

# BioServe Space Technologies

A NASA Center for the  
Commercial Development of Space

## Annual Report 1992

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# **BioServe Space Technologies**

## **A NASA Center for the Commercial Development of Space**

### **Annual Report 1992**

#### **I. Executive Summary**

##### **1.A. The BioServe CCDS**

BioServe Space Technologies, a NASA Center for the Commercial Development of Space (CCDS), was established in 1987. As is characteristic of each CCDS designated by NASA, the goals of this commercial center are aimed at stimulating high technology research that takes advantage of the space environment and at leading in the development of new products and services which have commercial potential or that contribute to possible new commercial ventures. BioServe's efforts in these areas focus upon space life science studies and the development of enabling devices that will facilitate ground-based experiments as well as the conversion of such to the microgravity environment. A direct result of BioServe's hardware development and life sciences studies, is the training of the next generation of bioengineers who will be knowledgeable and comfortable working with the challenges of the space frontier.

To accomplish its mission, BioServe is headquartered at the Department of Aerospace Engineering Sciences at the University of Colorado, Boulder, where it is led by Dr. Marvin Luttgies. Luttgies is assisted by Dr. Michael Robinson, Associate Director of Engineering; Dr. Louis Stodieck, Associate Director of Technical Affairs; Mr. John Berryman, Associate Director of External Affairs; and, Ms. Beverly Evans, Project Management.

Dr. Terry C. Johnson, University Distinguished Professor and Director of the Center for Basic Cancer Research, leads the life sciences efforts at BioServe's sister university, Kansas State University in Manhattan. He is assisted by Ms. Pat Adams, Administrative Assistant; Dr. Richard Gerren, Project Engineer; and, members of the BioServe Life Sciences Committee that include Dr. S. Keith Chapes, Dr. Brian Spooner, Dr. James Guikema, Dr. Larry Williams, and Mr. Kenneth Buyle.

BioServe Space Technologies fosters the participation of academia, business and government in increasing private-sector interest and investment in commercial space-related activities. The interaction of these entities encourages U.S. economic leadership and stimulates U.S. competitiveness in promising areas of research and development.

##### **1.B. Commercial Interactions**

BioServe Space Technologies maintains a unique position with regard to the assistance it provides to life sciences industries. BioServe brings a fully integrated engineering/life sciences core of personnel to industrial consortia wanting access to the space environment. Industrial affiliates are provided with quality science, consultation, engineering and applications support. Furthermore, they are provided with spaceflight

expertise in almost all life sciences arenas, and they can readily access flight-qualified generic space hardware to quickly begin their commercial development programs.

BioServe is committed to successful programs that facilitate the development of commercial products not currently envisioned. Our faculty scientists, engineers, and students are providing a meaningful, commercially responsive service to industries. To verify the quality of this service, BioServe is helping select industries develop exciting and highly visible new products in the areas of bioprocessing, biomedical models, and closed agricultural systems.

This CCDS has purposefully elected to work on product development that, by analysis, would appear to benefit most from access to the microgravity environment. This selection process produces relatively large amounts of developmental exclusivity. Such exclusivity tends to increase technical risks while decreasing competitive risks. This same developmental exclusivity for most products maximizes the technology transfers from NASA to the private sector. Thus, BioServe provides an environment where commercial products meet little commercial competition, yet derive maximum benefits from the NASA investments in a quality spaceflight program.

The development of specific projects or processes comes both from within BioServe and from interests expressed in specific experimental or process capabilities on the part of existing or potential industrial affiliates. Approval of the process or project is driven by the private sector in the sense that projects or processes that do not have potential commercial relevance or future applications are dropped.

A BioServe-developed project arises from the Center's belief that before industry can be expected to commit substantial resources to space-borne life sciences research and development, it must be shown that the Center has the ability to successfully, quickly and affordably address the industry's technical concerns. The performance of a series of significant, pioneering demonstration projects in the life sciences arena is the best way of proving this to the private sector. The major areas of technical emphases listed below were chosen as a result of our interactions with our industrial affiliates, and our own understanding of what some of the life sciences opportunities in space can include.

## **1.C. Technical Emphasis**

There are four major research project categories associated with the BioServe Space Technologies Center. During the past year a brisk level of activity was associated with each project, and all four include both ground-based studies and experiments carried out in the microgravity environment. Selected summaries of research advances, both from experiments conducted at 1-G, as well as experiments carried out in the reduced-gravity program on KC-135 and shuttle craft, will be presented.

During the past year the Center's four major research project categories included:

### **1.C.1. Biomaterials Processing**

This category is comprised of studies at the cellular and subcellular levels of both plants and animals, and includes experimental systems involving tissues, genetic alterations and regulation, molecular assembly, enzymes liposome formation, cell growth and differentiation and viral protein interactions.

There are many potential benefits from these studies with regard to the future of life sciences in the microgravity environment. Certainly, an understanding of molecular and cellular interactions will be key to commercial development of biologicals both in space and on earth, and many of these studies serve as the necessary preliminary *stepping stones* to manifesting experiments aboard the shuttle and Space Station Freedom. Downstream commercial consequences of these activities include the evolution of new



materials - artificial joints, skin, eye lenses, heart valves, sponges, membranes etc. - that could significantly impact the health care industry.

## **1.C.2. Biomedical Models**

These studies include research with intact animal systems, at the physiological level, including bone metabolism, regulatory events associated with the immune system and lipid metabolism.

There is reason to believe that earthly disease states may have counterparts in space. It is known that astronauts, for example, lose bone mass in a fashion that bears some similarities to the ways post-menopausal women lose bone mass - but in space, astronauts do so some fifty times faster than women do on earth. Thus it may be that space can serve man as an "accelerated laboratory" for animal model studies of human disorders. It is also known that in the absence of gravity, physiological systems are "unloaded", and can be studied without the disturbing effects of gravity. Bone mass loss, kidney function, muscular and neural deconditioning, alterations in the immune system and cardiovascular studies are all being evaluated as candidates for space-borne biomedical models of disease.

## **1.C.3. Closed Agricultural Systems**

Studies in this category include those with intact plants, microorganisms, events associated with symbiotic nitrogen fixation and water purification technology.

Humans will need to carry their atmosphere, food and water with them when they leave the Earth. BioServe is actively investigating biological avenues for the production of oxygen and food in space, as well as the purification of water and management of wastes.

## **1.C.4. Enabling Hardware Development**

BioServe plays a unique role in hardware development and certification for studies to be conducted in the life sciences in the microgravity environment. A suite of general purpose scientific apparatus, designed to accomplish a variety of life sciences research tasks in space, is in production at BioServe. These range in size and complexity from the Fluids Processing Apparatus (FPA), to the BioProcessing Modules, and the Commercial Generic Bioprocessing Apparatus (CGBA), all of which have now flown and performed well in the microgravity environment (shuttle missions STS-43 and STS-50).

BioServe is also an important part of a pan-CCDS effort to develop the COMET (COMmercial Experiment Transporter). COMET is a long duration satellite that will be launched by an expendable launch vehicle. BioServe is managing the development and use of COMET's Recovery System, as well as developing both the Plant Module for Autonomous Space Support (P-MASS) and the Animal Module for Autonomous Space Support (A-MASS) payload capabilities for COMET.

## **1.C.5. Education and Technology Transfer**

Although not a research project *per se*, a primary focus of the Center is the education and training of a new kind of space professional. These workers will embody the skills of both the life scientist and the engineer, and will provide leadership in the national space commercialization effort. Nearly 70 undergraduate and graduate researchers participated in the BioServe CCDS at CU and KSU. In addition 13 postdoctoral fellows were involved with ground-based and microgravity experiments associated with this CCDS. Many of the students who have worked in BioServe now hold professional positions in NASA, with NASA contractors or in large health-related companies. Testimony to the constructive interaction

between engineering and life sciences is found in three trainees with aerospace engineering degrees from the University of Colorado who currently are continuing their education and contributions to BioServe in the Division of Biology at Kansas State University.

## II. Technical Report

Research efforts during the year, at both CU and KSU, have been characterized by a significant amount of activity and an increased amount of interaction. Although each investigator is an expert in his or her field of study, the multidisciplinary approach that characterizes BioServe's goal of cooperation between engineers and life scientists, continues to provide a powerful team approach for ground-based and micro-gravity applications. It is clear to us that the achievements described below were only possible by the maintenance of fully interactive support of both ground and flight programs by scientists, staff and students at the University of Colorado at Boulder, and Kansas State University at Manhattan.

The following descriptions are brief summaries of the ongoing research programs during the year. Many of these scientific advances already have been published in peer-reviewed scientific journals, and presented before regional, national and international meetings, and details are available in the publications cited in Appendix III.

### II.A.1. Ground-Based Models and Studies

During the past year a wide variety of microbial, plant and animal cell model systems have been developed by BioServe researchers for present and future studies of the potential impact of the space environment on cellular function. These models are applicable to events associated with infectious disease, immune cell function, cell proliferation and differentiation, genetics, protein assembly, membrane structure, physiology and photosynthesis, tumorigenesis, and future applications of the space environment for commercial products including pharmaceuticals and materials for biomedicine and agribusiness. Many of these model systems already have progressed to a stage where they have been adapted to experiments in the reduced gravity environment. Virtually all of the studies briefly described below, selected to illustrate the broad range of life science activities and talents at BioServe, were published or *in press* during the past year.

#### II.A.1.a. Drosophila melanogaster as a Model System for Assessing Development under Conditions of Microgravity. *Michael K. Abbott, Richard B. Hilgenfeld, and Robin E. Denell.*

More is known about the regulation of early developmental events in *Drosophila* than any other animal. In addition, its size and short life cycle make it a facile experimental system. Since developmental perturbations have been demonstrated when both oogenesis and embryogenesis occur in the space environment, a strong rationale is provided for using this organism for the elucidation of specific gravity-sensitive developmental events.

#### II.A.1.b. Eye Development and the Appearance and Maintenance of Corneal Transparency. *Gary W. Conrad.*

Embryonic development of the eye, including the cornea, depends on the appearance and steady maintenance of intraocular pressure. The eye is a gravity-sensitive organ, as evidenced by changes in pupil diameter during parabolic flight. The cornea is largely a paracrystal of extracellular matrix. The extent to which it will polymerize normally in microgravity has yet to be determined.

**II.A.1.c. Microtubules as Key Cytoskeletal Elements in Cellular Transport and Shape Changes: Their Expected Response to Space Environments.** Gary W. Conrad and Abigail H. Conrad.

Application of reference standard reagents to alternatively depolymerize or stabilize microtubules in a cell that undergoes very regular cytoskeleton-dependent shape changes provides a model system in which some expected components of the environments of spacecraft and space can be tested on Earth for their effects on the cytoskeleton. The fertilized eggs of *Ilyanassa obsoleta* undergo polar lobe formation by repeated, dramatic, constriction and relaxation of a microfilamentous band localized in the cortical cytoplasm and activated by microtubules.

**II.A.1.d. Studies on Muscle Deconditioning.** Ellis Gayles, Steve Simske, and Marvin Luttgies.

The thrust of the muscular deconditioning research over the past year has been in five major areas: 1) The use of whole body electromagnetic fields to alleviate the effects of the muscle deconditioning induced by tail-suspension. The work over the past year has focused on determining the effects of both 8Hz and 10Hz fields in alleviating aspects of the suspension-induced muscle degeneration. The results obtained suggest that 8Hz fields, applied to the whole body of the suspended mouse, and inducing subthreshold stimulation of the muscles, alleviate much of the otherwise present muscle degeneration; 2) A comprehensive examination of the effects of tail-suspension on the hindlimb muscles of male and female mice of three separate strains; the Balb-C, C57BL-6, and the DBA2 strains. These studies will help determine the extent to which suspension results from general or specific effects; 3) Muscles were recovered from mice subjected to tail-suspension, false suspension (equipped for suspension but allowed to touch the cage floor with their hindlimbs), and pair-feeding with or without false suspension. The contribution of dietary, unloading and stress effects to the overall suspension effects on the muscle system will be discerned; 4) Hindlimb calf muscles are collected from mice subjected to sciatic nerve crush to determine if sciatic nerve crush is a useful model for muscle degeneration; and, 5) Mice that are tail-suspended are "anaerobically" exercised during the suspension period to determine if short periods of such exercise may abrogate the effects of tail-suspension induced muscle changes.

**II.A.1.e. Macrophage Binding of Cells Resistant and Sensitive to Contact-Dependent Cytotoxicity.** Charles F. Rosenkrans, Jr. and Stephen K. Chapes.

Macrophage binding and killing of F5b cells was compared to the binding and killing of P815 mastocytoma cells and to several other nontransformed and transformed cell lines. Formalin fixation of elicited or activated macrophages did not affect binding of F5b or 3T3 cells but did abrogate binding of P815 cells. However, formalin fixation abrogated resident macrophage binding of F5b and 3T3 cells. Therefore, depending on the type of macrophage or target cell, formalin fixation may affect binding. Only the binding of P815 cells was dependent upon activation; macrophage binding of target cells F5b and 3T3 was not. Even though macrophages bound F5b and 3T3 cells, macrophages only mediated contact-dependent cytotoxicity against F5b cells. Macrophages did not kill 3T3 cells. Experiments also compared macrophage binding and killing of the uv-light-induced tumor cell lines 1422, 2237, and 2237a46. Only the cell line 2237a46 was susceptible to contact-dependent killing. Both 1422 and 2237 cells were resistant. In contrast, cell lines 2237a46 and 1422 were bound by activated macrophages while 2237 cells were bound poorly.

**II.A.1.f. Glucocorticoid Effects on Immune Cell Activation by Staphylococcal Exotoxins and Lipopolysaccharide.** Stephen K. Chapes, Karen M. Kopydlowski, Sherry D. Fleming, and John J. Iandolo.

Experiments were conducted to determine the effects of physiologically elevated corticosterone on the activation of macrophages and T cells. These studies find that the elevation of corticosterone does not affect the expression of membrane receptors on macrophages and does not affect the activation of macrophages to produce cytokines. In contrast, elevated corticosterone levels correlate with enhanced T cell proliferation to both mitogens and superantigens.

**II.A.1.g. Murine Macrophage Activation by Staphylococcal Exotoxins.** *Sherry D. Fleming, John J. Iandolo, and Stephen K. Chapes.*

Investigations were carried out to determine the ability of staphylococcal enterotoxins A and B, exfoliative toxins A and B, and toxic shock syndrome toxin 1 to activate macrophages. All of the toxins tested had the potential to stimulate tumoricidal activity in peritoneal macrophages from lipopolysaccharide-responsive C3HeB/FeJ mice. In contrast, none of the toxins activated cytotoxicity in lipopolysaccharide-unresponsive macrophages from C3H/HeJ mice. Toxin stimulation of monokine secretion was also studied. Staphylococcal enterotoxin A, and toxic shock syndrome toxin 1, and both exfoliative toxins triggered C3HeB/FeJ macrophages to secrete tumor necrosis factor alpha, but enterotoxin B induced only marginal amounts of tumor necrosis factor. All of the toxins used stimulated interleukin-6 production by macrophages from both strains of mice. Nitric oxide is produced in response to the exfoliative toxins only by the lipopolysaccharide-responsive macrophages. These results suggest that macrophages respond differently to several staphylococcal exotoxins.

**II.A.1.h. Studies on the Metabolism and Mechanical Properties of Bone.** *Steve Simske, Jerry Broz, Tom Schmeister, and Marvin Luttes.*

Several aspects of bone metabolism, and the mechanical properties of bone, were pursued. The use of localized pulsed electromagnetic fields was demonstrated as a means for the prevention of suspension-related lower bone stiffness in mouse tibiae. The fields were apparently effective in preventing the loss of calcium from the tibiae. In addition, the use of whole body electromagnetic fields was employed as a means to alleviate the skeletal effects of suspension in mice. Two fields in particular were observed to have an effect on bone, one generally described as reducing the effects of suspension, the other as magnifying. A reduced growth in bone mass during suspension was linked with lower mechanical strength and stiffness in the femora, tibiae and humeri of suspended mice in comparison to controls. This difference was measurable for 3-pt flexure testing rates ranging from 0.1-10 mm/min. The reduced growth in bone mass during suspension was linked with lower cortical thickness throughout the diaphyses (long central portions) of the femora. The effects of suspension on bone mass and geometry were observed throughout the long bones (femora, tibiae, humeri), and only small changes in material properties were observed. These findings indicate that suspension causes reduced growth without whole bone material changes. A comprehensive examination of the effects of tail-suspension on the femora, tibiae and humeri of male and female mice of three separate strains; the Balb-C, C57BL-6, and the DBA2 strains. Bones were recovered from mice subjected to tail-suspension, false suspension (equipped for suspension but allowed to touch the cage floor with their hindlimbs), and pair-feeding with or without false suspension. From these experiments, stress effects, and lesser so dietary and unloading effects, accounted for the measured tail-suspension induced bone effects.

**II.A.1.i. Antipeptide Antibodies That Can Distinguish Specific Subunit Polypeptides of Glutamine Synthetase from Bean (*Phaseolus vulgaris* L.).** *Xiaoyin Cai, Ralph L. Henry, Larry J. Takemoto, James A. Guikema, and Peter P. Wong.*

The amino acid sequences of the  $\beta$  and  $\gamma$  subunit polypeptides of glutamine synthetase from bean (*Phaseolus vulgaris* L.) root nodules are very similar. However, there are small regions within the sequences that are significantly different between the two polypeptides. The sequences between amino acids 2 and 9 and between 264 and 274 are examples. Three peptides ( $\gamma_{2-9}$ ,  $\gamma_{264-274}$ , and  $\beta_{264-274}$ ) corresponding to these sequences were synthesized. Antibodies against these peptides were raised in rabbits and purified with corresponding peptide-Sepharose affinity chromatography. Western blot analysis of polyacrylamide gel electrophoresis of bean nodule proteins demonstrated that the anti- $\beta_{264-274}$  antibodies reacted specifically with the  $\beta$  polypeptide and the anti- $\gamma_{264-274}$  and anti- $\gamma_{2-9}$  antibodies reacted specifically with the  $\gamma$  polypeptide of the native and denatured glutamine synthetase. These results showed the feasibility of using synthetic peptides in developing antibodies that are capable of distinguishing proteins with similar primary structures.

**II.A.1.j. Subunit Analysis of Glutamine Synthetase Isozymes from Root Nodules of Tepary Bean (*Phaseolus acutifolius* Gray).** *Pat D. Green and Peter P. Wong.*

Native polyacrylamide gel electrophoretic analysis of purified glutamine synthetase (GS) from root nodules of tepary bean (*Phaseolus acutifolius* Gray) revealed as many as 17 isozymes. The banding of the isozymes showed two distinct regions (designated GS<sub>1</sub> and GS<sub>2</sub>). Analysis by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) demonstrated that the isozymes from each region were composed of two different subunit polypeptides. One of the polypeptides was specific to a particular region, and the other was common to both. The specific polypeptide in GS<sub>1</sub> had an isoelectric point (pI) of 6.2 and a molecular weight (mol. wt.) of 43,000 Da. The specific polypeptide in GS<sub>2</sub> had a pI of 5.2 and a mol. wt. of 42,000 Da. The common polypeptide had a pI of 5.5 and a mol. wt. of 42,000 Da. When the individual isozymes were cut from the gel and subsequently analyzed by sodium dodecyl sulfate (SDS) PAGE or 2-D PAGE, the results revealed that each isozyme was composed of varying proportions of two of the three polypeptides. GS holoenzyme consists of eight subunit polypeptides. Since the polypeptides from each region were of two kinds, random combination of the polypeptides should generate different isozymes with varying proportions of them. The findings of this study support this hypothesis.

**II.A.1.k. Development and Use of Domain-Specific Antibodies in a Characterization of the Large Subunits of Soybean Photosystem 1.** *Ralph L. Henry, Larry J. Takemoto, Jenifer Murphy, Greg L. Gallegos and James A. Guikema.*

The molecular architecture of the soybean photosystem 1 reaction center complex was examined using a combination of surface labeling and immunological methodology on isolated thylakoid membranes. Synthetic peptides (12 to 14 amino acids in length) were prepared which correspond to the N-terminal regions of the 83 and 82.4 kDa subunits of photosystem 1 (the PsaA and PsaB proteins, respectively). Similarly, a synthetic peptide was prepared corresponding to the C-terminal region of the PsaB subunit. These peptides were conjugated to a carrier protein, and were used for the production of polyclonal antibodies in rabbits. The resulting sera could distinguish between the PsaA and PsaB photosystem 1 subunits by Western blot analysis, and could identify appropriate size classes of cyanogen bromide cleavage fragments as predicted from the primary sequences of these two subunits. When soybean thylakoid membranes were surface-labeled with N-hydroxysuccinimidobiotin, several subunits of the complete photosystem 1 lipid/protein complex incorporated label. These included the light harvesting chlorophyll proteins of photosystem 1, and peptides thought to aid in the docking of ferredoxin to the complex during photosynthetic electron transport. However, the PsaA and PsaB subunits showed very little biotinylation. When these subunits were examined for the domains to which biotin did attach, most of the observed label was associated with the N-terminal domain of the PsaA subunit, as identified using a domain-specific polyclonal antiserum.

**II.A.1.l. Characterization of Photosystem 1 Chlorophyll *a/b*-Binding Apoprotein Accumulation in Developing Soybean Using Type-specific Antibodies.** *Ralph L. Henry, Trent Armbrust, Greg Gallegos and James A. Guikema.*

The structure and supramolecular assembly of the soybean photosystem 1 (PS1) chlorophyll *a/b*-binding antenna (LHC 1) was examined. The subunit composition of LHC 1 in soybean was identified and the accumulation of individual subunits during light-induced assembly was followed. Four LHC 1 subunits were observed, at 23, 22, 21 and 20.5 kDa. Partial sequence information by amino-terminal sequence analysis was obtained, and the 20.5, 22 and 21 kDa subunits were classified as being encoded by type I, II, and IV chlorophyll *a/b* binding protein genes, respectively. Antisera against LHC 1 subunits were used to follow the accumulation of individual subunits during the light-initiated transition from etioplast to chloroplast. Several points are noteworthy. First, monospecific antibody against the 22 kDa subunit decorated a 25 kDa peptide in etiolated tissue, which declined during maturation. This decline correlated with the light-induced appearance of mature 22 kDa peptide, suggesting a precursor product relationship. Second, the same antibody identified a 22 kDa protein in mature corn, but not a larger band in etiolated corn, suggesting that LHC 1 accumulation is regulated differently between species before the onset of

chlorophyll biosynthesis. Third, the mature 22 kDa subunit appeared somewhat later than the other LHC 1 peptides during greening, implying that this subunit is less intimately associated with the PS1 core than are the subunits appearing earlier in development.

**II.A.1.m. Plastids: Dynamic Components of Plant Cell Development.** *James A. Guikema and Greg L. Gallegos.*

The gravitropic bending of maize roots, as a response to reorientation of the root within a gravitational field, was examined for sensitivity to exogenous applications of the cytoskeletal inhibitor, cytochalasin D. Agar blocks were impregnated with this inhibitor, and were applied either to the root cap or to the zone of root cell elongation. Root growth was normal with either treatment, if the roots were not repositioned with respect to the gravitational vector. When untreated roots were placed in a horizontal position with respect to gravity, a 40 degree bending response was observed within one hour. This bending also occurred when cytochalasin D was applied at high concentrations to the zone of root cell elongation. However, when cytochalasin D above 40  $\mu\text{g/ml}$  was applied to the root cap, roots lost the ability of directional reorientation within the gravitational field, causing a random bending.

**II.A.1.n. The Role of a Cell Surface Inhibitor in Early Signal Transduction Associated with the Regulation of Cell Division and Differentiation.** *Terry C. Johnson, Daniel J. Enebo, Philip J. Moos, and Heideh K. Fattaey.*

Serum stimulation of quiescent human fibroblast cultures resulted in a hyperphosphorylation of the nuclear retinoblastoma gene susceptibility product (RB). However, serum stimulation in the presence of  $9 \times 10^{-8}$  M of a purified bovine sialoglycopeptide (SGP) cell surface inhibitor abrogated the hyperphosphorylation of the RB protein and the subsequent progression of cells through the mitotic cycle. The experimental results suggest that the SGP mediated its cell cycle arrest at a site in the cell cycle that was at the time of RB phosphorylation or somewhat upstream of the modification of this regulatory protein of cell division. Both cells serum-deprived and serum stimulated in the presence of the SGP displayed only a hypophosphorylated RB protein, consistent with the SGP-mediated cell cycle arrest point being near the G1/S interface.

**II.A.1.o. The Role of Microtubules in Contractile Ring Function.** *Abigail H. Conrad, Avelina Q. Paulsen, and Gary W. Conrad.*

During cytokinesis, a cortical contractile ring forms around a cell, constricts to a stable tight neck and terminates in separation of the daughter cells. At first cleavage, *Ilyanassa obsoleta* embryos form two contractile rings simultaneously. The cleavage furrow (CF), in the animal hemisphere between the spindle poles, constricts to a stable tight neck and separates the daughter cells. The third polar lobe constriction (PLC-3), in the vegetal hemisphere below the spindle, constricts to a transient tight neck, but then relaxes, allowing the polar lobe cytoplasm to merge with one daughter cell. Eggs exposed to taxol, a drug that stabilized microtubules, before the CF or the PLC-3 develop, fail to form CFs, but form stabilized tight PLCs. Eggs exposed to taxol at the time of PLC-3 formation develop varied numbers of constriction rings in their animal hemispheres and one PLC in their vegetal hemisphere, none of which relax. Eggs exposed to taxol after PLC-3 initiation form stabilized tight CFs and PLCs. At maximum constriction, control embryos display immunolocalization of nonextractable  $\alpha$ -tubulin in their CFs, but not in their PLCs, and reveal, via electron microscopy, many microtubules extending through their CFs, but not through their PLCs. Embryos which form stabilized tightly constricted CFs and PLCs in the presence of taxol display immunolocalization of nonextractable  $\alpha$ -tubulin in both constrictions and show many polymerized microtubules extending through both CFs and PLCs. These results suggest that the extension of microtubules through a tight contractile ring may be important for stabilizing that constriction and facilitating subsequent cytokinesis.

II.A.1.p. **Effect of Select Media Supplements on Motility and Development of *Eimeria Nieschulzi* in vitro.** Steve J. Upton and Michael Tilley.

A technique was developed to examine the effects of exogenous substances on apicomplexan sporozoite motility *in vitro*. Sporozoites of *Eimeria nieschulzi* were placed in the top compartments of blind well chemotactic chambers and separated from potential chemoattractant/chemokinetic agents by 8.0- $\mu$ m pore size Millipore filters. After 3 hr incubation, the number of sporozoites migrating through the filters was assessed using a hemacytometer. Results revealed that several substances, especially albumin and fetuin, enhanced motility of coccidial sporozoites. Cell culture assays supported these data, with higher numbers of parasites found in cultures supplemented with albumin and/or fetuin.

II.A.1.q. **The Tribolium Homeotic Gene Abdominal is Homologous to Abdominal-A of the Drosophila Bithorax Complex.** Jeffrey J. Stuart, Susan J. Brown, Richard W. Beeman, and Robin E. Denell.

The *Abdominal* gene is a member of the single homeotic complex of the beetle, *Tribolium castaneum*. An integrated developmental genetic and molecular analysis shows that *Abdominal* is homologous to the *Abdominal-A* gene of the bithorax complex of *Drosophila*. *Abdominal-A* mutant embryos display strong homeotic transformations of the anterior abdomen to parasegment 6, whereas developmental commitments in the posterior abdomen depend primarily on *Abdominal-B*. In beetle embryos lacking *Abdominal* function, parasegments throughout the abdomen are transformed to PS6. This observation demonstrates the general functional significance of parasegmental expression among insects, and shows that the control of determinative decisions in the posterior abdomen by homeotic selector genes has undergone considerable evolutionary modification.

II.A.1.r. **Production and Characterization of Monoclonal Antibodies to Budgerigar Fledgling Disease Virus Major Capsid Protein VP1.** Ali Fattaey, Laurel Lenz and Richard A. Consigli.

Eleven hybridoma cell lines producing monoclonal antibodies (MAbs) against intact budgerigar fledgling disease (BFD) virions were produced and characterized. These antibodies were selected for their ability to react with BFD virions in an enzyme-linked immunosorbent assay. Each of these antibodies was reactive in the immunofluorescent detection of BFD virus-infected cells. These antibodies immunoprecipitated intact virions and specifically recognized the major capsid protein, VP1, of the dissociated virion. The MAbs were found to preferentially recognize native BFD virus capsid protein when compared with denatured virus protein. These MAbs were capable of detecting BFD virus protein in chicken embryonated cell-culture lysates by dot-blot analysis.

II.A.1.s. **Early Events of Polyoma Infection: Adsorption, Penetration and Nuclear Transport.** Richard A. Consigli, John I. Haynes, Jr., Deching Chang, LaDonna Grenz, and Donald Richter.

Polyoma virions have different attachment proteins which are responsible for hemagglutination of erythrocytes and attachment to cultured mouse kidney cells (MKC). Virion binding studies demonstrated that MKC possess specific (productive infection) and nonspecific (nonproductive) receptors. Empty polyoma capsids have hemagglutination activity and bind to non-specific MKC receptors, but they are not capable of competing for specific virion cell receptors or preventing productive infection. Isoelectric focusing of the virion major capsid protein, VP1, separated this protein into six species (A through F). These species had identical amino acid sequences, but differed in degree of modification (phosphorylation, acetylation, sulfation and hydroxylation). Evidence based upon precipitation with specific antisera supports the view that VP1 species E is required for specific adsorption and that D and F are required for hemagglutination. The virion attachment domain has been localized to an 18 kD fragment of the C-terminal region of VP1. Monopinocytotic vesicles containing  $^{125}$ I-labeled polyoma virions were isolated from infected MKC. A crosslinker was used to bind the MKC cell receptor(s) covalently to VP1 attachment protein, and a new 120 kilodalton band was identified by SDS-PAGE. An anti-idiotypic antibody prepared against a neutralizing polyoma monoclonal antibody was used to identify a putative 50 kilodalton receptor protein from a detergent extract of MKC, as well as from an MKC membrane preparation.

**II.A.1.t. The Use of Additive and Subtractive Approaches to Examine the Nuclear Localization Sequence of the Polyomavirus Major Capsid Protein VP1.** *Deching Chang, John I. Haynes II, John N. Brady, and Richard A. Consigli.*

A nuclear localization signal (NLS) has been identified in the N-terminal (Ala<sup>1</sup>-Pro-Lys-Arg-Lys-Ser-Gly-Val-Ser-Lys-Cys<sup>11</sup>) amino acid sequence of the polyomavirus major capsid protein VP1. The importance of this amino acid sequence for nuclear transport of VP1 protein was demonstrated by a genetic "subtractive" study using the constructs pSG5VP1 (full-length VP1) and pSG5Δ5'VP1 (truncated VP1, lacking amino acids Ala<sup>1</sup>-Cys<sup>11</sup>). These constructs were used to transfect COS-7 cells, and expression and intracellular localization of the VP1 protein was visualized by indirect immunofluorescence. These studies revealed that the full-length VP1 was expressed and localized in the nucleus, while the truncated VP1 protein was localized in the cytoplasm and not transported to the nucleus. These findings were substantiated by an "additive" approach using FITC-labeled conjugates of synthetic peptides homologous to the NLS of VP1 cross-linked to bovine serum albumin or immunoglobulin G. Both conjugates localized in the nucleus after microinjection into the cytoplasm of 3T6 cells. The importance of individual amino acids found in the basic sequence (Lys<sup>3</sup>-Arg-Lys<sup>5</sup>) of the NLS was also investigated. This was accomplished by synthesizing three additional peptides in which lysine-3 was substituted with threonine, arginine-4 was substituted with threonine, or lysine-5 was substituted with threonine. It was found that lysine-3 was crucial for nuclear transport, since substitution of this amino acid with threonine prevented nuclear localization of the microinjected FITC-labeled conjugate.

**II.A.1.u. Phosphorylation of the Budgerigar Fledgling Disease Virus Major Capsid Protein VP1.** *John I. Haynes II and Richard A. Consigli.*

The structural proteins of the budgerigar fledgling disease virus, the first known nonmammalian polyomavirus, were analyzed by isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The major capsid protein VP1 was found to be composed of at least five distinct species having isoelectric points ranging from pH 6.45 to 5.85. By analogy with the murine polyomavirus, these species apparently result from different modifications of an initial translation product. Primary chicken embryo cells were infected in the presence of <sup>32</sup>P<sub>i</sub> to determine whether the virus structural proteins were modified by phosphorylation. SDS-PAGE of the purified virus structural proteins demonstrated that VP1 (along with both minor capsid proteins) was phosphorylated. Two-dimensional analysis of the radiolabeled virus showed phosphorylation of only the two most acidic isoelectric species of VP1, indicating that this posttranslational modification contributes to VP1 species heterogeneity. Phosphoamino acid analysis of <sup>32</sup>P-labeled VP1 revealed that phosphoserine is the only phosphoamino acid present in the VP1 protein.

**II.A.1.v. Evidence of Zinc Binding by Two Structural Proteins of *Plodia interpunctella* Granulosis Virus.** *C. Joel Funk and Richard A. Consigli.*

This laboratory previously reported the possibility of cation involvement in the *in vitro* dissociation of the *Plodia interpunctella* granulosis virus nucleocapsids. This current study found zinc associated with both granulosis virus nucleocapsids and granulin by atomic absorption analysis. A blotting assay with <sup>65</sup>Zn<sup>2+</sup> specifically identified the radioactive cation as binding to two viral structural proteins, granulin and VP12. These findings indicate that zinc may have a critical role in maintaining virus stability.

**II.A.1.w. Localization of Extracellular Matrix Components in Developing Mouse Salivary Glands by Confocal Microscopy.** *Patricia Hardman and Brian S. Spooner.*

The importance of the extracellular matrix (ECM) in epithelial-mesenchymal interactions in developing organisms is well established. Proteoglycans and interstitial collagens are required for the growth, morphogenesis, and differentiation of epithelial organs and the distribution of these molecules has been described. However, much less is known about other ECM macromolecules in developing epithelial



organs. Confocal microscopy was used to examine the distribution of laminin, heparan sulfate (BM-1) proteoglycan, fibronectin, and collagen types I, IV, and V, in mouse embryonic salivary glands. Organ rudiments were isolated from gestational day 13 mouse embryos and cultured for 24, 48, or 72 hours. Whole mounts were stained by indirect immunofluorescence and then examined using a Zeiss Laser Scan Microscope. It was found that each ECM component examined had a distinct distribution and that the distribution of some molecules varied with culture time. Laminin was mainly restricted to the basement membrane. BM-1 proteoglycan was concentrated in the basement membrane and also formed a fine network throughout the mesenchyme. Type IV collagen was mainly located in the basement membrane of the epithelium, but it was also present throughout the mesenchyme. Type V collagen was distributed throughout the mesenchyme at 24 hours, but at 48 hours was principally located in the basement membrane. Type I collagen was distributed throughout the mesenchyme at all culture times, and accumulated in the clefts and particularly at the epithelial-mesenchymal interface as time in culture increased. Fibronectin was observed throughout the mesenchyme at all times.

#### **II.A.1.x. Collagen in Organ Development. Patricia Hardman and Brian S. Spooner.**

It is important to know whether microgravity will adversely affect developmental processes. Collagens are macromolecular structural components of the extracellular matrix (ECM) which may be altered by perturbations in gravity. Interstitial collagens have been shown to be necessary for normal growth and morphogenesis in some embryonic organs, and in the mouse salivary gland, the biosynthetic pattern of these molecules changes during development. Determination of the effects of microgravity on epithelial organ development must be preceded by crucial ground-based studies. These will define control of normal synthesis, secretion, and deposition of ECM macromolecules and the relationship of these processes to morphogenesis.

#### **II.A.1.y. Endochondral Bone Formation in Embryonic Mouse Pre-Metatarsals. Brenda J. Klement and Brian S. Spooner.**

Long term exposure to a reduced gravitational environment has a deleterious effect on bone. The developmental events which occur prior to initial bone deposition will provide insight into the regulation of mature bone physiology. This laboratory has characterized a system in which the events preceding bone formation take place in an isolated *in vitro* organ culture environment. Studies show that cultured pre-metatarsal tissue parallels development of pre-metatarsal tissue in the embryo. Both undergo mesenchyme differentiation and morphogenesis to form a cartilage rod, which resembles the future bone, followed by terminal chondrocyte differentiation in a definite morphogenetic pattern. These sequential steps occur prior to osteoblast maturation and bone matrix deposition in the developing organism. Alkaline phosphatase (ALP) activity is a distinctive enzymatic marker for mineralizing tissues. This activity has been measured throughout pre-metatarsal development and results have shown where in the tissue it is predominantly found and that this is indeed the mineralizing isoform of the enzyme.

#### **II.A.1.z. Characterization of the Major Cyanogen Bromide Fragment of Alpha-A Crystallin. F. Ifeanyi and L. Takemoto.**

Alpha crystallin from the bovine lens has been digested with cyanogen bromide, and the major fragment (CB-1) has been purified using reverse phase HPLC. Characterization of this fragment by Edman degradation and antisera to synthetic peptides indicates that it originates from alpha-A crystallin, but lacks the N-terminal methionine and the last 35 amino acids from the C-terminus of the molecule. The purified CB-1 fragment binds as well as native alpha crystallin to lens membrane, but is unable to self-assemble into the correct size of high molecular weight oligomeric complexes characteristic of the intact alpha-A chain. Together, these results demonstrate that the alpha-A chain is comprised of at least two functional domains, one of which is involved in binding of alpha-A crystallin to lens membrane, and another which is necessary for correct self-assembly of the molecule into high molecular weight oligomers.

**II.A.1.aa. Age-dependent Deamidation of the Major Intrinsic Polypeptide from Lens Membranes.** *L. Takemoto and T. Emmons.*

The Major Intrinsic Polypeptide (MP26) of lens membranes contains an asn-gly- sequence, which has been shown in other proteins to be particularly susceptible to spontaneous deamidation. To determine if the asparagine residue of this sequence undergoes age-dependent deamidation *in vivo*, antiserum to a synthetic peptide containing the sequence was used to monitor purification of a tryptic peptide containing this sequence from fetal versus mature bovine lenses. The peptide from fetal lenses contained the -asn-gly- sequence, while the peptide from mature lenses contained an -asp-gly- sequence, demonstrating that age-dependent deamidation of this asparagine residue was occurring in the lens.

**II.A.1.bb. Identification of a Major Continuous Epitope of Human Alpha Crystallin.** *L. Takemoto and T. Emmons.*

Human lens proteins were digested with trypsin or V8 protease, and the resulting peptides resolved on a C<sub>18</sub> reverse phase column. Fractions from this column were probed with polyclonal antiserum made against the whole alpha crystallin molecule. Peptides in the seropositive fraction were purified to homogeneity, then characterized by mass spectral analysis and partial Edman degradation. The tryptic and V8 digests contained only one seropositive peptide that was derived from the C-terminal region of the alpha-A molecule. To determine the exact boundaries of the epitope, various size analogues of this region were synthesized and probed with anti-alpha serum. Together, these studies demonstrate that the major continuous epitope of the alpha-A chain includes the sequence KPTSAPS, corresponding to residues 166-172 of the human alpha-A crystallin chain.

**II.A.1.cc. Oxidation of the N-Terminal Methionine of Lens Alpha-A Crystallin.** *L. Takemoto, J. Horwitz, and T. Emmons.*

Antiserum against the N-terminal peptide of bovine alpha-A crystallin has been used to monitor purification of two different sero-positive peptides (i.e., T1a and T1b) from a tryptic digest of bovine lens proteins. Both these peptides have similar amino acid compositions, but peptide T1b has a molecular weight 16 atomic mass units larger than T1a, suggesting posttranslational oxidation. Analysis of ionization fragments of the T1b peptide by mass spectrometry demonstrates that this difference in molecular weight is due to the *in vivo* oxidation of the N-terminal methionine residue of the alpha-A crystallin molecule.

**II.A.1.dd. The Ability of Lens Alpha Crystallin to Protect Against Heat-Induced Aggregation is Age-Dependent.** *J. Horwitz, T. Emmons and L. Takemoto.*

Alpha crystallin was prepared from newborn and aged bovine lenses. SDS-PAGE and tryptic peptide mapping demonstrated that both preparations contained only the alpha-A and alpha-B chains, with no significant contamination of other crystallins. Compared with alpha crystallin from the aged lens, alpha crystallin from the newborn lens was much more effective in the inhibition of beta<sub>L</sub> crystallin denaturation and precipitation induced *in vitro* by heat. Together, these results demonstrate that during the aging process, the alpha crystallins lose their ability to protect against protein denaturation, consistent with the hypothesis that the alpha crystallins play an important role in the maintenance of protein native structure in the intact lens.

**II.A.1.ee. Involvement of the N-Terminal Region in Alpha-Crystallin-Lens Membrane Recognition.** *F. Ifeanyi and L. Takemoto.*

Previous studies have demonstrated that  $\alpha$ -crystallin binds specifically, in a saturable manner, to lens membrane. To determine the region of the  $\alpha$ -crystallin molecule that might be involved in this binding, native  $\alpha$ -crystallin from the bovine lens has been treated by limited digestion with trypsin, to produce  $\alpha$ -A molecules with an intact C-terminal region, and a nicked N-terminal region. Compared to intact  $\alpha$ -crystallin, trypsin-treated  $\alpha$ -crystallin binds less avidly to lens membrane, suggesting that the N-terminal

region of the  $\alpha$ -A molecule may play a key role in the recognition between lens membrane and crystallin.

**II.A.1.ff. An Assay for Intermolecular Exchange of Alpha Crystallin.** *S. Gopalakrishnan and L. Takemoto.*

An affinity column of alpha crystallin linked to cyanogen bromide activated Sepharose has been developed to study exchange of alpha subunits. Alpha crystallin bound to the Sepharose-alpha (Seph-alpha) complex was dissociated with 8 M urea, followed by quantitation using HPLC reverse phase chromatography. The time course of binding at 37°C shows a hyperbolic binding pattern reaching equilibrium between 6-18 hrs. Under these conditions, binding of beta and gamma crystallins to the same matrix was less than 10% of the alpha values, as was binding of alpha to glycine-coupled Sepharose. This assay was used to demonstrate changes in the subunit exchange of alpha crystallins present in the high molecular weight versus lower molecular weight aggregates of the human lens. Taken together, these results show that this binding procedure is both a specific and reproducible assay that can be used to study intermolecular interactions of the alpha crystallins.

## **II.A.2. Microgravity Studies**

BioServe scientists have maintained a brisk and aggressive stance with regard to developing and successfully flying life science experiments in the microgravity environment. Within the relatively short period of BioServe's existence, an enviable record of KC-135 flights, sounding rocket missions and shuttle craft studies have been assembled. This past year has been no exception and an increasing number of experiments, involving both animal and plant species, and which were designed and developed under ground-based conditions, have found their way to being manifested for reduced-gravity experimentation.

**II.A.2.a. Fluid Dynamics of Bone in Reduced Gravity (STS-43).** *Jerry Broz and Steve Simske*

The effects of spaceflight on the fluid dynamics of bone samples were investigated onboard the Space Shuttle mission STS-43 flown August 2-11, 1991. Bone samples were flown in 1/8" by 5/8" wells within the BioServe/ITA Materials Dispersion Apparatus (BIMDA). Undecalcified (normal) bone samples (n=4) taken from mouse humeri were exposed to EDTA, an agent that decalcifies bone. The samples flown were compared to samples tested on earth (for the same length of time and same EDTA concentration) to determine if the lack of convective fluid flow under microgravity conditions alters the EDTA decalcification process. Percent mineral loss, however, was similar under microgravity ( $17.5 \pm 3.0\%$ ) and normal gravity ( $14.2 \pm 6.7\%$ ) conditions.

Bone samples (also from mouse humeri), which had already been fully decalcified (n=2), were exposed to collagenase, which breaks down the collagen matrix. The samples were washed after flight and weighed to determine the percent collagen solubilized. These samples were also compared to samples tested under normal gravity. The percent collagen solubilized was similar for bone sections exposed to microgravity ( $7.2 \pm 3.9\%$ ) and normal gravity ( $6.0 \pm 1.6\%$ ) conditions.

These experiments provide preliminary data which indicates the fluid environment differences in microgravity do not affect bone demineralization or collagen removal in vitro.

**II.A.2.b. Brine Shrimp Development in Space: Ground-Based Data to Shuttle Flight Results (STS-37 & 43).** *Brian S. Spooner, Lynnette DeBell, Laura Hawkins, Janet Metcalf, James A. Guikema, and James Rosowski.*

The brine shrimp, *Artemia salina*, has been used as a model system to assess microgravity effects on developing organisms. Following fertilization and early development, the egg can arrest in early gastrula

as a dehydrated cyst stage that is stable to harsh environments over long time periods. When salt water is added, the cysts can reactivate, with embryonic development and egg hatching occurring in about 24 h. A series of larval molts or instars, over about a two-week period, results in the adult crustacean. We have assessed these developmental events in a closed syringe system, a bioprocessing module, in ground-based studies, and have conducted preliminary in-orbit experiments aboard the Space Shuttle Atlantis during the flights of STS-37 and STS-43. Although the in-flight data are limited, spectacular degrees of development have been achieved.

#### II.A.2.c. **Wetting in Reduced Gravity (KC-135).** *Alex Hoehn.*

Multiple phase fluid systems have altered characteristics in a reduced gravity environment. In normal gravity, the location of different density fluids and solids may easily be controlled. In reduced gravity, surface tension becomes the dominant force that determines the location and shape of liquids in gas-liquid-filled containers.

Tests in June 1991 and May 1992 used a simulated hydroponic plant growth system. Fluid motion and behavior were observed and recorded with a 35 mm slide film camera and an 8 mm video camera. The roots of a lettuce plant were partially submerged into a colored nutrient solution. The fill rate (ratio of air/liquid volumes) could be changed. In nominal and hyper-gravity, the liquid was on the bottom of the container, the roots, partially submerged, partially in air, were relatively dry (no fluid wicked up the roots). In a reduced gravity environment, all available liquid was rapidly taken up into the air space between the roots, displacing all air. This resulted in a pyramid-shaped fluid column around the roots, not allowing any direct communication between the roots and the air. With the increase of fill rate (more liquid), the corners/edges of the cubic container also were wetted. Finally, only a free-moving air bubble remained, and in no cases was the air bubble able to penetrate the system of rootlets.

To better understand the underlying mechanisms and to theoretically model the water uptake by the roots (capillary uptake), smaller fluid containers with 'root models' were developed. These containers could be mounted in front of a new free-floating camera assembly developed for use onboard the KC-135. This assembly, which provides back and side light, contained an 8 mm video camera and mounting capabilities for a variety of samples in the field of view (macro-mode up to 1" distance). The fluid containers were made from acrylic sheets (plexiglass). Acrylic and wooden rods were inserted into the chamber as single rods or as an array of rods in various shapes and patterns. Liquid bridges as observed with 'real' plant roots were observed only when the rods were spaced in an array with less than 3 mm spacing (5 mm diameter rods or less). Single rods or rod arrays spaced at larger distances than 3 mm did not wet or establish liquid bridges. Experimental fluids were water, glycerine, detergent and a liquid culture of *E. coli* bacteria. The material of the rods as well as the surface treatment were of little influence. The array of narrowly spaced rods allowed for efficient fluid uptake/acquisition and rapidly established a liquid bridge between the rods and around the centrally located rods. The height of the liquid column was only limited by the amount of fluid available and the physical constraints of the container.

#### II.A.2.d. **Wicking in Reduced Gravity - Test Hardware and Objective (KC-135).** *Alex Hoehn and Safwan Shah.*

Transport of liquids in a porous material is mainly driven by capillary action, but is limited by gravity in a vertical direction. Two experiments were developed to investigate wicking and transport characteristics of porous materials in variable gravity environments. The first experiment measured the location and distribution of liquids within a porous material by means of a distributed sensor array and has been flown on KC-135 flights in May and June of 1992. The second experiment investigated the actual transport mechanisms within the porous material and has undergone laboratory testing in preparation for a September 1992 flight.

The plant nutrient monitoring system consists of sets of emitter - sensor electrodes. Each emitter emits at a distinct frequency, which is picked up by all sensors distributed throughout the system. The amplitude

and amplitude changes together with the geometric location of the sensors are an indication of the amount of liquid present between any of the sensor-emitter pairs. The sensor-emitter pairs were tested in a Rockwool™ block, a fibrous material used in hydroponics. Different amounts of water were used as a test fluid (0%, 25%, 50%, 75% and 100% of total volume was water). The recorded signal, multiplexed for all sensors, is de-multiplexed for analysis. A Fast Fourier Transform yields the amplitudes for each emitter frequency. Correlation of amplitudes to different sensor-emitter pairs allows the determination of bulk fluid location within the block as well as fluid movements as a function of changing gravity environments.

The experiment to investigate transport mechanisms within porous materials is still in the development stage. Under the microscope, water transport by capillary action and bridging between the fibers of a porous material could be observed. The fluid was delivered and retrieved by means of syringes or a peristaltic pump. The porous materials investigated were Rockwool™, porous metals, fibrous sheets of cloths and Vermiculite™ (expanded rock). The experiment design is being continued for the September, 1992 KC-135 flight and concentrates on quantifying the amount of fluid being transported as a function of gravity.

#### **II.A.2.e. Human Vestibular Experiments (KC-135).**

During the weeks of May 4-8 and June 8-12, BioServe continued a series of experiments on the NASA KC-135 reduced gravity aircraft investigating the effects of reduced gravity and low-level electrical stimulation on the human vestibular system. Low-level electrical stimulation is directed to the vestibular system through skin surface electrodes placed on the bony protrusion of the skull behind each ear (the mastoid process). In normal gravity, using this electrode configuration, as the stimulation is turned on, subjects respond by leaning or swaying with the stimulation current. In other words, with the stimulation, it is as if one is being tilted or pushed to one side or the other. In May of 1992, BioServe became the first to investigate the effects of this type of electrical stimulation of the vestibular system in reduced gravity. It has been thought that electrical stimulation may hold some promise as a treatment for vestibular disorders such as vertigo or motion sickness.

During the eight KC-135 missions flown in May and June, a total of five test subjects from BioServe participated in the studies. Test subjects were asked to perform a number of vestibular function tests including visual target acquisition tests and a number of variations of a past-pointing test. Eye motion data were obtained using electro-oculographic (EOG) techniques. EOG data were stored in digital form on video tape using a recording system BioServe configured for the reduced gravity flights. This data acquisition system first flew on the KC-135 in February of 1991 with much success. This system allows for eight channel recording at high sampling rates for an extended amount of time (limited only by the length of the video tape). During the May and June flights, the data acquisition system operated without anomalies recording hours of flight data.

Vestibular function tests were performed in nominal and reduced gravity both with and without electrical stimulation. Although analyses of the test data are continuing, preliminary results of the tests may indicate that although some level of vestibular system control is lost on entry into reduced gravity, electrical stimulation can be used to offset the deficit.

#### **II.A.2.f. Bacteroid Formation of Nitrogen-Fixing Microbes in the Reduced Gravity Environment (KC-135). James E. Urban.**

The challenge of providing food for space travellers during interplanetary voyages is spurring intensive research in basic biological processes. Although a suitable life support system has yet to be developed, it has become clear that any effective system will include a means of biological nitrogen fixation. Probably the most effective way to fix nitrogen in a life support system is to employ the bacterial-plant interaction occurring in the *Rhizobium*-legume symbiosis.

Bacteroids, the developmentally altered form of bacteria which actually fix nitrogen, are induced to form when specific proteins on the bacterial surface bind certain small molecules produced by the legume host. Experiments for measuring binding of one bacteroid-inducing molecule, acetylsalicylic acid, were conducted during two days of microgravity flights on the NASA KC-135 aircraft. The experiments involved initiation of cell/substrate interaction followed by entrapment, fixing, and washing of exposed cells on the surface of a membrane filter. Upon return to earth the amount of acetylsalicylic acid bound during the microgravity episode was assayed. The results showed that binding in microgravity was 32% higher than binding in simultaneously conducted experiments on the ground, confirming that steps involved in the induction of bacteroids inside legume nodules can proceed in the absence of gravity.

**II.A.2.g. Effects of Microgravity on Phagocyte Respiratory Bursts and Signal Transduction (KC-135).** *S.K. Chapes and J.W. Armstrong.*

Murine peritoneal neutrophils (PMNs) were stimulated during parabolic flight on the NASA KC-135. The PMNs were activated by two stimuli that activate protein kinase C (PKC) and respiratory bursts; phorbol ester and f-met-leu-phe. The superoxide ( $O_2^-$ ) response was significantly enhanced by microgravity compared to ground controls with both stimuli. Cells were assayed for the amount of polymerized actin (F-actin) following stimulation. Microgravity did not have a different effect on cells compared to those stimulated at 1 x g. Microgravity also did not have an effect on normal tyrosine phosphorylation in response to PMA. When peritoneal macrophages were analyzed for CSF-1-induced protein phosphorylation during microgravity, no differences were observed compared to 1 x g controls. These data suggest that the enhanced  $O_2^-$  response of PMNs during microgravity is not dependent upon more efficient actin polymerization or tyrosine phosphorylation-dependent signal transduction events. Furthermore, certain kinase activities appear to be unaffected by microgravity in at least two distinct phagocyte populations.

**II.A.2.h. Retention of Water by Plant Roots Under Reduced Gravity (KC-135).** *P.P. Wong and J.A. Guikema.*

Retention of water by roots of various species of plants under conditions of 1-G and reduced gravity. The plants studied included bean, rice, sunflower, lima bean, Tepary bean and white clover. Different species of plants showed varying water retention under reduced gravity when compared to 1-G controls. Bean, lima bean and white clover retained significantly more water under reduced gravity. In contrast, rice retained less water under reduced gravity in comparison to measurements at 1-G. Water retention by Tepary bean and sunflower were more similar under the two gravity vectors.

**II.A.2.i. Collagen Self-Assembly and Directed Orientation (USML-1).** *Todd Bergren and Louis Stodieck.*

The Directed Polymerization Apparatus, experimental hardware developed and built by BioServe Space Technologies, was used aboard the USML-1 mission to study the effects of weak electric currents on the self-assembly process of collagen. Collagen, a fibrillar protein, is the most prevalent protein in the body. Collagen fibers, their cumulative physical characteristics the result of specific organizational patterns, give tendons and ligaments their strength, skin its resiliency, the cornea and lens of the eye their clarity, cartilage its ability to support compressive loads, and serve in bone as a scaffold on which calcium salts are deposited. Collagens are also found throughout the animal kingdom, their molecular structures changing little from species to species. Because it is so ubiquitous, collagen tends to induce very little immunogenic response when surgically implanted.

Collagen fibers can, under the appropriate conditions, be solubilized and reformed in the laboratory. These reconstituted fibers, because they do not cause a great immune response in the recipient and can act as a scaffold on which new, autogenous structures can be built, are attractive as an alternative to the synthetic materials now being used in reconstructive surgery. Unfortunately, the organization of in vitro fibers, and therefore the physical properties of constructs made from those fibers, cannot be controlled as well as that of collagen produced in vivo.

Samples appear to have assembled completely, with no obvious detrimental effects from having been stored at ambient temperature for several days. The electrically treated samples appear, at first glance, to be slightly more transparent than the control volumes, however this has yet to be quantified. Turbidometric and microscopic analysis will reveal any organizational changes wrought upon the collagen samples by the microgravity environment.

**II.A.2.j. Effects of Space Flight on the Action of Tumor-Necrosis Factor-Alpha-Induced Cell Lysis (USML-1).** *Stephen Keith Chapes.*

Tumor necrosis factor-alpha is an important regulatory and immunological hormone. It is important in the control of lipoprotein lipase, an enzyme involved in fat storage. Tumor necrosis factor-alpha (TNF) is also involved in the direct cytolysis of virus-infected cells (e.g. pox-, adeno- and herpes simplex virus-infected cells). Cytolysis 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 lysis of the cell, either by nuclear degradation or by dissolution of the plasma membrane. There have been some suggestions that clinorotation and microgravity can affect cellular and biochemical processes in cells. Because TNF is dependent upon the activation of several second messenger systems, and may be important for host destruction of virus-infected cells, it is important to determine whether TNF-mediated cellular lysis is affected by space flight. Therefore, an experiment was designed to determine whether TNF-mediated cytotoxicity is different in microgravity than in a 1 x g environment.

To avoid the complication of virus use in the shuttle orbiter, the LM929 cell line was used as the target cell in these experiments. LM929 cells were attached to Cytodex 3 microcarrier beads and placed in fluid processing apparatus (FPA). In flight, the cells were exposed to TNF for 24 hrs. The cells were then fixed and the number of cells remaining on beads was quantitated upon return to earth. Cellular proliferation was also quantitated by the presence of 10uCi of [<sup>3</sup>H]-thymidine during the TNF exposure period. Basically if cell death was to occur, there would be no [<sup>3</sup>H]-thymidine incorporation by the LM929 cells. Cell death was compared by ground controls and additional cells were incubated in space in the absence of TNF to determine spontaneous cell death.

Samples have now been analyzed and the data show that TNF inhibited the incorporation of [<sup>3</sup>H]-thymidine into LM929 cells 22-59% in simultaneous ground controls. In contrast, there was no inhibition of [<sup>3</sup>H]-thymidine in two FPAs flown in space and only 3% inhibition in the third FPA. Previous ground experiments found that [<sup>3</sup>H]-thymidine incorporation was inhibited between 44% and 21%. Therefore, the data for the simultaneous ground controls for this experiment were within expected ranges. Two possible conclusions can be drawn: 1) Space flight inhibited the cytotoxic activity of TNF on LM929 cells; or, 2) the FPAs that were flown in space were subjected to circumstances that did not allow for TNF-mediated cytotoxicity. Interestingly, the amount of [<sup>3</sup>H]-thymidine that was incorporated in space samples was higher than that for the simultaneous ground controls. This suggests that the cells were alive and that the cells did not have any difficulty in incorporating label in space. However, the reasons for the observed increased efficiency of space samples in incorporating [<sup>3</sup>H]-thymidine are unclear; especially since the simultaneous ground controls came from the same pool of cells. Additional flight experiments will need to be done to confirm these intriguing data.

**II.A.2.k. Proteolipid Membrane Formation and Encapsulating Efficiency During Spaceflight (USML-1).** *Dale E. Claassen, Brian S. Spooner, and Jacque van Twest.*

This experiment tested for microgravity effects on the assembly and reconstitution of an integral membrane protein, connexin-43. During space flight, protein reconstitution was performed by removing detergent (by dilution and adsorption) from mixed micelles of phospholipid and the connexin-43 protein. The proteo-liposomes were then returned to earth for analysis.

Results from the assay for functionally-reconstituted connexin-43 indicate that successful incorporation of connexin-43 occurred during space flight. Currently the proteo-liposomes are being immuno-gold labeled with antibodies specific for connexin-43. Analysis of these labeled proteo-liposomes by electron microscopy will allow us to quantitate and compare reconstitution efficiency in microgravity with ground controls.

#### II.A.2.l. **Biological Self-Assembly of Viral Protein (USML-1).** *Richard A. Consigli and Deching Chang.*

The major capsid protein (VP1) of polyomavirus was produced in *E. coli*. The recombinant viral protein was purified from the bacterial lysate by immunoaffinity chromatography. This purified non-infectious viral protein was used for assembly experiments in microgravity. Two experiments were performed to determine: 1) if VP1 capsomeres in the presence of  $Ca^{2+}$  will form capsid-like structures; and, 2) if a combination of capsomeres and capsids in the presence of  $Ca^{2+}$  will enhance the formation of capsid-like structures.

The space and ground-based experiments were identical as to instrumentation, reagents and reaction time. The experiment (dialysis) was for 12 days and samples were not fixed. Electron microscopic grids were prepared upon sample arrival. Possible conclusions indicate that microgravity may be causing changes in VP1 folding - swelling;  $Ca^{2+}$  is not functional because  $Ca^{2+}$  binding domains are altered due to changes in protein; and,  $Ca^{2+}$  may not function normally in microgravity.

#### II.A.2.m. **Seed Germination in Microgravity (USML-1).** *Alex Hoehn, Kirsten Abrahamson, Shawn Gomez, Michael Voorhees, and Marvin Luttes.*

In order to understand the effects of microgravity on the growth of leguminous plants, 24 fluid processing apparatuses (FPAs) were flown. Twelve FPAs contained seven alfalfa (*Medicago sativa*) seeds each and twelve contained nine seeds each of clover (*Trifolium repens*). Comparable ground controls were prepared as well. The seeds were loaded into an inert fibrous substrate (Rockwool™) where growth was initiated on orbit by transferring distilled water into the test chamber. After predetermined times, the sprouts were fixed with glutaraldehyde to terminate the experiment.

Based on our initial analysis, alfalfa seed germination rates for both flight and ground samples were 87%. For clover, the rates were 95% of flight samples and 90% of the ground samples. Of the seeds that began germination 19% had only a broken seed coat, 25% displayed a visible root, 1% displayed a visible shoot, and 55% displayed both a visible root and shoot. Additionally, the orientation of roots and shoots displayed a marked difference between flight samples and those on the ground. Flight samples were characterized by roots having random orientations (as opposed to roots going "downward" as is expected on Earth). This observation highlights the importance of gravitropism for plants on Earth.

Studies will be extended by computer-aided scanning pictures of the sprouts. Measurements of root and shoot length, cross section area and volume will be calculated. Histology of the individual sprouts will begin when scanning has been completed. This step entails the analysis of cell size and shape, number and size of starch granules per statocyte, as well as determining the existence of *Rhizobia* on the roots of the sprouts.

#### II.A.2.n. **Cytoskeletal Disruptive Agents; Resource Partitioning in Plant Tissues; and, Gravity Effects on Wild-Type and Agravitropic *Mellilotus alba* (USML-1).** *James A. Guikema, William Odom, Greg Gallegos, Emmanuel Hilaire, Trent Armbrust, M. Juergensmeyer, and Jeff Westberg.*

These experiments extended prior work performed on STS-37 and STS-43 in which seeds were germinated during spaceflight for subsequent cellular examination postflight. The GBA flight hardware should permit a greater flexibility in experimental manipulations and in achieving the following goals: 1) to validate the FPA as a useful device in which to monitor plant growth dynamics in space; 2) to obtain a developmental profile of seedling germination, as a way to compare wild-type and an agravitropic mutant



of *Melilotus alba*; 3) to examine the effects of cytoskeletal disruptive agents on directional root development in microgravity, in comparison with similar experiments in unit gravity; and, 4) to monitor carbohydrate resource partitioning in microgravity and calcium incorporation into plant cell walls.

These experiments have direct utility for space-based CELSS programs. An understanding of plant root growth dynamics will help define the experimental parameters to monitor in evaluating plant rooting media and plant nutrient delivery systems, and in the construction of plant growth units which permit plant growth to maturity. The experiment began when treatment fluids were added to dry seeds or to young seedlings. Termination and the biological sample were stabilized by the addition of fixative. The samples were returned to Kansas State University where they are undergoing postflight analysis.

#### II.A.2.o. **Reduced Gravity Effects on *E. coli* Growth and Adaptation (USML-1).** *David Klaus.*

In previous space flight experiments using *E. coli*, changes have been observed in cell genetic composition, population growth rate, immunological properties, morphology and viability. These studies, however, have formed inconclusive and often contradictory results. The underlying role that gravity plays at the cellular level is not fully understood. Gravity may act directly on subcellular regulatory mechanisms or indirectly on the fluid growth medium. Since *E. coli* is a well documented organism in general microbiology and is part of the normal human flora, it provides a practical model for study of cells in space.

This knowledge is important in development of a Closed Ecological Life Support System (CELSS) for extended duration spaceflight missions, as well as for crew health issues as related to potentially altered bacterial-vectored diseases. Commercial opportunities exist within the scope of genetically engineering bacteria strains in the reduced gravity environment for pharmaceutical or agricultural purposes, then propagating the cells indefinitely with the return of only a small sample.

Based on post-flight optical density measurements and cell counts, the cells grown in space consistently reached a higher saturation density on days 1, 2 and 3 of termination than their ground based counterparts.

The densities reached by the cells after one or more days do not indicate any differences as a result of switching between ribose and glucose in either flight or 1-g sets.

Post-flight growth assessment of the returned viable flight and ground samples was initiated approximately 48 hours after landing. Flight cells grown post-flight in glucose medium grew at the same rate under 2-g centrifugation as did their ground counterparts. The flight cells, however, reached the log phase of growth sooner than the ground cells under 1-g undisturbed (24°C); and exhibited an even earlier difference under slow clinorotation mixing (24°C).

Under constant shaking (37°C), the ground cells grew at an accelerated rate in glucose compared to their flight counterparts. Under 1-g (37°C), the ground cells also appeared to exhibit a slightly earlier log phase of growth compared to flight cells. All comparable sets of flight and ground post-flight growth samples reached similar saturation densities.

Electrophoresis separation of proteins for the post-flight viable samples and both FPA sets fixed on day 1 has been completed. Initial observation of the gel does not indicate any difference in molecular weight bands present between comparable flight and ground samples. The remaining samples fixed on days 2 and 3 are frozen in buffer for near future gel runs.

#### II.A.2.p. **Bactericidal Activity of Tri- and Pentalodide Resin Disinfectants Under Conditions of Microgravity (USML-1).** *George L. Marchin.*

The purpose of this experiment was to test the relative bacterial kill-power of the two resin disinfectants in microgravity. The configuration of the FPA included a few resin beads in the sample chamber with a

predetermined amount of exchangeable iodine. The "activator" is essentially a bacterial suspension in aqueous suspension. The fixative is aqueous sodium thiosulfate sufficient to reduce all available iodine on the beads. Two FPAs were used: one for triiodide and one for pentaiodide. Once in orbit, the FPAs were activated for a time and then terminated. After the samples were recovered, the suspensions were compared for: viable cells, iodine associated irreversibly with cells (neutron activation analysis), and some cellular distribution of iodine. Parallel ground studies evaluated these parameters at unit gravity.

Results indicated that the tri- and pentaiodide materials killed 91.875% and 95.000% under unit gravity and 39.375% and 86.975% on USML-1. In both cases the pentaiodide resin was a more potent disinfectant. Less kill under conditions of microgravity was probably due to a trivial event, i.e., sticking of the resin beads to the FPA septa because of the Dow-Corning high vac lubricant. Under these conditions the only occasional collision of bacteria with resin beads would magnify the pentaiodide's more potent bactericidal activity. Some contamination of FPA samples was experienced with other bacteria introduced during FPA loading. In the future, experiments like this could be used to evaluate contact times, resin effects on different microorganisms, and perhaps strategies for iodine/iodide removal and its effect on disinfection efficiency. Such experiments have value for long-term space flight and necessary disinfection procedures that will be required.

**II.A.2.q. The Influence of Microgravity on Cellular Events Associated with Proliferation and Differentiation (USML-1).** *Philip Moos, Dan Enebo, Heideh Fattaey, Richard Gerren, and Terry C. Johnson.*

This experiment was designed to test the effectiveness of a naturally occurring cell growth regulator, CeReS-18, in the microgravity environment. Biological activity by this inhibitor requires binding to a specific cell surface receptor which subsequently induces an intracellular cascade of signal transduction events.

Nonadherent insect cells (Sf9), shown to be sensitive to the inhibitory action of CeReS-18 in ground-based experiments, were used as the subject cell line. The first day in orbit  $9 \times 10^{-8}$  M of CeReS-18 was introduced to the Sf9 cells which were then incubated at 22°C until cell growth was terminated with a dilute solution of glutaraldehyde (final concentration 2%) after 96 hours.

The experiment was designed to allow two to four doublings of the Sf9 cells in the reduced gravity environment. Controls to measure the total number of Sf9 cells in the FPAs at the initiation of the experiment on orbit showed that only 25% of the subject cells survived the journey from the KSU campus to KSC and then to orbit. As a result of the reduced cell density, and the slower rate of cell division at these concentrations, the Sf9 cells completed just more than one doubling during the four days.

Cell enumeration upon recovery and analysis in the laboratory indicated that the CeReS-18 inhibitor did reduce cell division, compared to flight controls that did not receive CeReS-18. Our preliminary analyses show that cell division was inhibited by 40% in the reduced gravity environment while total cell cycle inhibition (100%) was mediated in ground-based experiments.

Whether the lessened effectiveness of the CeReS-18 in the reduced gravity environment was a reflection of an altered receptor binding step and/or subsequent signal transduction events is uncertain at this time. It also is possible that the lower rate of cell doubling, resulting from the reduced number of cells at the initiation of the experiment, played a role. Further ground-based studies, to discriminate between these possibilities, are planned, and additional flight opportunities will be necessary to substantiate these preliminary observations.

**II.A.2.r. Activation of T Cell Lymphokine Genes with Monoclonal Antibodies to the T Cell Receptor (USML-1).** *Joseph S. Murray and Tony Schountz.*

The goal of these experiments was to examine the activation of CD4 T lymphocytes during space flight. Data from Spacelab-1 demonstrated a profound decrease in tritiated thymidine incorporation by human T

lymphocytes exposed to the mitogen concanavalin-A. Previous work in this lab and others suggests that lymphokine gene expression and thymidine incorporation are independently regulated at the level of a single T cell clone. Comparisons of the activation of T cells with concanavalin-A versus direct stimulation of the T cell antigen receptor by monoclonal antibodies were made. Such studies may reveal new information about T cell activation and could lead to novel drugs for diseases such as AIDS.

The recovery of intact cells was slightly increased for all of the flight FPAs versus ground-based FPAs; and the percentage of intact cells recovered from in-flight concanavalin-A exposure was significantly higher than the ground control. This result is intriguing if the percentage of intact cells reflects T cell proliferation, as these data would not indicate decreased activation. The relative levels of lymphokine mRNA in these cells should clarify this issue, and measurements currently are being made.

**II.A.2.s. Fibrin Assembly in Microgravity and Magnetic Fields (USML-1).** *Konrad W. Pollmann, Marvin W. Luttes, and Louis S. Stodieck.*

When the blood plasma protein fibrinogen is acted upon by the enzyme thrombin, it is irreversibly converted to fibrin monomers. Fibrin monomers assemble into protofibrils, which then aggregate to form a network of fibrin fibers. Fibrin clots play a crucial role in hemostasis, thrombosis, infection, 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 in gynecology fibrin glues have been used to mend ruptured membranes. Strong fibrin fibers are envisioned as excellent suture material. Furthermore, a detailed knowledge of fibrin assembly and the influence of microgravity on this process will be important to many similar systems in biophysics and biochemistry.

A preliminary analysis of the transmissivity curves indicates that one of the fibrinogen solutions had partially precipitated out prior to experiment activation. The other curves imply clear solutions at sample initiation and show good repeatability for all the flight experiments. Fibrin assembly kinetics seem to be similar in micro- and nominal gravity. Small differences in lag and clotting times were noticed, however, and are under further evaluation.

The macroscopic appearance of the flight clots without inserts is similar to their ground controls. Three of the four magnet samples show strong evidence for movement of the magnets after clot formation, which compacted the gel above and below the magnets. More detailed analysis is necessary to determine whether this affected the primary sample portion exposed to the magnetic field. The other clot shapes showed differences between flight and ground samples that are attributed to collapsing of the gel due to gravity. The absence of gravity driven sedimentation and the long clotting times (2-4 hrs) chosen for the experiment should have enhanced the diffusional freedom of the protofibrils and are expected to further affect various microscopic clot properties. This is especially true for the clots assembled under the influence of magnetic fields. The samples will be analyzed for fiber thickness, alignment and branching, and for pore size and clot strength.

**II.A.2.t. Brine Shrimp Development in Space (USML-1).** *Brian S. Spooner, Lynnette De Belle, James Rosowski, Laura Hawkins, and Janet Metcalf.*

This study to assess the effects of microgravity on whole animal development was an extension of proof-of-concept experiments conducted aboard STS-37 and STS-43. FPAs, containing brine shrimp cysts in the A chamber, were activated by addition of salt water from the B chamber, and terminated by addition of fixative from the C chamber. Of 4 developmental FPAs, one was fixed immediately when chamber C contents entered the A-B chamber upon activation, but data was obtained from the other 3 FPAs. Of 5 cellular FPAs, it is still unclear as to why one was inexplicably not terminated. At recovery, 87 fixed brine shrimp were developed that had undergone development in space. The hatch rate was approximately

40%, the expected value based on ground data and previous flight results. This lab is in the process of analyzing the shrimp by a variety of light and electron microscopic methods.

#### II.A.2.u. **Protein Crystal Growth in Microgravity (USML-1).** *Paul Todd and Michael Sportiello.*

A small number of low-gravity protein crystal growth experiments has resulted in crystals with shape "habit" that differs from that of their counterparts crystallized at 1g. In particular, solid forms may dominate over dendritic growth, and monoclinic may dominate over solid forms. Experiments were designed to test the hypothesis that protein crystal growth habit in low gravity differs from that at 1g, using hen egg lysozyme, a standard protein for crystal-growth studies. On the ground this protein forms tetragonal crystals at low salt concentration and monoclinic crystals at high salt concentration.

The growth habit of lysozyme crystals seems to be approximately the same in low gravity and at 1g. When protein was crystallized with 7% salt (final concentration 4%), 100% of crystals were tetragonal in both cases. When protein was crystallized with 10% salt (final concentration 5.7%), less than about 1% of crystals were tetragonal in both cases. When protein was crystallized with 15% salt (final concentration 8.6%), less than 1 crystal in 1,000 was tetragonal in both cases. It is concluded that a change in crystal growth habit does not occur due to low-gravity crystallization of lysozyme, and crystal nucleation in low gravity occurs mainly by the homogeneous mechanism.

Note was also made of the locus of nucleation. On earth most crystals are found attached to the vessel wall, either due to heterogeneous nucleation on the solid surface or to sedimentation and subsequent adhesion to the solid surface. Both cases are considered undesirable. In contrast, most crystals grown in low gravity were found suspended in the mother liquor and not attached. This observation implies that nucleation is homogeneous in space, not requiring a solid surface, and that crystals do not sediment to the vessel wall during growth. Although organic crystals were grown from solution on Skylab using closed vessels some 20 years ago, very few protein crystals have been grown in low gravity in closed vessels; the hanging-drop method is most widely practiced, so it was necessary to determine whether or not crystals would be found attached to the walls after they had nucleated and grown in flight.

#### II.A.2.v. **Bacteroid Formation and Binding in Microgravity (USML-1).** *James E. Urban.*

As part of the cellular array of experiments, four replicate samples of lyophilized *Rhizobium trifolii* cells were mixed with medium containing succinic acid within several hours of attaining orbit. Ground-based experiments showed that mixing the lyophilized cells with medium allowed the cells to resume growth and to be induced to swell by the presence of the succinic acid. At day 4, growth and/or swelling was stopped in one sample by adding formaldehyde fixative to the reaction mixture. On day 8 another was stopped, and on day 11, the two remaining samples were fixed.

As part of the molecular array of experiments, three similar replicate samples were activated on the day after attaining microgravity and on the second day all three were fixed with formaldehyde. Upon their return to KSU from space, some cells from each sample were examined under phase-contrast microscopy while other of the cells were affixed to microscope slides. The cells on the slides were dried, lightly heat fixed, Gram stained, and photographed under 1,000 x magnification. The photographic images were then projected onto a screen and cell length, width, and general shape determined. Ground based controls using the same cells and media were similarly examined.

The particular cells used in this series of experiments did not swell to the maximum extent that has been observed, but within the range of swelling in these experiments, no difference could be detected between cells that were exposed to succinic acid in microgravity or under normal gravity. These results suggest that the enhanced binding detected in microgravity induces normal swelling processes and further demonstrates that the events of nodulation through cell swelling do occur under the microgravity conditions of spaceflight.

**II.A.2.w. Miniature Wasp Development in Microgravity (USML-1).** *Mike Voorhees and Marvin Luttgies.*

Little is known about the effects of microgravity on the growth and development of animals. However, understanding these aspects is crucial to the design of bioregenerative life support systems which will utilize a variety of biological organisms. In order to facilitate this understanding, research is being conducted on miniature wasps, specifically the entomophagous hymenoptera *Spalangia endius*. This species is a natural fly predator which stings fly pupae and inserts its egg to grow within the pupal casing. This life cycle, as well as the wasp's small size (1-3 mm in length), lends itself well to space research. The development of its eggs can be controlled merely by providing access to fresh fly pupae at the specified time. Furthermore, the maturation time is approximately 28 days, relatively short when compared to other organisms. Because fly maggots are efficient processors of human waste, they are likely candidates for use in CELSS, although adult flies would not be desirable. Miniature wasps might therefore be employed to control fly populations.

Flight research has included transport of pupal wasps onboard both STS-43 and STS-50. Additionally, on STS-50 unstung fly pupae were to be exposed to adult wasps using BioServe's Fluid Processing Apparatus. In this way, early stage development could be induced entirely in microgravity. Upon return of the samples to Earth, comparison could then be made both in terms of gross morphology and behavioral characteristics. Behavioral studies are effective in analyzing differences in neurological development.

While ground based data collection on wasps grown in nominal gravity has shown statistically significant phototropism and negative gravitropism, the STS-50 experiment experienced problems in both hardware and preflight mortality. Therefore, behavioral comparisons for flight samples were not obtainable.

**II.A.2.x. Effects of Microgravity on the Legume-Rhizobium Nodulation Process (USML-1).** *Peter P. Wong.*

Clover plants are small enough to survive for at least 10 days in the FPA. This laboratory has two strains of *Rhizobium*, TA1 and T24, that can nodulate the clover plants. However, strain T24 produces a toxin that specifically kills strain TA1. As a result, strain TA1 is a poor competitor for nodulating the plant when compared to T24. Ground-based experiments showed that by inoculating clover plants with the weak competitor, strain TA1, first for 5 days, then inoculated with T24, the first batch of the nodules was induced by TA1, which can be identified by antibodies and genetic markers. The nodules developed later were induced by T24, which also can be identified.

With regard to the experimental sequence on USML-1, on day 4, clover seeds in the FPA were irrigated with an N-free nutrient solution containing cells of strain TA1. The seeds germinated and seedlings developed. On day 11, the clover seedlings were inoculated with strain T24, which should kill off strain TA1. Upon returning to earth, the seedlings were planted in agar and examined for nodules induced by the two strains. Results show that reduced gravity had no effect on the germination of clover seeds. The seeds on USML-1 and the seeds on the ground both had 80% germination. In addition, the clover seedlings developed normally in reduced gravity. One week after returning to earth, 80% of the plants had visible nodules. The nodules were on the root sections that had developed at the reduced gravity conditions. Based upon these preliminary studies, this laboratory concludes that reduced gravity has no effect on the initiation and early development of the nodulation process.

**II.A.2.y. Effects of Low Gravity on the Lymphocyte Response (USML-1).** *Marian L. Lewis.*

The purpose of this experiment was to gain information on mediator-induced response of human peripheral blood lymphocytes in the FPAs. This preliminary test conducted on USML-1 at 20°C is the precursor to a subsequent experiment, to be flown at 37°C, in which monoclonal antibody will be used to activate the cells. Well-documented findings from spaceflight experiments over the past ten years indicate that one of the most detectable and consistent effects of altered gravity is blunted response of human T-

lymphocytes to Con-A. Mechanisms are not understood, nor have the response of many of the mediators of lymphocyte activation been investigated in low-g.

Initial results indicate that the cells remained sufficiently viable in the FPAs throughout the test profile of 4 days. Cell counts showed that approximately one to two million cells per ml were subjected to the mediator and the viability was about 60% when cells were fixed after 4 days. Post-flight analysis of the cells indicates that no markers for activation were evident, as expected, for this test at 20°C. However, the ground control for cell activation at 37°C showed the expected markers for activated cells and the DNA profile, shown by propidium iodide staining and flow cytometry, for cells moving into S and G<sub>2</sub> + M phases. The DNA content of the flight cells indicated that the majority of cells were in the G<sub>1</sub> or G<sub>0</sub> phases as expected for non-proliferating cells. Analyses of the ground controls for this flight are still in progress.

In conclusion, five of six FPAs functioned nominally in this test. One leaked slightly and one malfunctioned when the septum did not slide between the A and B chambers upon activation. It was also found that cells were healthy and numbers and viabilities remained high throughout the test including the 48 hrs pre-activation (pre-launch time plus on-orbit time before activation) and 4-day test. DNA profiles and cell surface marker assays showed that the cells were not activated in microgravity in the FPA's held at 20°C in this test.

#### II.A.2.z. **Formation of 3-Dimensional Bacteriorhodopsin Gel in Microgravity (USML-1).** *Robert Birge and Dershawn Govender.*

An important example of incorporating biomolecules in molecular electronic devices, which lies at the nucleus of this project, is the use of the 26 kD photosynthetic protein called bacteriorhodopsin. The protein functions as a chemiosmotic proton pump in the membrane of a micro-organism called *Halobacterium halobium*. When this purple colored membrane protein absorbs sunlight, it creates a chemical potential that is used to drive ATP hydrolysis and thus creating energy for the organism. When bacteriorhodopsin absorbs a photon of the correct energy, it undergoes a complex photocycle consisting of numerous excited states and intermediates that span the visible region of the electromagnetic spectrum. The memory storage device that has been proposed utilizes the photochemical switch between the bR and M states to encode a binary logic network within a three dimensional cube. The following list summarizes why bacteriorhodopsin was chosen to carry out optically coupled information storage and processing: 1) long term stability of the protein to thermal and photochemical degradation; 2) high forward and reverse quantum yields permits the use of low intensity laser beams for switching between states; 3) large two photon absorptivity enables the use of orthogonal colinear laser beams to store data in three dimensions; 4) a large spectral change that accompanies the switch between M and bR allows accurate assignment of the state the memory is in; 5) the use of monitoring the fast photovoltage during formation of M and bR in the assignment of the state within the irradiated volume; and, 6) the ability to orient and immobilize the protein in high optical quality water soluble polymers ensuring stability and the detection of the photovoltage. This last property of bR is the most vital aspect of making these memories and involves precise experimental procedure and technique. This laboratory has had moderate success in achieving (6), but there needs to be more basic research done in this area in order for memories described above to be reliable. It is hoped that microgravity experiments will provide the added information in order to enhance the experimental procedures for making more homogeneous and reliable data storage media.

The project specifically entailed the construction of oriented bacteriorhodopsin protein molecules immobilized in a polymer matrix to be used as a media for a three dimensional optical random access memory. The memory employs the intersection of two colinear orthogonal laser beams to address the volume within the protein-polymer matrix. The three dimensional addressing capability lies in the ability to change the position of the intersecting laser beams in the x, y and z dimensions. Presently, modern computer architecture integrates closely packed parallel two dimensional circuitry which is limited not only to x and y dimensions.

The goal for the first microgravity experiment was to try to polymerize the bR-polymer matrix in an efficient and non-destructive manner in which to preserve the homogeneity of the media. Initial results under microgravity conditions have shown to be successful. Proper polymerization was achieved and it was possible to monitor the sample efficiently with the onboard spectrophotometer. The polymerization will be used as a foundation for possible future experiments where both magnetic and electric fields can be applied to achieve orientation. In order to carry out these experiments on a space shuttle and due to the novelty of the experiment, it is necessary to design new apparatus in order to encase the experiment to achieve the desired properties. The information obtained from the last shuttle flight will be used to advance the experimental techniques to the final stage and to optimize the making of homogeneous memory media.

**II.A.2.aa. Cardiac Gap Junction Channeling Activity in Space (USML-1).** *Dale E. Claassen, Jacque van Twest, and Brian S. Spooner.*

This experiment investigated the effects of long periods of microgravity on cardiac gap junction channel function. Gap junctions are protein channels that link cells and allow intercellular communication necessary for normal tissue function. In this experiment, gap junction channeling was measured in liposome-reconstituted heart membranes using an enzyme-substrate signalling system. Prior to launch, cardiac gap junction membranes from chicken were inserted into liposomes containing an entrapped enzyme. After 1 day in microgravity, a membrane-impermeable colored substrate was added to the liposomes. If functional gap junction channels are present in the liposome membrane, the substrate passes through the membrane and reacts with the enzyme to produce a non-colored substrate, which is detected spectrophotometrically.

The initial results from this experiment reveal a considerable decrease (>50%) in heart gap junction channeling activity during spaceflight, as compared to identical ground controls. All components of the channeling assay are continuing to be analyzed.

### **II.A.3. Enabling Hardware Studies**

BioServe has continued to develop a wide range of experimental hardware that will be applicable to space life sciences. The details of the design, fabrication and flight certification are described in other documents. It is generally realized that the development of enabling hardware, suitable for a wide range of subcellular, cellular and intact animal and plant species, remains a key requirement for a successful future of space life sciences. Many of the hardware designs are tested under ground-based conditions, flown on the KC-135 aircraft - whenever feasible - and graduated to shuttle flights as accommodations and experimental protocols become available. The BioServe hardware program is ever-evolving with new enabling devices being planned, and others modified for improvements.

**II.A.3.a. Iodine Microbial Control of Hydroponic Nutrient Solution.** *Timothy L. Stroup, Steven H. Schwartzkopf, and George L. Marchin.*

The space transportation system uses a triiodide quaternary ammonium strong base resin to prevent microbial contamination of the crew's drinking water. Current plans for Space Station Freedom use the STS resin for microbial control in drinking water. Another use for this water is in the "salad machine" to grow vegetable plants hydroponically. These experiments demonstrate that leaf lettuce (*Lactuca sativa*) grown in nutrient solution treated with the triiodide resin and its next higher homologue, pentaiodide, result in greatly reduced growth or death. The triiodide and pentaiodide treatments reduced plant fresh weights to 0.2% and 0.04% of the controls respectively. Tissue analysis by neutron activation showed an iodine concentration of 0.47% to 0.6% in the experimental plants. Nutrient solution analysis showed an average residual concentration of 38 and 65 mg/l iodine at the end of the 30 day experiments for triiodide

and pentaiodide treatments respectively. Consequently, these resins should not be used alone in conjunction with plant growth systems.

**II.A.3.b. Preliminary Assessments of Fluid Transfer Induced by the Operation of Sliding Diffusion Wells in Microgravity.** *Konrad W. Pollmann, Louis S. Stodieck, and Marvin W. Luttgies*

Diffusion dominated experiments in microgravity avoid disturbances by double-diffusive convection and sedimentation, a benefit that has been extensively used for protein crystal growth in space. The same effect also offers manufacturing and research opportunities for many other biochemical systems such as assembly of supermolecular structures (fibers, membranes, virus capsids, etc.) or evaluation of double diffusion in complex liquids. Based on the scientific, operational and flight qualifying criteria for space experiments, the BMA has been optimized to fulfill the needs of diffusion dominated biochemistry in space. A series of KC-135 experiments was conducted in order to verify the quality of the diffusion interface in the sliding wells of the BMA, and to study the mass transfer that is induced by the operation of the wells themselves.

Water/glycerol mixtures at different viscosities were tagged with a pH indicator (bromothymol blue) and filled into clear lexan sliding wells. Close-up video and photography allowed direct observation of the mixing behavior at different sliding speeds and conditions. After the end of the flight, the fluids were retrieved and the total fluid transfer was measured. The pictures and videotapes show the diffusion interface to have a slight S-shaped deformation, the exact shape of which depends on the relative importance of viscous or inertial forces (i.e. the Reynolds number). Even at slow sliding velocities, the second sliding step always induces some non-diffusive fluid transfer. The short low-gravity time period aboard the KC-135 (about 25 sec) did not permit a complete evaluation of this effect at slow velocities. However, a 2-D computer simulation is intended to substitute the missing data. The good repeatability of the experiment is anticipated to allow development of a compensation for this induced transfer in order to facilitate quality diffusion measurements.

**II.A.3.c. Change of Radiative Energy Output from a Halogen Bulb in Reduced Gravity.** *Alex Hoehn.*

The light or radiant energy output from a filament bulb is a function of the temperature of the filament. This temperature is mainly determined by the electrical characteristics of the system (voltage, current, resistance). In addition, the filament temperature of gas-filled lamps, such as tungsten-halogen bulbs, is influenced by internal convection currents between the hot filament and the cooler glass enclosure. This convection current is used to re-deposit evaporated tungsten onto the filament, increasing the life-time of the halogen bulbs. In order to establish such a regenerative convection-driven transport of tungsten, the temperature of the glass enclosure has to be above 250°C. The convection current within the bulb enclosure is assumed to be a function of gravity, and changes in gravity will therefore change the thermal characteristics and the light output of the bulb.

An experiment was designed for use onboard the NASA KC-135 reduced gravity research plane. A six volt/10 watt tungsten-halogen bulb was connected to a constant voltage power supply. The light output was measured with six sensors, each measuring at a specific wavelength (20 nm bandwidth interference filters) between 400 nm and 800 nm in the visible portion of the spectrum. The lamp was turned on continuously and the light output, as well as electric variables, were measured during 40 parabolas of reduced/increased gravity. Reduced gravity episodes ( $2 \times 10^{-2}$  Earth gravity) were in the order of 20 seconds (30 seconds for Martian and Lunar parabolas), and increased gravity episodes were typically up to 1.8 times Earth gravity.

The light output was shown to be a function of the gravity environment. With a decrease of gravity, the lamp output increased (brighter, typically 5-15% depending on wavelength). At the same time, the lamp current decreased due to higher filament resistance at higher temperature. During increased gravity episodes, the light output decreased (lower temperature). These changes are related to the increase/decrease of natural convection within the bulb as a function of a decrease/increase of gravity,



respectively. In the reduced gravity environment of space, the gas-filled Tungsten-Halogen bulb will deliver more light and reduce electric power consumption.

Another experiment was designed to investigate the possible impact of the reduced gravity environment on the regenerative Halogen cycle (re-deposition of evaporated tungsten) and therefore the life-time of such a bulb. In this case, the lamp was operated during reduced gravity episodes (80 times 20 seconds of reduced gravity), but no tungsten deposits could be detected on the interior wall of the glass. It is believed that the available time in reduced gravity was not sufficient to show these deposits.

#### II.A.3.d. **A-MASS KC-135 Flight** *Ellis Gayles, Steve Simske, Mark Edwards, and Alex Hoehn.*

The A-MASS (Animal Module for Autonomous Space Support) underwent initial microgravity testing onboard the KC-135 June 9-12, 1992. Four mice were flown onboard the A-MASS model, which consisted of the cage system, the food and water supply system, the fan system, the fecal collection system and the electroluminescent lighting system. A number of important observations were made:

1. The electroluminescent light sheets worked well, and were not ostensibly affected by reduced gravity. No perceived alteration in light intensity was observed for these sheets during reduced gravity episodes.
2. The fan system provided a linear airflow through the cage area. Waste products (feces) were directed to the opposing end of the cage by the fan system, and collection of waste within the cage was not observed.
3. The force required to advance the cage system (thus providing new food and water for the mice) was not increased under conditions of reduced gravity.
4. The mice demonstrated an increase in fecal production during the flight periods. Prior to the onset of the KC-135 flight parabolas, the mice moved about the cage freely; however, subsequent to the onset of the parabolas, the mice remained in one place within the cage region. The mice were apparently traumatized by the flights (two times gravity, followed by reduced gravity, repeated up to 50 times) enough so that they did not recover to behavioral normalcy until after landing. No eating or drinking and little motional behavior was observed during the flights. Although the reduced gravity episodes were brief (30 seconds in length), the experiment suggests that mice will exhibit "frightened" behavior for a measurable period of time during the launch and reduced gravitational adaptation stages of future flights, such as the COMET.

#### II.A.3.e. **P-MASS KC-135 Flight**

The KC-135 z-acceleration signal (1 V = 1 g) was used for the first time, May 5-8, 1992. Some interface problems were experienced at the beginning that could be resolved. KC-135 signal requires that the measuring device has at least 1 Megohm input impedance. BioServe built a voltage follower using an op-amp. The remaining problem was that the op-amp, as well as the Polycorder, maintained their high input impedance only when powered on; when turned off, their input impedance changes and so does the displayed gravity level in the airplane (Polycorder drops from 1.8 Megohm to 38 kOhm; the op-amp drops from several Megohm to 1 Megohm). Two op-amps were used; one for each data acquisition system for the following days. No more problems were experienced. Op-amps have to be turned on all of the time while connected to the airplane acceleration signal to maintain high input impedance. This needs some refinement and a new box.

P-Mass was successfully interfaced with and flown onboard the KC-135, May 5-8, 1992. For this flight, only visual observation was noted (water drainage from oversaturated growth media, overall performance). No problems could be observed resulting from high or low g-levels during the short experimental intervals. At the start of the experiment, the P-MASS walls were completely covered with water condensation on the inside, which could successfully be removed by evaporation and air circulation during

the 40 parabolas. Future experiments could include air flow visualization and possibly bulk water orientation/redistribution (simulation of excess water forced out of the media during COMET launch).

The two P-MASS fans were mounted on a frame with 3 inch spacing between the fans, same orientation of rotation and powered by a 12 VDC battery pack. After turning fans on, this assembly was carefully released in low gravity. The intention was to observe any spin-up of the frame with respect to the rotors. Probably due to the large mass of the battery pack, such a spin-up could not be observed in a reproducible manner. Another fan (hand-held, battery-powered cooling fan) was used for comparison, and showed an almost instantaneously spin-up of the base with respect to the rotor. This fan produced a large thrust and could propel itself through the cabin (P-MASS fans moved very slowly if at all). With a smaller and lighter battery pack, experiments should be reflown and tested with counterrotating and non-counterrotating fans, and additional calibrations at the laboratory will be needed.

#### **II.A.3.f. FPA - Fluid Processing Apparatus onboard KC-135: Fluid Mixing and Two-Phase Fluid Behavior in Reduced Gravity Environments.** *Alex Hoehn and David Klaus.*

The Fluid Processing Apparatus (FPA) is a syringe-like fluid container used in the Generic BioProcessing Apparatus (GBA), which was successfully flown during the First United States Microgravity Laboratory (USML-1), in June/July 1992. Fluids are contained in a glass tube and separated by rubber septa. When depressing a plunger, the septa move and allow controlled mixing when the individual septa slide past a bypass in the glass tube. Chemical/biological reactions can be started by injecting an activation fluid (second chamber) in the process chamber (first chamber). Later on, the reaction can be terminated by injecting another fluid from the third chamber into the process chamber.

For some applications, the FPAs contain two-phase fluids, air and liquid (bacteria or brine shrimp with air space for oxygen supply). In a reduced gravity environment, the orientation and location of the fluids is different than in nominal gravity. Surface tension becomes the major force controlling fluid behavior. Parametric studies onboard the NASA KC-135 Reduced Gravity Research Plane were undertaken to characterize fluid behavior, orientation and manipulation possibilities as a function of fluids (water, glycine, oil, bacteria solution, alcohol, detergent, etc.) and surface treatment of the FPA (greased, cleaned or surface treated glass: wetting/non-wetting).

For most fluids, the location of the air space within the FPA reaction chamber could successfully be controlled by slight centrifugal movements. This is important for the optical density measurements of the fluids in the Generic BioProcessing Apparatus, due to the location of the optical sensors with respect to the reaction chamber. Sigma Cote™ and vacuum grease, both used to treat the glass walls for easier movement of the rubber septa, resulted in very flat fluid menisci. The solution containing bacteria, however, showed completely different fluid characteristics. The liquid was always wetting the glass container, trapping the air space into a single bubble in the center of the reaction chamber. This reaction was almost independent from surface treatments. The same fluid behavior was observed for brine shrimp cultures onboard USML-1 in June/July 1992. It is assumed that metabolic by-products of these living organisms cause this change in fluid characteristics.

In addition to observations of two-phase fluid systems, fluid mixing experiments were conducted, injecting different fluids into the reaction chamber. These experiments included different density fluids (oil into water, water into oil, air into water, water into air, high/low viscosity fluids into each other). Results and differences in fluid mixing efficiencies were documented on 8 mm video tape. All observations used a specially designed video camera mount with autonomous lighting capabilities (back light and side light). The camera assembly could be free-floated during reduced gravity with the experimenter or mounted to the surface of the plane. In free-floating mode, a very low residual gravity and very low vibration environment could be achieved. Fluid manipulation techniques, such as centrifugation or shock disturbances, could only be done in the free-floating mode.

### **II.A.3.g. The Directed Polymerization Apparatus (USML-1): Preliminary Assessments. Todd Bergren.**

The Directed Polymerization Apparatus, or DPA, a piece of hardware designed and built by BioServe personnel to study the polymerization of collagen in microgravity, was successfully flown in the Glovebox Facility on board the USML-1 mission. The hardware performed as expected and the collagen samples have been returned to BioServe labs for analysis.

Collagen is a major structural protein in the body, can be easily manipulated in the laboratory, and does not cause as strong an immune response, when implanted, as the synthetic materials now in use. These properties make collagen attractive as a material for prosthetics such as artificial tendons, skin and blood vessels. However, to approach the strength of the natural structures they are to replace, the artificial implants must be made of highly organized collagen, something not currently possible in the lab. We believe that in the quiescent fluid environment of microgravity, the organization of assembling collagen could be controlled with the application of a small forcing function such as an electric current. It was for this purpose that the DPA was designed. Within it, collagen is allowed to polymerize under the influence of a small electric current while in microgravity. It is hoped that the lack of gravity-driven flows in space will allow the production of highly organized collagen products.

Seven of the eight samples flown produced "plugs" of completely polymerized collagen that appear to the naked eye to be uniform in both color and texture. Microscopic examination confirmed these gross observations; magnification revealed gels of remarkable homogeneity, with no observable variations in the density of the polymerized material. The optical transmissivity of the samples was also measured; it did not vary significantly between samples. The collagen plugs were also examined with a polarizing microscope. No areas of birefringence, an indicator of organization visible under polarized light, were observed using this technique. We anticipate that examination of properly stained thin sections will better highlight the organizational patterns of the collagen samples.

Further testing to be completed includes both histologically examination and mechanical testing of the samples. The samples produced under the influence of the electric current will be compared to the non-electrified controls. When the hardware itself is returned, it will be used to complete ground tests. The ground samples will be analyzed and compared to the flight collagen. Photographs taken of the DPA during both flight and ground test will be examined for evidence of differences in polymerization rates.

### **III. Education and Training**

BioServe Space Technologies is at the leading edge of space life sciences research and hardware development. To maintain this position, BioServe personnel are committed to preparing for the future by training today's students to be tomorrow's space life scientists. Through BioServe's unique composition of engineers and life scientists, environments and opportunities are created that encourage student input and technology transfer, and that generate new knowledge. To this end, BioServe Space Technologies offers unique and innovative training programs that cultivate the development of scientific and engineering skills, focusing on both ground-based and microgravity studies.

As a demonstration of our training efforts it should be noted that during the past year alone, 13 postdoctoral fellows participated in BioServe research programs. Nearly 45 graduate students were involved in our experimental efforts and 24 undergraduate students were active participants (Appendix II). Trainees have been involved in research design, hardware development and certification, parabolic flight missions, preparation and recovery of sounding rocket and shuttle flight payloads, presentations at regional and national meetings and often were co-authors of scientific publications that appeared in professional journals during the year. Many of these students are now among the professional staffs of NASA, space contractors and biomedical companies. We remain firm in our belief that by encouraging student participation in these types of opportunities we are investing in our future, and the future of our nation.

In addition to our student training efforts, BioServe scientists continue to make many space life science outreach presentations to community, school and youth groups. Scientists have also made numerous presentations to industrial and professional space organizations.

A new graduate student research opportunity developed just last year, provides opportunities for our trainees to participate in the research efforts at JSC. Through the cooperative efforts of USRA, our students can now obtain an experience that otherwise is not available in academic communities. In addition, BioServe currently is pursuing a new agreement with one of the nation's largest pharmaceutical industries whereby our bioengineering and life science trainees will have an opportunity to participate in internship positions in the field of space life sciences and hardware design consistent with future pharmaceutical applications in the microgravity environment.

Both graduate and undergraduate students have received honors and awards for their scientific achievements during the past year and their accomplishments stand as a testimony to their dedication to the improvement of space life sciences.

*BioNexus*, BioServe's quarterly newsletter, continues to serve as a communications vehicle and provides an update for commercial participants, potential industrial affiliates and individuals interested in the activities of the CCDS at both the Colorado and Kansas campuses. In addition, a new series of regular communications has been developed to more frequently reach out to the commercial participants and to provide timely information about flight opportunities and research results.

#### **IV. Conclusions**

During the past year BioServe Space Technologies has continued to mature, and the interactions among aerospace engineering and life science personnel has provided an unusual strength to our nation's future in space. The Center blends a powerful and multidisciplinary program that includes ground-based research at the cutting edge of the biological sciences, microgravity experiments in the plant and animal sciences, and flight hardware development that already has served numerous investigators within the Center, our commercial colleagues, scientists at NASA field centers and participants at other universities. BioServe has played a key role in the development of COMET including the designs for the P-MASS and A-MASS facilities that are associated with plant and animal payloads for the commercial flyer.

BioServe's flight record is enviable and now includes several hundred parabolas on the KC-135 aircraft associated with the NASA reduced gravity program, several sounding rocket flights and three shuttle missions (STS-37, STS-43 & STS-50). Planning is underway for an expansion of our reduced gravity experiments, and our faculty scientists, trainees and staff continue to provide our industrial participants with a unique '*window of opportunity*' to reach the microgravity environment.

These activities have attracted several new companies to BioServe. In addition, several patents for flight hardware and biologicals, associated with the BioServe initiative, currently are being pursued.

One of our most important legacies for the future is associated with the training of undergraduate students, graduate students and postdoctoral fellows. We continue to emphasize the importance of the development of a new cadre of life scientists and engineers that are both knowledgeable and comfortable with the opportunities ahead for the commercialization of space.

We look forward to the challenges of these programs in 1993, and remain convinced that BioServe Space Technologies will continue to play an important role in the service of the NASA Office of Commercial Programs.

## **Appendix I:**

### **The BioServe Center for the Commercial Development of Space**

#### **Roster of Participants and Supporters**

Abbott Laboratories  
Alza Corporation  
Aquatic Products International  
Ball Aerospace Systems Group  
Barbara Davis Center for Childhood Diabetes  
Boeing Aerospace Company  
Carlton Technology  
Center for Space and Advanced Technology  
CETUS/Chiron  
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Development Planning and Research Associates, Inc.  
Diagnostic Concepts International  
Exabyte  
Florida Space Port  
Fluke Instruments  
Gelman Sciences, Inc.  
Granteur, Inc.  
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IatroMed, Inc.  
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Lockheed Missiles and Space Co.  
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Naval Research Laboratory  
OmniData International, Inc.  
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RanTek, Inc.  
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Spalding Industries  
SynchroCell, Inc.  
Syracuse University  
University of Alabama, Huntsville  
University of California, Riverside  
University of Colorado  
University of Colorado Health Sciences Center  
University of Rochester, NSCORT  
WTC Industries, Inc.



## Appendix II:

### The BioServe Center for the Commerical Development of Space

#### Personnel:

##### Administration:

|                                  |  |
|----------------------------------|--|
| Marvin W. Luttges, Ph.D., CU     | Director of BioServe                       |
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| Louis Stodieck, Ph.D., CU        | Assoc. Dir., Technical Affairs             |
| Michael Robinson, Ph.D., CU      | Assoc. Dir., Engineering                   |
| John Berryman, CU                | Assoc. Dir., External Affairs              |
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| James A. Guikema, Ph.D., KSU     | Scientific Steering Committee              |
| Brian S. Spooner, Ph.D., KSU     | Scientific Steering Committee              |
| Larry G. Williams, Ph.D., KSU    | Scientific Steering Committee              |

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Alex Hoehn, Ph.D., CU (ESA)  
Margaret Juergensmeyer, Ph.D., KSU  
David Klaus, Ph.D., CU  
Brenda J. Klement, Ph.D., KSU  
Mary Kranz, M.S., CU  
Mark Miller, M.S., CU  
Art Mills, Ph.D., KSU  
Ralene R. Mitschler, Ph.D., KSU  
Philip Moos, Ph.D., KSU  
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**Graduate: (cont'd)**

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Marla Pyle, Ph.D., KSU  
Don Richter, Ph.D., KSU  
Beth Scaglione, Ph.D., KSU  
Tom Schmeister, M.S., CU

Safwan Shah, M.S., CU  
Derek Shipley, M.S., CU  
Jay Spivak, Ph.D., CU  
Michael Voorhees, M.S., CU  
Keith Woods, Ph.D., KSU  
Jeff Zoelle, Ph.D., KSU

**Undergraduate:**

Trent S. Armbrust, KSU  
Marcia Bertsch, KSU  
Paul D. Fey, KSU  
Scott M. Hane, KSU  
Gabrielle Hanek, KSU  
Laura Hawkins, KSU  
Richard Hilgenfeld, KSU  
Suzanne Kraus, KSU  
Laurel Lenz, Jr., KSU  
Jeffrey Lickteig, KSU  
Kevin L. Mapes, KSU  
Kimberly Miner, KSU

David Moore, CU  
Ralph Najarian, KSU  
John P. Nichols, KSU  
Bryan T. Nolt, KSU  
Trang Pham, CU  
Keith Pharris, CU  
Amy M. Robinson, KSU  
Matthew Sylte, KSU  
Brian C. Timberlake, KSU  
Hoang-Danh Van, KSU  
Brenda Westhoff, KSU  
Teresa Young, CU



## Appendix III:

### The BioServe Center for the Commercial Development of Space

#### Publications

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## **Appendix IV:**

### **The BioServe Center for the Commercial Development of Space**

#### **Presentations**

Abrahamson, K.S., Lisec, J.T., Derby, J.A., Simske, S.J., and Luttgies, M.W. (1991) Effect of 6 day weightlessness on leguminous sprouts. *ASGSB Bulletin*, 5: 59.

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Appendix V:

USML-1, STS-50, Microgravity Experiments

The BioServe Center for the  
Commercial Development of Space

| Title   | Principal Investigators  |
|---|--|
| <i>Collagen Self-Assembly and Directed Orientation on Board USML-1</i>                | Todd Bergren<br>Louis Stodieck   |
| <i>The Directed Polymerization Apparatus</i>  | Todd Bergren   |
| <i>Effects of Spaceflight on Tumor Necrosis Factor-alpha-Induced Cell Lysis</i>       | S.K. Chapes  |
| <i>Proteolipid Membrane Formation and Encapsulating Efficiency During Spaceflight</i> | Dale E. Claassen<br>Brian S. Spooner<br>Jacque van Twest                               |
| <i>Biological Self-Assembly of Viral Protein</i>                                      | Richard A. Consigli<br>Deching Chang   |
| <i>Seed Germination in Microgravity</i>   | Alex Hoehn<br>Kirsten Abrahamson<br>Shawn Gomez<br>Michael Voorhees<br>Marvin Luttgies |
| <i>Cytoskeleton Disruptive Agents: Effects on Plant Growth in Microgravity</i>        | James A. Guikema<br>William Odom<br>Greg Gallegos                                      |
| <i>Resource Partitioning in Plant Tissues During Development</i>                      | James A. Guikema<br>Emmanuel Hilaire<br>Trent Armbrust                                 |
| <i>Gravity Effects on Wild-type and Agravitropic <u>Melilotus alba</u></i>            | James A. Guikema<br>M. Juergensmeyer<br>Jeff Westberg                                  |
| <i>Reduced Gravity Effects on <u>E. coli</u> Growth and Adaptation</i>                | David Klaus  |

| Title   | Principal Investigators   |
|---|---|
| <i>Bactericidal Activity of Tri- and Pentaiodide Resin Disinfectants Under Conditions of Microgravity</i> | George Marchin  |
| <i>The Influence of Microgravity on Cellular Events Associated with Proliferation and Differentiation</i> | Philip Moos<br>Dan Enebo<br>Heideh Fattaey<br>Richard Gerren<br>Terry C. Johnson          |
| <i>Activation of T Cell Cytokine Synthesis with Monoclonal Antibodies to the T Cell Antigen Receptor</i>  | Joseph S. Murray<br>Tony Schountz   |
| <i>Fibrin Assembly in Microgravity and Magnetic Fields</i>  | Konrad Pollmann<br>Marvin W. Luttgess<br>Louis Stodieck                                   |
| <i>Brine Shrimp Development in Space</i>  | Brian S. Spooner<br>Lynnette De Belle<br>James Rosowski<br>Laura Hawkins<br>Janet Metcalf |
| <i>Protein Crystal Growth in Microgravity</i>   | Paul Todd<br>Michael Sportiello   |
| <i>Bacterioid Formation and Binding in Microgravity</i>   | James E. Urban  |
| <i>Miniature Wasp Development in Microgravity</i>   | Mike Voorhees<br>Marvin Luttgess  |
| <i>Effects of Microgravity on the Legume-Rhizobium Nodulation Process</i>                                 | Peter P. Wong   |
| <i>Effects of Low Gravity on the Lymphocyte Response</i>  | Marian Lewis  |
| <i>Regeneration of Transected Planaria During Spaceflight</i>   | Dena Rashkover<br>Ramzi Nassar<br>James Ralph   |
| <i>Formation of 3-Dimensional Bacteriorhodopsin Gel in Microgravity</i>                                   | Robert Birge<br>Deshawn Govender  |
| <i>Cardiac Gap Junction Channeling Activity in Space</i>  | Dale Claassen<br>Jacque van Twest<br>Brian Spooner  |