shrimp were analyzed using light, phase-contrast, differential interference, fluorescence, transmission electron and scanning electron microscopy.

B. Results:

Data from one of the flight FPAs was lost as a result of fixative being introduced during activation. From the remaining flight FPAs, 87 fixed brine shrimp were obtained. The hatch rate for the cysts was approximately 40% which was expected based on ground data and previous flight results. Larval morphology determined from scanning and transmission electron micrographs were not found to differ between flight and ground organisms. The major effect of microgravity was found to be an accelerated rate of development (Fig. 9). As early as 2.25 days after initiation of growth, space flight brine shrimp development was accelerated by one full stage (Spooner et al, 1993).

C. Conclusions:

Based on the preliminary results, it is clear that brine shrimp development during space flight occurs and can be assessed using the FPA hardware. Remarkably, space flight was found to significantly increase the rate of brine shrimp development without altering the organism size or morphology. Additional flight and ground research is being directed at understanding the mechanism for the accelerated development.

VIII. Seed Germination and Early Plant Morphology in Microgravity

Dr. Marvin Luttges

Microgravity provides a unique research environment for the study of gravitational effects on plants otherwise not possible on Earth. In addition, plants would play a central role in future bioregenerative life support systems. Such life support systems will be needed if long-term space exploration is to be undertaken in a cost effective manner. Before higher plants can be routinely grown in reduced gravity, gravitational effects on plant growth and performance must be understood. This investigation (see also: Hoehn, 1993; Hoehn et al, 1992) uses a 'whole plant' approach and focuses on characterizing the effects of microgravity on seed germination and early plant morphology of two legumes, alfalfa (Medicacgo sativa) and white clover (Trifolium repens). The experiments were a continuation of studies performed during STS-37 and STS-43 experiments (Abrahamson et al, 1991).

Leguminous plants are capable of obtaining required nitrogen from the air through a symbiotic relation with rhizobia bacteria. Legumes therefore do not require synthetic nitrogen fertilizer and may be particularly useful in the development of larger bioregenerative life support systems. Further, knowledge gained from the study of legumes may lead to the development of methods to transform other plant species, such as wheat, to be less reliant on fertilizer. The use of space flight to help produce new plant materials can be extended to those plants currently used as sources of pharmaceuticals, such as taxol and vinca alkaloids.

A. Methods:

A total of 24 FPAs (12 alfalfa, 12 clover) were used for the seed germination experiment aboard STS-50. Each FPA was loaded with either seven alfalfa and nine clover seeds. The seeds were inserted four millimeter deep into an inert, fibrous plug (14 mm diameter, 20 mm long). The seeds were oriented such that the emerging root would grow deeper into the substratum and the shoot would eventually emerge into the air space above the substratum. Seed germination was initiated at different times on orbit by manually injecting 2.5 ml of distilled water from chamber B into chamber A. Based on the activation times, a profile of plant growth in microgravity for day 2, 3, 4, 5, 7, and 10 was established (two individual FPAs for each species and each time step). After activation, the FPAs were stowed near

a fluorescent lamp in the Spacelab module to provide minimal light levels of approximately 15 μmol m^{-2} s^{-1} during the growth period. On flight day 12, all samples were visually inspected and video images documented plant growth up to that time. All 24 samples were fixed by injecting 1 ml of glutaraldehyde fixative from chamber C into chamber A, thus terminating growth and preserving cellular arrangements in their microgravity state for subsequent analyses. Identical experiments were conducted on ground, closely duplicating environmental conditions in the Spacelab module.

Following sample return, seedlings and non-germinated seeds were evaluated for germination rates, growth orientation, overall plant geometry and mass. Light microscopy analysis of roots and shoot evaluated cell size and organization as well as possible infections by nitrogen-fixing bacteria.

B. Results:

Germination: Some FPAs supported little germination due to the lack of water wicking into the growth substrate. All other flight FPAs showed similar germination rates when space flight samples were compared to ground samples (Alfalfa: 96% flight and 86% ground; Clover: 94% flight and 90% ground; corrected for the five non-viable FPAs). Successful plant development, defined as the growth of a root and shoot with leaves, was lower than the germination rates, but again comparable between flight and ground (Alfalfa: 63% flight, 44% ground; Clover: 60% flight, 68% ground). The low rates when compared to open field tests (approximately 85%) indicate a possibility of water logging, especially for the microgravity samples where water distribution is not controlled by gravity and moisture around the seeds is assumed to be higher than for ground samples (Shah et al, 1993).

Orientation: Despite the attempt to orient seeds within the growth substratum, several roots emerged from the substratum and grew into the air space above. Analysis showed that either the seeds were not oriented appropriately (or had turned during insertion and/or launch) or that the emerging roots had responded to different resistance within the substratum and curved out of the medium. Flight samples showed an increase in directional changes while most ground samples had grown parallel to the gravity vector with only minimal deviations. The deviation from a straight, vertical growth direction of ground experiments was also seen in response to different substratum densities and resistance to the growing plant.

Mass and Plant Morphology: Growth curves for both alfalfa and clover are similar for flight and ground (Fig. 10). No significant differences can be found between the plants that successfully germinated and developed into a seedling (mass, total length, root length, shoot length). Most seeds had developed the hypocotyl hook characteristic for dicotyledons. Older plants that had emerged above the substratum (day 5, 7 and 10) and that were exposed to light had successfully straightened the hook and started to de-etiolate. Clover showed more de-etiolation (dark green leaves, hooks straightened) than alfalfa (some

greening, fewer hooks straightened). Relatively more clover plants had straightened the hypocotyl hook than alfalfa plants under the given low light conditions.

Growth Curves: Due to the relatively large number of samples (84 alfalfa, 108 clover), growth curves could be calculated with good accuracy. Growth slowed down after day 5, indicating a depletion of oxygen and build-up of toxic levels of metabolic byproducts in the hermetically sealed FPA. An improved FPA design now removes metabolic byproducts and enlarges the available oxygen volume for growing plants. Comparison with follow-on studies aboard STS-54 and STS-57 (dark-grown seedlings) showed reduced plant lengths on STS-50 even under the low light conditions. The reduction of growth was readily attributed to a decrease in hypocotyl length in lighted samples where there were similar root lengths for dark and low-light grown samples. Microscopic examination of leaf structure showed that the space flight materials exhibited advanced differentiation compared to matched ground controls.

C. Conclusion:

Despite the constraints of the FPA (hermetically sealed, no gas exchange, low light), the FPA has been successful in supporting large numbers of developing seedlings in a microgravity environment. Direct effects of gravity on gross plant morphology could not be observed. Flight and ground samples show similar mass and geometry (Fig. 10). Differences at the microscopic level were in evidence. Some minor changes in germination rates between flight and ground samples are most likely a response to changes in moisture distribution in the microgravity environment (secondary gravity effect). During the STS-57 mission, a new growth substratum was evaluated to avoid possible water logging while still providing uniform and sufficient moisture to the seeds. Photomorphological expressions were similar for flight and ground samples. However, the light intensities were most likely too low for a more successful phototropic orientation of all emerging seedlings. Prediction of the direction of root-shoot emergence and appropriate seed orientation was only successful in approximately 60% of all seeds because of directional changes imposed by the non-uniform fibrous growth substratum. In the new substratum tested during STS-57, more than 95% of all seeds were oriented successfully, enabling better postflight analyses.

Together with plant morphology data on lettuce seedlings from STS-57, three major plants of importance to bioregenerative life support systems have been characterized in a microgravity environment. With these data, an important step in the design of higher plant bioregenerative life support systems for space has been achieved. Related Rosy Periwinkle germination and morphological studies indicating that plants with pharmacological significance can also be supported. And, plant primary tissue culture experiments have also been supported in the FPAs.

IX. Effects of Gravity on the Growth and Development of *E. coli*

Dr. Louis Stodieck

The effects of gravity at the cellular level are neither well characterized nor understood. Because the bacterium *E. coli* has been extensively studied in general microbiology and is part of the normal human flora, it provides a good model for studying the effects of gravity on single cells. The average mass of an *E. coli* cell is such that the sedimentation rate due to gravity is on the same order of magnitude as randomly occurring Brownian motion (P. Todd, 1989). This implies that, by mass, *E. coli* is at the theoretical limit of being influenced by gravity in a fluid environment. Knowledge gained from empirical studies will be needed for developing bioregenerative life support systems and for understanding changes occurring in the human-microbe relationship during space flight which have potential health related consequences (Pierson et al, 1993). In addition, since E. coli is typically the workhorse of cloning and genetic engineering, it may be possible to exploit the microgravity environment to produce novel pharmaceutical or agricultural processes.

Previous space flight experiments using *E. coli* have reported alterations in genetic composition, immunological properties, morphology and viability, and in particular, general increases in population growth rate have been noted. At least one study has generated contradictory results, however. The *E. coli* study on USML-1 was evolved from initial experiments flown as part of the BIMDA payload on STS-37 and STS-43 and has since been continued on STS-54 and STS-57 (Klaus et al., 1993).

A. Methods:

Stock cultures of *E. coli* (ATCC 4157) were grown in a minimal growth medium supplemented with either glucose or ribose. Flight and ground FPAs were loaded from common stock solutions. Growth was initiated approximately 12 hours after launch by adding a concentrated sugar source to the *E. coli* cultures which were initially maintained in the minimal medium without sugar to minimize metabolic activity. Growth was terminated at 1, 2, and 3 days after initiation using ethanol. One FPA was kept viable for the full mission. On-orbit growth rate was sampled daily using the optical density capability of the GBA. Temperature controlled, matched ground FPAs were processed simultaneously. Growth rates and protein composition were compared between flight and ground samples.

B. Results:

E. coli cell population densities achieved after 1, 2, 3, and 14 days of growth were consistently higher in the space flight samples than in corresponding ground controls (Fig. 11). Combining these results with comparable data from the four other missions shows the increase in growth in space to be statistically significant ($p < 0.05$) for a total of 38 samples (19 flight, 19 ground), with cell counts averaging 88% higher. Cultures originally grown in one sugar source (glucose or ribose) and subsequently introduced to the alternate sugar exhibited increases in growth of up to 257% over ground

controls, and were consistently higher than for non-switched samples. Gel electrophoresis analysis from the USML-1 samples indicated an apparent difference in expressed structural proteins between flight and ground *E. coli* samples.

C. Conclusion:

The increased growth rates observed are consistent with most previous investigations and were shown to be statistically significant. The additional increase observed when the bacteria were introduced to an alternate sugar source suggests the metabolic adaptation process was enhanced in space. The electrophoresis data indicating a change at the transcription or translation levels will be further investigated.

X. Fibrin Assembly in Microgravity and Magnetic Fields

Dr. Konrad Pollmann

Fibrinogen is acted upon by the enzyme thrombin to form fibrin monomers. These monomers assemble into protofibrils which then aggregate to form a network of fibrin fibers. In addition to stopping bleeding, fibrin clots play a crucial role in infection, vascular disease, inflammation, wound healing and tumor growth and are of ongoing interest in medical research. Early medical applications of fibrin films and foams as wound-covering membranes and operative blood sponges were replaced by silicone-based materials but might attract renewed interest in the future. Current applications include fibrin spray glue to stop diffusion bleeding and to cover suture lines and to mend ruptured membranes. Strong fibrin fibers are envisioned as excellent suture material.

Microgravity may serve as a unique environment to form novel materials based on fibrin fibers and is the focus of this investigation (Pollmann et al, 1993). Magnetic fields were used to induce orientation of the aggregating fibers as a means of producing stronger materials. By demonstrating the utility of microgravity in forming such materials, the biomedical applications for fibrin might be extended.

A. Methods:

Fibrin clot formation in microgravity was initiated by mixing a thrombin solution in chamber B with fibrinogen in chamber A approximately 16 hours following launch. Four out of 10 FPAs contained small but powerful rare Earth magnets providing magnetic fields of up to 0.7 tesla. One sample contained a geometric dummy to assess the influence of the magnet shapes on the gel structure. Rates of assembly were monitored by transmissivity measurements in the GBA processor. Upon experiment completion, approximately 20 hours following initiation, glutaraldehyde fixative was added to the samples. After landing, the samples were retrieved and returned to our lab for analysis.

B. Results:

Initial analysis of the transmissivity curves indicated that one of the fibrinogen samples had partially precipitated from solution prior to experiment activation (Pollmann et al, 1993). The other curves revealed clear solutions at sample initiation and showed good repeatability for all the flight samples. Within experimental error, fibrin assembly kinetics seem to be similar in micro- and nominal gravity. Scanning electron micrographs revealed significantly greater homogeneity of the arrangement of fibrin fibers in space flight samples (Fig. 12). In contrast, ground samples were found to have numerous cavities and regions of dense fiber networks. Flight fibers were measured to be only about 50% of the diameter of ground produced fibers (Fig. 13). However, this difference may have been caused by slight differences in the temperature conditions experienced by the flight and ground samples during fiber aggregation.

C. Conclusions:

The absence of gravity driven sedimentation and the long clotting times chosen for the experiment should have enhanced the diffusional freedom of the protofibrils and are expected to affect various microscopic clot properties. Current analyses are expected to reveal these affects.

TABLE 1 - GBA INVESTIGATIONS FLOWN ON USML-1

Commercial Opportunity		Experiment/Product Development	Samples
Biomedical	Immune Disorders	Lymphocyte Induction Process	6
	Immune Disorders	T-Cell Induction Test Model	3
	Immune Disorders	Macrophage Induction Process	6
	Pharmaceuticals	Virus Capsid Product	2
	Pharmaceuticals	Liposome Products	3
	Drug Testing	Regeneration Test Model	6
	CELSS and Drug Testing	Brine Shrimp Test System	11
	Cancer	Inhibitor Protein Test Model	10
	Cellular Disorders	Gap Junction Processes	2
Closed Agriculture	Seed Storage	Seed Germination Products	24
	Pharmaceuticals	Seed Plant Products	6
	Tissue Regulation	Seedling Processes	10
	Drug Testing	Miniature Wasp Test System	3
	Crop Products	Rootlet Infection Processes	4
	Biotechnology	Bacterial Products and Processes	7
	Biotechnology	Bacterial Products and Processes	7
	Microbial Control	Pentaiodide Product Testing	5
Biomaterials	Drug Development	Protein Crystal Morphology Products	3
	Drug Development	Osmotic Dewatering Testing	2
	Data Mass Storage	Bacteriorhodopsin Biomatrix Products	2
	Synthetic Implants	Fibrin Clot Materials	10
	Synthetic Implants	Collagen Materials	DPA

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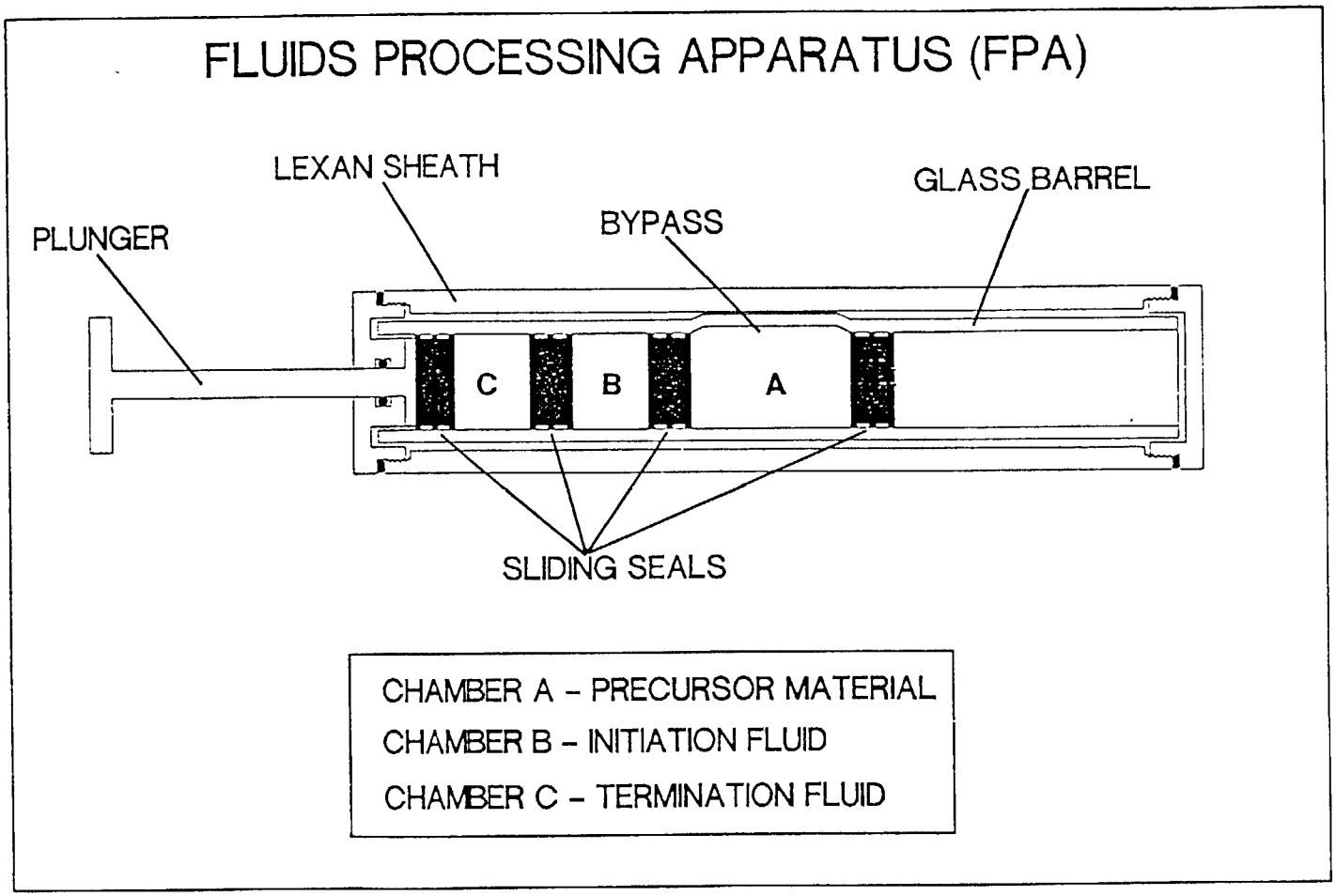


Figure 1 The FPA is a self-contained "test tube" which can hold up to three fluids (liquids or gases) of approximately 7 mls total volume Once on orbit, the fluids can be sequentially mixed to initiate and terminate a sample process, respectively. As shown, the FPA has two levels of containment, 1) the inner glass barrel and septa and 2) the outer polycarbonate sheath with endcaps and O-rings.

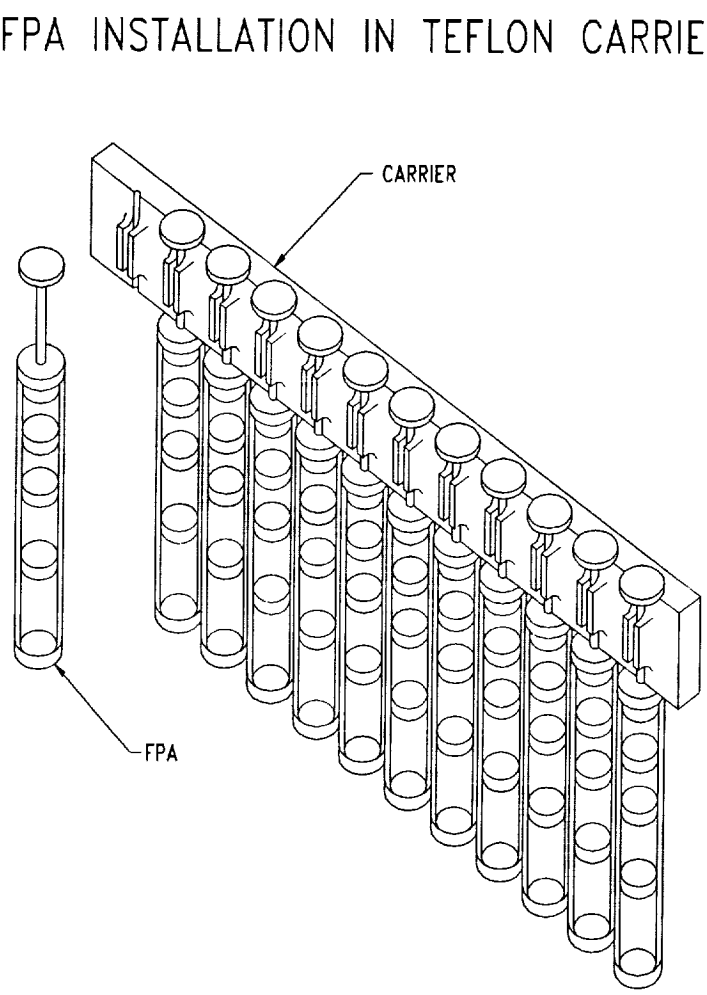


Figure 2 For USML-1, FPAs were processed in sets of 12. A rack carrier was used to hold the FPAs and the whole assembly placed in a plastic bag for stowage. A total of 11 sets of 12 (132) were flown.

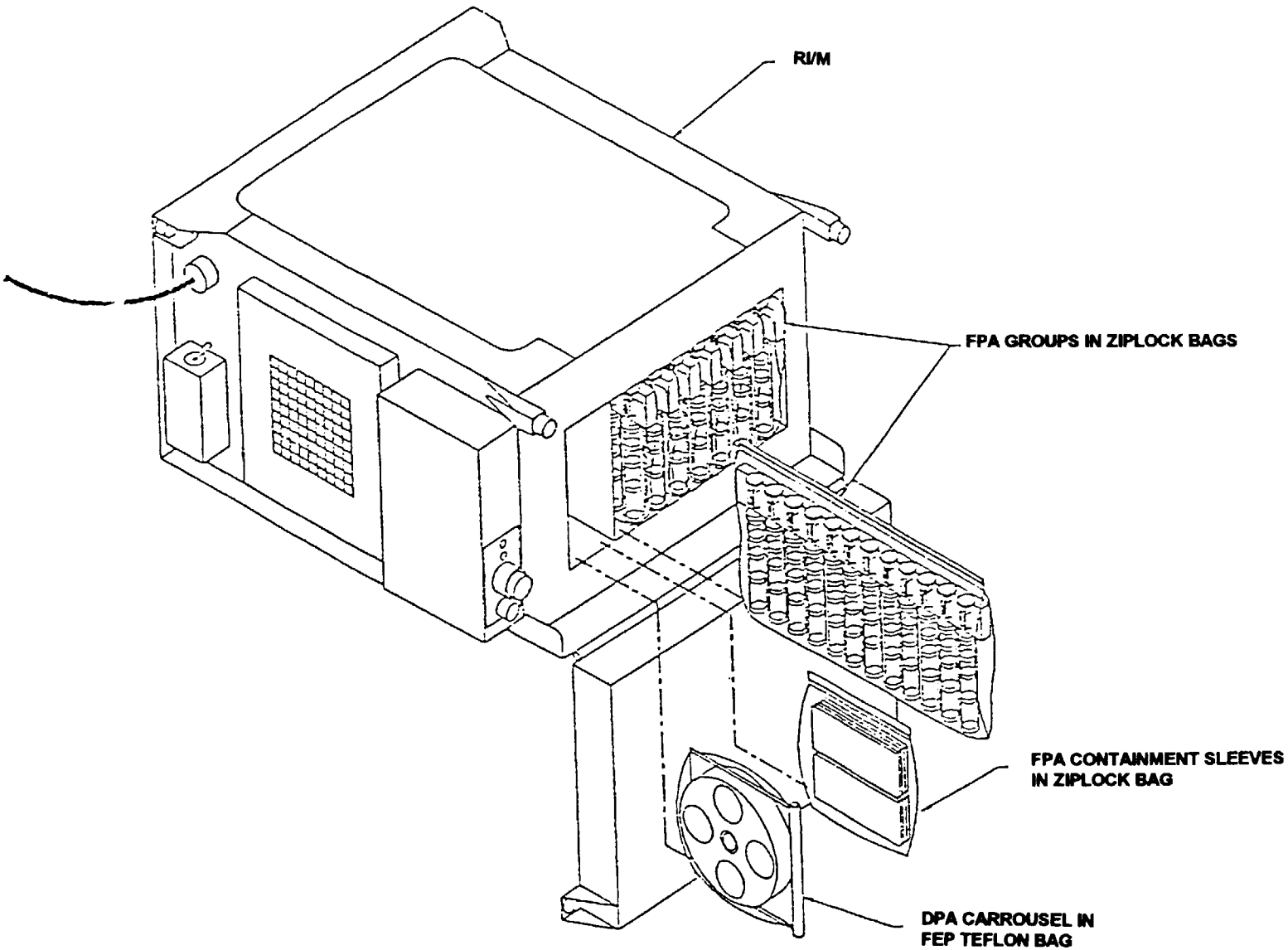


Figure 3 A NASA supplied Refrigerator/Incubator Module (R/IM) was installed in the Orbiter middeck for temperature controlled sample storage. The R/IM held 8 sets of FPAs at a constant temperature of 22 °C.

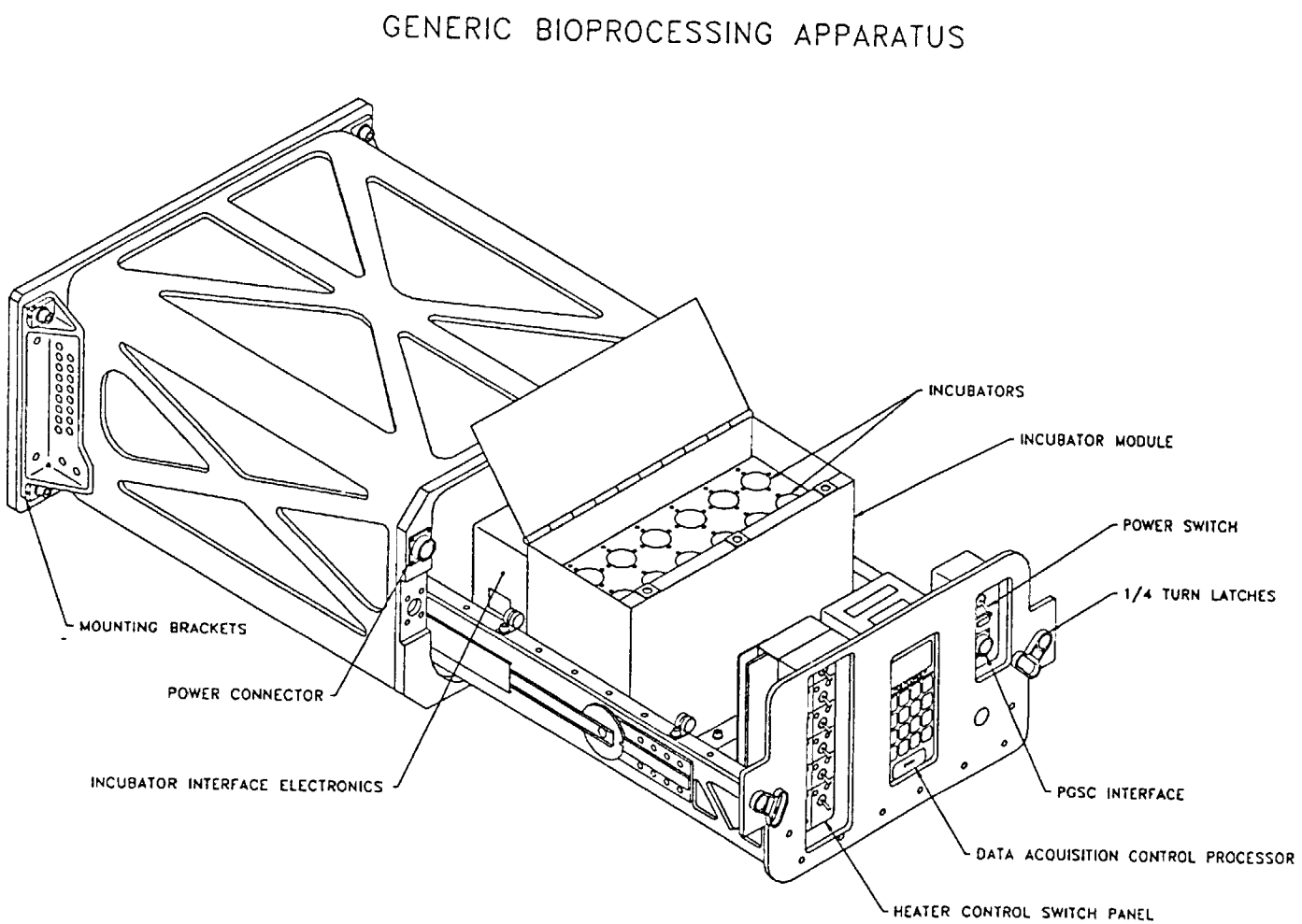


Figure 4 The GBA module was developed to provide spectroscopic measurements from 12 FPAs simultaneously. Temperature controlled incubator tubes were instrumented with LED illumination sources (565 nm) and optical sensors which fed signals to a datalogger (Omnidata Polycorder 700). Data collected on orbit could be transferred to the Payload General Support Computer for backup and downlink to ground personnel.

FLUID PROCESSING APPARATUS

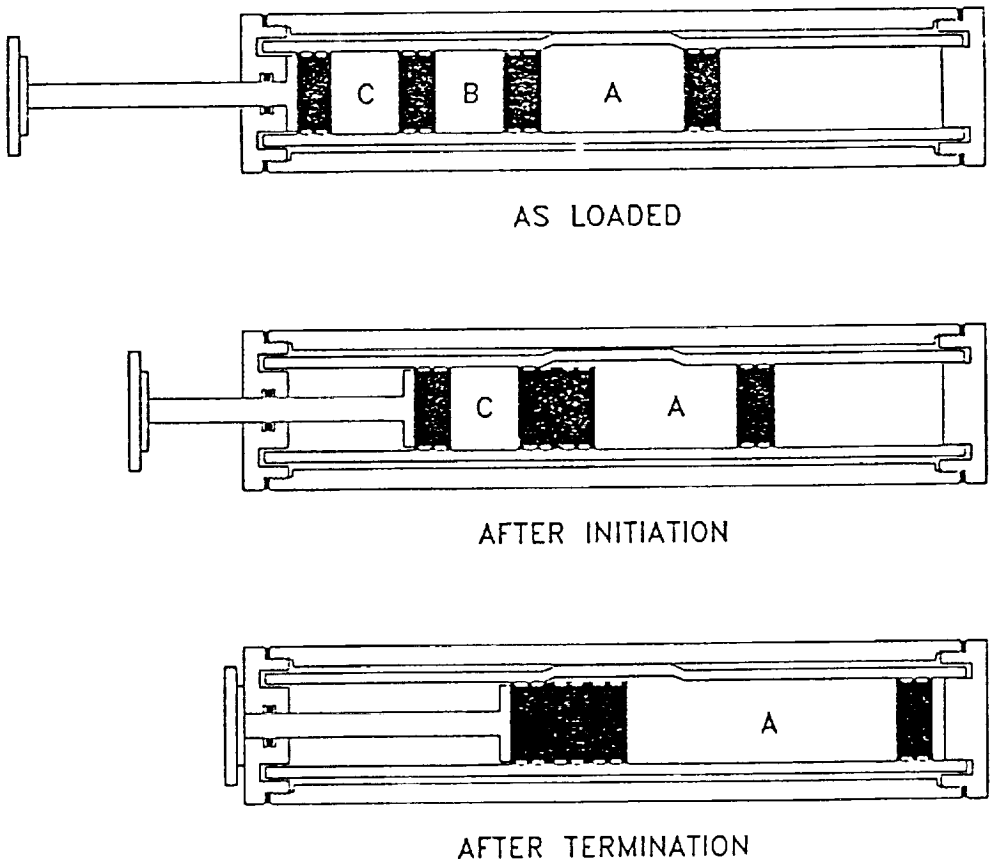


Figure 5 A sample process is typically initiated on orbit by depressing the plunger to transfer the fluid from the B chamber into the A chamber. Similarly, termination can be accomplished by further depression of the plunger to transfer the contents of the C chamber into the combined A/B chamber. An FPA can also be configured such that either the initiation step, termination step or both are omitted for additional flexibility.

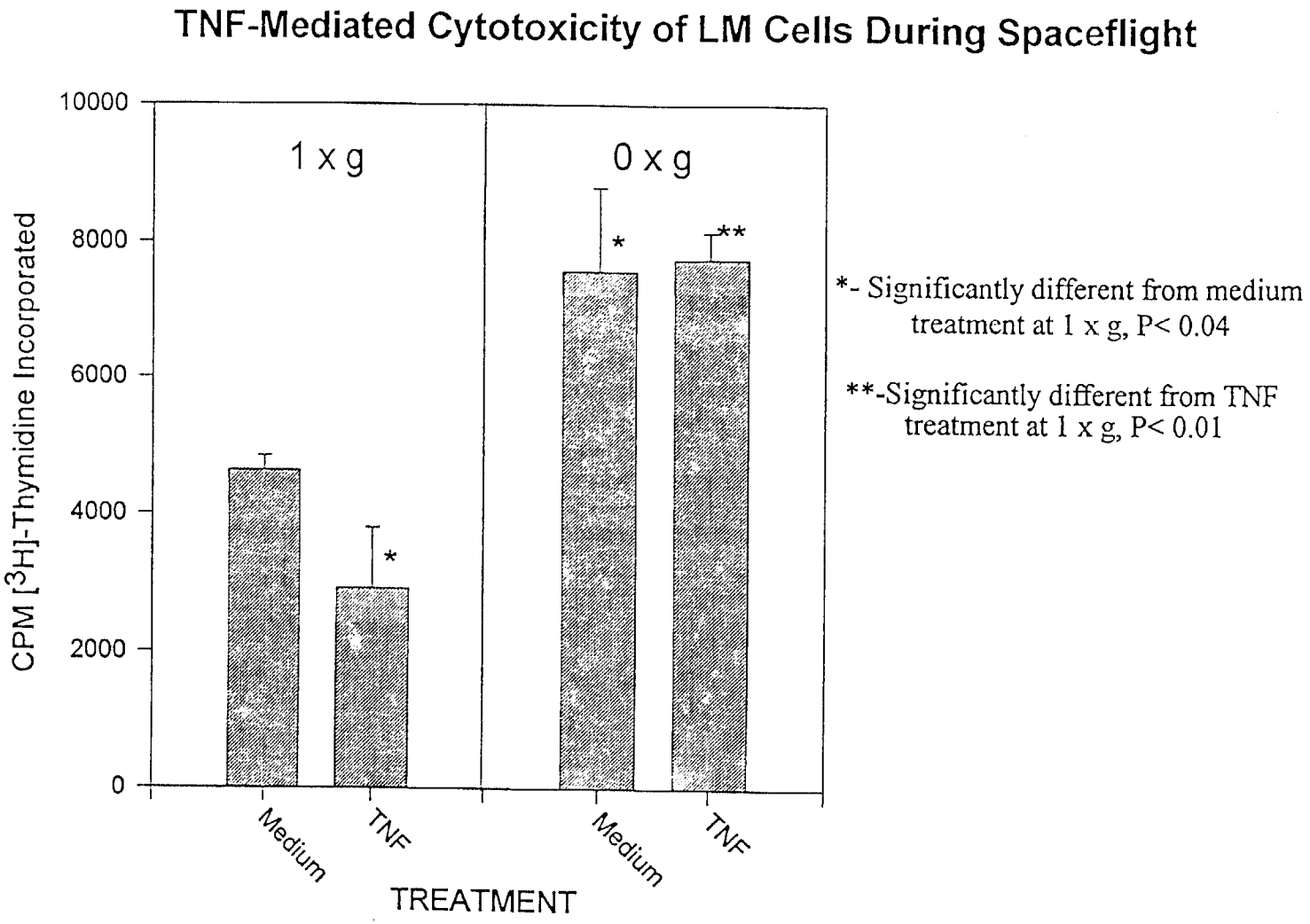


Figure 6 LM929 cells were incubated with [^{3}H]-thymidine in the presence or absence of TNF. As shown, TNF significantly decreases the amount of thymidine uptake in 1xg as expected. In contrast, TNF activity is inhibited in microgravity. Also of interest, thymidine uptake was increased in microgravity exposed cells. (From Chapes et al, 1993.)

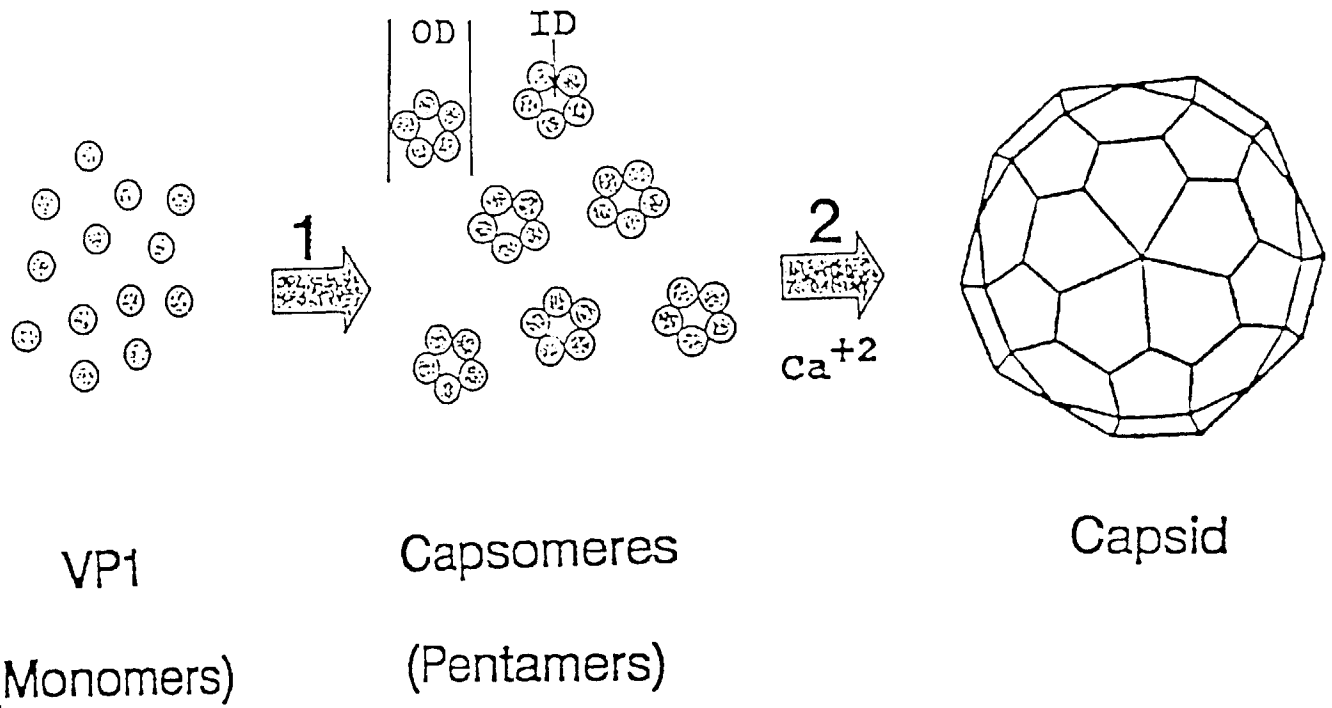
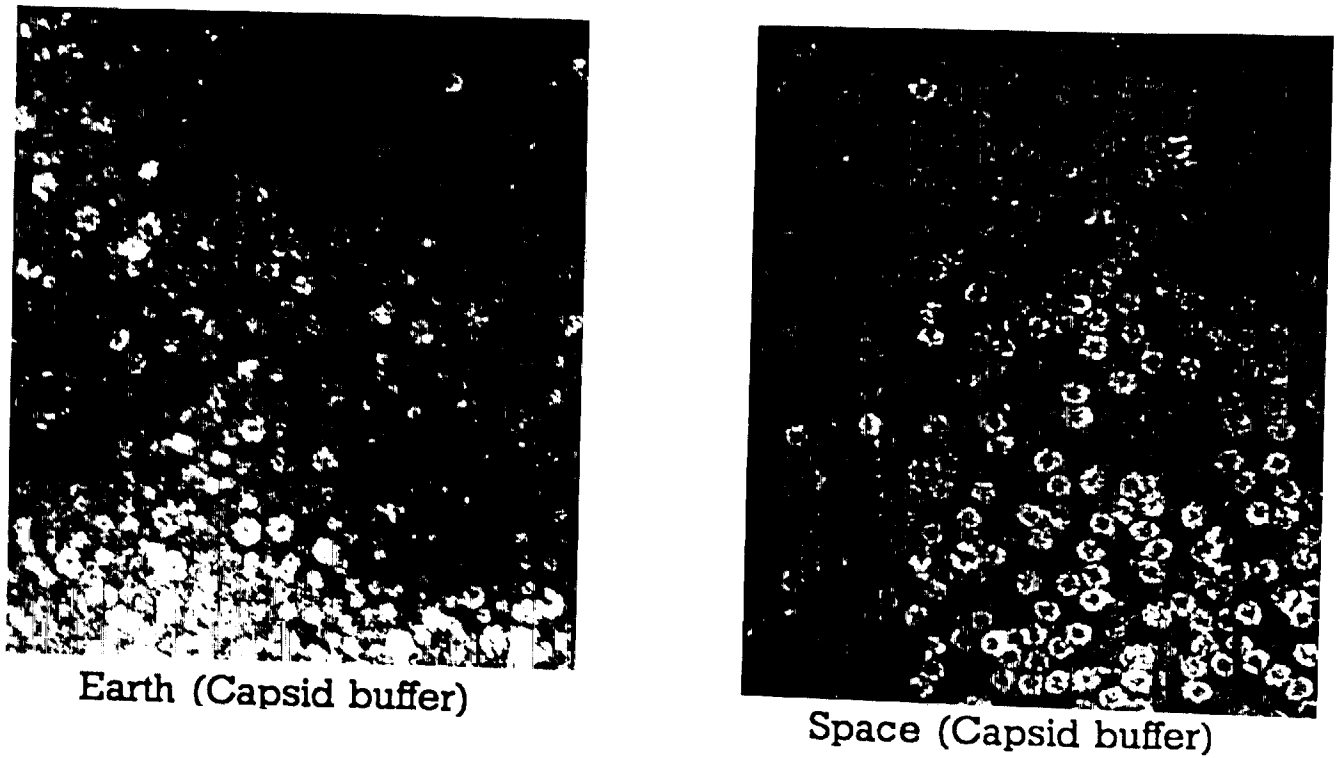


Figure 7 Recombinant, purified virus capsid protein, VP1, will self-assemble into intact virus capsids. The assembly process occurs in two steps, 1) VP1 assembles into smaller capsomeres (approx. 10 nm diameter) and 2) the capsomeres assemble, in the presence of Ca^{2+}, to form capsids (approx. 42 nm diameter). (From Chang et al, 1993.)

GROUND

FLIGHT



A

B

Figure 8 Transmission electron micrographs of ground (A) and flight (B) samples of assembly products from capsid protein VP1 from mission STS-37. Three major differences between the micrographs are apparent, 1) flight capsomeres are present with much greater homogeneity and yield, 2) flight capsomeres are swollen compared with ground controls, and 3) capsid structures are absent from flight samples. (From Chang et al, 1993.) An investigation on USML-1 was done to confirm the results and begin to determine the mechanism underlying the profound differences.

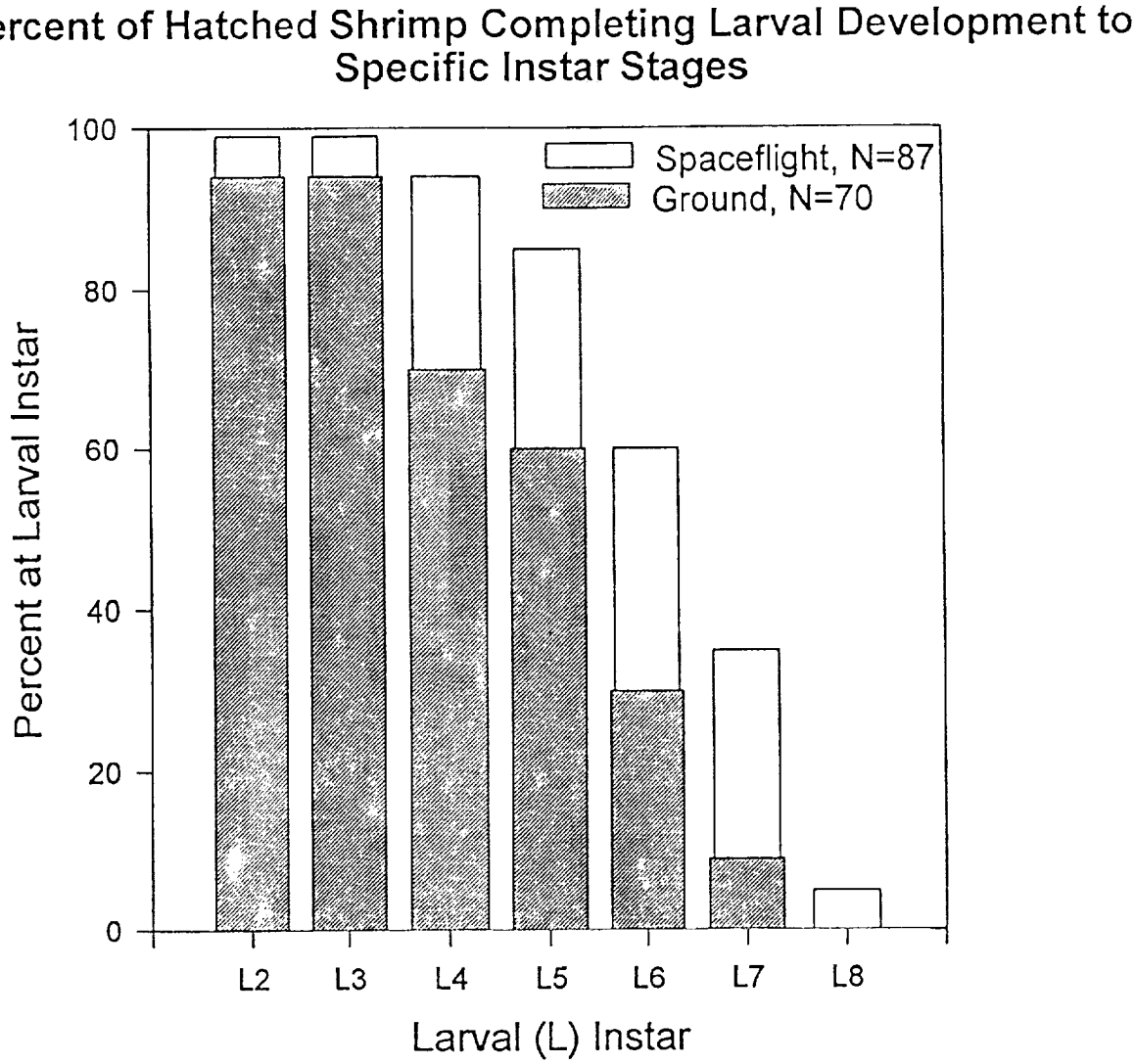


Figure 9 Brine shrimp were hatched on orbit and allowed to develop for 7-10 days. Based on microscopic analysis, the number of shrimp that reached each instar (moulting stage) were determined. Compared with ground controls, significantly more brine shrimp developed to later instar stages suggesting that development occurs more rapidly in microgravity. (From Spooner et al, 1993).

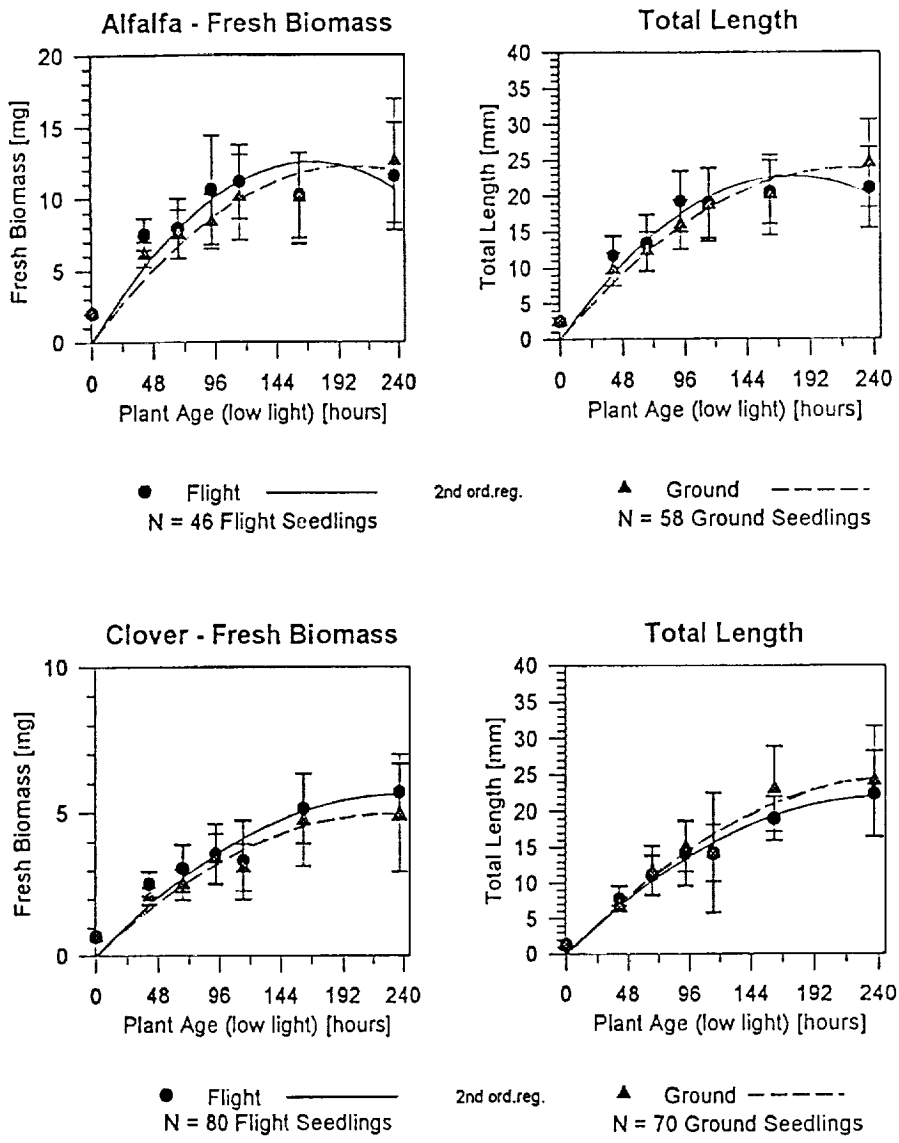


Figure 10 Clover and alfalfa seeds were germinated and grown for periods from 2-10 days on orbit on USML-1 and more recently on STS-54 and STS-57. Growth curves are plotted for the two species for both mass and length. No differences in mass or length of flight versus ground sprouts were observed. However, flight sprouts were found to have significantly greater mass and length committed to the root compared to shoot. (Data not shown.) (From Hoehn, 1993).

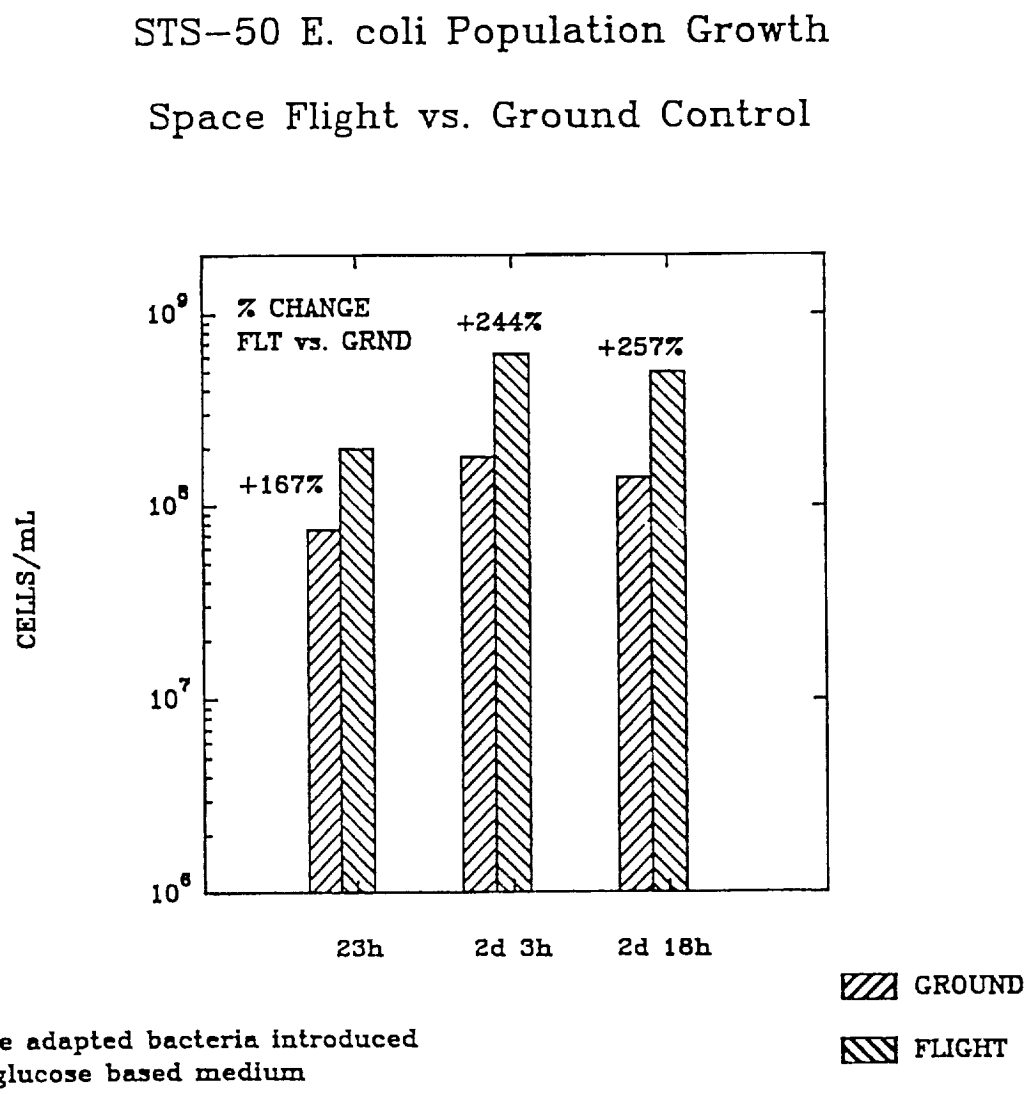


Figure 11 Comparison of *E. coli* samples grown in space vs. matched ground controls. Bacteria from a common ribose-based starting stock were subsequently introduced into a glucose-based medium and allowed to grow for 1 to 3 days to evaluate metabolic adaptation. As shown, cultures grew to significantly higher cell densities during space flight.

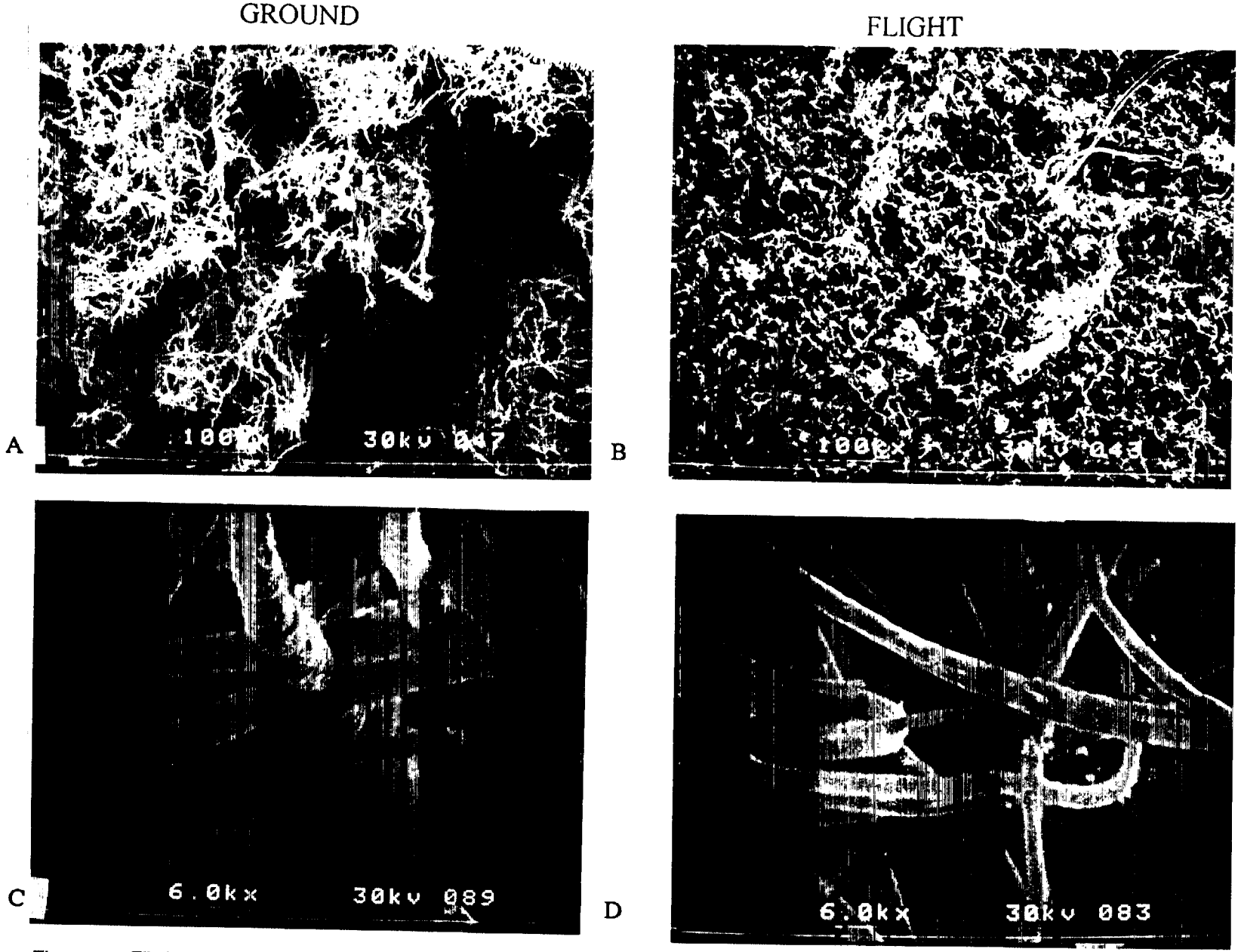


Figure 12 Fibrin materials assembled on orbit (B,D) and ground (A,C) were prepared for scanning electron microscopy at 100x (upper) and 6000x (lower) magnifications. Fibrin formed in space was found to be much more homogeneous than equivalent ground controls. Further, fibers from flight samples exhibited significantly smaller diameters. (From Pollmann et al, 1993.)

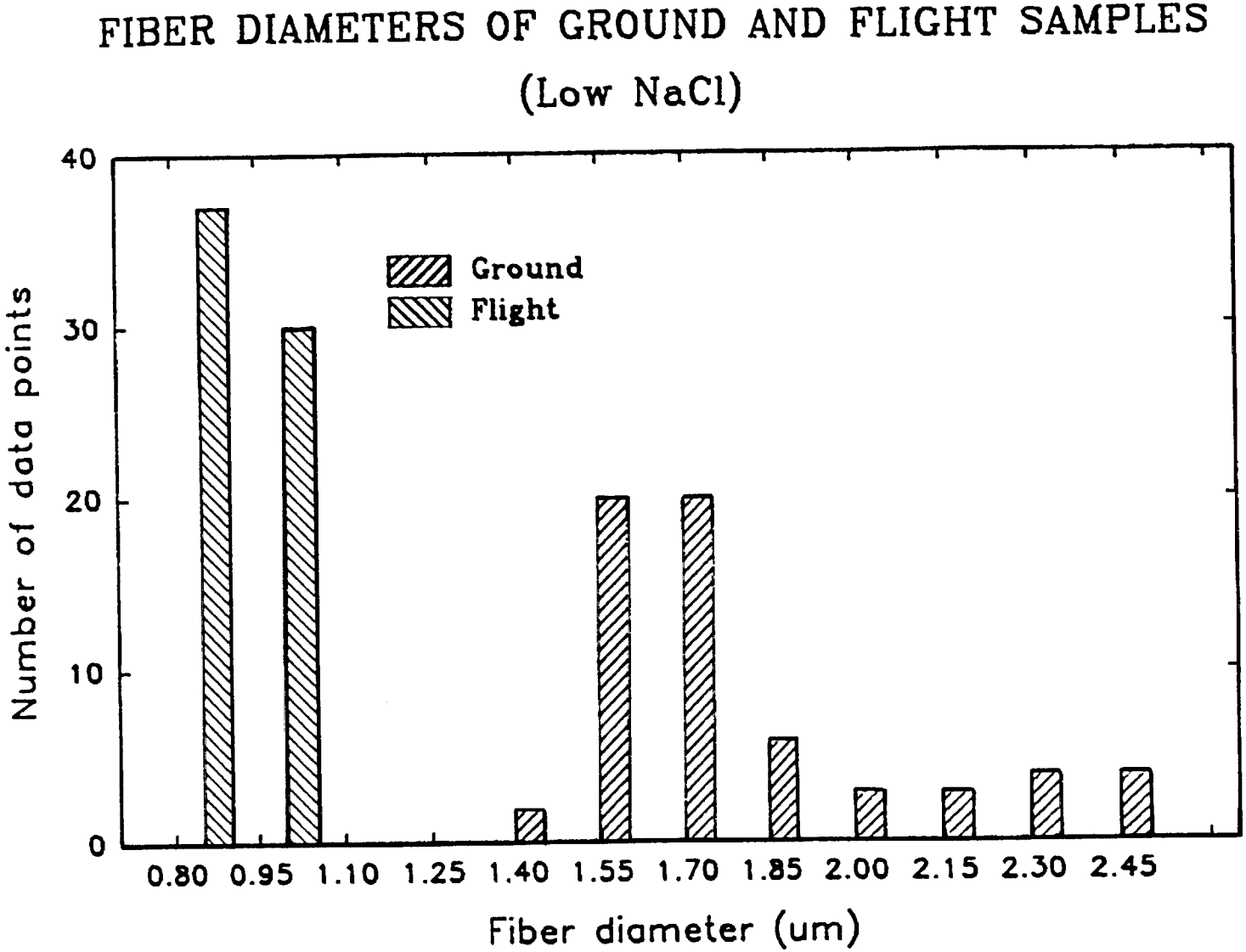


Figure 13 Fibrin fiber diameters were measured from high magnification scanning electron micrographs (6000x) and plotted as a size distribution for both flight and ground samples. Flight samples exhibited significantly smaller diameters of more homogeneous distribution. (From Pollmann et al, 1993.)

Discussion

(Speaker: M. W. Luttges, University of Colorado)

Question: *Does your data indicate a quicker maturity level (for organisms) in space ?*
Answer: Would you believe I really did not know that until I put this talk together. I think you are right. It may be just for what we have selected. I just told my colleagues just give me some of your data, I will probably screw it up but I want to give a summary of what we have been up to. And only when I started going through it did I recognize that they had that one thing in common, there are other things that happen, but they have that one thing in common that they do seem to mature much more quickly.

Question: *Has anybody else observed that effect ?*
Answer: I don't think anybody has had this data before, actually. Especially across such a wide range of materials.

Question: *How many different species were involved in the flight experiment ?*
Answer: Well we have got brine shrimp, we've got the miniature wasps, we've got 4 kinds of plants, we've got the bacterial system, and we've got some of the actual cell differentiations. If you think about it, the mouse L cells, the epithelial cells, also showed enhancing, and I really haven't gone back to the PI's and said, "okay, never mind the other stuff you are doing, give me information on just this one thing". I haven't done that, quite honestly. I'll do that but it looks pretty exciting.

Question: *On the collagen experiment could you say a little bit about combing capabilities of the flight specimens compared to ground and also the diameter of the material ?*
Answer: The diameter of the collagen fibers as you may or may not know depends on the concentration of the starting material that you have and it also depends on the ionic concentration. I sort of briefly touched on that because, basically, we could fill the whole front of the room with those interactions because it is not only that, but it is the thermal reaction. There is not a nice linear relationship between any of those variables. Okay, that having been said, the range of diameters can be as low as 0.06 of a micron all the way up to several microns (in some conditions), very large fibers, and this is without combing. I don't think anyone has reported this, and it has only occurred in space flight. Now with combing, that is something that actually would not work to be quite honest. You take these magnetozomes from the bacteria and then what we are really trying to do is organize the collagen during the polymerization process. We have used Couette flows to try to align all the fibers parallel to one

another so that cross-linking would give rise to really strong material. But something is wrong with the Couette flow because if you think about it unless you stop the rotation of the fluid against itself or against the plate or however you do it, at the right time, it is going to end up shearing the very material you are trying to produce, unless you happen to have a happy circumstance where you can shut if off at the right time. So we have tried variations on that type of thing and you always run into this trait. So we got interested for other reasons in the magnetozomes, in a size scale point of view. We just take the collagen, let it form and at the same time start from a homogeneous distribution of magnetozomes in the solution and just pull them down with just a small magnet. And we have done magnetic effects on collagen before trying to see whether 0.5, 0.8 tesla would give rise to some organization. We have got some results (ground based) on that. Quite to our amazement, because we have not done as much ground based work as we should have, all the flight samples that came back showed a very nice organized combing. The ground samples that we ran seemed to have enough counteractive flow that it disrupted some of the collagen that we got. It was one of those cases, like being little bit lightening struck, when it actually worked like it was supposed to without adequate planning.

Question: *This is about the e-coli samples that grew faster in the space environment. There are many strains of e-coli some of which have a tendency to flocculate. Could you comment on that ?*
Answer: These don't flocculate. They are selected on the basis of size with Paul Todd, based on some of the work that he had done, and he does a whole bunch of biophysical calculations. He thinks physics is important. But they are right from a size point of view, the size that makes sedimentation about on the same order of magnitude as Brownian motion; they don't go very far, at least in the nonflagellated ones. The flagellated ones are different. I don't remember their little identities in terms of all their little numbers. We do some work with Coors on yeast. They are very interested in what products you get with sedimenting yeast as opposed to non-sedimenting flocculating yeast. There, you are talking about gargantuan cells that really do sediment. They are interested in that from an obvious point of view. But this has long been the case, for 2000 years, or so, the people have had a difficult time looking at flocculation and sedimentation in terms of what that meant to a life cycle of different organisms. I know what you are sort of getting at, and it is an interesting question. We selected these bacteria just to get around those issues.

Question: *Essentially, you spend about as much energy in space flight as you do on the ground. It is hard for me to believe that, but do you have any thoughts on that ?*

Answer: I have never understood those kinds of things. I don't know where people come up with those kinds of things. I guess you could put people in a little baggy, and then do metabolic studies to test such concepts.

Question: *Assuming the data is right, and I think it is because somebody did something called a heavy water study, another subject I know nothing about, but if John Charles or somebody is here, maybe he could talk about that. But let's assume that the data is good would you have any theories on why that might be?*

Answer: This is a trap isn't it. I think that for every advantage there is that you could imagine, from a basic passive physics point of view, there are disadvantages in terms of what the organism has to do to make up for it; for example, it was talked about earlier, there is a cephalic fluid shift in space. Well that seems good for now. Maybe the heart does not have to work as hard. That is fine, except that the normal consequence of moving around in a normal gravitational environment, is that, passively, your muscles take the blood that has sedimented, if you will, in the lower extremities and push it up through muscle activity through one-way valves. That is a pretty efficient thing to do, as opposed to using a pressure head from the heart to deliver all the way down from the arteries to the capillary beds, where you have no pressure head hardly anymore, and now you have to use other kinds of mechanisms. It is just an example for everything you get for free you probably have to make up for that in some way. You can go down a whole bunch of things I think in terms of global levels, systemic levels, and even molecular levels that show that, that is really the case. I guess I don't find that terribly surprising. We are also doing some stuff on immune systems on STS-60 on rats. This has got to be one of the most intense studies done on animals. So you have almost everything but the squeak being analyzed by a variety of people, all experts in the area, so we will be able to correlate muscular skeletal things to metabolize things at a cellular level to hormonal changes to cardiovascular things, and that will be pretty interesting. I could probably better answer your question after that.

Question: *Do you have a proposed mechanism for the human necrosis factor difference between ground base and flight ?*

Answer: I don't. Dr. Chase has actually gone further and shown that it was the protein kinase-C. The question is why is there this ineffectiveness of the protein kinase-C ? His speculation right now, as near as I can tell, is that there may well be some buoyancy effects in the membrane of cells themselves that are different from a terrestrial cell as opposed to a space flight cell. In other words, in terms of how much of the molecule is really inserted (and that is sort of what he is looking at) and he has got one of his colleagues that is doing fluorescent labeling of different parts of the molecule to stick into the

membrane to different extents, they are going to be able to fly these different markers and see whether or not there is any believable information there in terms of how buoyant the lipid environment is.

Question: *Are you doing any macrogravity studies? In a centrifuge?* Isn't that a less expensive approach?

Answer: No. We are not doing that right now. We are proposing to do that with animals on the centrifuge out at Ames in the future.