ABSTRACTS
SESSION A: DICK YOUNG SYMPOSIUM

[1] PLANT CELLS IN SPACE: WHAT HAVE WE LEARNED? A.D. Krikorian. Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, NY 11794-5215

Plant cells grown in vitro, especially those that are embryogenic, afford valuable opportunities for both applied and basic research. For example, there is little doubt that CELSS will profit immensely by incorporating a tissue culture component into its overall strategy. Before that becomes possible, however, one has to know how to grow plant cells in the space environment and to understand limitations. Plant cultures at different levels of development have allowed the relationship of development under the special conditions of space to be probed. As expected there is a risk associated with growth in space but that risk can be reduced and rendered acceptable with knowledge. I have proposed and reasserted that perturbations such as chromosomal and nuclear abnormalities (genome shock), accelerated aging and reduced growth observable in some space-grown samples and not in others, are due to a combination of factors including the biological "status" of the systems and the way things are grown and exposed to and, ultimately, the way they "experience" what may be called "space-specific stress". Apparently these stresses can be minimized but not completely eliminated. A unifying hypothesis that has already been tested in several parts but not comprehensively shows that the data obtained from several missions, including the latest from an extended duration exposure on Mir are consistent with the hypothesis. The findings and recommendations should provide a framework for utilizing embryogenic plant systems in space towards practical ends. The practice of growing somatic embryos in space is moving out of the age of empiricism and on to a logical basis; it is ceasing to be an art, and becoming a science. Supported by NASA.

[2] NORMAL TADPOLES FROM AMPHIBIAN EGGS INVERTED BY CENTRIFUGATION. Steven D. Black, Ashley Crutchfield, Melissa Murphy, Teresa Swayne, Dept. of Biology, Reed College, Portland, Oregon.

Eggs of *Xenopus* laevis and many other amphibians contain a gradient of yolk platelets along the animal-vegetal axis. Small platelets predominate in the animal hemisphere, and a boundary between medium and large yolk platelets exists near the equator. The blastopore forms at this boundary at the beginning of gastrulation, in the vegetal hemisphere.

Does this boundary have a role in determination of the position of the blastopore, or does cortical information predominate? Past experiments using 1g to invert the egg suggested a strong tendency to form the blastopore in the original vegetal hemisphere. The present experiments, however, used centrifugation at 20g to achieve a more complete inversion of the yolk gradient, and the blastopore formed in the original animal hemisphere in 96% of egg clutches. Normal tadpoles formed and developed into frogs with gametes, so the inverted gastrulation is functionally normal. These data support the idea that the position of the blastopore depends on the position of the egg's internal contents rather than cortical determinants.

Earth’s biosphere consisted solely of single-celled life for at least 80 percent of its more than 3.8 billion year (Ga) lifetime. Life was remarkably sophisticated even prior to 3 Ga ago. It could perform photosynthesis and consume organic matter, and it could oxidize and reduce a variety of chemical substrates to obtain energy. It lived in a world where volcanism and hydrothermal activity were more pervasive than today, and where the atmosphere was rich in CO₂ and very poor in O₂.

The transition to the modern world witnessed the aggregation and stabilization of larger continents from smaller ones, the rise of O₂ and decline of CO₂, and the proliferation of life across vast expanses of ocean, coastlines and land. The episodic oxidation of the environment between 2.5 and 0.5 Ga ago created new opportunities for the development of the microbial ancestors of plants and animals. This close correspondence between the planetary controls on O₂ levels and the biological evolution leading to multicellular life is an elegant demonstration of the intimate relationship between the evolution of Earth and its biosphere.


For humans to live in the environment of space permanently, we must develop a bioregenerative life support system and not take all the life sustaining resources with us. Both physical/chemical and bioregenerative life support systems can remove CO₂, recover water, and recycle solid waste, but only bioregenerative systems produce food. Research and technology development efforts and human testbed evaluations currently are focused on developing sufficient research and engineering data to allow for the utilization of higher plants as the primary component in a bioregenerative life support system. These tests and experiments are identifying crop cultivars, determining controlled chamber conditions for optimum plant growth, evaluating technologies that will minimize energy and volume use, investigating microbial stability and trace contaminant issues, and developing food processing equipment. Flight experiments are investigating the impacts of microgravity on plant photosynthesis and metabolism, the delivery of water and nutrients to plant roots, and the methods of handling water and gasses in plant growth chambers, in general, so that plants will grow normally.


Although the Viking results may indicate that Mars has no life today, there is direct geomorphological evidence that, in the past, Mars had large amounts of liquid water on its surface — possibly due to a thicker atmosphere. From a biological perspective the existence of liquid water, by itself motivates the question of the origin of life on Mars. One of the martian meteorites dates back to this early period and may contain evidence consistent with life. From studies of the Earth’s earliest biosphere we know that by 3.5 Gyr. ago, life had originated on Earth and reached a fair degree of biological sophistication. Surface activity and erosion on Earth make it difficult to trace the history of life before the 3.5 Gyr timeframe. Ecosystems in cold, dry locations on Earth — such as the Antarctic — provide examples of how life on Mars might have survived and where to look for fossils.
OPTIMIZATION OF INTRACANOPY LIGHTING FOR HYDROPONICALLY GROWN COWPEA IN CONTROLLED ENVIRONMENTS. J.M. Frantz and C.A. Mitchell, Dept. of Horticulture, Purdue University, West Lafayette, IN

A major source of power consumption in controlled-environment crop production for regenerative life support in space will be plant-growth lighting. Methods developed to minimize this source of power consumption will improve the efficiency of crop production through more efficient management of non-renewable resources. One such method uses “intracanopy lighting” in which plants are allowed to grow through multiple levels of low intensity lamps to irradiate the understory that normally is shaded when traditional overhead lighting is used. Early results with cowpea (Vigna unguiculata L. Walp ‘TR7D-941-1’), a legume species with edible leaves, pods, and/or dry beans, indicate a significant potential for increased energy-use efficiency. Incorporation of reflectors, optimization of plant density within stands, and manipulation of lamp geometries for more efficient irradiation while maintaining low power consumption are the focus of present experiments. Computer-generated light maps in three-dimensional space were used as a predictor for lamp placement to obtain maximum light interception. The productivity parameters harvest index, edible yield rate (EYR = gDW m⁻² day⁻¹), yield efficiency rate (YER = gDW edible m⁻² day⁻¹), energy conversion efficiency (ECE = EYR [gDW non-edible]⁻¹), energy conversion efficiency (ECE = EYR [kWh h⁻¹]), and energy partition efficiency (EPE = YER [kW h⁻¹]) express the costs of edible biomass production in terms of the spatial, temporal, energetic, and non-edible biomass penalties. Research supported, in part, by NASA grant NAGW-2329.

MODULATION OF LIGNIN (AND LIGNIN-LIKE) FORMING PROCESSES DURING NORMAL, COMPRESSION AND HEARTWOOD DEVELOPMENT IN CONIFER. M.K. Chang, A.M. Amorela, D.L. Bedgar, L.B. Davin and N.G. Lewis, Institute of Biological Chemistry, Washington State University, Pullman, WA

Lignin (and putative lignin-like) forming processes are thought to be modified during development of normal, compression and heartwood secondary xylem. These changes can involve either the preferential biosynthesis of different monolignols (coniferyl and p-coumaryl alcohols), or alterations in the range of phenylpropanoid products generated. Together these metabolites account for in excess of 30% of all organic carbon circulating in the biosphere.

This study had two objectives: first, to define the biochemical basis for preferential biosynthesis of p-coumaryl and coniferyl alcohols, and, second, to define the precise enzymology of monolignol coupling reactions involved in different tissues. For the first objective, cell suspension cultures were induced to form the lignin monomers, with assays being conducted to determine metabolite levels (intra- and extra-cellular), enzyme activities and transcript levels of genes of interest. Using a combination of HPLC, biochemical assays, and northern blots, it has been possible to establish a relationship between [hydroxy]cinnamate build-up and monomer composition. In the second objective, a combination of tissue-printing, in situ hybridization and subcellular localization studies revealed the location of a dirigent protein involved specifically in control of monolignol coupling in differentiating xylem, root tissue and developing phloem, respectively. A new paradigm is hence emerging which links the formation of these various metabolic events to precise, distinctive tissue-specific processes.

Funding for this study was supported by NAG100164.

PLASTID SEDIMENTATION KINETICS IN ROOTS OF WILD TYPE AND STARCH-DEFICIENT MUTANTS OF ARABIDOPSIS. S.A. MaciCleary and J.Z. Kiss. Department of Botany, Miami University, Oxford OH

Plastids in specialized cells are hypothesized to be involved in gravity perception in plants. When a root is re-oriented, movement and/or sedimentation of these dense bodies within the root-cap columella cell cytoplasm are believed to elicit a cascade of cellular events which results in gravitropic curvature. Recent research in this laboratory has shown that a starchless mutant is less gravitropic than its wild type (WT) and that two reduced-starch mutants (51% and 60% of WT) have an intermediate response to gravity (Kiss et al. 1997, Plant Cell Physiol. 38:518-525). Thus, the degree of gravitropism is decreased in the total amount of starch per cell. In this study, we assayed plastid position and movement in the WT and three starch-deficient strains. Plastid sedimentation in the columella cells of Arabidopsis roots was measured by rapid vertical position and angular movement around the center of the cell (center of area). Four-day-old seedlings were reoriented 90°, fixed at various times (0, 0.5, 2, 5, 10, 30, 60 minutes), and then embedded in Quetol resin. A vertical control was also fixed. One micrometer median longitudinal sections of the root cap columella cells were visualized under brightfield optics and images were captured and analyzed with an image analysis program. Qualitative observations of the WT show that some plastids sediment within 30 seconds and that the majority in 2-5 minutes, which is less than the presentation time of 5.3 minutes. These data are consistent with a plastid-based statolith theory. Work is in progress on the localization of the plastids of the starch-deficient strains. Discrimination of the kinetics of plastid movement in plants with differing abilities to perceive gravity would be valuable in evaluating any proposed gravitropic mechanisms, including the starch-statolith theory. (Financial support was provided by NASA grant NAG 2-1017.)

OSMOSENSING PATHWAY ACTIVATION BY LOSS OF TURGOR PRESSURE. Q. Zhao, B. Smith, J. Doll, K. Fang, and M.C. Gustin. Dept. of Biochemistry & Cell Biology, Rice University, Houston, TX

The response of cells and organisms to gravitational acceleration - or its absence - depends on various sensory mechanisms, pressure sensors, etc., whose function is still poorly understood. To investigate the molecular mechanisms of pressure sensing, we have analyzed how a yeast osmosensing MAP kinase cascade called the HOG pathway is activated by an increase in medium osmolarity. Specifically, we are interested in defining the physical forces and biochemical mechanisms responsible for HOG pathway activation by osmotic upshock. Our working hypothesis is that loss of turgor pressure, induced by collapse of the osmotic gradient, is the activating signal. A competing hypothesis, that cell shrinkage activates the HOG pathway, seems less likely because maximal activation of Hog1p, measured as an increase in tyrosine phosphorylation of this MAP kinase, is induced by small increases in extracellular osmolarity that have no obvious effect on cell volume. To further test the turgor-sensing hypothesis, we investigated whether a pore-forming toxin that lets out small solutes and thereby decreases turgor pressure can also activate the HOG pathway. Amphotericin B induced a time- and dose-dependent increase in Hog1p tyrosine phosphorylation. Treatment with 1 g/ml amphotericin B for 30 min induced a 3-fold increase in Hog1p activation with <5% decrease in cell viability and no detectable release of cellular ATP. This Hog1p activation correlated with a partial collapse of the cellular osmotic gradient. HOG pathway mutants showed increased sensitivity to the pore-forming K1 killing toxin, suggesting that HOG pathway activation is physiologically important. The HOG pathway utilizes two osmosensors acting in parallel, Snlp/Ssklp and Sho1p, either of which is sufficient for high osmolarity activation. Interestingly, amphotericin B activation of the HOG pathway appears to be mediated primarily by Snlp/Ssklp, suggesting important differences in transduction mechanisms by these two different sensory proteins. (Supported by NASA: NAGW-5007)
[10] AUXIN-INDUCED GENE EXPRESSION DURING THE GRAVITROPIC RESPONSE OF TOMATO HYPOCHOTYLs. A. Madlung, F. Behringer, A. Nebenfuhr, and T.L. Lomax. Department of Botany and Plant Physiology and Center of Gene Research and Biotechnology, Oregon State University, Corvallis, OR

The focus of our interest is the signal transduction pathway that mediates plant responses to gravity. To understand the mechanism of gravitropic signal transduction we are using a tomato mutant, *lucy-2*, which has shoots that show a phytochrome-dependent positive gravitropic response.

A central goal in our work is to test the Cholodny-Went theory which states that the hormone auxin is redistributed unequally towards the lower side of the stem of a gravistimulated plant. We have used auxin-responsive genes of the *LirLAA* gene family to monitor auxin activity in gravistimulated tomato seedlings. 10-day-old, light-grown plants were gravistimulated. After the hypocotyls were bisected and total RNA was extracted and reverse transcribed to make cDNA, semi-quantitative RT-PCR was performed to detect auxin-induced differences in gene expression between the upper and lower halves.

Results show higher *LirLAA* expression on the lower side of gravistimulated wild-type tomato seedlings after 2 hours of gravistimulation. This stimulation of auxin-responsive genes on the lower side of the stem is compatible with the Cholodny-Went theory of differential auxin redistribution. Comparisons with the *lucy-2* mutant will be discussed. (Supported by NASA award No. NAGW-3716 to T.L.L.)


Plants are the foundation of the proposed Controlled Ecological Life Support System (CELSS) which will be utilized in future long-term missions. In a functional CELSS, plants would be responsible for the production of edible biomass, oxygen evolution, and clear water production as well as psychological benefits for crews separated from earth for long periods. If plants in these systems could be grown under low O2 concentrations, their productivity might be increased due to a reduction of photorespiration resulting in increased crop yields, and the O2 necessary for CELSS could be reduced. Photosynthesis and gas exchange in plants occur in the leaf. Consequently, if atmospheric composition were altered, any changes in plant structure would most likely be reflected in the leaf. In this study, changes in the stomatal density, epidermal cell size and internal leaf structure of *Arabidopsis thaliana* grown under low oxygen atmospheres was investigated. *A. thaliana* (L.) Heynh. var. 'Columbia' was exposed to five different O2 atmospheres (2.5%, 5%, 10%, 16%, 21%) with ambient CO2 in N2 in sealed, polypropylene bags receiving 200 μmol mol-1 PAR at 25°C for 28 days. Leaves were examined using scanning electron and light microscopy and statistical analysis was performed. As O2 concentration was decreased, stomatal density increased significantly on the adaxial leaf surface. However, the increase in stomatal density on the abaxial surface was significant only in the lowest O2 treatment (2.5%). Overall leaf structure was also affected with an increase in palisade layers and a loss of differentiation between the mesophyll layers with low O2. A change in chloroplast density within the palisade cells in low O2 treatments was also observed. In addition, the number and size of the leaf epidermal cells appears to be altered. These data indicate that an oxygen-sensitive process is involved in leaf development and that oxygen may be an important factor in the control of overall plant development. (Supported by NASA Space Biology grant #NAGW-3759 and by the Louisiana Space Consortium.)

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Mammary metabolic activity was measured in ten pregnant rats flown for 9 days aboard STS-70. Glucose oxidation to CO2 increased in flight animals as compared to control animals at day 20 of gestation (ASGSC Bulletin 10(1):55, 1996). It is unclear what caused this increased activity. The objective of this study was to determine if changes in mammary metabolic activity paralleled changes in mRNA expression of the milk protein, β-casein. Mammary tissue that had been collected from rats aboard STS-70, flight delayed synchronized controls, vivarium control, and vivarium non-laparotomized control were used to isolate RNA. Mammary tissue was frozen from four rats per treatment on day 20 of gestation and from six rats per treatment after parturition.

RNA was extracted from mammary tissue using acid guanidine thiocyanate-phenol-chloroform. The isolated RNA was electrophoresed in an agarose gel and transferred to a nylon membrane. Each membrane was probed with β-casein cDNA which was radio-labeled with 32P-dCTP by random priming. The blots were exposed to film and the bands were measured on a densitometer to compare the treatments. An 850 bp transcript was observed which is consistent with the expected size for β-casein mRNA. The β-casein mRNA was expressed in all treatments. Higher levels of expression was observed in mammary tissue from pregnant animals subjected to spaceflight compared to controls. This is consistent with premature lactogenesis. (Supported by NASA grant NCC 2 870 and HELIX program mini-grant)


Tail suspension is a model for disuse osteopenia in mice as it unloads hindlimbs and induces osteopenia due to reduced bone formation. IGF-1 stimulates bone formation by increasing osteoblast activity. This study investigated the ability of IGF-1 to mitigate suspension-induced osteopenia.

Sixty-day old C57BL/6J (Jackson Labs, Bar Harbor, ME) mice were assigned to 4 groups (n=11 to 12; suspended or non-suspended controls: saline or IGF-1). Oxytetracycline injections were administered 14 (20 mg/kg), 8 (20 mg/kg), and 2 (10 mg/kg) days prior to sacrifice to enable quantitative histomorphometric evaluation. After the 2 week treatment, all groups were sacrificed and skeletal tissues harvested. Left femora, tibiae, and humeri were measured for physical properties (length, medial-lateral diameter, and dorsal-ventral diameter); mechanically tested in three point flexure; and weighed after heating for 48 hours at 105°C (dry mass = organic + mineral). Strength and displacement energy at elastic, maximum, and failure, and stiffness were calculated from mechanical testing data. Bone mechanical properties for suspended mice were lower (p<0.05) than for controls. Suspended mice experienced approximately 32% lower femur stiffness and 26% lower femur maximum strength. The effects of IGF-1, combined with suspension, on mechanical and physical properties were mixed. IGF-1 treated, suspended mice experienced 16.6% higher stiffness values and 8.5% higher femoral maximum strength values than saline suspended animals. IGF-1 treated, suspended animals also experienced significant increases in femur dry mass and lateral diameter, 6.3% and 4.6% respectively, and a 1.5% increase in humerus length. Full effects of combining suspension and IGF-1 will be determined after completion of compositional analysis and quantitative histomorphometry.
CLENBUTEROL PHARMACOKINETICS IN HINDLIMB SUSPENDED RATS: GC-MS DETERMINATION. J. Nichol, B. Smith, D.A. von Deutsch, D.E. Potter, E. Chidebelu-Eze, L.E. Wineski, and D.F. Paulsen. Musculoskeletal Research Group, Space Medicine and Life Sciences Research Center, Morehouse School of Medicine, Atlanta, Georgia.

Extending the duration of manned spaceflight increases the likely need for medical interventions to assure optimal crew performance. More information is needed about spaceflight effects on pharmacokinetics (i.e., patterns of drug distribution and metabolism) to optimize the efficacy of medications given during a mission. The β-adrenergic agonist, clenbuterol, is a potential pharmacologic countermeasure to microgravity-induced musculoskeletal atrophy. In the rat hindlimb-suspension model, hindlimb unloading is accompanied by a headward shift of body fluids, simulating microgravity exposure. Three groups of pair-fed animals (suspended, non-suspended, and tethered) were treated for 2 weeks with either 1 mg/kg clenbuterol (Cb) or an equivalent volume of vehicle (PBS) by subcutaneous injection in a 2-days-on-2-days-off treatment regimen. Serum and tissues were collected for extraction and analysis of Cb content. Tissues were homogenized. Samples were alkalized, extracted with ethyl acetate, and centrifuged. The organic phase was isolated, evaporated under nitrogen gas, and derivatized with N-methyl-N-(trimethylsilyl) trifluoroacetamide:trimethylsilane. GC-MS (gas chromatography-mass spectrometry) was accomplished using a fused silica capillary column (30m x .530mm OD) on an HP 5890/5972 GC-mass spectrometer. The instrument was focused in single-ion monitoring mode to measure fragments of m/z 335 (Cb) and 431.20 (internal standard), with a dwell time of 100 ms for each mass range. Recovery efficiency was verified from all tissues and serum samples with the aid of an internal standard. Cb was detected in serum, liver and kidney of treated, suspended rats. Traces were found in kidney and liver of treated, tethered animals. Tissue levels of Cb were approximately 20% that found in serum. Cb concentration in serum from non-tethered, non-suspended rats was higher than in other groups. Serum from suspended rats contained the lowest Cb levels. This preliminary report of GC-MS detection of Cb levels in both biological fluids and tissues suggests hindlimb suspension has detectable effects on Cb pharmacokinetics. Supported by NASA NCCW-0083 and NIH RR03034).


Gravity alters local blood pressure within the body so that arterial pressures in the head and foot are lower and higher, respectively, than that at heart level. Furthermore, vascular responses to local alterations of arterial pressure are probably important to maintain orthostatic tolerance upon return to Earth after space flight. However, it has been difficult to evaluate the body’s arterial pressure gradient due to the lack of noninvasive technology. This study was therefore designed to investigate whether finger arterial pressure (FAP), measured noninvasively, follows a normal hydrostatic pressure gradient above and below heart level during upright posture and 30° head down tilt (HDT). Seven healthy subjects gave informed consent and were 19 to 52 years old with a height range of 158 to 181 cm. A Finapres device measured arterial pressure at different levels of the body by moving the hand from 36 cm below heart level (BH) to 72 cm above heart level (AH) in upright posture and from 36 cm BH to 48 cm AH during HDT in increments of 12 cm. Mean FAP decreased by 85 mmHg transitioning from BH to AH in upright posture, and the pressure gradient calculated from hydrostatic pressure difference (pgh) was 84 mmHg. In HDT, mean FAP decreased by 65 mmHg from BH to AH, and the calculated pressure gradient was also 65 mmHg. There was no significant difference between the measured FAP gradient and the calculated pressure gradient, although a significant (p=0.023) offset was seen for absolute arterial pressure in upright posture. These results indicate that arterial pressure at various levels can be obtained from the blood pressure at heart level by calculating pgh + an offset. The offset equals the difference between heart level and the site of measurement. In summary, we conclude that local blood pressure gradients can be measured by noninvasive studies of FAP. (Supported by NASA grants 199-80-02-05 and 199-26-12-34).
SESSION B: CONCURRENT POSTER SESSION I
STUDENT LIFE SCIENCES TRAINING PROGRAM I

The 1997 Space Life Sciences Training Program was made up of thirty-nine students from the United States and two from Canada. The students selected one of four research emphasis groups centered around projects related to: Environmental monitoring and research; life science spaceflight hardware development and operational medicine, advanced life support research, and space biology research. The students spent over half of their time during the six week program in the field or laboratory with most of the rest of the time spent in lectures and tours. The lecturers included NASA personnel (including astronauts), NASA contractor personnel, and representatives from universities and other agencies. The students also utilized a local university library for literature searches; were trained in the use of the NASA Life Science Data Archive; participated in a Space Station Construction Project; and visited Epcot, Brevard Community College Planetarium and Observatory, and Sea World. The students also took tours of Kennedy Space Center, Cape Canaveral Range Operations Control Center, Cape Canaveral Space Museum, and U. S. Space Camp. The students earned five hours of college credit from Florida A & M University, who administers the program for NASA. To earn their grade, each student was required to submit and present a technical paper on their specific emphasis research project during the final week of the program. (Work performed under NASA contract: NAS10-12180)


Of forty-two undergraduate college students chosen to participate in the six-week Space Life Sciences Training Program during the summer of 1997, nine of these students comprised the Ecology Group of the program. Under the supervision of their respective primary investigators, these students executed a number of varied research projects which addressed various questions pertaining to ecological systems found in the immediate vicinity of the Kennedy Space Center and in surrounding areas. Research conducted by the Ecology Group included the following: (1) harvesting of plant biomass from scrub habitats in order to develop fuel models to predict fire and smoke behavior under predicted weather conditions, (2) monitoring of scrub regrowth in areas of prescribed burns and assessing the influence of fire-maintenance of scrub habitats on the survival of the Florida scrub jay, (3) characterization of biogenic volatile organic compounds found in local plant species, (4) collection from local flora of biochemical and biophysical data to be used in remote sensing applications, and (5) collection of sea grasses in order to characterize and quantify pigments found in this aquatic vegetation. At the end of the program, the students submitted a final paper on their research and also orally presented their findings in a final seminar. These ongoing studies of NASA have long-term ramifications for addressing environmental concerns and provide useful information for the preservation and management of different types of ecological systems.


Students in the six-week seminar and laboratory program were involved in the first testing of compost usage in the KSC-ALS Breadboard Project and the effects of human-associated bacteria in hydroponic systems. Students ran several lab-scale composting studies: combining human waste (simulated) with inedible plant material, using plant material as a growth matrix, either alone or as a supplement to other solid substrates, and leaching the composted material to reuse the nutrients in a hydroponic study. Gas-exchange measurements were also conducted on these plants. Students worked on microbial monitoring studies, observing the microbial effects of using gray water (human hygiene water) in a hydroponic system and the microbial and fungal invasion of the rhizosphere community of wheat. Engineering students redesigned a growth chamber for more efficient temperature and humidity control. Finally, one student looked at modeling sodium flow (from urine and soap) in a bioregenerative life support system. The students presented the results of their efforts both in a seminar at the end of the program and in a research paper.


Students in this 6 week program were involved in science research and engineering activities pertaining to spaceflight experiment development. Research efforts included: protocol development for spaceflight experiments studying planaria regeneration within BRIC (Biological Research In Canisters); an investigation of the effects of different light quality treatments (white, red, green, blue) on Ceratophyllum demersum L. (an aquatic angiosperm) pigmentation composition; development and analysis of pre- and post-flight aquatic specimen transportation methodologies for the CEBAS (Closed Equilibrated Biological Aquatic System) middeck payload; characterization of the freezing gradient profile in the BRIC-60 spaceflight hardware; redesign, improvement and testing of various components within the Microgravity Plant Nutrient Experiment (MPNE) spaceflight hardware; design and development of an expert system for the cultivation of plants in space; and a double-blind clinical drug trial evaluating the merits and side effects of three anti-nausea drugs as potential countermeasures for motion (or space) sickness.

At the end of the course the students submitted research papers on their projects and participated in a two-day seminar during which they made formal oral presentations before their peers and personnel associated with the course at FAMU, the Life Sciences Support Facility at KSC, and NASA. We gratefully acknowledge the contributions and assistance of those individuals who made this summer experience possible; specifically the NASA and FAMU managers of SLSTP, and the Dynamac Corporation and Biometrics Corporation employees who generously contributed many hours of their time mentoring these student projects.
[20] NASA SPACE LIFE SCIENCES TRAINING PROGRAM: EVALUATION OF COUNTERMEASURES FOR MOTION SICKNESS. J. M. Hollier1, K. A. Townsend2, C. S. Watt2, L. M. Tumyan3, and D. Woodward4. Xavier Univ., of Louisiana, 1College of Arts and Sciences, 2College of Pharmacy, New Orleans, LA; 3North Carolina State University, College of Agriculture and Life Sciences, Raleigh NC; 4Occidental College, Los Angeles, CA; 5Bionetics Corporation, KSC, FL.

We compared the effectiveness of cinnarizine, dimenhydrinate, and ondansetron to placebo as countermeasures for motion sickness. The medications were administered four hours prior to the motion sickness susceptibility test in double blind fashion. Each subject received the four drugs in randomized order. Motion sickness was induced by 15° off vertical rotation. Level of discomfort due to motion sickness and due to medication side effects, as well as heart rate and lateral eye movement were recorded during the test. The drugs were compared for average effectiveness, and also to identify the number of subjects who benefited most from each drug. Results suggest that using a test such as this to select the best medication for each individual from a small group of effective drugs may be more productive than attempting to identify a single most effective drug. Eye motion varied markedly between subjects and was not a reliable indicator of susceptibility to motion sickness. Heart rate appeared to be increased by anxiety and was sometimes remarkably decreased during motion sickness. A special acknowledgement goes to NASA, Florida A&M University, and Dynamac Corporation for their programmatic support.

[21] CHARACTERIZATION OF THE FREEZING GRADIENT IN BRIC 60 FLIGHT HARDWARE. M. L. Korwin1, R. D. Elms1, and P. A. Currier1. 1Dept of Ceramic Engineering and Materials Science, Alfred Univ, 2Dept of Biochemistry and Biophysics, Texas A&M Univ, and 3The Bionetics Corporation, Kennedy Space Center. The Space Life Sciences Flight Team performs duties which include the recommendation and support of flight hardware to PI's. One type of flight hardware is the Biological Research In Canister (BRIC) hardware. The freezing of BRIC-60s prior to reentry into the earth's atmosphere is essential to halting biological growth of the specimens contained within. To obtain results that can be considered consistent from specimen to specimen and from ground unit to flight unit, all biological processes should be halted at the same moment. It has therefore been deemed necessary to characterize the rate of freezing within the volume of the BRIC-60 and if different canister orientations in the G1, freezer affect this rate. The modeling of the freezing differential of a BRIC on earth will give information that will allow engineers to predict how a BRIC freezes in microgravity. The four orientations of loading the canister into a G1, freezer will be: 1-Vertically, handle up; 2-Vertically, handle down; 3-Horizontally, handle out; 4-Horizontally, handle in. These four tests will yield data that will be used to determine which orientation is the most efficient and most uniform for freezing on earth. Canisters were loaded in four different orientations with nine 60 mm petri dishes containing agar and Arabidopsis seeds. This setup is representative of an experiment to be launched in spring 1998. This experiment defines freezing as reaching a temperature at or below -70°C. Preliminary results show the outside wall of the canister experiencing temperatures in excess of 100°C colder than the interior volume. For each orientation, the petri dishes contained in the canister did not experience the freezing temperature of -70°C until approximately 45 minutes after the interior wall of the canister. The petri dishes at the top and bottom of each canister froze approximately 45 minutes sooner than the others, indeed creating a freezing gradient. Future work should include studying the different effects of freezing rate on various tissues.


Students in the six-week program were involved in a myriad of research and engineering projects germane to plant space biology. These projects included: Evaluation of the impact of microgravity (or altered gravity) on the relative levels of AGPase expression in soybean and corn, in addition to growth, carbohydrate metabolism, and hormonal levels and transport in a variety of plants, particularly wheat, corn and soybean; Evaluation of the impact of spaceflight conditions, such as elevated carbon dioxide levels, on alterations of lignin, peroxidases, and invertase activity and composition in wheat; Evaluation of nutrient delivery systems and arrangements of red and blue LEDs, to provide baseline data for the Lunar/Mars Life Support Project; Evaluation of the stomatal behavior in Arabidopsis under space flight conditions; and Design modifications of a porous tube plant nutrient delivery system. The students presented their findings during a seminar at the end of the program, in addition to writing a technical paper.

[23] CHARACTERIZATION OF BOUND AND SOLUBLE INVERTASES IN WHEAT GROWN UNDER ELEVATED/SUPERELEVATED CO2 LEVELS AND RED LIGHT EMITTING DIODES. J. Ho1 and D. Bishop1. 1University of Western Ontario, Canada, and 2Utah State University, Logan, UT.

Invertase is the primary enzyme involved in the conversion of sucrose to glucose and fructose; and sucrose availability is the main regulator of its activity. Plant invertases may change in response to the spaceflight environment of increased CO2 levels (400 ppm, 1000 ppm and 10,000 ppm) and the narrow spectral range (660 nm) of red light emitting diodes (R-LED). Sanwro et. al. 1995 found decreases in photosynthesis and changes in associated carbohydrates in wheat grown under R-LED. Wheat seeds, cv. Super Dwarf were prepared as described by Bishop et. al. and planted in a nutrient porous tube system developed by Dreschel et. al. The tubes were maintained in environmentally-controlled CO2 chambers designed by Piastuch et. al. and the plants were harvested after 14 days. Invertases were localized by direct staining of root and leaf tissues and visualized with light microscopy. Soluble and bound invertases were extracted from roots and leaves, and invertase activity was measured both spectrophotometrically and using activity stains to see changes on SDS-PAGE gels. In roots and leaves, invertase was qualitatively localized to the phloem in the vascular system. There was an increase in bound (cell-wall associated) invertase activity of 10 000 ppm plants compared to ambient (400 ppm) controls. Soluble (cytoplasmic) invertase activity was found to be highest in the roots and leaves of 1000 ppm plants. Examination of carbohydrate-associated enzymes under spaceflight conditions may contribute to understanding carbohydrate metabolism in space-grown plants.

(Supported by CSA SLSTP and NASA Grant NGT 10-52609.)
INTERACTIONS BETWEEN ETHYLENE, STARCH METABOLISM AND GROWTH IN CLINOROTATED AND STATIONARY SOYBEAN PLANTS. J.S. Lanham¹, K.L. Hiltz², and M. Sanwo³. ¹Muhlenberg College, Allentown, PA, ²Florida A&M University, Tallahassee, FL., ³National Research Council

In a previous space shuttle experiment (STS-63), soybean seedlings grown in microgravity produced less starch and higher levels of ethylene compared to ground controls. To investigate whether there is an interaction between ethylene, starch metabolism and growth, soybean seeds (Glycine max [L.] Merr.) were germinated and grown in sealed canisters under clinorotated or stationary conditions. A set of plants under clinorotated and stationary conditions was treated with silver thiosulfate (STS), an inhibitor of ethylene action. In another set of plants, ethylene was chemically scrubbed from the canisters with KMnO₄ (Purafil®, Dourville, GA). These plants were compared to untreated controls.

In the stationary STS-treated cotyledons, starch concentrations were 40% lower than untreated stationary controls, suggesting that the STS-treatment affected starch metabolism. In the canister of clinorotated plants with the ethylene scrubber, starch concentrations were 25% greater than in untreated stationary and clinorotated cotyledons. The clinorotation, STS and ethylene scrubbed treatments all influenced root weight, lateral root formation, and shoot:root ratios, indicating an interaction between ethylene, growth, and starch metabolism under clinorotated and stationary conditions.
SESSION C: CONCURRENT POSTER SESSION II
STUDENTS POSTER COMPETITION (cont.)

During August to December 1996, SuperDwarf wheat, Triticum aestivum L., plants were grown onboard the Russian Space Station, MIR. Germination was 60% and seedling growth was vigorous, individual plants produced 5-8 tillers. From the final harvested samples, spikelets, florets, pistils and stamens were excised, dehydrated with increasing concentrations of ETOH:acetone, transferred into 100% tetramethylsilane (TMS) and evaporated overnight. Tissue samples were mounted onto circular aluminum stubs with an inert biological glue and 12.6 to 21.0 μm of gold and palladium applied to enhance contrast and density. SEM examination of MIR wheat spikelets and florets showed that the pistils, stamens and lodiculae developed and expanded normally prior to anthesis after which the pistils, stamens and lodiculae became dry, collapsed and withered; the anthers did not dehisce. We are continuing to seek clues concerning the failure of seed production under the microgravity of space.

(Supported: NASA Grant NCC 2-831 and the Utah Agric. Exp. Station).


As an Arabidopsis root pushes downward through its substrate, it tends to form a three-dimensional spiral pattern due to an endogenous nutation, which can interact with responses to environmental stimuli to determine the overall shape of the root. Through the use of an image-digitizer system and custom analysis routines, the period for this nutation was found to be 11.8 ± 1.5 hours. This spiral growth pattern is due to differential growth in both the central elongation zone (CEZ), approximately between 400 mm and 800 mm from the root tip, and the distal elongation zone (DEZ), approximately between 200 mm and 400 mm from the tip. However, the differential growth patterns in these two regions are different: large magnitude curvature, which drives the nutation, originates in the CEZ, while the DEZ is the source of an opposing growth pattern, which dampens the nutation.

Growing the root on an agar surface, as opposed to growing it in a solid volume of agar, has little effect on nutation. Thus, nutation can account for the periodicity of skewing in roots growing along an inclined plane (see Rutherford and Masson, Plant Physiol 111: 987). However, skewing is typified by cell file rotation (CFR) in the root's epidermis, whereas CFR is not a characteristic of root nutation. This implicates mutation-driven thigmotropism, which does show CFR, as a major determinant of skewing. (Supported by NSF IBN-9416015, NASA NAGW-4522, and the NASA/NSF Network for Research on Plant Sensory Systems.)

[27] GRAVITROPISM OF THE INFLORESCENCE STEMS IN STARCH DEFICIENT MUTANTS OF ARABIDOPSIS. S. Weise and J. Z. Kiss. Dept. of Botany, Miami University, Oxford OH.

Previous work in this laboratory has assayed the gravitropic response of both the roots and hypocotyl of a wild-type (WT) Arabidopsis thaliana and three starch-deficient strains. The time course of curvature of the inflorescence stems of Arabidopsis WT (strain Wassilewskija), a starchless mutant (ACG 21), and two reduced starch mutants (ACG 20 and ACG 27) were used to study gravitropism. The plants were grown in cubes of rockwool, a porous silica-based substrate. The plants were then reoriented in the dark by rotating the rockwool cube 90 degrees so the inflorescence stems were approximately perpendicular to the gravity vector. The plants were photographed initially after reorientation and then at regular intervals for 8 hours. Short inflorescence stems (1.0 - 2.9 cm) were less responsive to the gravistimulus than were the long stems (3.0 - 6.0 cm). In both data sets, the WT initially had the greatest response and the starchless mutant had the least response, while the reduced starch mutants exhibited an intermediate response. Growth rates for all four strains were approximately equal. Approximately 8 hours after reorientation, all of the strains returned to a position parallel to the gravity vector. It appears that statoliths play an important initial role in gravitropism, accelerating the response of the inflorescence stem. However, after longer time intervals, the three mutant strains had a full gravitropic response similar to that of the WT, which indicates that in flower-stalks, the longer term response to gravity is independent of the total mass of the statoliths. (Financial support was provided by NASA grant NAG 2-1017 and the Summer Scholar Program at Miami University.)

[28] CONSTRUCTION AND SCREENING OF A CERATOPTERIS GAMETOPHYTE cDNA LIBRARY TO SELECT GENES DIFFERENTIALLY EXPRESSED DURING GRAVITY-RESPONSIVE PERIOD. D.J. Eastburn, A. Chatterjee, and S.J. Roux. Department of Botany, Univ. of Texas, Austin.

Our lab is interested in the biochemical, physiological, and molecular mechanisms by which external stimuli such as light and gravity promote signal transduction events and adaptive responses in plants. We have found that gravity directs certain aspects of early polar development in germinating spores of Ceratopteris richardii, a homosporous fern that grows in tropical and subtropical regions of the world. The gravity-directed event is the downward migration of the spore cell nucleus from a central position to a lower position, a migration crucial for the asymmetric first cell division and subsequent development of the rhizoid and prothallus. The polarity of this migration is set by gravity during a definable period 5-10 h before the nucleus actually migrates. Using both Differential Display PCR and Northern analyses, we have identified 18 partial cDNAs (all in the 300-500 bp range) that are differentially expressed during the period when gravity sets the polarity of nuclear migration. We wish to study whether any of the genes represented by these cDNAs are crucial for the gravity sensing that occurs during the period when they are differentially expressed. To aid in this study a gametophyte cDNA library was constructed from 5 μg of mRNA isolated from Ceratopteris. The unamplified library has a titer of 5.0 x 10⁶ pfu and has been confirmed to have at least some near full-length clones.

From our initial characterization of this library, we conclude that it is of sufficient quantity to serve as a valuable resource for selecting more full-length versions of genes that are differentially expressed during the critical gravity-responsive period of germinating Ceratopteris spores. (Supported by NASA grants NAGW 1519 and NAG10-0202).

Vertically oriented roots generate symmetrical patterns of electrical current with current flowing out of the central elongation zone (CEZ) and into the distal elongation zone (DEZ) and meristem. Upon gravistimulation current begins to flow out of the DEZ on the top and into the DEZ on the bottom (Behrens et al., 1985; Planta 163: 463-472). This is consistent with the observation that cells along the top of the DEZ become hyperpolarized following gravistimulation (Plant Cell Physiol 31: 457). In order to begin to characterize the nature of these currents and their possible role in root gravitropism, we have examined the effects of the anion channel inhibitor, NPPB [5-nitro-2-(3-phenylpropylamino)-benzoic acid] on the kinetics of root gravitropism. Roots of Arabidopsis seedlings (4-6 d old, Columbia) growing on 1% agar containing 1% sucrose were covered with agar containing 35 μM NPPB. Root growth and gravicurvature were measured using a video digitizer system with custom software capable of measuring overall growth rates along opposite sides of the root as well as the changing angle of overlapping sequential root sections during curvature. NPPB either increased the latent period of gravitropic curvature [controls = 20 min, treated = 400 min] or inhibited the response completely. Although the growth rate of NPPB-treated roots was reduced by 20-30% compared with controls, this did not appear to account for the strong effects of NPPB on gravitropism. In roots that showed a gravitropic response in the presence of NPPB, the rate of curvature was greatly reduced in comparison with control roots. Our results indicate that anion channels may play a role in the initial signaling or motor mechanism causing gravitropic curvature in roots. (Supported by NSF Grant No. IBN-9416015, by NASA Grant No. NAGW-4522, and by the NASA/NSF Joint Program in Plant Biology, Network for Research on Plant Sensory Systems.)


The Advanced Separator (ADSEP) was designed for studying gravity-dependent features of biphasic aqueous extraction. It is also applicable to any low-volume chemical experiment requiring multiple steps involving active mixing of fluids (e.g. batch crystallization of biological macromolecules or changing of medium and fixing of cell cultures). Experiments utilizing or testing ADSEP were performed on space shuttle mission STS-77 and a Conquest sounding rocket. Successful experiments were performed in the areas of cell culture and protein crystal growth; however, it was necessary to make modifications in the hardware to achieve biphasic extraction experiments in microgravity. Although ADSEP functioned for this purpose on the ground, gravity-dependent phenomena interfered with space flight operation. A useful gravity-independent separation technology, magnetically and electrically driven multistage separations based on the ADSEP design is under development, and separations of magnetic particles on the basis of their susceptibility have been achieved.

(Supported by: SHOT, Inc, NASA grant: NAGW-1197, and the Colorado Institute for Research in Biotechnology (CIRB).)


To understand further the bone loss astronauts experience in spaceflight, osteoblasts from normal 17-day old chick embryos were cultured and flown aboard shuttle flight STS-77 from May 19-29, 1996. Osteoblasts grown in MEM + 10% FBS were attached to Thermaxx coverslips coated with type I collagen. MEM was changed to DME when cells reached confluence and cultures were then supplemented with β-glycerophosphate and ascorbate before being loaded into Fluid Processing Apparatus (FPA) hardware. Each FPA, a space flight-qualified device, was used in a 2-chamber configuration to separate a coverslip and its surrounding media from a parafilm-aldheyde-sucrose solution, applied to fix and terminate the cells 3 h or 3 d into flight. Post-flight the cells (flight and control groups) on coverslips were dehydrated and embedded in LR White resin for examination by transmission electron microscopy. Other cells attached to coverslips were critical-point dried after dehydration and sputter-coated with gold for scanning electron microscopy. Transmission or scanning microscopy revealed that the osteoblasts fixed 3 h into flight exhibited different and attachment patterns on coverslips and were more vacuolated than their control counterparts. Three h flight cells displayed a twirled appearance of substrate attachment, whereas control cells were arranged in a more parallel manner. After 3 days in microgravity, cells were fewer in number, of an apparent different shape, and even more vacuolated than controls. These observations with a chick culture model would suggest that the extreme launch environment and/or low gravity rapidly and adversely affect osteoblasts in terms of their phenotype, growth and development. Results may also indicate that forces encountered by humans at launch and in microgravity may exert similar effects on bone. (Supported by NASA: 97-GSRP-015)

[32] EFFECTS OF FLUID SHEAR STRESS ON BASIC FIBROBLAST GROWTH FACTOR RELEASE FROM HUMAN AORTIC SMOOTH MUSCLE CELLS. D.N. Rhoads, S.G. Eskin, and L.V. McIntyre. 'Cox Laboratory for Biomedical Engineering, Rice University, and 'Texas Biotechnology Corporation, Houston, TX

Smooth muscle cells can experience fluid shear stress from interstitial flow, as well as blood flow upon endothelial denudation caused by cardiovascular interventions. Previous work has shown that fluid shear stress affects the growth and metabolism of human aortic smooth muscle cells (hASMC). We hypothesize that basic fibroblast growth factor (bFGF) may be involved. The objectives of this study were to examine whether fluid shear stress can affect the release of basic fibroblast growth factor (bFGF) from smooth muscle cells. An in vitro parallel plate flow channel system with recirculating medium was used to expose hASMC (passage 6 to 10) to defined physiological levels of shear stress (1 to 25 dynes/cm²). Conditioned media samples were collected from cells exposed to flow or static conditions. Additionally, after exposure to shear stress, cells were treated with a heparin solution to liberate bFGF bound to the cell surface and surrounding matrix. Media samples and heparin treatment samples were assayed for bFGF content using a quantitative sandwich ELISA, and amounts were normalized with respect to cell number. Results indicate that after 15 minutes of exposure to shear stress (25 dynes/cm²) conditioned media contains bFGF at levels 12 times greater than those found in media from static controls (p<0.0001). Shear stress exposure at 1 and 5 dynes/cm² for 15 minutes also caused significant increases in bFGF media levels. Analysis of heparin treatment samples indicates that increasing levels of shear stress cause increasing levels of bFGF at the cell surface.

These studies indicate that fluid shear stress can mediate the release of bFGF from hASMC. bFGF is known to stimulate smooth muscle cells upon binding to its receptor, FGFR-1. The possibility of shear stress-mediated changes in FGFR-1 expression is also under investigation. (Supported by: NASA NSCRT grant, NIH grant HL18672, and Texas Biotechnology Corporation.)
[33]

IMMUNOLOGICAL STRESSORS INHERENT TO MURINE SPACEFLIGHT EXPERIMENTATION. M.J. Pecaut¹, S.J. Simske¹, R.J. Zimmerman¹, K.T. Nguyen¹, M. Fleschner¹ ¹BioServe Space Tech, Dept of Aerospace Engineering, Univ of Colorado, Boulder, ²Chiron Corporation, Emeryville, CA, ³Behav. Neuroscience, Dept of Psychology, Univ of Colorado, Boulder

There are several aspects inherent to spaceflight which must be fully explored to truly understand the results of a typical space shuttle life science experiment. Animals flown aboard the shuttle are exposed to a number of acute and chronic stressors: launch and landing loads, cephalic fluid distribution shifts, unloading of the limbs, and exposure to a novel environment. Each of these stressors may affect the immune system in different ways.

Experiments were designed to examine these stressors. Anti-orthostatic tail suspension was used to simulate the chronic stress of microgravity, unloading of the limbs, and cephalic fluid distribution shifts. A large centrifuge was used to simulate the launch and landing loads of a typical shuttle mission. Sprague-Dawley rats (n=6-8 per group) were exposed to one of the following stress regimens: 10 days of tail suspension, Landing only (via centrifuge), Launch+10 days in home cages, Launch+10 days in home cages+Landing, Launch+10 days of tail suspension+Landing, and the various controls. The results of these experiments were compared to those from animals flown aboard the Space Shuttle Endeavor for STS-77 (12 flight, 16 ground controls). In all cases, splenocytes were removed and labeled with antibodies against CD4, CD8, CD11b, and αβTCR for flow cytometry. Changes in splenic cytotoxic/suppressor (TCR+/CD8+), T-cells, splenic helper (TCR+/CD4+) T-cells, and neutrophil/macrophages (CD11b+).

Lymphocyte and macrophage subpopulations did not change following tail suspension, suggesting that this model does not mimic the changes seen in the flight animals. This also suggests that the chronic stress of spaceflight may not be responsible for the changes seen in splenocyte subpopulations following spaceflight. Similarly, the launch and landing loads simulated in a centrifuge did not reproduce the large changes seen on STS-77, suggesting there is something inherent to spaceflight that cannot be mimicked on the ground.

Funding was provided by NASA Grant NAGW-1177.

[34]

RESPONSES OF RHESUS MONKEYS TO 2G. L.K. Barger, T.M. Hoban-Higgins, and C.A. Fuller. Section of Neurobiology, Physiology & Behavior, University of California, Davis.

Organisms respond to alterations in the gravitational environment. Among the affected physiological systems is the Circadian Timing System (CTS). The CTS coordinates an animal’s physiology and behavior, ensuring that the body is in the proper state for anticipated activities. Dysfunction of the CTS has been correlated with sleep and psychological disorders. We undertook this study to elucidate the responses of multiple circadian rhythms of a primate to an altered force environment. Six male rhesus (Macaca mulatta) were housed individually on a 6.0 m diameter centrifuge. Water was available ad lib through a lixit system. A pelletized diet was provided through the Psychomotor Test System (PTS), developed at Georgia State Univ.

Husbandry was performed one hour each day on a non-24 hour schedule. Data were collected for: 2 weeks (1G), 3 weeks (2G), then 2 weeks (1G). Each animal was implanted with a telemetry transmitter to measure heart rate and body temperature; these data were stored on microcomputer. Drinking counts (number of contacts with the lixit) were summed and stored in 10 minute bins, also on a microcomputer. Performance was monitored using the PTS. This video based multi-task system presented 8 different tasks to the subject in random groups of five. The animal selected which task it would complete from those offered. After all five tasks had been completed (or an experimenter-designated amount of time had elapsed), a new group of five tasks was presented. There were several physiological alterations associated with the onset of 2G. The circadian rhythm of body temperature decreased in amplitude during the first week at 2G, while the phase of the body temperature rhythm was delayed during the period of hypergravity. However, heart rate rhythm, while it also decreased in amplitude at the onset of 2G, did not show a change in phase over the study. The effects of 2G on performance varied greatly between animals; one animal was unaffected while another took 8 days to return to baseline. The mean of performance recovery was 4 days. The results indicate that circadian rhythms persist, but are affected by exposure to 2G in the rhesus. (Supported by NASA: NAG5-4320 to CAF & UT95-036 to LKB)
SESSION C: CONCURRENT POSTER SESSION II
STUDENT LIFE SCIENCES TRAINING PROGRAM II
[35] TEMPORAL CHANGES IN THE MICROBIAL ECOLOGY OF REFRIGERATED GRAY WATER. M.J. Walker¹, K.L. Cook¹, and K.M. Reuther². ¹Department of Biology, Oral Roberts University, Tulsa. ²Dynacorp Corporation, Kennedy Space Center, FL.

The proposed goals of NASA for the next few decades include a permanent space station, renewed missions to the Moon, and a long term mission to Mars. Due to payload constraints, a bioregenerative life support system is necessary. Such a system would recycle air, nutrients, and water, and would provide a large portion of the astronauts' food requirements. Personal hygiene water from showering and laundry (gray water) is proposed to be introduced into the plant nutrient solution of a recirculating hydroponic system. The presence of human associated bacteria in gray water is a concern due to the possibility of their survival in the rhizosphere of the hydroponically-grown crops. If a build-up of these microbes were to occur, the results could be detrimental and compromise the success of the mission. Refrigeration has been proposed as both a means of storage until usage and as a possible means of killing these potential pathogens. The microbial population of a gray water sample taken from a single shower was tested at 0 hours, 5 hours, 1 day, 3 days, and 7 days of refrigeration. By utilizing epifluorescent microscopy, the total population of microbes was enumerated. Selective and differential media were used to track the population shifts of coliforms, as well as gram-positive and gram-negative bacteria. An overview of the microbial community throughout the test time was produced, carefully noting the dominant organism in all replicates. Pseudomonas aeruginosa was dominant in nearly all samples, and Bacillus spp. became increasingly numerous as refrigeration time increased.

[36] IS A HYDROPONIC PLANT'S LEVEL OF RESISTANCE TO PYTHIUM AFFECTED BY THE COMPLEXITY OF RHIZOSPHERE BACTERIAL COMMUNITIES? R.S. Levy¹, R. Rodriguez², D. Jenkins³, J. Garland⁴, K. Hendricks⁵. ¹Dept. of Psychobiology, Binghamton University-SUNY, Binghamton, NY 13902, ²Dept. of Biology, UC-Davis, Davis, CA 95616, ³Dept. of Biology, University of Illinois at Springfield, Springfield, Ill. 62794, ⁴Dynacorp Corp., Mail Code DYN-3, Kennedy Space Center, FL, 32899.

Long term colonization of Mars will require the use of plant systems. The growth of plants also means there is a potential for plant pathogens to invade the system. Many researchers have theorized that more complex communities of bacteria in the plant rhizosphere will confer better resistance to the plant than a less complex community of bacteria. In order to test this hypothesis, we extracted bacteria from a soil sample and made dilutions (10⁻², 10⁻⁴, 10⁻⁶, 10⁻⁸) of the resultant slurry. Wheat plant rhizospheres were then inoculated with either the full strength community or one of the dilutions. Within the rhizosphere, the bacterial communities grew to the same density while still allowing for the preservation of each dilution’s unique diversity. The communities were then harvested and a determination of complexity level was made using BIOLOG, a tool used to measure phenotypic expression. Only the Full Strength community, labeled High Complexity and the 10⁻⁸ dilution, labeled Low Complexity, were subsequently used. Wheat plant rhizospheres were then, once again, inoculated, either with the High Complexity, Low Complexity bacterial communities or a sterile control solution. After either 0 or 1 week of growth, the plant pathogen, Pythium, was introduced into the system. Data will be presented showing the results of the different complexity treatments and their interactions, if any, with time of pathogen invasion.

This project was done in conjunction with the Space Life Sciences Training Program (SLSTP) held at Kennedy Space Center and the Faculty Fellowship program, also held at Kennedy Space Center.

[37] DEVELOPMENT AND ANALYSIS OF PRE AND POST-FLIGHT AQUATIC SPECIMEN TRANSPORTATION. R.D. Elms¹, M.L. Korwin², and P.A. Currier³. ¹Dept of Biochemistry and Biophysics, Texas A&M Univ, ²Dept of Ceramic Engineering and Materials Science, Alfred Univ, ³The Bionics Corporation, Kennedy Space Center.

The duties of the Space Life Sciences Missions Operations Team include the pre and post-flight transportation of various types of plant and animal specimens to and from Kennedy Space Center in support of space shuttle experiments. The method of aquatic specimen transportation utilized by some Principal Investigators was found to produce unacceptable mortality rates and stress levels. Therefore, it was determined that development of a more acceptable and successful method of transporting these specimens was needed. A possible alternative method was evaluated by comparing the results of shipping specimens by the standard method (breathing bags in a polyfoam packer box) and by using a vented and stratified or compartmentalized polyfoam packer box. The specimen of interest was Xiphophorus helleri, a livebearing swordtail fish. Mortality proportions and water quality assays were used as a measure of induced stress on the specimens as a result of the shipping procedure. The water quality assays include analysis for levels of ammonium, nitrite, carbon dioxide, oxygen and pH. In addition, temperature was measured during the shipping process by the use of HOBO sensors. Preliminary results indicate a lower mortality rate for the stratified boxes. At this time it also appears the stratified boxes provide a more stable environment for gas exchange as shown by more consistent total CO₂ levels.

(Supported by NASA and SLSTP)

[38] FREEZING GRADIENT OF PETRI DISHES IN THE BRIC - 60 FLIGHT HARDWARE. J.E. LeBret¹, M.L. Korwin², M. Shao³. ¹School of Engineering, Gonzaga Univ, ²Dept of Ceramic Engineering and Materials Science, Alfred Univ, ³The Bionics Corporation, Kennedy Space Center.

In studying biological experiments, it is important for statistical information and consistency to study materials frozen at approximately the same time. Consistency in biological experiments is essential, since biological processes progress very quickly. The biological processes need to be stopped uniformly so that the observer is looking at the specimens exposed to micro-gravity for the same time period. This will help the observer obtain controlled results. The purpose of this experiment is to examine the freezing gradient of the petri dishes in the BRIC - 60. It is hypothesized that the freezing gradient of the petri dishes in the BRIC - 60 will be approximately uniform.
[40] CHANGES IN LIGNIN, H₂O₂, AND PEROXIDASE PRODUCTION OF WHEAT GROWN IN SIMULATED SPACEFLIGHT CONDITIONS. B.P. Lucey and D.L. Bishop. University of Vermont, Burlington, VT. and Utah State University, Logan, Utah. Plant Space Biology, Dynamac Corporation, KSC, FL.

Peroxidases are involved in the conversion of phenoxy alcohols, with H₂O₂, to produce phenoxy radicals which undergo a non-enzymatic condensation reaction to generate lignin. Plant levels of lignin, H₂O₂, and peroxidase may change in response to super-elevated CO₂ (SECO₂) levels present during spaceflight. The effects of these three CO₂ levels were examined: 400 ppm, 1000 ppm, and 10,000 ppm. It is hypothesized that the observed changes in plant lignin, H₂O₂, and peroxidase levels due to SECO₂ can be minimized or alleviated by the manipulation of the spectral environment through the use of red light emitting diodes (R-LEDs). Triticum aestivum L. cv. Super Dwarf wheat seeds were prepared as described by Bishop et al. and planted in a microporous tube nutrient delivery system. The wheat was grown in a computer-controlled environmental growth chambers. The plants were harvested after a 14 day growth period. Lignin, H₂O₂, and peroxidases were stained for on the root, sheath, and leaf tissues, and visualized at 100x using light microscopy. After extracting the peroxidases from the roots and the leaves, peroxidase activity was measured spectrophotometrically with guaiacol and H₂O₂ substrates, and with activity stains on native polyacrylamide gels. Lignin, H₂O₂, and peroxidases were found to be localized, by deposition, accumulation, and activity respectively, in the vascular cylinder. A decrease in peroxidase specific activity was found in SECO₂ and R-LED grown plants as the CO₂ concentration increased from 400 ppm to 10,000 ppm. Furthermore, H₂O₂ and peroxidase specific stains were found to be co-localized with sites of active lignin deposition. Peroxidase isozymes increased in size in SECO₂ grown plants. Deciphering the effects of SECO₂ and R-LEDs on lignin production may aid researchers in growing plants in space.

[41] THE EFFECTS OF CLINOROTATION AND CENFRIGUATION ON ADP-GLUCOSE-PYROPHOSPHATE GLUCOSE EXPRESSING INGERMINATING MAIZE. S.R. Bagchi and K. Johnson. Dept. of Biology, Indiana University-Bloomington, Bloomington, IN.

The starch synthetase hypothesis states that starch storing plastids, called amylplasts, are involved in gravity sensation in plants. Stato- linths, gravity sensing amyloplasts, are organelles that mediate the perception of gravity by sedimentation in a gravitational field. The cytokinins of 6 of 6 clinorotated (by gravity simulation) soybean have significantly decreased starch concentrations while centrifuged soybean plants (hypergravity simulation) have significantly increased starch concentrations compared to vertically rotated control plants. ADP-glucose-pyrophosphorylase (AGPase), one of many starch metabolism enzymes, is rate limiting in the biosynthesis of starch in plant tissue. More importantly, all of the starch biosynthesis enzymes only AGPase activity is significantly affected by space flight. A consistent trend observed in plants grown in altered gravity is a direct relationship between gravitational force and ADP-glucose-pyrophosphorylase activity. For example, germinating soybean cotyledon AGPase activity was 19% higher in centrifuged plants and 37% lower in clinorotated plants compared to vertically rotated control plants. The clinostat trend has been reproduced during actual spaceflight. Since AGPase is affected by altered gravity conditions, the next step in elucidating the mechanism by which starch granules sense gravity is to analyze AGPase at the transcriptional level.

An interesting model in the study of AGPase gene expression is plants in maize. Starch stored in the kernel is utilized in germinating maize, thus it is thought that the starch synthesis biophyway is largely inactivated in these plants. Studying the effects of altered gravity on AGPase gene expression in maize would provide data on starch biosynthesis complementary to that of soybeans which would allow for a much broader analysis of the mechanism of starch granule gravity sensation in plants. In this experiment, total RNA was isolated from five day old maize kernels and hypnotocyt was probed with cDNA coding for an AGPase subunit in order to assess AGPase gene expression in these plants.


Previously conducted space-flight experiments have shown that microgravity has a dramatic influence on plant growth, development, and composition. Among a myriad of other effects, plants grown in space flight have exhibited shorter root lengths and a distribution of root hairs shifted toward the tips. Even subtle changes in the levels of the hormone auxin can initiate developmental effects such as those seen in space flight. Possible changes in auxin concentration or increases in the tissue sensitivity or response to auxin caused by an altered gravity environment could play a distinct role in the effects seen in space-grown plants. Tomato seedlings were grown on a horizontal clinostat in this study, in order to identify the effects of an altered gravity environment on the concentration of auxin. A GH3-GUS transgene system was employed to study this process. The GH3-promoter sequence, an auxin inducible gene, provides an indication of auxin concentrations in the tomato hypocotyls. The GUS gene functions as a reporter gene, by coding for β-glucuronidase which stains blue in the presence of the substrate 5-bromo-4-chloro-3 indolyl glucuronic acid (X-Gluc). After growing the tomato seeds on the clinostat and in vertical controls, the hypocotyls were harvested, inverted, and put into an agar containing auxin. Additional hypocotyls were placed into agar without auxin as controls. The hypocotyls were then placed back into their original clinostat or vertical positions for 24 hours before being placed in the X-Gluc substrate. Results have indicated that hypocotyls grown on the clinostat show a distinctly more intense staining than the vertical controls, with both naturally and externally induced auxin levels. This suggests that hypocotyls grown in the clinostat exhibit a higher concentration of auxin in these tissues or an increase in the tissue’s sensitivity and response to auxin.
DESIGN MODIFICATION OF THE POROUS TUBE PLANT NUTRIENT DELIVERY SYSTEM PLANTING PROCESS. V.D. Payne¹, and D.L. Bishop. ¹Mechanical Engineering, Fort Lewis College, Durango, CO; ²Biology Dept., Utah State University.

The Porous Tube Nutrient Delivery System (PTPNDS), a hydroponic system involving hydrophilic, microporous ceramic tubes, is presently undergoing design modifications of the planting process in the Plant Space Biology Laboratory at Kennedy Space Center. The present planting process was lengthy and tedious. Current experiments are focused on introducing planting cassettes and root separators into the system with the goals of reducing total planting time and enabling individual plant harvest and root studies. This user-friendly system would make planting and harvesting easier for astronauts on the shuttle or for those living in a colony on the Moon or Mars. The system provides minimum damage to the seed, as well as a higher percentage of germination and minimum contamination. Flight-approved materials under investigation for use as the separators and cassettes are nylon and polyethylene. Materials being tested as separators and cassettes that are not yet flight-approved include fiber (separator only), vinyl, and lucite. Cellulose acetate is being tested in two different forms as a medium for germination because of its ability to provide air and nutrient solution to the seed. In half of the cassettes, the cellulose acetate was left as a plug and inserted into the cassette. In the other half of the cassettes, the outer casing was removed and a small amount of tissue was loosely wrapped around the seed and placed in the planting cassette. Glucose glue, a previously untested planting method, is also being tested as a feasible way of germinating seeds in direct contact with the porous tube. Plant germination and growth may be affected by both the cassette and separator materials and orientation. Some non-flight-approved materials have been used in this experiment, but may be changed to flight-approved materials and tolerances in the design will be made less to ensure that the system will be stable during launch and can be utilized in the microgravity environment of space.
SESSION D: SYMPOSIUM ON MOLECULAR APPROACHES IN GRAVITATIONAL BIOLOGY RESEARCH

[44] EFFECTS OF MICROGRAVITY ON OSTEOSTBLAST GROWTH. M. Hughes-Fulford and V. Vincent Department of Medicine, Univ. of California San Francisco and Department of Veterans Affairs Medical Center, San Francisco

Serum deprived mouse osteoblasts (MC3T3-E1) were flown on STS-76. The osteoblasts (200,000) were grown on coverslips in plunger boxes. Cells were examined for changes in gene expression during growth activation and cell morphology. Cells were loaded in the Biopack plunger boxes 18 hours before launch. Cells were activated in the Biopack incubator under microgravity conditions 19 hours after launch. Cells were harvested at 3 and 29 hours after growth activation. There were 4 samples per data point for each gene or morphology data collection. At 24 hours remarkable changes in elongation of nuclei and actin cell cytoskeleton were seen in the 0-G samples when compared to 1G flight samples. In addition, there were changes in fetal calf sera induced growth activation gene expression between the 0-G flight and 1-G flight samples. The microgravity activated cells after 29 hours had increased c-fos and osteocalcin mRNA when compared to flight 1-G controls (p < .01). The growth associated gene, Cox-2 mRNA was decreased in the microgravity activated cells when compared to 1-G flight controls (p < .01). Cox-1 was not detected in any of the samples. There were no significant differences in the expression of histone or actin mRNA between the 0-G and 1-G samples. These data suggest that the quiescent osteoblast is slow to growth activate in microgravity which may be a factor in bone loss in microgravity. (Supported by NAGW-1244 and NAG-2-981 and NAG-2-1086 and the Department of Veterans Affairs)

[45] GRAVITY RESPONSES IN MUSCLE CELLS. H.H. Vandenburgh, Dept. of Pathology, Brown University School of Medicine and The Miriam Hospital, Providence RI.

Long-term manned space flight requires a better understanding of skeletal muscle atrophy resulting from microgravity. Atrophy most likely results from changes at both the systemic level (e.g., decreased circulating growth hormone) and locally (e.g., decreased myofiber resting tension). Differentiated skeletal myofibers in tissue culture have provided a model system over the last decade for gaining a better understanding of the interactions of exogenous growth factors, endogenous growth factors, and muscle fiber tension in regulating protein turnover rates and muscle cell growth. We have shown in ground-based studies that tissue cultured skeletal myofibers are stimulated to hypertrophy from increased tension through a G-protein dependent mechanism, and atrophy when tension is decreased. Tension partially regulates muscle protein turnover rates by altering the secretion of autocrine growth factors such as insulin-like growth factor-1 (IGF-1) and by modifying the sensitivity of myofibers to systemic factors such as glucocorticoids. When flown in space aboard the Shuttle's Space Tissue Growth Module, the tissue cultured muscle fibers detect both the loss of gravity and the reloading effects of 1x g. While total cellular metabolism and total protein degradation rates are not altered during 9 days in microgravity, total protein synthesis rates are significantly reduced and result in myofiber atrophy. One g reloading of the muscle cells postflight significantly increases total protein synthesis rates and the synthesis rates of myosin heavy chain, fibronectin, and collagen. Tissue cultured muscle cells can thus "sense" changes in gravity and provide a valid model to study countermeasures. Based on our results and the results of others, IGF-1 is an attractive growth factor which may assist in attenuating skeletal muscle atrophy in space. Our laboratory is developing a new cell-based delivery system for this and other potential therapeutic factors. (Supported by NASA Grant NAG2-914)
[46] THE MOLECULAR BASIS OF WALL EXTENSION IN GROWING PLANT CELLS. D.J. Cosgrove. Department of Biology, Pennsylvania State University, University Park, PA 16802.

Plant cells are encapsulated within a tough polymeric wall composed of cellulose microfibrils bonded to a matrix of complex polysaccharides and structural protein. The wall is strong enough to withstand the large tensile forces generated by cell turgor, while at the same time is plant enough to extend by a process of polymer creep. A pH-dependent mechanism of wall extension, known as "acid growth", is catalyzed by expansins. The role of expansins in cell enlargement will be reviewed, with a focus on the mechanism of gravitropism and other gravity-dependent growth responses in stems and roots. Other possible mechanisms of wall extension will be briefly summarized, and the prospects for further dissection of the biochemistry and genetics of wall extension and cell enlargement will be presented.


Gravitropism defines the mechanisms by which plant organs use gravity as an environmental cue to define the vector of their growth. In roots, gravity sensing occurs in the root tip, while the differential growth response occurs in the distal and main elongation zones. We are studying the mechanisms of gravity sensing and early phases of gravity signal transduction in roots, using molecular genetic strategies in Arabidopsis thaliana. We have identified Arabidopsis thaliana mutants affected in root gravitropism without alterations in their ability to respond to exogenous auxin (Masson et al., 1993, ASGB Bull. 7: 26-27). agr1 mutant seedlings develop an abnormal gravitropic response in both roots and hypocotyls. Physiological studies have shown that agr1 mutant seedlings are as sensitive to exogenous auxin and ethylene as wild type, and contain starch in their root cap columella cells. The ARG1 locus was mapped on the South arm of chromosome 1, and cloned using a combination of chromosome walking and complementation by DNA transformation strategies. Interestingly, the ARG1 protein carries a dmal-like domain at its carboxyterminus, and a coiled-coil domain conserved with proteins known to bind actin or tubulin filaments at its carboxyterminus. We have also isolated seven new AGR1 alleles. Agr1 mutations confer an altered gravitropic response in the roots, as well as a slight alteration in hypocotyl gravitropism early after germination. Interestingly, we found that some agr1 mutant alleles also confer an increased resistance to exogenous ethylene. agr1 was shown to be allelic to eir1 (Romain et al., 1995, Genetics 139: 1393-1409). The ARG1 locus was mapped on the South arm of chromosome 5, and cloned by chromosome walking. We are in the process of characterizing its molecular structure, as well as its pattern of expression. We will describe the molecular characterization of ARG1 and AGR1, and discuss their potential function in root gravitropism and ethylene response.


Plant transposons, originally discovered in maize by Barbara McClintock, have been adapted for use as mutagens in recent years. We have developed a transposon mutagenesis system based on the Activator Dissociation Ac Ds transposon family. The tagging Ds transposon 1) is immobile in the absence of a transposase source, 2) carries a bacterial aphD gene used to identify its presence, 3) contains a promotorless -glucuronidase (GUS) gene and 4) is inserted into an herbicide-resistant acetolactate synthase gene which functions only after excision of the transposon, providing a means of identifying plants in which transposition has occurred. The source of transposase is an immobile truncated Ac element closely linked to a negative selectable marker and resides in separate plant lines from those containing the tagging transposon. Transposition is initiated by crossing transposon and transposase plants and transposed elements expressing the GUS gene are selected in the F2 progeny. Among many lines that exhibit root-specific GUS gene expression, two of particular interest express the GUS gene either throughout the root cap or confined to the columnellar cells and the quiescent center. Both tagged genes have been cloned and are being characterized, as are their promoter regions. To examine the consequences of root cap cell ablation throughout the plant, the promotors are being used to drive expression of the diphtheria toxin A chain gene in transgenic plants. We will describe the morphological and physiological consequences of the genetic ablation experiments.
SESSION E: PLANT RESPONSES IN MICROGRAVITY AND GRAVITY
[50] STATICs AND KINETICS OF STATOLITH POSITIONING IN CRESS ROOT STATOCYTES (BION-11 MISSION). R. Laurinavičius1, D. Švėgdienė1, A. Sievers2, B. Buchen3 and M. Taïrbekov4. 1Institute of Botany, Vilnius, 2Institute of Botany, Bonn and 3Institute of Biomedical Problems, Moscow.

To estimate the force balance exerted on statoliths of gravising cells under different mass accelerations, two experiments with cress roots on Bion-11 satellite in an automatically operating centrifuge have been performed. (1) Cress roots grew under permanent acropetal acceleration at 1g for 25 h, and were subsequently exposed to microgravity for 0, 6, 12 and 24 min. They were then fixed in 4% buffered glutaraldehyde and the mean position of amyloplasts in the statocytes has been determined morphometrically by light and electron microscopy. The statoliths moved in proximal direction, i.e. opposite to the direction of the previously applied acceleration. After 24 min in microgravity, the position of amyloplasts, expressed as mean distance (% of total cell length) from the distal cell wall, changed from 27.0±1.5% in roots grown at 1 g to 38.1±2.5% after transition to microgravity, but was still smaller than after continuous growth in microgravity (48.1±2.1%). (2) Cress roots after 25 h growth under permanent acropetal accelerations of 0.005, 0.01, 0.1 and 1 g were fixed and analysed as described in (1) in order to determine the range of the elastic force of the cytoskeleton that is exerted on Earth while keeping the statoliths in their normal position. In contrast to the horizontal gravitropic stimulation of cress roots where a threshold acceleration was determined to be about 0.003 g, the acropetally directed centrifugal force which was still capable to affect the spatial distribution of statoliths was more than ten times greater. Even an acceleration of 0.1 g was not sufficient to balance the elastic force of the cytoskeleton and to position the statoliths at the distal cell pole.

The mean position of amyloplasts was 40.6±2.0% under acropetal centrifugation at 0.1 g instead of 27.0±1.5% at 1 g.

(Supported by the Deutsche Agentur für Raumfahrtangelegenheiten, DARA, Bonn)


Seeds of WT Arabidopsis, two reduced-starch strains, and a starchless mutant were grown in microgravity during the STS-81 flight of the Space Shuttle. This experiment was included in the European Space Agency’s Biorack module, which is a laboratory with incubators, centrifuges, video-cameras, fixation devices, and a glove-box. Dry seeds were launched, and the mission specialists activated the experiment by hydrating the seeds. Germination was greater than 90% for seeds in microgravity as well as the controls. Flight seedlings were smaller (60% in total length) compared to control plants grown on the ground and to control plants on a rotating clinostat. Seedlings that developed in space had two morphological features that distinguished them from the two groups of controls: a greater density of root hairs and an anomalous hypocotyl hook structure. During the flight, hypocotyls of WT seedlings responded to a unilateral 60-min stimulus provided by a 1-g centrifuge while those of the starch-deficient strains did not. In addition, hypocotyls of seedlings grown in space were less sensitive to gravity compared to those of plants on the ground. Nevertheless, the strain with the greatest amount of starch responded to the stimulus given in-flight, and, therefore, these data support the starch-statolith model for gravity sensing. This is the first spaceflight study to compare the response of a WT and mutant strains to a unilateral 1-g stimulus from an on-board centrifuge. (Financial support was provided by NASA grant NAG 2-1017.)


To test whether microgravity affects changes in starch metabolism and/or starch composition we examined by means of magnetograviphoresis starch grains extracted form cotyledons and hypocotyls of 5 day old space grown soybean (Glycine max L.) seedlings frozen in orbit (BRIC-03 experiment) and from parallel ground controls. Starch grains from space grown tissues were 20-50% smaller in diameter than that from the ground controls. The particles were measured in a 150 μm glass capillary, positioned in the gap between magnetic poles of a magnetic system. When magnetic field was off, the grains sedimented under the gravity force, and the sedimentation velocity (v_{sed}) was measured with a videomicroscope. When magnetic field was on, the particles moved upwards (v_{up}) under the action of the ponderomotive magnetic force near the upper edge of the gap. The ratio (v_{up} + v_{sed})/v_{sed} is proportional to the ratio Δ /Δ0, where Δ - difference of magnetic susceptibilities of the particle and the medium, Δ0 - difference in their densities. The proportionality coefficient is a device constant. The term (v_{up} + v_{sed})/v_{sed} of starch grains from cotyledons was ca. 15% higher for space grown tissue. Densities of the particles were determined by centrifugation in metrizamide gradients and found to be similar for all samples (1.37 to 1.38 g/cm^3). Therefore the observed difference in (v_{up} + v_{sed})/v_{sed} of cotyledons was probably due to a difference in magnetic susceptibility, indicating potential alterations in chemical composition of starch grains formed in space. (Supported by NASA grant NAG10-0190 and NASA contract NAS10-12180).
[53] MECHANOTRANSDUCTION MOLECULES IN PLANT GRAVISENSORY RESPONSES: AMYLOPLAST/STATOLITH MEMBRANES CONTAIN A β1-INTEGRIN-LIKE PROTEIN. T.M. Lynch1, P.M. Lintilhac1, and D. Domozych1. 1Botany Department, University of Vermont, Burlington, VT 2Department of Biology, Skidmore College, Saratoga Springs, NY

It is hypothesized that the sedimentation of amyloplasts within growing root cells is the mechanism by which plants sense gravity. Statolith sedimentation, with its ability to generate weakly mechanical signals, is a legitimate means for organisms to discriminate the direction of the gravity vector. However, it has been demonstrated that starless mutants with reduced statolith densities maintain some ability to sense gravity, calling into question the statolith sedimentation hypothesis. Here we report on the presence of a β1 integrin-like protein localized in amyloplasts of tobacco NT-1 suspension culture and callus cells. Two different antibodies to the β1 integrin, one to the cytoplasmic domain and one to the extracellular domain, localize in the vicinity of the starch grains within amyloplasts of NT-1. Biochemical data reveals a 110 kd protein immunoprecipitated from membrane fractions of NT-1 suspension culture indicating size homology to known β1 integrin in animals. This study provides the first direct evidence for the possibility of integrin mediated signal transduction in the perception of gravity by higher plants. An integrin mediated pathway, initiated by starch grain sedimentation within the amyloplast, may provide the signal amplification necessary to explain the gravitropic response in starch depleted cultivars. This work was partially funded by NASA grant No. NAGW 3604


Ponderomotive forces acting on amyloplasts in high gradient magnetic fields (HGMF) were used to test whether displacement of amyloplasts by high gradient magnetic fields (HGMF) affect the gravireponse mechanism of wildtype (WT) and lazy-2 mutant seedlings. Hypocotyls of the lazy-2 gravitropic mutant of tomato (Lycopersicum esculentum Mill.), cv. Atsia Craig exhibit negative gravitropic response similar to WT, but positive gravitropism in red light. Four d old plants with straight hypocotyls were selected and the tips of their hooks were placed in a HGMF near the edge of a magnetized ferromagnetic wedge (V(H)=10^8 Oe/cm) and mounted on a 1-rpm clinostat in darkness. After 4 h 85% of WT hypocotyls (n=50) and of dark grown mutant seedlings (67%, n=40) curved toward the wedge. When the seedlings were exposed to red light for 1 h prior to and during the application of the HGMF, 78% (n=40) of the WT seedlings curved toward the stronger field, but the direction of curvature of the majority of the lazy-2 seedlings changed such that 75% (n=40) curved away from the stronger field zones. Magnetic susceptibility of amyloplasts of both varieties was measured by magnetograviphoresis, density - by centrifugation in metrizamide gradients. Microscopic observations have shown displacement of amyloplasts in statocytes of both WT and lazy-2 under the action of HGMF similar to displacement due to gravity. The data indicate, that both types of tomato seedlings respond to amyloplasts it did not alter the response of the mutant indicating that the mutation affects the gravireponse mechanism. Our observations lead us to conclude that HGMF do not alter biological functions unrelated to amyloplast displacement. (Supported by NASA grant NAG10-0190).

[55] DIFFERENTIAL GENE EXPRESSION ASSOCIATED WITH A GRAVITY RESPONSE IN Ferns. A. Chatterjee, D.J. Eastburn, and S.J. Roux. Department of Botany, University of Texas at Austin. Ceratopteris richardii is a tropical fern which has become a useful model system for studies of plant growth and development due to the ease with which genetic studies can be carried out on it. We recently reported that in single-cell spores of Ceratopteris there is a defined period during which gravity orients the direction of nuclear migration and subsequent rhizoid growth. Using this model system, we employed Differential Display Reverse Transcriptase-PCR (DDRT-PCR) to look for differentially expressed cDNA's during the critical period of gravity responsiveness. Numerous differentially expressed partial-length cDNAs were found using this method. However these cDNAs were all smaller than 500 bp and comprised mainly of 3' untranslated sequences, so they revealed little about the identity of the proteins they encoded. To overcome this limitation, two approaches have been taken. One uses 5' RACE to obtain sequences 5' of the cDNAs. The other uses the differentially expressed cDNAs as probes to screen a cDNA library of Ceratopteris gametophyte genes. The RACE approach has thus far provided an additional 700 bp of sequence for one of the cDNAs, so that 320 amino acids at the C terminus of the protein encoded by this gene could be deduced. Unfortunately, this partial sequence does not have sufficient sequence similarity to any known protein in the database to confidently assign its identity. Another round of 5' RACE is being carried out to further extend this cDNA and learn more about the structure of the protein it encodes. The library screening approach is in progress, and the results of this assay will be presented and discussed. (Supported by NASA grants NAGW 1519 and NAG10-0202).


The signal transduction pathway that mediates plant responses to gravity can be modulated by light. To understand how light modulates gravitropic signal transduction we are using a tomato mutant, lazy-2, which has shoots that show a phytochrome-dependent positive gravitropic response. Because at least five different phytochrome genes are expressed in tomato, it is of interest to know which of the phytochromes are involved in reversing graviresponse. We have begun to address this question by constructing double and triple mutants in which lazy-2 plants lack either phytochromeA (phyA), phyB1 or both phyA and phyB1 and observing gravitropism under different light conditions. Under glasshouse lighting, shoots of lazy-2 plants deficient in phyA, phyB, and both phyA and phyB1 grow downward, indicating that another phytochrome(s) is sufficient to induce bending. When seedlings are grown under low fluence far-red light, in which phyA is normally active, lazy-2 plants lacking phyA no longer bend downward, whereas lazy-2 plants lacking phyB do bend downward. When grown under low fluence red light, lazy-2 plants deficient in phyA, phyB, and both phyA and phyB1 bend downward. phyB1 is active under low fluence red light, as demonstrated by increased hypocotyl elongation, but apparently is not required for reversing bending in lazy-2 plants. These results indicate that phyA is required for bending under low fluence far-red light, but that in other light environments at least one other phytochrome participates in inducing reversed graviresponse of lazy-2 plants. (Supported by A NASA Space Biology Research award (to FJB) and NASA Life Sciences grant No. NAGW-3716 (to T.L.L.).

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SESSION F: CONCURRENT POSTER SESSION III
ANIMAL DEVELOPMENT, GROWTH AND GENETICS
[57] CELL PROPERTIES IN EARLY XENOPUS EMBRYO ARE ALTERED BY NOVEL GRAVITATIONAL CONDITIONS. S. Huang, P.H.Z. Wang, K.E. Johnson and W.L. Wei. Department of Anatomy and Cell Biology, The George Washington University, Washington, DC.

Our study reports the effect of gravity on autonomous differentiation, and inducing and responding properties of the blastomeres. Fertilized eggs were 90° rotated or subjected to microgravity simulated with a horizontal clinostat just before cortical rotation during first cleavage. To examine the change of autonomous differentiation properties, blastomeres were explanted at 16-cell stage and cultured in simple salt solution. Elongation and staining for muscle-specific antibody (12/101) were assessed when control embryos reach stage 37/38. We found that 1) ventral vegetal blastomeres take the place of dorsal animal blastomeres, elongating and differentiating with a dorsal marker in the rotated embryo; 2) the frequency of elongation and dorsal fate differentiation is much higher in rotated ventral vegetal blastomeres (100% elongation and dorsal differentiation) than in the normal dorsal animal blastomeres (50% elongation and 17% dorsal differentiation); 3) elongation and dorsal differentiation of dorsal animal blastomeres is increased in the clinostated embryos. These results indicate that autonomous differentiation of blastomeres is enhanced under novel gravity. To examine the change of inducing property, blastomeres from the 90° rotated embryo were transplanted to the ventral vegetal side of the normal embryo to see if secondary axis is induced. Ventral vegetal blastomeres from the rotated embryo, but not from the normal embryo, were able to induce secondary axis. To examine the change of the responding property, vegetal blastomeres were labeled and transplanted to "retinoic" position of host embryos and the contribution of the labeled blastomere to retina was assessed. Normal vegetal blastomeres transplanted to "retinoic" region do not change their endodermal fate, but vegetal blastomeres from the rotated embryo change their fate to contribute to retina. These results indicate that cell properties are altered under novel gravity. Preliminary in situ hybridization results of a putative dorsal determinant, Vgl1 mRNA, suggest that redistribution of Vgl1 mRNA under novel gravity may be involved in these cell property changes (Supported by NAGW-4516).

[58] EGG ROTATION DURING AVIAN EMBRYOGENESIS. P.Y. Hester1 and K. Boda2. 1 Dept. of Animal Sciences, Purdue University, West Lafayette, IN and 2 Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Ivanka pri Dunaji, Slovakia.

Exposing chicken embryos to the space environment caused death during earlier stages of embryogenesis, while older chicken embryos developed normally. Though success rate has been low compared to earth-bound controls, quail embryos have successfully completed embryogenesis in orbit. Microgravity's role in the death of the embryos will not be known until a centrifuge is employed in orbit. Since avian eggs in microgravity have not been turned, or turned too infrequently or inappropriately, the objectives of the current ground-based study were to determine the effects of frequency and orientation of turning on embryogenesis. Quail embryo viability was not affected by incubating eggs horizontally with daily rotation (4X) as compared to controls. A decrease in frequency of egg rotation caused a concomitant linear decrease in hatchability for both quail and chicken eggs (p < 0.01). Fertile chicken eggs were more adversely affected by lack of egg rotation than quail eggs (p < 0.05). Gas exchange and nutrient distribution may be facilitated in unturned quail eggs as compared to chicken embryos because of its smaller egg size. The shorter distance between a settling unturned quail blastoderm relative to the shell surface may increase the probability of survival for quail as opposed to chicken embryos. (Supported by NASA (ARC): NAG 2-1001.)

[59] EMBRYONIC SKELETAL TISSUE DEVELOPMENT IN THE NASA MICROGRAVITY BIOREACTOR. B.J. Klement, J.Li, and B.J. George. Musculoskeletal Research Group, Space Medicine and Life Sciences Research Center, Morehouse School of Medicine, Atlanta, GA.

In culture, embryonic mouse pre-metatarsal mesenchyme explants undergoes a series of developmental events including cartilage formation, terminal chondrocyte differentiation, matrix mineralization and an increase in explant length. We have studied development of pre-metatarsal tissue in the NASA microgravity bioreactor high aspect ratio vessel (HARV). The pre-metatarsal explants were cultured in the HARV for 8, 10 or 14 days at 14 rpm. The length of the explants and the amount of alkaline phosphatase activity were measured and compared to controls. Alkaline phosphatase activity was used as a marker of terminal chondrocyte differentiation. The data show that the pre-metatarsal explants cultured in the bioreactor were shorter than the controls. After 8 and 14 days of culture, the pre-metatarsals cultured in the bioreactor were 16% (225 m shorter, and 17.5% (284 m) shorter than the controls, respectively. However, explants cultured the first 8 days in standard lab dishes followed by 6 days of bioreactor culture grew to the same length as the controls. The explants cultured in the bioreactor also showed a much lower level of alkaline phosphatase activity than controls. At 8 days of culture, control cultures expressed an activity of 244 IU/explant, while the bioreactor cultures had an activity of only 18 IU/explant. At 10 and 14 days of culture, the controls expressed 385 and 492 IU/explant respectively, while the bioreactor cultures expressed only 0.7 and 43 IU/explant, respectively. The explants that were pre-cultured in standard laboratory dishes for 8 days prior to bioreactor culture, displayed a slightly higher level of activity, 79 IU/explant, which was 83% less than the controls. These data show a similar trend to data gathered from pre-metatarsals that were cultured in microgravity on the space shuttle. (Supported by NASA: NCCW-0083)

[60] EXPOSURE TO EITHER HYPO- OR HYPERGRAVITY ATTENUATES HEART DEVELOPMENT IN CULTURE. P. Lwigel1, J. Denning1, A. Juhl1, W. Norton1, B. Spooner1, and D. Wiens2. Dept of Biology, Univ N Iowa, Cedar Falls, 2 Southeastern Louisiana Univ, Hammond, and 3 Div of Biology, Kansas State Univ, Manhattan.

Exposure to altered gravitation may disturb the macromolecules comprising the cytoskeleton-cell surface-extracellular matrix (ECM) interface of embryonic cells. Early development of organs such as the heart depends on dynamic interactions across cell surfaces. For example, fibronectin (FN), a glycoprotein that links the ECM to the cytoskeleton through the integrin receptor at the cell surface, is known to be necessary for normal heart development. Thus altered gravity may perturb organogenesis.

We cultured endodermal-mesodermal precardiac explants dissected from chick embryos in a high aspect ratio bioreactor vessel to simulate microgravity (μG), or in a short arm centrifuge (NASA-Ames research Center) at 1.4 and 2.0 G, for 18 hours of heart development. Bioreactor μG did not alter the size or morphology of the explants, but did significantly reduce the proportion that developed contractions by at least 20%. Immunostaining of explant sections with antibodies to FN followed by color thresholding in digitally captured images of cross-sections showed that it also significantly reduced the length of immunostainable FN present in basement membrane by 42%, though not the total amount of FN staining. Exposure to hypergravity dramatically abolished cardiac development, including contractions and vesicle morphogenesis. These effects of altered gravity are being correlated with changes in ultrastructure. Taken together, the results indicate strong sensitivity of cardiomyogenic development involving FN to alteration of gravity. (Supported by NASA NAGW-4992 and NAG5-3751)
SESSION F: CONCURRENT POSTER SESSION III
ANIMAL STRUCTURAL SYSTEMS & MUSCLE PHYSIOLOGY I

For normal skeletal muscles, velocity of shortening correlates directly with whole muscle, myosin ATPase activity. However, in a variety of unloading models, single fiber studies show that increased shortening velocity is not always accompanied by a detectable increase in fast myosin. An alternative possibility is that speeding is due to decreased packing density of contractile filaments. This was assessed in atrophic soleus muscle fibers by quantifying the packing density of myosin thick filaments and actin thin filaments in 8 male subjects before and after 17 day bedrest. Pre-bedrest needle biopsies were taken from the left soleus muscles and post-bedrest samples from the right for electron microscopy. Packing density was measured in cross sections by computerized digitizing morphometry and normalized for sarcomere length determined on the same fibers recut in longitudinal section. Thin filament density (3.044±0.355/μm², ± S.D.) decreased 20% in the I band (2.426±0.333/μm²) and by 24% (2.559±0.170 vs 1.948±1.91/μm²) in the overlap region of the A band. This reduced average number of thin filaments surrounding a thick filament from 6 to less than 5 and increased the estimated mean distance from thick to thin filaments by 4.5 nm. The decrease in packing density appears sufficient to account for the 34% increase in velocity measured on single fibers expressing only slow myosin in the same biopsies. Thus, a disproportionate loss of thin filaments during muscle fiber atrophy appears to increase the velocity of shortening which compensates for reduced force output.


Clenbuterol (Cb) is a β-adrenergic agonist known to enhance muscle hypertrophy and diminish atrophy in various species, including humans. Most rat studies have been carried out in immature (fetal, neonatal and juvenile) animals whose muscles are more plastic with regard to fiber type and myosin heavy chain (MHC) isoform expression. In this study, we begin characterizing the effects of this potential countermeasure to microgravity-induced muscle atrophy on the muscles of mature rats, in which fiber type and MHC expression are more stable. Mature, male, Sprague Dawley rats (6 months old; > 300 g) were subjected to standard hindlimb suspension for two weeks and treated in a 2-days-on-2-days-off regimen of subcutaneous Cb injections (1 mg Cb/kg body weight)(designated CbS). Pair-fed controls included vehicle treated suspended rats (VsS), Ve and Cb treated “tethered” rats which were subjected to the hindlimb-suspension apparatus but whose hindlimbs were not unloaded (VsT, CtT). Four muscles were collected from the experimental and control animals' hindlimbs: soleus, extensor digitorum longus, gastrocnemius, and plantaris. All groups showed some weight loss over the experimental period (body weight loss: S>T>NS). All muscles in clenbuterol-treated animals showed increases in raw weight over untreated controls. When muscle weights were expressed as mg muscle wt/kg body wt, significant clenbuterol-induced increases were seen in all muscles and groups examined except the soleus of suspended animals. Here muscle weight increase failed to compensate for clenbuterol-induced decreases in body wt loss. The results suggest that clenbuterol preferentially enhances growth, and limits atrophy, in the fast, type II-MHC-containing muscle fibers. Analyses of MHC expression and protein content in these muscles are underway to test this hypothesis. (Supported by NASA NCCW-0083 and NIH GM08248 and RR03034).


The longitudinal growth rate (LGR) was measured in tibia of rats from four spaceflight experiments (PSE 1, 3 and 4 and PARE 3) and from two models of skeletal unloading (hindlimb unloading and sciatic neuromyotony) using the flurochrome labeling technique. Additionally, the effects of a 11-day spaceflight (PSE 3) on gene expression for cartilage matrix proteins were determined by Northern analysis of total cellular growth plate RNA. The absence of an effect of skeletal unloading on LGR was consistently observed regardless of the age, strain, sex, gonadal status, duration of unloading (4-11 days) or method of unloading. These negative results contrast with the decreases in steady-state mRNA levels for Type II collagen (-33%) and aggrecan (-53%) detected in the flight rats. These results indicate that skeletal unloading has no effect on the rate of bone elongation. However, the observed changes in gene expression raises concern that spaceflight may result in changes in the composition of extracellular matrix which could have a negative impact on the mechanical properties of cartilage.

(Supported by the Mayo Foundation and NASA NGW 4963.)
SESSION F: CONCURRENT POSTER SESSION III
ANIMAL REGULATORY PHYSIOLOGY

[64]
PREDICTION OF AN INCREASE IN THE BODY MASS OF RATS DURING EXPOSURE TO SPACEFLIGHT. C. E. Wade¹, L. A. Baer² and R. M. Ortz¹. ¹NASA Ames Research Center Moffett Field CA, ²Marine Biological Laboratory, Woods Hole, MA. Exposure of rats to spaceflight has been reported to increase, not change or decrease body mass. Following spaceflight we recently found body mass to progressively decrease for 3 d. Mass measurements are usually obtained upon return from spaceflight after 4 or more h of exposure to 1 G, a period of time in which significant mass loss could occur. This led to speculation that body mass may be increased during spaceflight. We therefore developed two models to predict body mass during spaceflight. The first was based on the above information as to the loss of mass immediately following spaceflight and the second was based on the reduction in body mass with exposure to hypergravity (minimum of 10 d). Analysis of available spaceflight data was also conducted to support the predicted values. Data were obtained from the published literature. Percent of control animals body mass (% mass) was correlated with time following spaceflight or to the G-level in the case of hypergravity. Percent mass was correlated (r=0.78, n=23, p<0.0001) with the time post-landing when mass was measured. The intercept at zero time equaled 103±1% (SE). Percent mass during exposure to hypergravity was correlated (r=0.89, n=40, p<0.0001) with the level of G exposure. The intercept at zero G was 104±1%. Both correlations predicted an increase in body mass of 3-4% during spaceflight (zero time or zero G). Recent inflight (SLS-2) measurements of body mass found an increase of 107% on day 6 (C=286±5 vs. F=306±5 g, n=15 per group, p<0.0001) and an increase of 109% on day 13 of flight (C=301±6 vs. F=327±6 g, p<0.0001). There was no difference in initial body mass between groups 6 days prior to launch: C=234±3 vs. F=228±3 g, p=0.23. The increase in body mass during spaceflight may be due to maintenance of food intake and activity in the presence of a reduced workload. The increase in body mass of rats may also be due to other alterations of metabolism that warrant further investigation during spaceflight. (Supported by: NASA #199-18-12-02)

[65]

Blood pressure and mesenteric resistance artery function were assessed in 9-week-old spontaneously hypertensive rats following an 18 day shuttle flight on STS-80. Blood pressure was measured twice, first in conscious animals using a tail-cuff method and then while the animals were anesthetized with 2% halothane in O2. Isolated vessel responses to cumulative additions of norepinephrine, acetylcholine, sodium nitroprusside, and calcium were measured within 17 hours of landing using a wire myograph system. Blood pressure was slightly reduced in conscious flight animals (p=0.056) but was significantly elevated (p<.001) above vivarium control group values following flight. Maximal contraction to norepinephrine was attenuated in the mesenteric vessels (p<.001) and relaxation to acetylcholine was reduced (p<.001). There was no difference between flight and control animals in the vessel response to sodium nitroprusside (p>0.05) or calcium (p>0.05). The pattern of results suggest that space flight impairs endothelial function which diminishes peripheral vascular responsiveness to vasoactive agents. As a result, the ability of the animal to make hemodynamic adjustments is compromised which may lead to orthostatic intolerance.

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[66] AQUAPORIN 1 IS DOWN-REGULATED IN RAT CHOROID PLEXUS AFTER ALTERED GRAVITY EXPOSURE AS WELL AS AFTER WATER DEPRIVATION AND SALT-LADING. C. Masséguet', M. Corcoran', C. Cercenac', L. Mani-Ponset, N. Daunton1, and J. Gabrion1. 1'URA CNRS 1448, Univ. PARIS VI, France, 2NASA Ames Research Center, Moffett Field, CA, USA. Cerebrospinal fluid (CSF) which is secreted in cerebral ventricles, is mainly produced by the choroid plexus. The water channel CHIP 28 or AQP3 seems to be involved in this process, due to its symmetrical distribution in the apical membrane domain. In adult rats adapted to actual (14-d spaceflight, SLS-2 experiment, STS-58) or simulated microgravity (14-d hindlimb-suspension), we noted that the loss of apical microvilli observed in choroid plexus was concomitant with a strong decrease and heterogeneous organization in the apical AQPI, shown with a polyclonal antibody directed against CHIP 28 (generous gift from Pr A. Verkman, Univ. California San Francisco, CA). Similarly, AQPI was reduced at the apical pole of choroidal cells in adult rats adapted for 14 days to hypergravity (2G and 3G). This most important down-regulation was observed in choroid plexus of rats chronically adapted to 3G. The protein was also immunochemically detected in choroid plexus of rats dissected 3-7 days after water deprivation and compared with results in rats following salt-loading (2% NaCl for 7 days) to assess effects of hypoosmolality. A reduced and heterogeneous immuno-reaction was noted in both conditions, but the most altered pattern was noted in water-deprived rats. In the experimental conditions known to impair water balance, as well as in altered gravity, a down-regulation of the AQPI was clearly induced. In contrast, AQPl reappeared at the apical pole in rats dissected 2d after readaptation to earth gravity (NIH-R1 experiment, STS-66) or 5h or 1d after water-administration at the end of a 7-d dehydration, demonstrating the up-regulation of the choroidal AQPI, in particular during restoration of water balance. (Supported by CNES: #94-224, 95-223 & 96-264; NASA: Gravitational Research Facility, 1999-62-13 & 1999-16-12-01).

[67] CHOROIDAL GUANYLATE CYCLASE (cGC) ACTIVITY IN RATS ADAPTED TO EITHER SPACEFLIGHT OR HYPERGRAVITY. C. Cercenac', S. Herbut', I. Poljakov2, N. Daunton', A. Malouvier1 and J. Gabrion1. 1'URA CNRS 1448, Univ. PARIS VI, France, 2NASA Ames Research Center, Moffett Field, CA, USA, 3IMASSA, Bretigny/Orge, France. Choroidal guanylate cyclase (cGC) activity regulates the cerebrospinal fluid (CSF) production. Receptor activation by atrial natriuretic peptide (ANP) increases cGMP levels and mediates an inhibition of the CSF secretion. After 17 days in space (STS 78-LMS experiment), cGMP contents, measured in choroidal plexuses excised from adrenaleonized (ADX) or sham-operated "Flight" rats, were compared with those from ground-based ADX or sham-operated control rats, in the presence or absence of ANP stimulation. ADX rats were supplemented with physiological doses of corticosterone and aldosterone. In sham-operated "Flight" rats, cGMP baseline was significantly increased (p<0.025) and no elevation in cGMP levels after ANP stimulation was observed. No differences were noted between ground control (Synchonous, SC, "vs" Vivarium Control, VC) sham-operated rats or between ADX "Flight" and VC rats. In contrast, we noted that the cGMP baseline was significantly increased in choroid plexus of SC rats (p<0.05), suggesting that SC housing conditions could involve a stress response, which is not readjusted in ADX rats by the implantation of pellets delivering corticosteroids at a constant rate. In parallel, we investigated the effects of hypergravity on cGC activity, by assaying cGMP in choroid plexuses of rats adapted to 1.03G, 2G and 3G. In contrast with flight results, choroidal cGMP baselines were slightly decreased (1.03G "vs" 2G, p<0.06) or unchanged (1.03G "vs" 3G). Conversely, cGMP contents were increased after ANP stimulation in the three conditions. The most important elevations were observed after 14 days under 3G (1.03G "vs" 3G, p<0.05). Considering that elevations in cGMP levels reflect low CSF secretion, increased cGMP baselines suggest a CSF reduction in spaceflight through an ANP-independent regulation. On the other hand, hypergravity, which do not modify cGMP baselines, increases cGMP levels after ANP stimulation, seeming to decrease CSF production through an ANP-regulated mechanism. (Supported by CNES: #96-264; NASA: Gravitational Research Facility, 1999-62-13 & 1999-16-12-01).

[68] MAINTENANCE OF CENTRAL BLOOD VOLUME DURING PROLONGED EXERCISE DID NOT ELIMINATE CARDOVASCULAR DRIFT. P.B. Raven, K.H. Bryant, S.A. Smith, R.G. Querry, and K.M. Gallagher. Dept of Integrative Physiology, Univ. of North Texas Health Science Center, Fort Worth, Texas.

Eight volunteer subjects performed one hour of dynamic leg cycling exercise at 65% of maximal aerobic capacity (VO2max) with: i) no intervention; and ii) maintenance of cardiac filling volume via continuous infusion of a 6% dextran in saline solution. In the control condition, mean arterial pressure (MAP) fell 15%, from 10 to 60 minutes of exercise. Conversely, heart rate rose 16% during this time period. While central venous pressure (CVP) decreased 7% from 10 to 60 minutes of exercise in the control condition presumably due to a greater percentage of cardiac output being routed to the cutaneous circulation. Indeed, this fall in central blood volume results in a lessened cardiac filling volume which is reflected by a concomitant reduction in SV and a compensatory increase in HR. When CVP, and thus SV were maintained, using continuous infusion of a 6% dextran in saline solution some progressive decrement in MAP remained (8%), presumably due to a fall in total peripheral resistance corresponding to cutaneous vasodilation. In addition, HR rose to the same extent (15%) during the hour of exercise with volume infusion as during the control condition. This data in conjunction with a concomitant rise in VO2, and rate of perceived exertion (RPE), which increase similarly during the two bouts of exercise, indicate that central command activation increased during the prolonged exercise despite maintenance of cardiac filling volume. Therefore, the increases in HR seen during the volume infusion exercise bout is indicative of the effect of central command without the influence of the compensatory response to a fall in SV.

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SESSION F: CONCURRENT POSTER SESSION III
CELL BIOLOGY I
[69] RETARDATION OF VASCULAR WOUND HEALING IN VITRO BY SIMULATED MICROGRAVITY IS MEDIATED BY C-MYC AND C-FOS. S.A. Harris-Hooker, G.L. Sanford and C.D. Melhado, Space Medicine & Life Sciences Research Center, Morehouse School of Medicine, Atlanta, GA.

Anticipated hazards for crew members in future long-term space flights may result in a variety of injuries including fractures, deep punctures or cuts. The microgravity environment of space may complicate the wound healing process. To mimic a capillary wound, we examined the response of endothelial cells to a demutated injury under simulated microgravity using a horizontally rotating clinostat. Cultures were adapted to clinostat rotation for 24 hr, an area denuded and the time course for cells to close the wound area measured. Denuded clinostat rotated cultures were also examined in the presence of antisense oligonucleotides (AS-oligos) to c-myc, c-fos, c-jun, galectin-1, or bFGF mRNA. Stationary control cells, treated similarly, completely closed the wound area by 6 hr. Clinostat treated cells failed to close the wound area within 24 hr. AS-oligos to c-myc or c-fos prevented the clinostat induced retardation in closing the wound area, where AS-oligos to c-jun or galectin-1 allowed the wound areas to close within 12 hr. AS-oligo to bFGF or c-myc had no effect. These studies indicate that simulated microgravity severely retards vascular wound healing; the underlying mechanism for this effect requires the expression of c-myc and c-fos. Our findings suggest that prolonged time for vascular wound healing maybe a major problem that could occur in long duration space flights. (Supported by NASA: FAR NAG9-852 and NCCW0085)

[70] FLUID SHEAR INDUCES MORPHOLOGICAL CHANGES IN ENDOTHELIAL CELLS IN THE ROTATING BIOREACTOR. D. Ellerson, C.D. Melhado, S.A. Harris-Hooker and G.L. Sanford, Space Medicine & Life Sciences Research Center, Morehouse School of Medicine, Atlanta, GA.

NASA’s biotechnology program in cell science is focused on developing the rotating bioreactor to provide a firm basis for space flight cellular experiments and for culture of cells in 3-dimensional structures. We used this system to examine the behavior of endothelial cells to fluid shear in a simulated microgravity environment. Cells were cultivated on cytokex-3 microcarriers in bioreactors rotating at 8 or 15 rpm. Controls (1 G) were maintained in spinner culture flasks. In both systems, microcarriers and cells remain uniformly suspended in the fluid. Scanning electron microscopy showed that cultures had both 3-dimensional cellular aggregates and single cell configurations. Rotating cultures at 15 rpm or higher produced a fluid flow that resulted in the elongation of cells, a rapid decline in cell numbers and a loss of cell microvilli and cobblestone morphology. Lower rotation allowed cells to maintain their phenotypic differentiation. These results demonstrate that shear forces will induce morphological changes in a simulated microgravity environment. (Supported by NASA: NCCW0085)

[71] THE EFFECT OF HYPERGRAVITY ON PROLIFERATION OF OSTEOBLASTS. M. Kirven-Brooks1, G. Meeker1, N. Searby2 and C. Wade2. 1Lockheed Martin Eng. and Sciences Co., 2NASA, Ames Research Center, Moffett Field, CA.

Centrifugation is used during spaceflight as a simulated 1-g control. Past experiments have used centrifuges of different radii, and future hardware development plans continue this trend. Results have indicated differences in cell responses between the 1-g centrifuge control and ground based controls at 1-g. Ground based and flight studies have indicated that isolated cell cultures respond to centrifugation with alterations in proliferation. To investigate the effect of centrifugation on cells, we examined the proliferation rate in cells exposed to 2 to 4 hours of acceleration on two different radius centrifuges, thereby supplying different angular velocities at the same g-levels. ROS 17/2.8 osteoblasts were subjected to centrifugation on the Hypergravity Facility for Cell Culture (HyFaCC), a long arm centrifuge (LAC: 2.6 m radius) and a Short Arm Centrifuge (SAC: 0.2 m radius). Proliferation rates were measured, up to 48 hours post-treatment, by evaluating cell counts and cell viability. Centrifugation to generate a 1.4 g force on the cells provided a baseline growth curve which indicated that growth curves for the SAC and LAC and each associated ground control incubator were identical. Results indicate that increased g, from 1.4 to 5.7 g, caused increased cell proliferation in both centrifuge types. More importantly, cells reached confluence earlier in the SAC than in the LAC. These results suggest that arm length/rotation rate influences cell proliferation and possibly other parameters. Therefore, to reduce the influence of centrifugation effects vs. true spaceflight effects, this factor should be considered in the design of experiments utilizing on-board centrifugation controls. (Supported by NASA: NAS2-14263.)
SESSION F: CONCURRENT POSTER SESSION III
BIOTECHNOLOGY I

[72]
DESIGN OF A LOW-COST CREW-DISTURBANCE ISOLATION SYSTEM. J. Zimmerman1, M. van Schoor2, J. de Luis3, B. Maters3, S. Sell1. 1Payload Systems, Inc; 2Milde Tech. Corp, Cambridge, MA. Vibrations and transient disturbances from intravehicular crew motion will corrupt the microgravity (μG) environment onboard International Space Station (ISS). Mitigation approaches proposed mostly involve isolation of sensitive equipment from surrounding vibrations. An alternative is reduction or mitigation of vibrations at their source. The Astronaut Dynamic Load Sensing and Mitigation system (ADLSM) design meets this need. ADLSM, an innovative high bandwidth 6 d.o.f. isolation unit, incorporates state of the art active and passive isolation, data recording, and audiovisual feedback for the astronaut. In ADLSM, closed-loop-controlled actuators will provide active isolation over specific problematic frequencies, while passive mechanisms attenuate disturbances over a broad frequency range. Preliminary performance studies indicate that the flight units will provide an order of magnitude reduction of forces transmitted to the ISS structure, and approximately 20-40% reduction of transmitted energy. Integral data recording circuitry will permit non-intrusive data collection over extended on-orbit periods: an event detection algorithm will engage the data recording function only during periods in which units are in use. Thus data collection will occur continuously without wasting power or memory. Should memory reach capacity at a point at which it is inconvenient for the crew to replace the storage disk, data from the current operation will be lost, but the isolation systems will continue to function without degradation of performance. Visual and audio indicators will provide astronauts with a direct measure of the forces and moments imparted by their motions, enabling them to speed their adaptation to the μG environment. This learning effect will lead to longer μG operations periods and lower crew-induced disturbance values not only locally to ADLSM sites, but over the entire station. ADLSM complements existing rack-level and experiment-level μG isolation systems, and offers to become an inexpensive yet invaluable tool in meeting the μG requirements of future ISS experiment payloads. ADLSM units likely will be ready for parabolic flight evaluation trials in 1998 and spaceflight evaluation trials in 1999/2000. (Fundied by NASA contract NAS9-97077)

[73]
TESTING THE MODIFICATIONS OF THE ANIMAL ENCLOSURE MODULE FOR NEUROLAB (STS-90). L.A. Baer1, M.K. Steele1 and D. Reiss-Bubenheim1. 1Lockheed Martin, Moffett Field, CA and 2NASA/Ames Research Center, Moffett Field, CA. The Animal Enclosure Module (AEM) houses animals in the shuttle middeck in support of NASA life science experiments. The AEM is currently qualified to support eight-150 gram or six-250 gram rats for 20 days, but does not allow for in-flight access of the animals and the waste filter has not been qualified to contain mouse waste odors. PI requirements for the Neurolab mission require access to the animals (adult Fischer 344 rats with head implants, Sprague Dawley rat dams with neonates and timed-pregnant ICR mice) during scheduled in-flight activities. In addition, the AEM must have a waste filter capable of containing mouse odors. To support this mission, modifications to the AEM, including the addition of access doors to the AEM’s lexan cover and an assessment of the current filter’s abilities to contain mouse odors, were made and tested. A test of the AEM’s accessibility was performed using rat dams and their neonates to verify that the animals can be accessed during scheduled in-flight activity days and to ascertain that modifications to the habitat will not affect the health and well-being of the animals. Access of the animals was successfully accomplished on test days 6 and 12. No anomalies were observed regarding the health and well-being of the animals throughout the test. Filter testing using timed-pregnant ICR mice demonstrated that the current AEM rat filter configuration could be used for the mission duration the mice would be in the habitat. It was determined that the current modifications made to the AEMs will be able to support the Neurolab mission. (Supported by NASA Space Life Sciences Payloads Office).

The Cell Culture Unit (CCU), currently in development under funding from the NASA Ames Research Center, is planned to serve as the facility for all cell growth experimentation onboard the International Space Station (ISS). As such, the CCU is being created to satisfy a broad spectrum of studies with diverse experiment designs. The CCU consists of: the Cell Specimen Environment Assembly, which holds up to 24 cell specimen chambers, 3, 10, or 30 ml capacity, and provides recirculation of media, additive delivery, and heat and gas exchange for all 24 chambers individually; the Automated Sampling Module, which permits up to 60 samples to be collected and stored under computer control; the Electronics Assembly, which contains all computer and signal conditioning electronics; the Video Microscopy Subsystem, which provides 40x or 200x optical magnification views of the specimen cultures via video downlink; and the Structural Containment Assembly, which supports all other hardware and provides interface mounting with the various ISS host systems. Each of these assemblies is clear and distinct interfaces with the others and with the ISS host systems. In the event of a failure, an entire assembly can be replaced with minimal impact on the other CCU subsystems. An example of the way the entire CCU design is organized around a principle of minimizing the probability of failures that could compromise an experiment. The CCU chambers are also designed to be removable off-orbit without violating biocountermeasure or bioisolation barriers.

The CCU successfully completed its payload requirements review in April 1997. Functional tests of several prototypes are underway to ensure that the CCU will safely support the incubation of multiple cell types, including plant, avian, mammalian, and bacterial lines, and that the prototype units are scheduled to commence ground-based science evaluation rounds in early 1998. Results of these studies will be reported in separate communications. (Funding: NASA contract NAS2-96001.)


Two investigations will be conducted aboard the first BPS flight, a technology verification test and a peer reviewed wheat physiology experiment. The objective of the BPS Technology Verification Test is to validate hardware functionality against design specifications developed to accommodate NASA science while minimizing technical, cost, and schedule risks for systems capable of operating in microgravity for durations of 90 days or more.

All four BPS chambers will be used to collect data to verify system functionality on orbit. Two chambers will be dedicated to technology verification. One chamber will be cycled through a combination of temperature and humidity levels. Wheat seedlings (v. Super Dwarf) will be used to maintain a transpirational load on this chamber during tests. The other chamber will be maintained at constant temperature, humidity and CO2 levels. Arabidopsis and miniature Brassica plants (two primary test species for space-based plant research) will be used to impose challenges specific to plant systems, including exhibition of multiple development stages (i.e. flowering and seed development), crew intervention for pollination (Brassica), and generation of debris (detached floral parts, etc.) which can move throughout the BPS flowpath in microgravity.

Evaluation categories include basic subsystem functionality, information acquisition (data, video, samples), operations and support (including plant manipulation and expendables servicing), component performance, and advanced technology tests. Criteria for evaluation will include general plant appearance and development, plant productivity, sensor data for all subsystems, mass flow numbers for water and CO2, video images, crew assessments, and postflight analysis of BPS components.


A series of tests were conducted growing Brassica rapa seedlings for two experiments using the Plant Growth Facility (PGF) in the Collaborative Ukrainian Experiment now scheduled for launch on STS-87 in November of 1997. One experiment (BPAC) initiates growth on-orbit and harvests most of the seedlings after landing to determine the effect of microgravity on the photosynthetic apparatus. Another experiment (BSTIC) launches 12-day-old seedlings to study the effect of microgravity on the reproductive process. Both experiments require the crew to add nutrient fluid requirements during the mission. Tests were conducted growing the plants under similar conditions expected during the space flight experiment to (1) characterize the effects of temperature, relative humidity and carbon dioxide on the growth and development of the seedlings, (2) determine the timing and amount of nutrient fluid required to be supplemented during the mission, and (3) verify crew procedures used conduct the experiments. The plants were grown in separate units called Plant Growth Chambers (PGC’s) which fit inside the PGF. Two PGC’s were each planted with 54 seeds, which were imibed the first day of the simulated mission. The other 3 PGC’s contained six 12-day-old seedlings. Plant growth measurements and analysis were conducted at the completion of the 16 day test. Results of the simulated tests confirmed that plants grown in the controlled environment of the PGF would meet the experimenters requirement for flight. (Funded under NASA Contract NAS10-12180 with Dynamac Corp.)
SESSION F: CONCURRENT POSTER SESSION III
SPACEFLIGHT EXPERIMENT RESULTS I
[77] EFFECT OF SPACEFLIGHT ON ULTRASTRUCTURE, CHLOROPHYLL AND CARBOHYDRATE CONTENT OF ARABIDOPSIS LEAVES. A. Kuang1, C.S. Brown2, S.W. Matthews1 and M.E. Musgrave1. 1Dept. of Plant Pathology & Crop Physiology, and 2Botany, Louisiana State Univ., Baton Rouge, LA, and 3Dynamac Corporation, Durham, NC.

Leaf structure and function under spaceflight conditions have received little study despite their important implications for biological life support systems using plants. Previous reports described disruption of the membrane apparatus for photosynthesis and a general decrease in carbohydrate content in foliage. During a series of three short-duration experiments on the space shuttle, we had an opportunity to examine attributes of Arabidopsis thaliana leaves. The plants were at the rosette stage at the time of loading onto the space shuttle, and received the same light, temperature, carbon dioxide and humidity regimes in the orbiter as in the ground controls. The experiments differed according to the regime provided in the headspace around the plants: this was either sealed (on mission STS-54); sealed with high levels of carbon dioxide (on mission STS-51) or vented to the cabin air through a filtration system (on mission STS-68). Immediately post-flight, leaf materials were fixed for microscopy or frozen in liquid nitrogen for subsequent analyses of chlorophyll and foliar carbohydrates. At the ultrastructural level, no aberrations in membrane structure were observed in any of the experiments. When air-flow was provided, plastids developed large starch grains in both spaceflight and ground controls. In the experiments with sealed chambers, spaceflight plants differed from ground controls with regard to measured concentrations of carbohydrate and chlorophyll, but the addition of air inflow eliminated these differences. The results point to the crucial importance of consideration of the foliage micro-environment when spaceflight effects on leaf structure and metabolism are studied. Sponsored by NASA grants NAG10-0075 and NAG10-0139 to MEM.

[78] MICROGRAVITY EFFECTS ON SUCCESSFUL FERTILIZATION IN BRASSICA RAPA. N.G. Guillory1 and M.E. Musgrave2. 1School of Forestry, Wildlife and Fisheries and 2Dept. of Plant Pathology & Crop Physiology, Louisiana State University, Baton Rouge, LA.

The successful development of seeds in Brassica rapa after pollination in microgravity was investigated. The results will help to clarify problems encountered in growing reproductively viable plants in the microgravity environment of space. The Brassica rapa plant was chosen for its relatively short life cycle and its edible qualities. Plants were pollinated aboard the KC-135A aircraft during short periods of microgravity. Also, pollen was collected from flowering Brassica rapa in microgravity for later transfer to ground-based control plants. The siliques of these plants were allowed to mature for 13 days under normal growing conditions and then dissected to determine the percentage of successfully fertilized ovules present for each treatment. Through statistical analysis of this data, it was determined that there was no significant difference in the success of fertilization following pollination in microgravity and pollination under normal gravity conditions. This indicates that for Brassica rapa, problems with the transfer of pollen would not be the cause of failure of developing ovules in a microgravity environment. Supported by grants from NASA (NAG10-0139, NAG2-1020), the Louisiana Space Consortium, and the LSU College of Agriculture. This undergraduate research on the KC-135 was possible because of an award from the Texas Space Grant Consortium.

[79] MICROGRAVITY EFFECTS ON COLLECTION AND TRANSFER OF POLLEN IN BRASSICA RAPA. M. Tabor1 and M. E. Musgrave1. 1Dept. of Agronomy and 2Dept. of Plant Pathology & Crop Physiology, Louisiana State University, Baton Rouge, LA.

Pollination is vital to seed development in self-incompatible systems such as Brassica rapa. Because Brassica rapa pollen grains are small, dry particles, problems involving static charges may arise during pollination in microgravity. Pollen grains from flowering Brassica rapa were collected and transferred to 1-mm square moist filter paper surfaces during the microgravity segments of the KC-135 flight parabolas and during the ground control. Pollen was subsequently eluted from the filter paper surfaces post-flight using 70% ethanol, concentrated by centrifugation, and counted under a dissecting microscope. Each treatment was replicated at least six times. Data analysis by t-test revealed that there was no significant difference in the quantity of pollen collected and transferred in the ground control and in microgravity. This observation indicated that manual pollen transfer can occur normally in this plant in a microgravity environment. The results suggest that pollen collection and transfer should not be a problem in the upcoming Mir and CUE experiments with Brassica. Supported by grants from NASA (NAG10-0139, NAG2-1020), the Louisiana Space Consortium, and the LSU College of Agriculture. This undergraduate research on the KC-135 was possible because of an award from the Texas Space Grant Consortium.

[80] ANTIBIOTIC RESISTANCE IN STATIONARY PHASE BACTERIAL CULTURES EXPOSED TO LONG TERM MICROGRAVITY. E.A. Juergensmeyer1 and M.A. Juergensmeyer2. 1Judson College, Elgin, IL 60123 and 2Kansas State University, Manhattan, KS.

Exposure to microgravity has been shown to increase the ability of bacteria to resist antibiotics. Resistance is spontaneously acquired and subsequently lost upon return to one gravity. In order to examine the ability of bacteria to acquire and maintain resistance to antibiotics in microgravity, stationary-phase cultures of Escherichia coli and Bacillus subtilis were flown for four months on the Space Station MIR. Upon return to Earth, these bacteria were challenged with a wide array of antibiotics of different modes of action. We have demonstrated that stationary phase cultures of E. coli and B. subtilis incubated in microgravity for four months do not acquire an increased ability to resist antibiotics that is evident when they return to one gravity. (Supported in part by NAGW-1197.)
[81] CIRCADIAN GROWTH RATE VARIABILITY OF LAMELLAR BONE IN RATS EXPOSED TO MICROGRAVITY. T.G. Bromage\(^1\), I. Smolyar\(^2\), S.B. Dony\(^3\), I.M. McMahon\(^4\), and F. Holton\(^5\).  
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Bone lamellae represent successive forming fronts during development in long bone width. We report on methods established to characterize the morphological consequences of gravitational factors on developing lamellar bone. Midshaft humerus thin sections from SLS-1 ca. 300 gm male Harlan (SpragueDawley) rats flown on the Space Shuttle, and their controls, were examined. Each group had a proscribed vital labeling regime of calcine and demeclocycline prior to and around launch, and at recovery. A thin section protocol was developed using dental bonding materials, resulting in polished (to 0.05 micron) 60-80 micron thick sections suited for both light (LM) and backscattered electron (BSE) microscopy. LM images of endosteal lamellae were obtained and processed. A mathematical model of lamellar bone was developed for the relatively unbiased quantification of lamellar growth rate, taking into account both the potentially anisotropic organization of bone and accuracy of the results.  
Polarized light and fluorescence imaging revealed a circadian rhythm to the lamella formation rate, hence lamellar bone growth rate could be analyzed with 24 hour resolution (this is the first reported calculation of a lamellar formation rate in the mineralized tissue sciences). Quantitative analyses indicate decreased widths between lamellae and, hence, decreased growth rates, at times of administrations of the vital labels. The resumption of pre-flight growth rates from a vital labeling just prior to launch, is short-lived in flight rats and lasting only several days compared to controls. Subsequently, the growth rate adopts a slow downward trend under conditions of microgravity. The circun-recovery period is characterized by a drop in bone formation following vital label administration and then a rise in the bone formation rate until euthanized. BSE imaging revealed no density dependent differences owing to microgravity. Research funded by NASA (IRP-95-101) and NSF (SBIR-9512373).

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While the effects of spaceflight on bone have been studied at the tissue-level, little is understood of the events occurring at the cellular level. Here we have used an established, conditionally immortalized osteostastic cell line, hFOB 1.19 (Harris et al., JMBR 10:178, 1995). Cells were cultured on cytodex, loaded on a cell culture module system (CCM), and flown on STS-80, a 17-day flight. Flight and ground-control (GC) cells demonstrated no difference in glucose utilization, procollagen (carboxylterminal propeptide) or prostaglandin E2 synthesis. Orbital spaceflight had no effect on steady-state mRNAs for osteogenin, alkaline phosphatase, IL-12, or TGF\(_B\). However, TGF\(_B\)-1a, IL-1a, IL-1b, and IL-6 mRNAs were transiently diminished. This demonstrates that orbital spaceflight does not significantly impact human osteostatic cell proliferation or differentiation. However, spaceflight may result in altered expression of skeletal signaling peptides which influence bone remodeling. (Supported by NASA NAG 2-896)

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Most laboratory animal studies to investigate the skeletal response to spaceflight have been performed in male rats. Recent studies suggest that sex hormones influence the skeletal response to spaceflight (Endocrinology 138:1567-1576, 1997). In this study, we determined the effects of a 14 day spaceflight (PSE-4) on cancellous bone in tibia (proximal metaphysis), femur (distal metaphysis and epiphysis) and vertebrae (L1, L2) of OVX'd rats. Spaceflight resulted in bone loss over and above that caused by OVX in all sites except the vertebrae, which was unchanged. The bone was associated with decreases in trabecular number; trabecular thickness was unchanged. In contrast to normal male rats, spaceflight had no effect on indices of bone formation and increased bone resorption. This study was supported by NASA grant NAGW-4963.

[84] RECOVERY FROM UTERINE SMOOTH MUSCLE HYERTROPHY FOLLOWING SPACE FLIGHT. M.C. Poole\(^1\), B. Jeaanson\(^1\), J. Zary\(^1\), H.W. Burden\(^1\), and J. R. Albers\(^2\). \(^1\)Dept of Anatomy and Cell Biology, East Carolina University, Greenville, NC and \(^2\)Dept of Psychology, Indiana University, Bloomington, IN.  
As part of a broader study examining effects of space flight on the female reproductive system, we quantified morphological changes of uterine smooth muscle. Rats were launched (STS 70) on gestation day 11 and recovered on day 20. Within 3 h of recovery uteri were removed from some animals, processed for microscopy, and quantified. Other animals were allowed to recover to term on gestation day 23 at which time their uteri were also quantified. Epoxy sections were cut and stained for light microscopy, and only sections possessing all 3 uterine layers, and lacking sectioning artifacts were chosen for analysis. Multiple overlapping photographs were taken at 160x from each section, printed at 720x, and assembled into large montages. Each montage was quantified using a 1 cm\(^2\) sampling grid, and the areas of morphological structures were expressed as cm\(^2\)/100 cm\(^2\) of tissue sampled. Our earlier analysis suggested that the uterine smooth muscle of the recovery animals hypertrophied during space flight although the changes were not statistically significant. However, the current analysis found that the smooth muscle volume density (i.e. area muscle/area myometrium) significantly (p < .02) returned to normal by day 23. Although not statistically different (p < .07), myometrial numerical density (i.e. # nuclei/muscle area) also increased as the hypertrophy declined. Thus, the smooth muscle of the myometrium had returned to normal size by term. However, the observation of an increased number of contractions required to expel fetuses at term in flight animals is consistent with the hypothesis that cell to cell communication between smooth muscle cells was altered by space flight and had not returned to normal by term. (Supported by NASA NCC 2-870.)

Echocardiographic measurements of astronaut cardiac function have documented an initial increase, followed by a progressive reduction in both left ventricular end-diastolic volume index and stroke volume. The investigators hypothesize that the observed reduction in cardiac filling may, in part, be due to the absence of a gravitationally dependent, intraventricular hydrostatic pressure difference that exists in the ventricle due to its size and anatomic orientation. This pressure gradient, which can be estimated to be 6660 dynes/cm² (= 5 mm Hg), promotes cardiac diastolic filling, but is absent in weightlessness.

This presentation reviews the use of an automated cardiovascular simulator to be flown on STS-85 as a Get Away Special payload in August 1997 to test this hypothesis. The simulator consists of a pneumatically actuated, artificial ventricle connected to a closed-loop fluid circuit with adjustable compliance and resistance elements which create physiologic pressure and flow conditions; a 40% glycerin in water solution simulates the viscosity of blood. The ventricle is powered by a miniaturized controller. Ventricular instrumentation includes high-fidelity, acceleration-insensitive, catheter-tip pressure transducers in the apex and base to determine the instantaneous ventricular pressures and ΔP₁ across the left ventricle (LVp₃-p₃). The ventricle is also instrumented with pressure transducers immediately upstream of the inflow valve and downstream of the outflow valve, and an ultrasonic transit-time flow probe downstream of the outflow valve. The experiment is microprocessor controlled with analog signals stored on a seven channel FM data tape recorder. On-orbit performance of the experimental protocol is initiated once the operational temperature range has been established. By varying the circulating fluid volume, ventricular function can be determined for varying preload pressures at a regulated, mean afterload pressure of 95 mm Hg. This variation in preload condition will permit the construction of a ventricular function curve for the microgravity environment for comparison to the ventricular function curve for the 1-G environment. If the proposed hypothesis is true, there will be a parallel shift to the right of the ventricular function curve of ~2 mm Hg for the microgravity condition.


Wheat, (Triticum aestivum L.), cv. Super-Dwarf, seeds were planted in nutrient-charged Balkan medium in the Svet greenhouse onboard the Russian Space Station, MIR. Seedlings were harvested at various stages of meiosis and stored in plastic bags containing 4% formaldehyde:1% glutaraldehyde or “Sorb-It silica.” Upon return to earth, pistils and stamens were excised and dehydrated with increasing concentrations of ETOH, transferred into acetone and embedded in Spurr's resin. Examination of semi-thin (1-2 μm) and thin (50-70 nm) sections of anthers and ovaries via light (LM) and transmission electron microscopy (TEM) showed that reproductive structures of MIR-grown wheat progressed similarly to greenhouse-grown reference plants. Development, however, ceased at the onset of anthesis.

(Supported: NASA Grant NCC 2-831 and the Utah Agric. Exp. Station).
SESSION F: CONCURRENT POSTER SESSION III
PLANT DEVELOPMENT, GROWTH AND GENETICS I
[87]
ROLE OF EARLY CELL DIVISIONS IN THE ESTABLISHMENT OF SOMATIC EMBRYO POLARITY FROM ORCHARDGRASS MESOPHYLL CELLS. A. Vasilenko, J.K. McDaniel and B.Y. Conger, Department of Plant and Soil Science, University of Tennessee, Knoxville.

Observations suggest that development of somatic embryos from orchardgrass leaf cells occurs from organized planes of division which in turn determines the way in which the new cross walls are aligned across dividing cells. The objective of the present study was to delineate the cellular organization of two to eight cell stage proembryos. Using the fluorescent dye, Hoechst 33258, we were able to investigate both the mitotic index and the cell division planes in early proembryos. These observations confirm the hypothesis of a single cell origin of somatic embryos directly from orchardgrass mesophyll cells. These analyses support our earlier concept that the plane of division leading to embryo formation is predominantly periclinal and that the maintenance of early embryo polarity is established during the first two or three cell divisions. Transient GUS expression in leaf cells bombarded with DNA (containing the uid A gene) coated tungsten particles was also used to follow the plane and direction of cell divisions. Microscopic examination of mesophyll cells 72 and 120 h after bombardment further support the single cell origin of somatic embryos.
(Supported in part by NASA Grant No. NAG10-0138.)

[88]
STRINGENT INDUCIBLE TRANSGENE EXPRESSION USING THE TOPO1-TETRACYCLINE PROMOTER IN PLANT CELLS. J. Love, G.C. Allen and W.F. Thompson, NSCORT, North Carolina State University, Dept. of Botany, Raleigh.

The use of tightly controlled transgenic promoters is essential to induce transgene expression at specific developmental stages, or if the transgenic product is deleterious to regeneration of the transformed organism. Recent developments in this area have produced a series of tetracycline regulated promoters which function well in transgenic plants (Gatz et al. Plant Journal (1992) 2: 397-404). Advantages of this system include strict control of transgene transcription and dependence on exogenous application of a compound which is absent in plant cells. One variant, the TOP10 promoter system, offers particularly stringent transgene control with very low levels of tetracycline. The drawback of this system, however, is that tetracycline represses transcription of TOP10 controlled transgenes, making it difficult to induce gene expression at a definite time.

A novel tetracycline analogue, compound GR33076X (Christ-Bal & Hoofit van Huisdouwen, Nucleic Acids Res. (1996) 24: 3900-04), has been shown to switch on TOP10-controlled transgene expression in bacteria and in animal culture cells. We are currently investigating the effects of GR33076X on the expression of TOP10-GUS in N1 plant cell culture cells. The use of this compound may enable us to modify the TOP10 promoter system into a stringent, inducible system ideal for transgene expression in plants.
(Supported by the NSCORT in gravitational biology at North Carolina State University)

[89]
REGULATION OF SWEETPOTATO STORAGE ROOT GROWTH IN HYDROPONICS. A.H. B.M. Witte1, J.H. Hil1, D.G. Mortley1, and D.Z. Douglas2. 1Dept. of Biological Sciences, California State University, Long Beach, CA. 2GWC Agricultural Experiment Station, Tuskegee University, AL.

A physiological model describing sweetpotato (Ipomoea batatas) growth was developed from 10 days of hydroponics research at Tuskegee University. At any time during development, sweetpotato tissue sink strength, the ability to grow, is determined by the difference between their current N concentration and critical N concentration (CNC). The lower CNC of storage tissues allows for more growth under limited N supply, than the high CNC of the photosynthetic tissues and the nutrient uptake tissues, because more assimilates can be added per g N. Leaves and fibrous roots of new sweetpotato cuttings have high N concentrations that decrease exponentially during growth. When the N concentrations in these tissues have decreased to their CNC, storage root initiation occurs, and leaf and fibrous root growth ceases. Total biomass growth remains constant because of storage tissue growth. CNC of the different plant tissues, and the balance of plant nitrogen uptake and photosynthesis determine storage root initiation and growth. Other environmental influences on storage root initiation and growth, e.g., temperature, are likely to work through their influence on nitrogen uptake and photosynthesis, and sweetpotato storage root initiation and growth can be manipulated through these two factors. This modeling project was supported by the H.B.C.U. Research Center Program of the NASA.
SESSION F: CONCURRENT POSTER SESSION III
PLANT PHYSIOLOGY I
[90] ACTOMYOSIN AS A MECHANISM FOR MOTION IN PLASTIDS. M.A. Juergensmeyer and J.A. Guikema, Kansas State University, Manhattan, KS

Movement of organelles within the plant cell is thought to be caused by a molecular motor protein. This protein would travel along the cytoskeletal network, positioning the plastid within the cell. Plastids tend to travel along actin filaments, and have been shown to co-localize with the motor protein myosin. However, the cellular architecture of the organelar motor has not been completely described to this point. Two possible configurations stand out: myosin in contact with the plastid envelope, moving the plastid along an actin filament by means of the myosin tail region embedded in the membrane; and actin in contact with the plastid envelope, either directly or via a bridging link, allowing a myosin aggregation to move the embedded actin along a cytoplasmic actin filament. Experiments will be described which attempt to remove either of the cytoskeletal elements from the plastid envelope using detergents or proteases; these experiments all destroyed the integrity of the plastid envelope before removing either cytoskeletal protein. We have therefore used the ability of ATP to cause myosin motion, and the tendency of low pH buffers to induce depolymerization of actin filaments to suggest that actin is the most likely protein present on the surface of the plastid. Supported by NASA grants NAGW-2328 and NAG10-0142.

[91] GRAVISTIMULATION INDUCES MEMBRANE-ASSOCIATION OF SUCROSE SYNTHASE. H. Winter1, J.L. Huber2, S.C. Huber3, 1Dept. Botany, 2Horticultural Science, North Carolina State University, and 3USDA-ARS, Raleigh, NC

Sucrose Synthase (SuSy) catalyzes a readily reversible cleavage of Sucrose with UDP into UDP-Glucose and Fructose, providing substrates for glycolysis and cell wall synthesis. SuSy is phosphorylated in vivo on Serine-15. Phosphorylation increases the affinity of SuSy for its substrates Sucrose and UDP without affecting Vmax. Gravistimulation of maize induces an increase in the amount and activity of SuSy associated with the plasmamembrane. Membrane bound SuSy can be released into the soluble fraction by phosphorylation in vitro. Incubation of clarified crude extract with alkaline Phosphatase increased the amount of membrane associated SuSy protein. This suggests a role for reversible phosphorylation in the subcellular localisation of SuSy. A membrane-associated SuSy could provide UDP-Glucose as a substrate for the cell wall synthesis in rapidly growing tissue like the elongating cells of graviresponding pulvin.

(Supported by the NSCORT in Gravitational Biology at North Carolina State University, NASA grant NAGW 4984)


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In floral inflorescence stems of Arabidopsis, the gravitropic response is rapid with a response within 30 min of stimulation resulting in a 90° bend within 2 h. Fine adjustment continues over the next several hours. The position of bending relative to the inflorescence tip is constant at different developmental stages, although the kinetics of the response varies with developmental stage of the inflorescence stem. Reorientation of a plant bent at a 90° angle leads to a new bend in this region but the site of response is closer to the apex. The goal of the research was to determine why the inflorescence stem shows a consistent gravitropic bending only in a defined region of the stem. The stems can be excised and still maintain their ability to respond to changes in gravity even if the apical meristem and lateral shoots are removed. The position of bending does not appear to be due to differences in amyloplast sedimentation since starch grains are localized to the inner cortex throughout the length of the stem and appear to reorient in all regions similarly. Likewise, auxin sensitivity or transport do not appear to control the position of bending since auxin transport capacity is constant down the inflorescence and bending in response to exogenous auxin can occur below the site of normal bending. It appears that the morphology of the stem is different in the region of bending. Fibers of cortical lignum develop only at sites below the point of bending. Cortical fibers are apparent in nonresponsive tissue but are not present in the responsive region. These results indicate that the responsive region of the Arabidopsis stem is most likely determined by a specific cellular and tissue structures and not by a difference in perception or transduction of a gravity stimulus. (Supported by the NSCORT in Gravitational Biology at North Carolina State University)
SESSION F: CONCURRENT POSTER SESSION III
PLANT GRAVITY PERCEPTION I
[93] AUTOTROPISM, AUTOMORPHOGENESIS, AND GRAVITY. F.D. Sack, B. Stankovic, and D. Volkmann. Department of Plant Biology, Ohio State University, Columbus, OH; Botanisches Institut, Universität Bonn, Germany.

Segments of organs that have undergone gravitropic curvature later straighten during the course of gravitropism or after the g-vector becomes randomized on a clinostat. Little is known about the mechanisms underlying these and perhaps related phenomena which have been described with various overlapping terms such as autotropism, autotropic straightening, automorphogenesis, automorphosis, automorphic curvature, and gravitropic straightening.

The types of phenomena that historically have been named by the above terms are reviewed critically with respect to an interaction with gravitropism. We suggest that the term “autotropism” should not be applied to the phenomenon of organ straightening that occurs during the course of gravitropism, since this straightening is part of a complex series of local growth adjustments overall through time, and since this phenomenon is not itself a tropistic response to a directional exogenous stimulus. It is suggested that the term autotropism should be used only for the phenomenon of organ straightening that occurs after the g-vector is randomized on a clinostat or withdrawn in the microgravity conditions of spaceflight. Usage of the term automorphogenesis is most appropriate for describing curvatures or orientations that result from morphological relationships such as in nastic curvatures. (Supported by NASA: NAG2-1023 to F.S., and by DARA: 50 9429 and MWF to D.V.)


The presence and features of gravitropism in moss protonema are known for three species, Ceratodon purpureus, Physcomitrella patens and Funaria hygrometrica. Secondary protonemata of Potta intermedia are also gravitropic when cultured in darkness. Here we describe Potta gravitropism with respect to kinetics, plastid orientation and sedimentation, and effects of inversion, and compare these features with those of the other species. Spore germination produces primary protonemata which give rise to the leafy moss plant, the gametophore. Culture of gametophores result in production of secondary caulonemata that usually arise at the leaf base but also originate from the leaf vein or the stem. Secondary protonemata that form in light are agavtritropic. However, caulonemata that form when gametophores are placed in darkness are strongly negatively gravitropic.

When these caulonemata are reoriented 90°, initial upward bending can be detected after 1 h. Protonemata reach the vertical within 24–48 h. Most inverted protonemata reach the horizontal 1 d after inversion and become upright after 48 h. Clear plastid sedimentation occurs 10–15 min after reorienting upright protonemata to the horizontal i.e. about 45–75 min before the start of upward curvature. This sedimentation takes place in a subsapical zone, a zone located in the same approximate position in all four moss species known to react gravitropically. Whereas Ceratodon protonemata possess a group of (omovedimenting) plastids in the apical dome, this zone is absent in the other species. As in Ceratodon, amyloplast sedimentation also takes place along the length of Potta protonemata; when these cells are inverted, plastids accumulate at the apical (lower) end of the sedimentation zone. However, these plastids do not fall into the apical dome indicating that factors in addition to plastid mass limit sedimentation and maintain zonation. These results support the hypothesis that amyloplast sedimentation functions in gravitropic sensing since sedimentation occurs before gravitropism in Potta and since the location and presence of a unique sedimentation zone is conserved in all four mosses known to be gravitropic. The effects of microgravity on the development and tropisms of Potta will be evaluated during the Cooperative Ukraine Experiment on STS-87 using hardware that will allow protonemata to be chemically fixed in situ.

[95] GRAVIPERCEPTION IS ABOLISHED IN SNAPDRAGON AND OAT SHOTS BY LaCl₃, A CALCIUM CHANNEL BLOCKER. S.C. Chang, H. Friedmann, F. Jansen, H. Sajadi, S. Philosoph-Hadas, S. Meir, and P.B. Kaufman. Molecular, Cellular, and Developmental Biology Group. Dept. of Biology, Univ. of Michigan, Ann Arbor, Michigan 48109-1048, USA; and Dept. of Postharvest Science of Fresh Produce, Institute for Technology and Storage of Agricultural Products, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet-Dagan 50250, Israel.

In an attempt to unravel how graviperception occurs in graviresponding monocot and dicot shoots, we discovered that the calcium channel blocker, LaCl₃, not only suppresses negative gravitropic curvature (upward bending) of gravistimulated snapdragon (a dicot) and oat (a monocot) shoots, but also, irreversibly blocks graviperception by causing loss of starch in the gravisensors (starch-containing chloroplasts). This clearly implicates a central role for cytosolic calcium in the gravity perception process in graviresponding monocot and dicot shoots. It is postulated that Ca²⁺ may be acting to enhance starch biosynthesis and/or repress starch degradation in chloroplast gravisensors in graviresponding shoots of these plants.

[96] GRAVITY-INDUCED CHANGES IN PHOSPHORYLATION OF INOSITOL CONTAINING LIPIDS FROM PLASMA MEMBRANES OF MAIZE PULVINI. I.Y. Perera, I.H. Heilmann and W.F. Boss. Dept. of Botany, North Carolina State University, Box 7612 Raleigh NC, 27695-7612

The phosphorylated inositol phospholipids, phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP₂) are important mediators of signal transduction and direct effectors of membrane and cytoskeletal proteins in mammalian cells. In higher plants and certain algae the PI pathway has been implicated in the early responses to external stimuli such as light, osmotic stress and fungal elicitors. However, to date, the possible involvement of the PI pathway in the gravity response of plants has not been clearly established. The pulvinus of the maize stem is an excellent model system because it is composed of non-growing tissue which, when stimulated by gravity, responds with different cell elongation. To determine whether the metabolism of inositol lipids changes during graviperception and/or response we examined the in vivo phosphorylation rates of PIP and PIP₂ in plasma membranes of pulvinum from vertical and gravistimulated maize. Within 10 min of gravistimulation we could detect an increase of 20-30% in the formation of PIP₂ in the lower half of gravistimulated pulvinum compared with the upper half. After several hours, however, the upper half showed a twofold increase in the levels of PIP and PIP₂ compared to the lower. This increase in PIP and PIP₂ persisted on to 2 days when gravitropic bending was clearly visible. These data suggest that the phosphoinositide pathway is involved in the early perception of the gravistimulus as well as the gravitropic response. This work is supported by the NSCORT in Gravitational Biology at North Carolina State University (# NAGW-4984) and a DAAD fellowship HSP III (to IH) financed by the German Federal Ministry of Education, Science, Research and Technology.

Cytoskeletal components such as actin (Heath, 1990,1995), tubulin, spectrin, and integrin (Kaminsky and Heath, 1995) are thought to be likely candidates for participation in gravity perception, transduction, and response in plants and fungi. A technique for the subapical micro-injection of vertical Phycomyces sporangiophores (SP) was developed using a Narishige microinjector system and utilized to introduce localized concentrations of cytoskeletal inhibitors to investigate cytoskeletal roles in gravitropism of the SP. Micropipets having ~3μm diameter tips were backfilled with PBS buffered solutions (pH 7.3) containing a 1.25M Rhodamine B tag and adjusted to the osmolarity of the fungal cytoplasm (495mosM). Vertical gravitropic sensitive stage IV SPs were injected 2-3mm below the sporangium under symmetrical white lighting. Injected and non-injected SPs were placed horizontally, injection site down, in a humid plexiglas chamber under nonphototropic 750nm light. Digital time-lapse video recording of the resultant gravicurvature was made with a high resolution black/white camera and Adobe Capture software. Video analysis of gravicurvature was executed with Ficovis 1.2 developed by the KC Physics Dept. Control studies of the effects of microinjection alone on gravicurvature of the SP indicate that injection, on average, increases the lag period to curvature onset by 30-60min while having no effect on resultant curvature and kinetics compared to uninjected controls.

Inhibitors of f-actin polymerization and depolymerization, cytochalasin B (500μM CB) and rhodamine-phalloidin (26μM RP) respectively, were examined in vitro. SPs microinjected with CB showed a 4hr delay to curvature induction with resultant curvature being unaffected while kinetics were increased after onset. Conversely, microinjected RP hastened curvature onset and dramatically increased rate of curvature and often resulted in higher degrees of resultant curvature.


Primary roots of rgr1 have reduced gravitropism, resistance to growth inhibition by auxin, fewer lateral roots, and a tendency to coil clockwise as viewed from the front (Simmons et al. Physiol Plant 93: 790). We used a video digitizer system to further characterize the mutant. Roots of rgr1 grow slightly slower than roots of wild type (Ws) on the surface of agar plates but grow 34% faster than Ws when plates are immersed in oxygenated liquid medium. In short term growth experiments (immersion) the mutant is 5-fold less sensitive to auxin than Ws (comparable to long term experiments with auxin incorporated into the agar). However, when the roots are immersed in auxin solution, the concentration of auxin required for 50% growth inhibition is 6-7 fold lower for both rgr1 and Ws than for long term experiments with the auxin incorporated into the agar. When gravistimulated by counterclockwise rotation to the horizontal, the response of roots of rgr1 is weaker than that of Ws and in both cases downward bending results primarily from growth inhibition along the lower side. When stimulated by rotating clockwise, roots of rgr1 initially bend upward, most likely a continuation of their clockwise coiling tendency. This upward bending is associated with reduced elongation on the upper side. We used the upward bending of roots rotated clockwise to examine the nature of the upward curvature/coiling response. Upward bending did not occur when the root was buried in a uniform agar medium or growing in humid air. Upward curvature was unaffected by the angle of incident light but was suppressed in the dark. These findings indicate that the upward bending/coiling response of roots of rgr1 is dependent upon the tactile environment of the root and on the presence of light. (Supported by NSF: IBS-9416015, NASA NAGW-4522, and by the NASA/NSF Network for Research on Plant Sensory Systems).
SESSION G: GROUND-BASED ANIMAL STUDIES
[100] A NOVEL LABORATORY APPROACH TO AQUATIC BIOGENERATIVE CLOSED-LOOP FOOD PRODUCTION SYSTEMS. V. Blum, M. Andriske, and D. Voeste. Ruhr-University Bochum, Faculty of Biology. C.E.B.A.S. Center of Excellence Bochum, Germany.

Based on the construction principle of the Closed Equilibrated Biological Aquatic System (C.E.B.A.S.), a novel combined animal-plant production system for mid-term operation in closed state up to two years was developed. It consists of the "classic" C.E.B.A.S. subcomponents: animal tank, plant cultivators, ammonia converting bacteria filter and data acquisition/control unit. The new approach is the utilization of three aquatic plant cultivators for different species. The animal tank has a volume of about 160 liters and is an "endless-system" surrounding a heat exchanger and the bacteria filter with volumes unit of about 1.5 liters each. A suspension plant cultivator (1 liter) for the edible duckweed Wolffia arrhiza is externally connected. The second plant cultivator is a meandric microalgal bioreactor for filamentous green algae. The third plant growth facility is a chamber with about 2.5 liters volume for cultivation of the "traditional" C.E.B.A.S. plant species, the rootless buoyant Ceratophyllum demersum. Both latter units are illuminated with 9 W fluorescent lamps. The animal tank contains the live-bearing teleost fish Xiphophorus helleri and the small pulmonate water snail Biomphalaria glabrata. The water temperature is maintained at 25°C and the oxygen level is regulated between 4 and 7 mg/l by switching on and off the plant cultivator illuminations according to a suitable pattern thus utilizing solely the oxygen produced by photosynthesis. The animals and the microorganisms of filter and biofilm provide the plants with a sufficient amount of carbon dioxide. Oxygen concentration, pH value, temperature and redox potential are on-line recorded. Ion concentrations and numbers of gersms in the system water are determined in the laboratory from samples taken by a special volume-compensating "sample removal module". A rotary pump produces a water flow of about 38 l/min. The paper provides detailed information on the system construction principle and the biological, physical and chemical data of the initial phase of the test run. ( Funded by DARA-grant WSSWB9319-3.)


To determine whether gravity influences the plane of bilateral symmetry in medaka embryos, we held zygotes in Nitex glued to the bottom of a plastic tissue culture dish containing 2% methyl cellulose in a balanced salt solution. The zygotes were placed with their animal-vegetal polar axis oriented vertically (vegetal pole upmost). At regular intervals during the first cell cycle, the zygotes were tilted 90° for 10 min and then returned to their original orientation. Control zygotes were not tilted. In 92-100% of the embryos tilted before a normalized time of 0.5 (where 1.0 represents the beginning of the first cell division), the embryonic shield (a marker of the dorsal side of the embryo) formed on the margin that had been lowermost when the zygote was tilted. In zygotes that were tilted later and in untitled controls, the position of the embryonic shield showed no preference with respect to gravity. To determine whether the effect of gravity was reversible, we tilted zygotes 90° in one direction for 10 min, returned them to their original position (vegetal pole upmost), tilted them 90° in the opposite direction, and returned them to their original position. In 80-97% of these embryos, the embryonic shield formed on the margin that was lowermost when the zygote was tilted the first time. To determine whether centrifugation could affect the position of the dorsoventral axis, we embedded zygotes in agarose and centrifuged them at 5g for 5 min. In 98% of the embryos, the embryonic shield formed on the margin that had been on the outwardly radial (centrifugal) side during centrifugation. We conclude that gravity can influence the plane of bilateral symmetry in medaka embryos. (Supported by NSF MCB-9316125.)


Many molluscan species sense gravity through paired statocysts, spherical organs with sensory receptor cells, separated by supporting cells, forming the wall of the cyst and dense bodies in the lumen, which sink to activate receptors cells at the bottom of the cyst. After hatching, larvae of the marine mollusc, Aplysia californica, are free-swimming, using cilia on the velum for propulsion. In the larvae, there is a single statolith which is supported by the cilia of all 13 receptors cells, distributed over the whole statocyst. This form is maintained through metamorphosis, which can be 60 to 100 days after oviposition. After metamorphosis, the animals become benthic, crawling rather than swimming and multiple statocyst are then produced in the supporting cells. This process continues throughout life with large adults having up to 1,000 statocyst. In contrast, the pond snail, Biomphalaria glabrata, does not go through metamorphosis and moves only by crawling as soon as they hatch from the spawn pack. In Biomphalaria, a single statolith is never seen: as early as 3 days after oviposition, multiple statocyst are seen and no specimen has been found with fewer than 3 statocyst. Additional statocyst are added until the snails reach a shell diameter of approximately 4 mm and the number of statocyst increases only slightly as they grow beyond this size. The average size of statocyst does continue to increase, indicating that further calcification continues in the cyst lumen. Thus, it appears that the single statolith, supported by the cilia of all 13 receptor cells, is available to monitor relatively rapid movement in 3 dimensions, whereas a group of statocyst settling to the bottom of the cyst is employed to sense direction in the more sedentary crawling animals. In Aplysia embryos, and post-metamorphic animals and isolated statocyst maintained at 2 to 5 g on a centrifuge, the size of the statolith and the size and number of statocyst is smaller than in 1 g controls. Adult Biomphalaria will be flown in the German CEBUS unit on the NeuroIBILITY Shuttle mission to study the production of statocyst in μ-g in snails conceived and developed in μ-g. (Supported by NASA: NAG 2-952 and NSF: IBN 9529136)

[103] EFFECTS OF CLINOROTATION ON SPIDER SURVIVAL, PREY CAPTURE, AND WEB STRUCTURE. A.M. Helmenstine, C.R. Grupka, E.A. Hudson. Dept. of Biology, Tusculum College, Greenville, TN.

The effects of clinorotation (~ 1 rotation/sec) were examined on the survival, prey capture, and web structure of Achaearanea tepidariorum (Araneae, Theridiidae) spiderlings and adult female spiders and Liriodectes mactans (Araneae, Theridiidae) and Araneus cormatus (Araneae, Araneidae) spiderlings. In one study, webs and behavior of clinorotated or vibrated spiders were compared to those produced by captive and wild siblings. Spiders were exposed to a 24-hr treatment, data recorded (webs destroyed, new Drosophila melanogaster provided), and the same treatment was resumed. In a subsequent study, each spider was compared to itself in permutations of 24-hr clinorotation, vibration, rotation, or captive control. In each case, spider survival and molting frequency/success were statistically unaffected by any treatment. Prey capture success and the sequence of prey acquisition were also unaffected, although web morphology was altered for all spiders as compared to wild controls. Clinorotated, vibrated, and captive control spiders produced similar webs. Webs produced in response to rotation (centrifugation) were different from webs characterized by captivity alone. A. tepidariorum and L. mactans assumed the normal dorsal-down, suspended posture and A. cormatus normal inverted posture, except when clinorotated. (Supported by a grant from the Appalachian College Association, Mellon Foundation, and an anonymous donor.)

Artificial gravity may be essential for maintaining bone and muscle integrity during very long duration space missions. A rotating space vehicle can provide artificial gravity by generating a centrifugal force that is proportional to the velocity of rotation squared times the radius (in radians). Thus, a level of 1g could be generated with 10 rpm and a radius of about 10 m or 1 rpm and a radius of about 1000 m radius. Short radius devices with a high rotation rate have several potential disadvantages including gravity gradients dependent on different positioning with respect to the rotation axis and significant Coriolis forces on linearly moving objects.

Coriolis forces are inertial forces that are proportional to the velocity of vehicle rotation and the object's linear velocity and that act perpendicularly to an object's movement direction. When one attempts to move about in a vehicle rotating at 10 rpm, one's head, arm, and leg movements are deflected by the Coriolis forces generated. However, with repeated movements adaptation can occur, often within 15 to 20 movements. On cessation of vehicle rotation, aftereffects in movement control of opposite sign are initially exhibited.

Quantitative descriptions will be presented of the 1) effects of Coriolis force perturbations on eye and leg movements during 28.6 rpm (eye) and 10 rpm (leg) rotation, 2) the adaptive changes that occur with repeated movements, and 3) the aftereffects that occur following rotation. These results will be discussed in the context of the feasibility of high rates of rotation for artificial gravity vehicles.

Supported by NASA grants NAGW-4031, NAGW-4375, NAGW-4374, and NAGW-4733.

[105] LONG TERM GROWTH, TOOTH DEVELOPMENT, AND REPRODUCTION OF RODENTS FED PURIFIED PASTE DIET. V.E. Strength1, J.D. Gossett2, D.C. Holley3, and A.H. Battles3.

In a multiple generation breeding study diet, groups of rats were fed either lab chow, KSC-25 paste (24% water by weight), or KSC-25 paste with supplemental water ad lib. Each subject was mated to another in the same diet group. Parent generation fertility was equivalent between groups, but F1 pups in the paste groups weighed significantly less than controls. The F1 litters were weaned onto the same diet as their parents. One female and male from each F1 litter was mated to a non-littermate in the same diet group. Five days after the F2 pups were weaned, reproductive stress was induced by mating the F1 pairs to produce a second F2 litter. Indicators of fertility and litter health were significantly lower for paste groups compared to controls, and the effects were magnified under reproductive stress. The paste group given supplemental water consumed significantly more water and was significantly less fertile under reproductive stress than the other groups.

In another study, incisor development of a group of rats maintained for 375 days on KSC-25 paste was compared to controls receiving lab chow and water. The lab chow pellets provided substantially more dense stimulation than KSC-25 paste. Yet there were no significant group differences in incisor length and color or in gum condition. The cages were configured to preclude chewing. Subgroups provided chew bars gnawed them but their dental health indicators were not significantly different. Observed tooth grinding by subjects may be sufficient to prevent gum disease and excessive incisor growth.

NASA rodent food bars are made with T93062 purified diet. A third study compared the capability of T93062 and KSC-25 to support reproduction. Four breeding groups were fed dry T93062, dry KSC-25, KSC-25 paste, or lab chow. No group differences were observed for a wide range of fertility and F1 litter health measures.

[106] THE EFFECTS OF DIMINISHED ABDOMINAL GROOMING ON MAMMARY METABOLIC ACTIVITY AND MILK PROTEIN EXPRESSION IN THE PREGNANT RAT. K.S. Snyder1, K. Plaut1, and J.R. Alberts2. 1Dept. of Animal Science, University of Vermont, Burlington; 2Dept. of Psychology, Indiana University, Bloomington.

Because nipple stimulation plays a necessary role in lactation, it has been questioned if it also contributes to mammary development during gestation. We were concerned that pregnant rats may be unable to perform ventral grooming during spaceflight and that this behavioral change could affect mammary development. We measured the effects of diminished abdominal grooming on mammary development in gestating rats. Functional development was measured by mRNA expression of the milk protein β-casein and by the oxidation of glucose to CO2 and its incorporation into fatty acids by mammary tissue in vitro. Gross morphology and histology were used for the effects on structural development. Sprague-Dawley rats were fitted with Elizabethan collars from days 11-20 of gestation to block self-grooming of the abdominal area; uncollared rats served as controls (n=6/treatment). On gestation day 20, mammary tissue was excised. In the collared rats, there was no significant difference in glucose oxidation to CO2 (384.7±54.0 nmoles/100 mg wet mammary tissue/3 hr) compared to the control group (386.3±44.3 nmoles/100 mg wet mammary tissue/3 hr) or glucose incorporation into fatty acids (53.3±14.0 nmoles/100 mg wet mammary tissue/3 hr). There was no significant difference in β-casein mRNA expression between treatment groups as determined by densitometric analysis after Northern hybridization. Additionally, there was no apparent effects of the collars on either the gross morphology or histology of the mammary glands, therefore, abdominal grooming during gestation does not affect the metabolic activity or structural development of the mammary gland in the rat. (Supported by NASA grant NCC 2 870.)


Skeletal unloading inhibits bone formation, decreases osteoblast number, and impairs the mineralization of bone in vivo. To determine whether skeletal unloading influences osteoprogenitor cells in vitro, bone marrow stromal cells (BMSCs) were isolated from the tibiae of rats which had been hindlimb-elevated (HLE) for 0 (control), 2 or 5 days. Cells were cultured for up to 28 days. RNA was isolated from some cultures, reverse-transcribed into cDNA, and analyzed by quantitative competitive PCR for expression of the following genes: osteocalcin (associated with well-differentiated osteoblast during mineralization); alkaline phosphatase (AP, associated with intermediate osteoblast differentiation), and c-fos (associated with osteoblast proliferation). Cultures from the 5 d HLE group had decreased levels of c-fos and osteocalcin mRNA and increased levels of AP mRNA compared to controls. Alizarin red staining revealed significantly reduced in vitro mineralization of cultures in the 5-d HLE group compared to controls. AP enzyme activity was also reduced in cultures from the 5-d HLE group. The number of adherent BMSCs isolated from control and 5-d HLE tibiae was virtually identical at the start of culture (days 3-5). However, the proliferation of BMSCs from the HLE group was reduced from days 7-28 compared to controls. In conclusion, we have demonstrated that 5 days of skeletal unloading leads to inhibited proliferation and differentiation of rat osteoprogenitor cells, and that these phenotypic changes are retained and manifest in culture. (Supported by NASA grant NAGW-4460.)
BONE, MUSCLE, AND HORMONAL CHANGES INDUCED BY HINDLIMB ELEVATION IN THE MATURE RAT. W. Dehority, B. Halloran, D. Bible, J. Harris, T. Curren, P. Kostenuik, E. Morey-Holton. University of California and Veterans Affairs Medical Center, San Francisco, CA, and the National Aeronautics and Space Administration-Ames Research Center, Moffett Field, CA.

Skeletal unloading induced by hindlimb elevation in the young growing rat inhibits bone formation, decreases bone and muscle mass, and diminishes serum 1,25 (OH)2D. To determine the effects of skeletal unloading on mature rats we hindlimb unloaded 6 month old male SD rats for up to 5 weeks. Body wt., thymus wt. and blood Ca2+ did not change. Bone formation rate at the TFJ and tibial midshaft decreased by 80% (p<.001) and remained below control levels throughout the 5 week unloading period. Total femur Ca decreased by 13% (p<.05) and the slope of change in total bone Ca over the 5 week period was significantly less (p<.001) reduced in the unloaded animals. Ssexo and gastrocnemius wts. were reduced by nearly 50% after 5 weeks of unloading. Serum PTH was unaffected by unloading whereas serum 1,25 (OH)2D decreased transiently (-62% at 1 week), then increased reaching a new steady state between 3 and 5 weeks that remained significantly below normal. These data indicate that the young growing rat where bone formation and serum 1,25 (OH)2D are only transiently affected, unloading in the mature rat produces a more chronic impairment of bone formation and 1-hydroxylase activity.


The objective of the study was to determine the effect of nitric oxide (NO) inhibition on the concentrations of inflammatory cells, muscle fiber injury, and apoptosis of inflammatory cells in rats subjected to 10 d of hindlimb suspension and 48 h of reloading. NO synthase inhibitor L-NAME (50 mg/ml) was administered 1 d prior to reloading and during 48 h of reloading via the drinking water. Immunohistochemistry showed significantly fewer neutrophils, ED1+ invaded fibers, and ED1+ and ED2+ macrophages in soleus sections for L-NAME treated rats (N=10) relative to water treated controls (N=10). In addition, inflammatory cell populations and ED1+ invaded fibers for L-NAME were not significantly different from ambulatory (N=4) and hindlimb suspended controls (N=4). The number of apoptotic inflammatory cells determined by TUNEL labeling was also significantly lower for L-NAME relative to water and similar to controls. The similar decrease in the total number of inflammatory cells and apoptotic inflammatory cells for L-NAME relative to water treated controls indicates that the reduction in apoptosis results primarily from a decrease in inflammatory cells. In conclusion, NO or one of its intermediates contributes to inflammatory cell-mediated fiber injury and plays no more than a minor role in the resolution of muscle inflammation by inducing apoptosis of inflammatory cells.

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Our previous studies have demonstrated that chronic exposure to 2G via centrifugation in rats results in a loss of circadian rhythm amplitude for heart rate, body temperature, and activity which recovers after ten to fourteen days. In addition, the ability of light to alter circadian rhythms is diminished. These results suggest that 2G exposure affects the pacemaker function of the suprachiasmatic nucleus (SCN) and the retino-hypothalamic tract (RHT) which is the primary visual pathway to the SCN. An important issue in gravitational biology concerns scaling effects where body mass can influence the biological effects to altered gravitational fields. For example, it is possible that a smaller animal, such as the mouse, will not exhibit the same physiological responses to chronic 2G exposure. Therefore, this study examined the effect of chronic 2G exposure on circadian rhythms in body temperature and activity of the mouse.

Mice (Mus musculus, 18-23 g) were implanted with a biotelemetry transmitter to record body temperature and activity. After recovery from surgery, mice were individually housed and placed on the centrifuge (12:12 light/dark cycle; food and water ad libitum). During the baseline period, all mice exhibited prominent circadian rhythms of temperature and activity. Mice were then exposed to chronic 2G via centrifugation for 3 wk. Mice exhibited an immediate decrease in daily mean body temperature and activity which recovered to a new steady state within 3 d. There was also a loss in circadian amplitude in both temperature and activity lasting for 7 d. Circadian amplitude recovered completely by 10 d. The recovery of circadian rhythms and mean daily body temperature and activity of mice was faster than previously observed in rats. These results are consistent with the principle of scaling in that the effect of 2G on the smaller animal (18 g) was less than that of the larger animal (300 g). However, the effect of 2G on the physiology and circadian rhythmicity of the mouse demonstrates that it is a useful animal model for gravitational biology studies. (Supported by NASA Grant NAGW-4552.)

HAIRCELL MECHANORECEPTORS IN THE VESTIBULO-TYMpanic ORGAN OF THE ORIENTAL HORNET (THE ISHAY ORGAN). J.S. Ishay1, E. Rosenzweig1, D. Kalicharan2 and W.L. Jongebloed3. 1Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, ISRAEL; 2Laboratory for Cell Biology and Electronmicroscopy, University of Groningen, Oostersingel, Groningen, The Netherlands.

We report herein on the presence of kinocilia underneath the cuticle of the Oriental hornet Vespa orientalis (Vespinae, Hymenoptera). These are located in the upper anterior region of the head, that is, beneath the frons and vertex plates and in the cuticular areas between the antennae, the ommatidia and the ocelli. This region of the head is positioned over a cavity recognized to be an acoustic box. Each kinocilium proceeds inwardly to the juncture point between its hair-cell and the cuticle and is thus directed against the earth’s gravitational force because the building hornet commences building of its comb with its head directed downwards. Between each group of hair-cells there is a photoreceptor within a translucent pore in the cuticle. The correlation between these two structures, namely, the hair cells and the photoreceptors, and their possible roles in gravity orientation during comb building and in the identification of sounds within the nest are discussed. V. orientalis is the first insect of all Insecta in which the presence of sensory kinetic cilia has been detected.
SESSION H: GROUND-BASED PLANT STUDIES

Gravitropic tip-growth in rhizoids and protonemata of characean algae is based on the structure and function of the actin and microtubule cytoskeleton, which has been examined by confocal laser scanning microscopy. Although the morphologically very similar positively gravitropic (downward growing) rhizoids and negatively gravitropic (upward growing) protonemata show opposite gravitropic response, no major differences have been detected in the rich three-dimensional distribution of actin and microtubules in both cell types. The apical zone, the site of gravitropic and graviresponse, is devoid of MTs that form a dense axially oriented network in the subapical zone; however, a unique, complex organized actin MF system has been found in the apical dome. Mainly axially oriented actin MF bundles converge into a spot-like actin array associated with the ER aggregate in the center of the Spitzenkörper. Fine actin MFs radiate fountain-like from this actin spot towards the apical plasma membrane, merge into thicker bundles and return in basal direction. Unlike microtubules, which have a static function in organizing the cytoplasmatic zonation and the arrangement of the actin cytoskeleton, the prominent actin cytoskeleton is directly involved in the gravitropism of rhizoids and protonemata. Manipulating the satolith position with centrifugation and laser tweezers revealed major differences in the action of actin in both cell types and since the actin cytoskeleton in rhizoids is not reoriented during the gravitropic response, in protonemata, however, is apparently more directly involved in the gravitropic response of protonemata, the opposite gravitropism in both cell types seems to be based on different properties and activities of the actin MFs regulated by associated proteins in a calcium-dependent manner. (Supported by a DFG Fellowship to M. Braun and by the project AGRAVIS by DARPA, Bonn, and Ministerium für Wissenschaft und Forschung, Düsseldorf)

[113] MECHANICAL BEHAVIOR OF ROOTS OF MAIZE RESPONDING TO TIP LOAD. P.S. Kuzema and P.M. Limithac, Botany Dept., Univ. of Vermont, Burlington, VT

The primary root’s emergence from the caryopsis and penetration of the soil are two critical events in seedling establishment. To insinuate itself into the soil, the root must perform two mechanical actions; first, it must align itself with the gravity vector, and second, it must create a path for itself into the substrate. Mechanical activity involved in this latter action was the subject of this study.

Corn caryopses were imbedded for 24h in distilled water and germinated in a fog chamber. Seedlings with roots 16mm long were mounted vertically in a specially made Teflon fixture actuated by a Vitrodyn V-200 mechanical testing system (Chatillon Instruments, Durham, NC); the seedling and testing actuators were kept at 100% relative humidity. The testing apparatus was run in load controlled mode, wherein a constant restoring force was applied to the tip of the elongating root. Elongation was measured by a linear variable displacement transducer.

Root elongation rate was insensitive to tip load up to ca. 12g (118mN). Above this value, elongation slowed dramatically or ceased. One model for this behavior portrays the root epidermis as a cylindrical skin with a large radius under low tensile stress holding in place cells with small radii experiencing relatively high tensile stresses in their walls. This model must be reconciled with the possibility of compressive stresses acting to counter yield stresses of the cell walls, as well as the experimental artifact of ethylene buildup in the root’s environment.

(Support for this work was furnished in part by NASA: NAGW-3604.)


A chimeric calcium/calmodulin-dependent protein kinase (CCaMK) with a catalytic domain, calmodulin-binding domain, and a neural visin-like domain was cloned and characterized (Patil et al., (1995) Proc. Natl. Acad. Sci. 92, 4797-4801; Takezawa et al. (1996) J. Biol. Chem. 271, 8126-8132). The mechanisms of activation of CCaMK by calcium and calcium/calmodulin were investigated using various deletion mutants. The use of deletion mutants of CCaMK lacking either one, two, or all three calcium-binding EF hands indicated that all three calcium-binding sites in the visinin-like domain were crucial for maximum kinase activity. As each calcium-binding EF hand was deleted, there was a gradual reduction in kinase activity from 100% to 4%. Another mutant (amino acids 1-322) which lacks both the visinin-like domain containing three EF hands and the calmodulin-binding domain was constitutively active, indicating the presence of an autoinhibitory domain around the calmodulin-binding domain. By using various synthetic peptides and the constitutively active mutant, we have shown that CCaMK contains an autoinhibitory domain within the residues 322-340 that overlaps its calmodulin-binding domain. Kinetic studies with both ATP and the GS peptide substrate suggest that the autoinhibitory domain of CCaMK interacts only with the peptide substrate binding motif of the catalytic domain, but not with the ATP-binding motif. The structural features of CCaMK and mammalian CaMKI were studied by homology modeling. These studies revealed that the kinase and calmodulin-binding domain of these two kinases are similar. However, the visinin-like domain of CCaMK may function as a calcium sensor element in mediating kinase activity. (Supported by NASA and NSF)


The TCH2 gene of Arabidopsis encodes a calcium-binding protein which shows 44% amino acid identity with calmodulin. Features that distinguish TCH2 from calmodulin, including an extended, more flexible linker region, the potential to form an internal disulfide bond, and an abundance of basic amino acid residues, indicate that the function of TCH2 is likely distinct from that of calmodulin. Expression of TCH2 is rapidly and transiently upregulated following touch, darkness, temperature shifts and osmotic shock. TCH2-GUS gene fusions are expressed at branch points, abscission zones, root tips, trichomes and hydathodes. Furthermore, TCH2-GUS expression is upregulated specifically in guard cells of plants subjected to touch, darkness or dehydration. This result suggests that TCH2 may have a role in the closure of stomata.

To gain insight into the biochemical and physiological roles of TCH2, we are producing the protein in E. coli to test its abilities to function in calmodulin activity assays and bind potential target proteins. In addition, we have generated and are analyzing transgenic plants engineered to produce altered levels of TCH2 protein. (NSF-COT grant NAGW-5007 is gratefully acknowledged.)
[116]
ELECTRON MICROSCOPIC ANALYSES OF SOMATIC EMBRYO INITIATION AND DEVELOPMENT FROM ORCHARDGRASS LEAF CELLS. B.V. Conger, A. Vasilenko and J.K. McDaniel. Department of Plant and Soil Science, University of Tennessee, Knoxville.

Somatic embryos from orchardgrass (Dactylis glomerata L.) leaf cultures have unique features that make them a convenient model for studying early events in plant embryogenesis. Ultrastructural studies of leaf segments confirm the initiation and development of embryos from single mesophyll cells. Starch accumulation and the presence of storage lipids are main characteristics of the embryogenic cells. Amyloplasts evolve from etioplasts and chloroplasts during culture in early embryogenic cells. Our observations show that amyloplast size is regulated with respect to stage of the somatic embryo development. Examination and analyses of two to multicellular stage proembryos support the single cell origin. These analyses also support our earlier hypothesis that the plane of the first division leading to embryo formation is predominantly periclinal and establishes polarity. Our observations further show that the embryos develop independent of surrounding tissue and that this independence is established early, perhaps before the first cell division.

(Supported in part by NASA Grant No. NAG10-0138.)

[117]
EFFECTS OF ROOT MODULE DESIGN ON GROWTH AND DEVELOPMENT OF PLANTS UNDER THE CONDITIONS OF LOWERED WATER POTENTIALS IN THE ROOT ZONE. N.M. Krivobok, Y.A. Berkovich, S.M. Krivobok, and S.O. Smolianina State Scientific Center IMBP, Moscow, Russia.

In a set of 5 tests growth of wheat (Triticum aestivum L.), cultivar Super Dwarf was studied in different root modules in which nutrient solution was supplied via porous membranes and seeds planted either on their surface or in perlite above. Titanium plates with pore size of 2 to 6 μm were used in test 1 and ceramic tubes 22 mm and 16 mm in diameter with the same pore sizes in tests 2 and 3, respectively. In test 2, plants grew in a 2.5 cm thick layer of perlite on top of the titanium plate, and in root module with a porous ceramic tube 16 mm in diameter buried under perlite in test 5. In tests 1 and 2, the superficial membrane area per one plant was about 17 cm²; in test 3 it was about 3 cm². Perlite volume per a plant made up approximately 40 cm³ in tests 4 and 5. Plants grew for 49 days at water potentials in the root zone of -0.4, -3.0, and -5.0 kPa. Given equal levels of water potential, dry mass and potential yield of plants were significantly higher in root modules with perlite than without. Thus, comparing with test 5, at -0.4 kPa dry mass of plants in tests 2 and 3 was by 38% and 73% less, respectively; in test 1 this parameter was by 65% lower than in test 4. Reduction of water potential invariably affected growth and potential yield in each test; in test 3 this correlation was the strongest. In test 5, gross losses in plant mass and yield broke out even at -5 kPa, and at -3 kPa when plants grew on membranes without perlite. Root module designs markedly modified the volumetric spread of roots. Thus, in tests 1 and 4 flat root mats were formed immediately on the surface of porous plates, whereas in tests 2 and 3 they were at the undersurface of the tubes and on the tube and root module bottom in test 5. The results can be used by designers of space greenhouses. (Supported by NASA: NAS-15-10110)

[118]
OPTIMIZATION OF LIGHT REGIME IN SPACE GREENHOUSES. Y.A. Berkovich. State Scientific Center IMBP, Moscow, Russia.

In the existing space greenhouses from 75 to 90% of power are expended for crop irradiation. The paper outlines methods how to make an optimal choice of photosynthetic active radiation intensity and diurnal photoperiod for a space greenhouse based on the criterion of maximum product of its proportion yield from a volume unit multiplied on effectiveness of the photosynthetic use of light. As was shown, this criterion allows a trade-off between optimal use of volume and power in space greenhouse. Consideration was given to the selection of optimal light parameters for different modes of greenhouse operation with account to the systems of habitable space modules. According to the data of ground experiments with light-dependent radish crops, optimal are PAR value 200 W/m² or continuous irradiation by incandescent lamps with water filter. Based on the laboratory studies of leaf cabbage yield at various age, optimal PAR levels and diurnal photoperiods are the following: age 15 days - 150 W/m² and 16 hr, age 25 days - 100 W/m² and 20 hr, age 30 days - 50 W/m² and 16 hr. Below the specified values of crop irradiation corresponding to its maximum energy effectiveness, the suggested optimization criterion doubles in step with a rise in PAR falling on the crop. The method makes possible programming of operational mode for lighting units of greenhouses designed for different intentions with reference to the data of ground plant studies. (Supported by NASA: NAS-15-10110)

[119]

The BPS was developed under the SBIR program to meet science, biotechnology and commercial plant growth requirements in the Space Station era. Each of the four BPS chambers has a volume of 4000 cc and independent control of temperature, humidity, lighting, CO₂, and active nutrient delivery. Chambers are sealed for gas and water vapor exchange measurements and have gas/liquid sample ports.

The BPS upper avionics bay contains exhaust ducting and is configured with two color video cameras per chamber. The lower avionics bay houses the computer and data acquisition system, power conditioning, and two fluid reservoirs. The computer is a fully equipped 486 with an integrated hard drive, ethernet port, and zip drive, RS-232/RS-422 serial ports, DIO capability, and a video frame grabber which stores images to the harddrive. The control software utilizes fuzzy logic for nonlinearly coupled temperature and humidity control. The software is modular to meet user defined command and data display needs and internal diagnostics allow operation with minimal crew time. The crew interface consists of a high resolution color display, key pad, and a video port for recording real time video via an external camcorder. The BPS chamber access bay mounts on a pull out slide drawer and integrates the plant chambers, lighting modules, nutrient delivery systems, temperature/humidity control systems, CO₂ supply, and a digital sensor data bus. The plant chambers can be removed by releasing a latch and pulling the chamber away from the drawer. A quick disconnect louver maintains a seal when the chamber is removed. The shoot/root chambers can be separated without tools. Modular design of all subsystems increases accessibility to components and allows user configured hardware options (e.g., nutrient delivery and lighting), or alternative chamber configurations, such as 2 tall or 2 wide chambers or a single large chamber.
SESSION I: SPACEFLIGHT ANIMAL STUDIES

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The "Phorbol" and "Isozyme" experiments were flown in the ESA Burack facility during the third and fifth Shuttle Mir docking missions. Both experiments examined the effect of microgravity on Protein Kinase C (PKC) quantity, localisation and translocation in response to phorbol esters which stimulate PKC. In the "Phorbol" experiment U937 cells were stimulated in flight with radiolabelled PD Bu, which both selectively activates and labels most PKC isoforms, permethylated in-flight with digitonin & fractionated postflight to yield cytosolic and particulate fractions. A DNA assay was performed to determine cell number. The amount of PKC per cell determined by this technique was correlated with the applied acceleration (highest in 1.4g ground, least in 0-g). A second set of U937 & purified human peripheral T-cells were stimulated with unlabelled PD Bu and the amount of PKC α, βI, βII, δ and ε isoforms determined by western blot. Results from western blot confirm the observed correlation between PKC quantity and gravity level obtained in the 3H-PD Bu assays. In the "Isozyme" experiment U937 and purified human peripheral T-cells were stimulated with phorbol esters or natural agonists. Postflight the quantity and sub-cellular localisation of each PKC isoform at various times following stimulation was determined by western blot. The results of both experiments support previous studies which indicate that PKC mediated signal transduction is sensitive to microgravity. (Supported by CNES; 96/241 & 97/071/6751)

[121] HORMONAL RESPONSE TO HUMAN SPACE FLIGHT ON THE SPACE SHUTTLE. T.P. Stein and M.D. Schluter. Dept. of Surgery, Univ. of Medicine and Dentistry of NJ, Stratford, NJ, 08084.

Microgravity perturbs the body’s homeostasis because of the loss of hydrostatic pressure, the conflicting inputs into the neuron-vestibular system and the lack of physical tension on the musculo-skeletal system. The objectives of his study were to measure the changes in urinary hormone excretion of hormones that have been shown by ground studies to be involved in regulating muscle protein content with the objective of assessing their relative importance to the infight muscle protein atrophy. We assayed C-Peptide, Cortisol, ACTH, T4, GH, IGF-1. PGE2 and PGF2α in the urine of the astronauts from the SLS1 and SLS2 missions. The principal findings (p<0.05 vs mean preflight value) were increases in C-Peptide and cortisol, no change in growth hormone, transient (over the end of the first week) decreases in ACTH, IGF-1 and PGE2 and chronic decreases (60%) in T4 and PGF2α. These observations suggest a down regulation of energy metabolism and the possible involvement of prostanooids in the muscle loss.


Our previous studies demonstrated that exposure to 2G and space-flight can affect circadian rhythms and neural function. An important issue in gravitational biology has been to determine whether 2G exposure may be a useful method for understanding responses of animals to flight and recovery. It is possible that the physiological responses to 2G exposure (an increase in 1G) is similar to that of an animal immediately after landing that had been adapted to space flight conditions (an increase in 1G). The immediate early gene protein, c-Fos, has become a useful biological marker for examining activation of neurons in the central nervous system. We previously showed that c-Fos expression in SCN neurons is correlated with effects of 2G on circadian function. This study compared the pattern of c-Fos reactivity with the CNS between rats exposed to 2G for one hour and adult rats within 3h after landing from 9 d of space flight.

Time pregnant rats (Sprague-Dawley, Taconic) were placed in LD12:12 with food and water ad libitum. At gestational age 12 (G11) flight dams were exposed to spaceflight for 9 d (STS-71), while a control group remained at 1G. Rats were examined either within 3 h or 2 d after landing. A separate group of rats was exposed to 2G via centrifugation for 1 h and immediately examined. Rats were sacrificed, brain prepared for histology, sectioned on 40 mm, immunohistochemically stained for c-Fos and counterstained.

The effect of recovery following space flight and 2G exposure on the pattern of c-Fos expression within the CNS was very similar. Both groups of rats exhibited elevated c-Fos within the paraventricular nucleus, vestibular nuclei, dorsal parabrachial nuclei, dorsolateral raphe, and locus coeruleus. These results suggest that the animals exposed to space flight for 9 d had adapted to the environment and the response following recovery is similar to that of a hypergravity exposure.

(Supported in part by NASA Grant NAGW-4552 and NCC2-886.)

[123] HEAD-DOWN BEDREST IS NOT A VERY GOOD MODEL FOR CALCIUM METABOLISM IN SPACEFLIGHT. C.E. Cann, C.D. Arnaud, M.E. Hammond, and S. Sanchez, University of California, San Francisco, CA

Head-down tilt bedrest has been used as a ground-based model to reproduce some of the effects of the body seen in space flight. While it may elicit some of the same measured responses, it is not clear that the mechanisms producing these results are the same as in microgravity. One mechanism thought to be responsible for bone loss in space is the lack of skeletal loading, and bedrest has been suggested as a good model to simulate this effect even though a gravitational vector is still present. We hypothesized that serum ionized calcium would increase rapidly as a result of increased bone resorption, that serum parathyroid hormone (PTH) would decrease in response, and that the increase in Ca++ and decreased PTH would persist as long as the stimulus (microgravity or bedrest) was maintained.

Six male volunteers underwent 17 days of head-down tilt bedrest to simulate the nominal profile of the LMS mission. Each subject was used as his own control with 2 weeks pre-bedrest and 2 weeks post-bedrest data collection. The flight experiment had 4 male astronauts as subjects, with data collection starting at L-90 and continuing to R+30. All serum samples were collected and analyzed in identical fashion for flight and bedrest.

Inflight, Ca++ increased 2% by 2 hours, fell slightly by day 2, then increased 2.6% and remained there for the mission. PTH decreased 25% early and 37% later. In bedrest, Ca++ increased 1% on the first day, fell below baseline by day 2, then rose to a sustained increase of 0.3% (ns). PTH did not change significantly. When normalized to pH 7.4, inflight Ca++ increased 6% over baseline while bedrest values increased only 1.2%. The expected negative correlation of Ca++ and PTH was present in the astronauts (r=0.31) but not in the bedrest subjects (r=0.16).

Bedrest did not elicit a sufficient rise in Ca++ to induce the adaptive response of decreased PTH seen in flight, suggesting that while the bedrest model may have some similarities to flight it cannot be used to investigate the underlying mechanisms causing bone loss in space.

(Supported by NASA: NAS9-18769)
[124] MICROGRAVITY INDUCES PROSTAGLANDIN G/H SYNTHASE AND INTERLEUKIN-6 GENE EXPRESSION IN NORMAL RAT OSTEOSTROPHOBLASTS: POTENTIAL ROLE FOR OSTEOCLAST ACTIVATION. Y. Kumei1, H. Shimokawa1, H. Katano1, E. Hara2, H. Akiyama1, M. Hirano1, C. Muka1, S. Nagaoa4, P.A. Whitson5 and C.F. Sam1. 1Tokyo Medical and Dental University, Tokyo, 2Kyoto Prefectural College of Medicine, Kyoto, 3Tory Research Center, Kamakura, 4NASDA/Tsukuba Space Center, Tsukuba, 5NASA/Johnson Space Center, Houston.

Normal osteoblasts obtained from adult male rat femur marrows were cultured for 5 days during a Shuttle-Scalable flight (STS-65). After collection of the culture medium, the cellular DNA and RNA were fixed on board. Microgravity induced a 5- to 136-fold increase of Prostaglandin E2 (PGE2) production in flight culture medium as compared to the ground controls. This increase of PGE2 production was consistent with a 3- to 10-fold elevation of inducible prostaglandin G/H synthase-2 (PGHS-2) mRNA, quantitated by RT-PCR. The induction for the constitutive isozyme PGHS-1 was less than that for PGHS-2. The IL-6 mRNA was also increased by 6- to 9-fold in microgravity as compared to the ground controls. Since PGE2 and IL-6 are both known to play a role in osteoclast formation and bone resorption, these data provide insight into molecular mechanisms of microgravity-induced bone loss. (Supported by ISAS Space Station Utilization Research Fund, Japan and NASA #106-30-12-40, USA)

[125] THE EFFECTS OF SPACEFLIGHT ON mRNA LEVELS FOR CYTOKINES IN PROXIMAL TIBIA OF Ovariectomized RATS. M. Zhang and R.T. Turner, Dept of Orthopedic Research, Mayo Clinic, Rochester, MN.

Bone resorption was elevated in ovariectomized rats during a 14 day orbital spaceflight over and above that caused by gonadal hormone deficiency (Endocrinology 138:1567-1576, 1997). Locally produced cytokines are believed to have an important role in normal as well as abnormal bone resorption. The purpose of the present study was to determine whether spaceflight results in altered expression of cytokines in cancellous bone. The mRNA levels for selected cytokines were determined in proximal tibial metaphysis using RNase protection assays. The message for interleukin 1 receptor antagonist, interleukins 1α, 10, and 12, macrophage migration inhibitory factor, and tumor necrosis factor-α was below the limit of detection for all groups. Interleukin 6 and transforming growth factor-β were expressed in bone but the mRNA levels for these cytokines were not altered by either ovariecctomy or spaceflight. Insulin-like growth factor 1 mRNA was increased following O VX and not influenced by spaceflight. There was a tendency for interleukin-1β message to be increased following ovariecctomy and this tendency achieved statistical significance following spaceflight. Finally, spaceflight resulted in an increase in the message level for interferon gamma in O VX rats. In summary, spaceflight results in increases in mRNA levels of two cytokines in O VX rats which have been shown to increase bone resorption.

Supported by NASA grant NAGW-4963.

[126] LACK OF AN EFFECT OF SPACE FLIGHT ON BONE MASS AND FORMATION IN RAPIDLY GROWING RATS. T.J. Wronski1, M. Li1, S.C. Miller2, Y. Shen1, B.M. Bowman1, and B.P. Halloran3. 1Dept of Physiological Sciences, Univ of Florida, Gainesville, 2Radiobiology Division, Univ of Utah, Salt Lake City, and 3Dept of Medicine, Univ of California, San Francisco.

To study the effects of space flight on bone, six male Sprague Dawley rats (6 weeks old, 165g body weight) were placed in orbit for 17 days in an animal enclosure module (AEM) aboard the space shuttle (LMS mission). A control group of 6 male rats was housed in a ground-based AEM. At recovery, the mean body weights for flight and control rats were 281±22g (SD) and 270±13g (NS), respectively. Several bones were processed undecalcified for histomorphometric analysis. Cancellous bone volume in the proximal tibial metaphysis was nearly the same in flight and control rats (6.9±2.7% vs. 7.2±2.0%, NS). Similarly, osteoblast surface, an index of bone formation, varied little between flight and control rats (25.5±3.8% vs. 27.2±4.7%, NS). Flight rats also exhibited normal levels of cancellous bone mass and bone formation in the lumbar vertebrae and femoral neck. In the tibial diaphysis, periosteal bone formation rate was identical in flight and control rats (0.05±0.01 vs. 0.05±0.01 mm2/d, NS). The results indicate that space flight has minimal effects on bone mass and bone formation in rapidly growing rats. These findings emphasize the importance of rat age, strain, and housing in planning space flight experiments. (Supported by NASA: NCC2-825)

[127] EPHYRA DEVELOPMENT IN POST-FLIGHT AND CONTROL ASEXUAL GENERATIONS OF Aurelia polyps. D.B. Spangenberg1, R. Schwarte1, and C. Philpott1. 1Dept of Pathology, Eastern Virginia Medical School and 2Clinical Investigations and Research, Naval Medical Center, Portsmouth, VA.

Groups of polyp end-pieces from strobilae of jellyfish which were microgravity exposed (SLS-1) and their controls were grown to full-sized polyps. Individuals were isolated and cloned by collecting their buds according to asexual generations to the third generation. They were fed once weekly. When sufficient numbers had been produced through budding, the polyps were induced to metamorphose with iodine and tested using the Aurelia Metamorphosis Test System. The numbers of arms, rhopalia, statoliths, and pulses were counted in at least 5 ephyrae per generation. Polaroid pictures were made to record the morphology of entire ephyrae. Although there were no statistical differences between generations with regard to mean numbers of rhopalia and arms, statolith numbers decreased and pulses increased generationally. Comparisons of clones with the general population group from which the clones were derived likewise revealed a decline of statolith numbers with succeeding generations. Morphological features are being analyzed to determine whether specific characteristics are transferred throughout generations through asexual reproduction and to determine whether different features developed in the clones through asexual generations of the flight animals as compared with controls.

Supported by NASA: NAG10-0178.
SESSION J: CONCURRENT POSTER SESSION IV
ANIMAL GRAVITY SENSING

[128]
CARDIOVASCULAR AND HEMODYNAMIC RESPONSES TO SHORT-TERM SIMULATED MICROGRAVITY. R.R. Socci, M. Wang, S. Caines-McKenzie, M. Theirry-Palmer, N. Emmett and M.A. Bayorh. Morehouse School of Medicine, Atlanta, GA.

Microgravity is known to induce orthostatic intolerance and baroreflex impairment. Cardiovascular and hemodynamic responses observed in the whole body suspension (30° head-down tilt; HDT) rat model mimic observations made during exposure to microgravity. We evaluated the effects of 24 hr simulated microgravity and the subsequent return to normal gravity on normotensive Sprague Dawley (SD) and hypertensive Dahl salt-sensitive (SS) rats (2 wks on 8% NaCl diet). An initial 22 mm Hg blood pressure (BP) rise occurred in normotensive SD rats from 87 ± 3 mm Hg and was sustained over the first 5 hrs. By 24 hrs, BP had decreased to 100 mm Hg. This BP rise may be stress-related, since parallel unsuspended controls did not show a significant (p<0.05) BP change over the same period. Upon release from suspension, BP initially decreased to 70 mm Hg and this was sustained at 6 hrs; whereas the parallel controls were unchanged.

Blood flow in the lower abdominal aorta was significantly higher in suspended animals than in controls, but not for the renal artery. Plasma concentrations of ionized calcium and glucose were significantly decreased with no change in hematocrit. Hypertensive SS rats had a basal BP of 130±10 mm Hg. Neither suspended nor control SS animals showed a significant BP change over the 24 hr period. Upon release from suspension, BP decreased to 105 mm Hg and recovered at 6 hrs., whereas their parallel controls did not experience a significant BP change. For both SD and SS rats, the suspended animals showed a significantly greater weight loss than the controls. These results indicate that dietary salt/sensitivity may protect against the post-suspension fall in BP. (Supported by NASA: NCCW-0083.)

[129]
INFLUENCE OF 7 DAY SIMULATED MICROGRAVITY ON CARDIOVASCULAR AND HEMODYNAMIC PARAMETERS. M.A. Bayorh, R.R. Socci, M. Wang, S. Caines-McKenzie, M. Theirry-Palmer and N. Emmett. Morehouse School of Medicine, Atlanta, GA.

Prolonged exposure to microgravity induces cardiovascular deconditioning and impairment of baroreflex activity partially as a result of fluid and electrolyte shifts. Several animal models have been developed to mimic these cardiovascular and hemodynamic responses. We examined the effects of 7 day simulated microgravity and the subsequent return to normal gravity on normotensive Sprague Dawley (SD) rats and hypertensive Dahl salt-sensitive (SS) rats (2 wks on 8% NaCl diet) using the tail-suspended rat model. In both suspended and control (prepared for suspension, but remained in a horizontal position) SD rats, blood pressure (BP) decreased 17 mm Hg during the first day and remained unchanged for the rest of the 7 day period. After the 7 day period, the BP of suspended hypertensive SS rats declined from 118 to 77 mm Hg, while that of the controls decreased from 107 to 58 mm Hg. When released from suspension, BP decreased to 60 mm Hg over 6 hrs, whereas the control BP was unchanged. Blood flows measured in the lower abdominal aorta and renal artery were not significantly different between suspended and control animals. For both SD and SS rats, the suspended animals showed a significantly greater weight loss than the controls. These data support the notion that the tail-suspended rat model can yield significant results that mimic human studies. Also, that salt-sensitivity/dietary salt may partially counter the orthostatic intolerance observed post-suspension. (Supported by NASA: NCCW-0083.)
[130] DYNAMICS OF 3-D MODEL OF THE OTOLITHIC MEMBRANE.
A V. Kondrachuk, NASA/Ames Research Center, CA

Spaceflight affects behavior of gravireceptor, which consists of the sensory macula and its otolithic membrane (OM, gelatinous membrane plus otocoria) which detects linear accelerations. Modeling approaches are essential to understand how these structures respond to gravity and alter function in altered environments.

The dynamic behavior of the otolithic membrane was analyzed using the analytical and computer (finite element analysis) 3-D models. The structural and geometrical parameters of the models correspond to the utricular membrane of guinea pig. The OM model comprised the three parts: otocorial, gel and supramacular (S) (boundary the epithelial surface) isotropic layers. All components of the OM were assumed to be viscoelastic (Kelvin body). The viscoelastic properties of the OM determine the specific times and frequency-dependent behavior of the local displacements of the membrane caused by the inertial time-dependent forces. Two dynamic regimes were analyzed: transient change of the acceleration and the harmonic oscillation of the membrane. The results of modeling were compared to published data (Fernandez & Goldenberg, 1976) to estimate dynamic storage and loss moduli of the OM gel layer. They were found to be close to the known moduli of the mucus:5-10N/m^2 at 1-10 Hz.

The transformation of the OM local displacements into the local deformation of the receptor hair cell bundles is mainly determined by the mechanical parameters of the S-layer (thickness - 10 mm, Young's modulus - 10 N/mm^2) that may provide preliminary filtering of the mechanical input. The elastic and geometrical parameters of the S-layer determines the normal modes (frequencies) of the OM. Due to the inhomogeneous otocorial distribution the lowest normal modes (40-80 Hz) may correspond to the twisting rather than linear oscillations of the utricular membrane. This may be the possible explanation of Barnes & Benson (1983) results of the centrifugal double-rotation experiments. (Supported by the NRC Award and Biocomputation Center at NASA/Ames Research Center).


Hsp25 was initially characterized as a molecular chaperone required for protection from stress and for the development of stress tolerance in cultured cells. In the present study, we explored the expression pattern of hsp25 in the adult mouse brain under normal conditions as well as under stress induced by experimental hyperthermia and hypoxia, conditions relevant to the space flight environment. Immunohistochemical analysis of brain sections revealed a surprisingly restricted pattern of constitutive expression of hsp25, limited to the facial, trigeminal, ambiguous and hypoglossal nuclei of the brain stem. Significant increases in the levels of hsp25 were observed in these same areas 2 hr after hyperthermia and after 2 and 8 hr of hypoxia. The induction of hsp25 was also detected in fibers of the facial and trigeminal nerve tracts. Surprisingly, no other area of the brain showed expression of hsp25, in either control or stressed animals. The highly restricted expression of hsp25 implies that this protein may have a specific physiological role in the orofacial motor nuclei, which govern precise coordination between muscles of mastication, pharynx, larynx, and face. Its rapid induction after stress further suggests that hsp25 may serve as a specific molecular chaperone in the motor nuclei and along their fibers under conditions of stress or injury. An extension of this analysis will be the effects of altered gravitational environments on hsp25 expression. (Supported in part by NASA NAGW-4462).

[132] FEASIBILITY STUDY OF A SHORT-DURATION BRIC-100 SPIDER FLIGHT EXPERIMENT. C.R. Grupka, E.A. Hudson, and A.M. Helmenstine, Biology Department, Tusculum College, Greenville, TN.

The feasibility of maintaining spiders within BRIC-100 canisters and late loading and early access protocols for a short-duration shuttle experiment were evaluated. Specifically, optimal spider species and quantity, prey species and quantity, relative humidity, barometric pressure, lighting, container composition and configuration, and means of preventing activity prior to launch and following landing were examined. Adult female Achaearanea tepidariorum and male and female Araneus cornutus were successfully maintained for 3 weeks in separate sealed 25x80-mm polystyrene-capped shell glass vials under conditions of 100% relative humidity and 730-mm Hg barometric pressure over a temperature range of 19-22°C. Ten live Drosophila melanogaster and 0.5-mL distilled water were added to each vial prior to sealing. Refrigeration (2°C) successfully prevented web construction and prey capture. An aluminum canister with dimensions of a BRIC-100 was configured to hold 20 polystyrene vials/spiders, a battery-powered environmental data logger, and insulation and a sealed cold-pack sufficient to prevent spider and prey activity for ~20 hours. The simulated BRIC-100 did not affect spider or fly survivability or orientation of spiders within vials. Spiders produced webs morphologically comparable to those produced in unsealed, unrefrigerated vials. Spiders successfully captured prey, molted, and produced egg sacs during and following treatment. Spiders exhibited no signs of hypoxia or starvation. No difference was observed between spiders maintained with normal lighting and spiders maintained in the dark. The spiders in this study do not routinely modify webs, so no treatment in anticipated to prevent web alteration during and immediately following landing. In laboratory studies, spider orientation could be deduced from web structure. (Supported by an Appalachian College Association/Mellon Foundation grant.)
SESSION J: CONCURRENT POSTER SESSION IV
ANIMAL STRUCTURAL SYSTEMS & MUSCLE PHYSIOLOGY II
[133] EFFECTS OF HEAVY-ION BEAM IRRADIATION ON OXIDATIVE ENZYME ACTIVITY OF SOLEUS MUSCLE FIBERS IN YOUNG AND OLD RATS. S. Taguchi, S. Ogo, S. Yamasaki, H. Senge and H. Okamoto, Grad. Sch. of Human & Environ. Studies, Kyoto Univ. Kyoto, Japan

The present studies were designed to determine whether heavy-ion irradiation affected metabolism in the deep or superficial region of skeletal muscles in young and old rats. Ten young (3 month old) and ten old (18 month old) Wistar female rats were assigned to irradiation and non-irradiation groups, respectively (n = 5, each group). Irradiation intensities were 2.5 Gy by LET around 42.35 KeV/μm using Heavy Ion Medical Accelerator in Chiba. At three months after irradiation, rats were sacrificed and the soleus muscle was removed. Cross-sectional samples of soleus muscle were stained for ATPase, succinate dehydrogenase, and α-GPD for histochemical analysis. After irradiation the soleus muscles of the young rats displayed a significant increase in the area of fast twitch fibers (FOG) and a concomitant decrease in area of slow twitch fibers (SO), when compared to the non-irradiated rats. However, there was no difference between the older rats in fiber type distribution between irradiated or non-irradiated groups. In addition, the deep region of soleus muscle from the irradiated rats had a decrement of oxidative (SDH) and glycolytic (α-GPD) activities. This decrement was greater in the young rats compared with the older rats. These findings suggest that heavy-ion irradiation affects the metabolism of single muscle fibers during the growing period of young rats.

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[134] EFFECT OF WEIGHTLESSNESS ON OXIDATIVE AND ANTIOXIDANT ENZYME ACTIVITY IN SKELETAL MUSCLES OF PREGNANT RATS. M.D. Lee1, G. Cervantes1, S. Bodine-Fowler1, and B. Giren1. Trega Biosciences, Inc. (formerly Houghten Pharmaceuticals, Inc.) and 2University of California at San Diego, San Diego, CA.

Although there is much information regarding the changes in skeletal muscle observed in hindlimb suspension models, it is still important to look for any differences between the effects seen in ground based models of weightlessness and space flight. We were able to obtain skeletal muscles from pregnant female rats that were in space for 9 days during gestation days 11 through 20. Citrate synthase, malondialdehyde, and superoxide dismutase levels were measured spectrophotometrically in the three skeletal muscles (soleus, tibialis anterior, and medial gastrocnemius). In examining the soleus muscle, we found that only superoxide dismutase levels were significantly (p < 0.05) lower in the flight tissues when compared to ground based controls. Citrate synthase and malondialdehyde levels showed decreasing but not significant trends. There were no significant flight-induced changes in oxidative or antioxidant enzyme activity observed in the tibialis anterior muscle. However, the medial gastrocnemius showed decreased levels of citrate synthase (p = 0.059), malondialdehyde (p < 0.05), and superoxide dismutase (p = 0.07), with only the malondialdehyde changes being statistically significant. These results indicate that space flight does result in significant changes in oxidative and antioxidant enzyme levels in pregnant rats. Results also show that these changes vary among different hindlimb skeletal muscles.


Microgravity conditions produce significant morphological changes, including shifts in fiber-type composition, in skeletal muscles in the hindlimbs. However, facial muscles (that insert into subcutaneous soft tissues rather than into bone) should not respond similarly because they are subject to different mechanical loading conditions. To test this hypothesis, we are comparing the fiber-type composition of the vibrissae-operating facial muscles in the adult rat under normal and simulated microgravity conditions. Experimental animals are maintained in a 30-degree head-down tilt position via a standard hindlimb suspension apparatus for 14 days. Fiber-type composition is determined via standard enzyme histochemical (myosin ATPase, NADH tetrazolium reductase and immunocytochemical (monoclonal antibodies specific for certain myosin heavy chain isoforms) methods. In normal controls, the facial muscles are composed of mosaics of types I, IIA, IIB, and IIX fibers. There are no α-cardiac fibers. All muscles share IIB as the predominant type. Preliminary data indicate that the facial muscles of suspended rats undergo a significant shift in composition that further emphasizes type IIB fibers, at the expense of the other types. This change appears to be different from that seen in the hindlimb muscles. (Supported by NASA 1NA4A438 and NIH GM08248 and RR03034).

[136] COMPARISON OF BONE MINERAL IN RATS WITH WEIGHT GAIN REDUCED BY DIET RESTRICTION OR SKELETAL UNLOADING. M. Navidi1, M. Moran1, T. Wang1, P. Milbury2 and S.B. Arnaud3. NASA/Ames Research Center, Moffett Field, CA and 2ESA Laboratories, Inc., Chelmsford, MA.

Growing rats have lower body weights (BW) and femoral bone mineral during exposure to a space flight model that unloads the hindlimbs than controls (Navidi et al. J. Appl. Physiol. 78:70-75, 1995). Since whole body weight as well as regional load are factors in generating the mineral content of weight-bearing bones, we investigated the role of reduced weight gain in the bone deficit due to unloading (S). We compared mineral content (BMC) in the femurs and humeri of 180g male rats fed diet restricted in calories (D) to rats of the same weight following a 2-week period of tail suspension. S animals and their pair-fed amputee controls (C) were fed AIN76A with 0.5% calcium and 0.6% phosphorus. Diets containing 75% of the calories supplied to S and C but enriched to provide normal dietary calcium and phosphorus were fed to D animals. BW were monitored biweekly; food and water daily. BMC was determined by the weight of ash in femur and humeri. Food intake was lower in D than S and C (11.1±0.1 vs 15.1±1.9 vs 14.3±0.2 g/d, p < 0.05). BW in D were equal to S which in turn were 5% lower than C (249±8 vs 243±11 vs 256±6 g, p < 0.05). Dietary calcium levels were the same in all three groups. Femoral ash weight was greater in D than S, and the same as in C (D = 0.24±0.02 vs S = 0.20±0.01 vs C = 0.24±0.02 g, p < 0.05). Mineral content in the loaded bone, the humerus, was the same in all 3 groups. In spite of identical food consumption in S and C, S showed 5% decrease in BW and a mineral deficit in the femur of 17%. Calcium intakes, restricted to 75% of S diets, were effective in reducing BW to the same level in D as in S, but there was no mineral deficit in D. After 2 weeks, there is no effect of reduced whole body weight gain in the regional mineral deficit from unloading. (Supported by NIH NIA-R44 AG13327-02 and NASA 199-26-12-02).
SESSION J: CONCURRENT POSTER SESSION IV
CELL BIOLOGY II
[137] EFFECT OF HYPERGRAVITY ON VASCULAR SMOOTH MUSCLE CELL WOUND HEALING. G.L. Sanford and J. Liu, Space Medicine & Life Sciences Research Center, Morehouse School of Medicine, Atlanta, GA.

The response of cells following wounding in an altered gravity environment has not been evaluated. We examined possible mechanisms involved in vascular smooth muscle cell response to wounding under hypergravity. Confluent vascular smooth muscle cells (SMC) were subjected to a denudation injury under normogravity (control) or hypergravity (6G) conditions. The time course of closing the wounded area was monitored and cultures were harvested at various times after injury to measure TGFβ and c-myc expression. Under 6G, SMC showed a clear difference in wound closure compared to controls: between 8 and 16 hr; cells had moved further into the wounded area by 8 hr and completely closed the wound by 16 hr. Control cells showed increased TGFβ expression with time of incubation where SMC under 6G increased expression only between 0.5 to 2 hr. Hypergravity induced the expression of c-myc between 0.5 and 2 hr after wounding. These findings indicate that hypergravity induces a transient increase in the expression of both c-myc and TGFβ prior to cells migrating into the wound area. (Supported by NASA FAR: NAG8-852)

[138] VECTOR-AVERAGED GRAVITY INDUCED CYTOSKELETON REARRANGEMENT IS MODULATED BY PROTOONCOGENE. C.D. Melhado, G.L. Sanford, and S.A. Harris-Hooker, Space Medicine & Life Sciences Research Center, Morehouse School of Medicine, Atlanta, GA.

Microgravity reached in a sounding rocket was shown to strongly decreases EGF-induced expression of protooncogenes (Rijken et al., Aviat. Space Environ. Med., 62:32-6,1991). These authors also reported a similar finding in the fast rotating clinostat which provides a "Vector-free" gravity environment by continuous averaging of the gravity vector. We conducted in vitro cellular studies examining protooncogene regulation of cytoskeleton organization during BAEC sheet migration. Confluent cultures treated with antisense oligonucleotide (AS-Oligo) and adapted to clinostat rotation (30 rpm) for 12 hr were demed and rotation continued for an additional 6 hr. Cultures were fixed, immunocytochemically stained and examined by enface confocal scanning laser microscopy. The cytofilament-dependent cell migration inhibited in the presence of vector-averaged gravity was enhanced by the AS-Oligo blockade of c-myc, c-fos expression. Under vector-averaged gravity, vimentin relocates from a uniform dense cytoplasmic distribution (control) to a diffuse filamentous network, and distinct juxtanuclear, perinuclear vimentin bundles distribution. Vector-averaged gravity also induces distinct parallel alignment of F-actin stress fibers. The migrated cells had distinct bipolar vimentin aligned with extensions, while the actin filament were disorganized and diffused. These studies imply that gravitational unloading exerts its effect by modulating the expression of growth regulatory genes (protooncogene) which regulates partial cytoskeletal disassembly, peripheral withdrawal and delayed regrowth of cytoskeleton. Our data further suggest that gravity alterations of mammalian cell migration differentially modulate distinctive signal transduction pathways. (Supported by NASA: FAR NAG8-852 and NCCW0085)

[139] MANIPULATION OF CALCIUM HOMEOSTASIS WITH CALRETICULIN. S.E. Wyatt, P.-L. Tsou, and D. Robertson. Department of Botany, North Carolina State University Raleigh, NC

Calcium is hypothesized to play a major role in the signal perception, transduction, and response mechanism in plants. In an attempt to dissect the role of calcium, we are using a reverse genetics approach to perturb Ca^{2+} homeostasis by altering the expression of the Ca^{2+}-binding protein calreticulin (CRT). CRT is an evolutionarily conserved luminal ER protein containing both a regulatory P-domain with two high affinity Ca^{2+}-binding sites and a high capacity, low affinity C-domain which binds up to 50 moles of calcium/mole of protein. In animal cells, overexpression of CRT increases the Ca^{2+} capacity of IP_{3}-sensitive Ca^{2+} stores (Bastianutto et al., J. Cell Biol. 130, 847) and decreases store-operated Ca^{2+} influx from the extracellular space (Mery et al. J. Biol. Chem. 271, 9332). In Xenopus oocytes, overexpression of the CRT P-domain inhibits IP_{3}-induced Ca^{2+} waves (Camacho & Lechleiter. Cell 82, 765). We sequenced a full-length maize CRT cDNA clone, and the encoded protein has all the characteristic domains of other known CRT's. We are generating a variety of experimental CRT constructs driven by constitutive or inducible promoters, with and without fusion to green fluorescent protein (GFP), and with the appropriate signals to localize the recombinant proteins to the ER. Initially, we have used these constructs to overexpress the full-length CRT and the C-domain in tobacco BY2 cells to determine their ability to alter Ca^{2+} homeostasis. The resulting transgenic cells are being imaged for intracellular localization of the recombinant proteins and to determine changes in free cytosolic Ca^{2+} concentrations. (Supported by the NSCORT in Gravitational Biology at North Carolina State University.)


Negative response to growth stimulation of lymphocytes in microgravity is consistent yet mechanisms remain enigmatic. An objective of the "TCCELL" experiment flown on the ESA Biorack Facility was to investigate mechanisms for negative lymphocyte growth response during space flight. Cells, launched in medium containing 2% serum, were growth stimulated20 hours after launch by increasing serum to 10% and raising the temperature from 20 °C to 37 °C in flight and simultaneous ground controls. After 4, 24 and 48 hours, cells were filtered from medium and cell-free medium was stored at -20 °C. Post-flight analysis of six replicate medium samples for each time point were tested for presence of Fas/APO-1 protein (a cell death factor) using a commercial ELISA kit. A time dependent increase in Fas/APO-1 in flown but not ground samples (P<0.005 InStat ANOVA) was evident. Medium from cells maintained in the 1g in-flight centrifuge showed the same time dependent increase in Fas/APO-1, however; values were an order of magnitude less. Fas/APO-1 protein was not increased in cells cultured in 2% serum medium or by simulated launch vibration. We conclude that a microgravity related increase in the cell death inducing protein, Fas/APO-1, and consequent apoptosis, is one mechanism by which space flight confers sensitivity and negative growth response to lymphocytes. This research was supported by NASA grant NAG2-985.
EVALUATION OF A MICROGRAVITY OPERATED CLINICAL HEMATOLOGY ANALYZER. S.J. Prow1, K.A. Gunter1, M.S.F. Clarke1, D.L. Feeback1, K. Krug Life Sciences, 2USRA, and 3NASA, Clinical Laboratories, SD3, Johnson Space Center, Houston, TX.

Hematological analysis for diagnosis of infection and anemia is highly desirable for the International Space Station. Most hematological analyses are performed using volumetric impedance. This type of analysis is unsuitable for space flight because of the behavior of fluids in microgravity and the toxicity of the chemicals involved. We evaluated the Quantitative Buffy Coat analysis method, developed by Becton-Dickinson Primary Care Division (Sparks, MD) for potential space flight use.

In this method, ~70 µl of whole (capillary or venous) blood is drawn into a mylar-wrapped, acrdine orange internally coated capillary tube. A calibrated float is inserted and the tube centrifuged for five minutes at 12,000 rpm. The tube is loaded into the analyzer, where the cell-serum interface layer (expanded by the plastic float) is scanned with red and fluorescent light. Transmittal and fluorescence signatures are evaluated, and the following values generated: Total Leukocyte count, % and # of Granulocytes, % and # of Lymphocytes/Monocytes, Hemoglobin, Hematocrit, MCHC, and Platelet Count.

We tested the system aboard NASA’s KC-135 aircraft under microgravity and hypergravity conditions. On the first flight, only the analyzer was evaluated due to space constraints. The analysis of the capillary tube worked well in all conditions, but the tubes floated out of the analyzer’s loading platform in microgravity. In collaboration with the manufacturer, we engineered several modifications to the system, and developed a method for mixing the loaded tubes in microgravity. On two subsequent KC-135 flights, both the modified analyzer and centrifuge performed normally, yielding reproducible results on two subjects. A modified pipette was used to mix blood in the tube. The entire procedure takes no more than 15 minutes and is very simple to learn. We plan to further evaluate the analyzer on future Space Shuttle missions.

USE OF A NASA BIOREACTOR IN ENGINEERING TISSUE FOR BONE REPAIR. P.J. Duke, D. Monufar-Solis, R. Grant, and G. Martin. Univ. of Texas Health Science Center, Dental Branch, Houston, TX, 77225.

Skeletal disorders, inherited or acquired, cost billions of dollars a year to treat, and cause untold suffering and heartache. Thus, there is intense basic and applied research in an effort to mold, replace, or repair bone. Because many bones form by endochondral ossification, cartilage can be used to repair bone, but its use has been limited in part by the amount of tissue available from conventional tissue culture systems. The NASA-developed bioreactors provide homogeneous distribution of cells, nutrients, and waste products, with less damaging turbulence and shear forces than conventional systems.

Cultures under these conditions have higher growth rates, viability, and longevity, allowing larger "tissue-like" aggregates to form, and open the possibilities of producing enough tissue for implantation. Using one of the commercially available bioreactors, we have grown cartilaginous aggregates from a cell suspension using the MPLB1 (Mouse Posterior Limb Bud-1) cell line. Cells were cultured for 100 days without ascorbate acid to allow proliferation without extensive matrix production. Aggregate size, monitored every 2-3 days, continued to increase throughout the experiment. Aggregate staining with Alcian blue indicated the presence of cartilaginous matrix, but light microscopy showed cells with appearance of pre-chondrocytes with only some metachromasia. Aggregates grown for 41, 62 and 68 days were implanted subcutaneously in the costal region of mice, using a system shown to support endochondral ossification. Implants were harvested on weeks 1, 2, 3, 4, 7, and 10. Endochondral ossification in these aggregates is under evaluation to assess their usefulness in bone repair.

Supported by TMC/NASA Subcontract NCC9-36.

Generation of an effective immune response requires that antigens be processed and presented to T lymphocytes by antigen-presenting cells, the most efficient of which are dendritic cells (DC). DC are present in only trace amounts in most tissues, but because they appear to represent a powerful new vehicle for vaccination against cancer and infectious agents, there has been an intense drive to establish the optimum conditions for their propagation in vitro. DC can be generated from human CD34+ progenitor cells in standard 2-dimensional (2D) cultures. However, we questioned whether this process might be improved within the 3D environment of the rotating bioreactors. We observed that the NASA bioreactors (including the HARV, STLV and RCCS-D vessels) did indeed support generation of DC; these DC were identified morphologically as large cells with elongated cytoplasmic processes, phenotypically as distinct from other leukocyte lineage expressing high levels of MHC Class II molecules (i.e. DC were CD3 11b 16 56 19 20 14 HLA-DR+), and functionally by their high allostimulatory activity. Some differences between DC generated in 2D versus 3D were observed: (a) while cells proliferated in both conditions, the overall cell expansion in 3D was 25-50% of that seen in 2D cultures; (b) 3D-DC had reduced phagocytic activity; (c) 3D-DC often displayed higher allostimulatory activity; and (d) 3D-DC expressed higher levels of some heat shock proteins. Since DC have been shown to lose antigen uptake capabilities and gain allostimulatory activity as they mature, our data suggest that bioreactor-generated DC may be more mature than those cultured in 2D. These observations are important since small numbers of more mature DC, rather than large numbers of less mature DC, may be more suitable for therapeutic purposes. Supported by subcontract NCC 9-36 under the Texas Medical Center-NASA/JSC Cooperative Agreement.


The Photosynthesis and Assimilation System Testing and Analysis (PASTA) experiment was funded for development by NASA Life Science Divisions Flight Program in response to NASA NASA 96-OLMSA-01A and will be a component of the technology verification testing of the Biomass Production System (BPS).

The objective of the PASTA experiment is to determine the effect of microgravity on photosynthesis and metabolism of Super Dwarf wheat in microgravity. Direct measurements of canopy gas exchange using a closed-system approach will permit CO2 and light response curves to be obtained from wheat canopies in space at different stages of development. The effect of μg conditions on electron transport processes associated with photosynthetic and respiratory gas exchange and subsequent carbon assimilation will be quantified post-flight. This experiment will be conducted in two plant growth chambers during the first flight of the BPS. One chamber will contain plants that have developed their photosynthetic apparatus under μg conditions (leaf blade present at launch), the other chamber will contain plants that will develop their photosynthetic apparatus under μg conditions (no leaf blade present at launch).

Photosynthetic measurements will also be conducted in the BPS wheat verification chamber being cycled through different relative humidity and temperature regimes in order to determine the impacts of differential vapor pressure deficit on photosynthetic characteristics under μg conditions.


The effects of chronic vibrations on specimens can, under some circumstances, produce deleterious experimental results. Gravitational Biology researchers using the Ames Short-Arm Centrifuge Facility require data on vibratory intensities and spectral content to assess the significance of such environmental effects on experimental results. The objective of the Short-Arm Centrifuge Vibration Characterization Tests is to empirically quantify steady-state vibratory amplitude and spectral content under several nominal operational settings representative of typical researcher-selectable conditions. Data are obtained with a sensitive single-axis accelerometer and a data-acquisition/processing/storage unit to digitize measured transducer signals in the time domain and convert data to the frequency domain via conventional fast Fourier transform (FFT) techniques. Data are acquired in 63 80-second runs, and spectral content plots between 0.5 and 100 Hertz are produced with consistent scaling to facilitate comparisons. Several operating conditions were repeated to ascertain common variances. Results show that facility environments are characterized by a broad-band spectral level of approximately 50.0 to 500.0 micro-g for most of 0.5 to 100 Hertz spectral range and narrow-band excitation peaks ranging from 1.0 to 50.0 milli-g, depending upon the particular equipment and operating condition being tested. Test results imply that: 1) acceptable experimental results can probably be obtained under typical operating conditions at specimen locations, 2) rotor balancing quality can readily affect vibration intensity on the centrifuge, 3) frequency modulation effects were more prevalent than initially envisioned on the rotating centrifuge and can significantly affect spectral energy distribution and density, and 4) spectral energy content has been sufficiently established under typical operating conditions so that proven vibration isolation techniques can be applied to attenuate higher frequencies (if required by researchers).

(Supported by NASA: NAS2-14263.)
SESSION J: CONCURRENT POSTER SESSION IV
SPACEFLIGHT EXPERIMENT RESULTS II
[146] DETERMINATION OF MICROGRAVITY EFFECTS ON POLLEN COLLECTION. M. Garber1 and M.E. Musgrave2. 1Dept. of Horticulture and 2Dept. of Plant Pathology & Crop Physiology, Louisiana State University, Baton Rouge, LA

Successful pollination of *Brassica rapa* under space flight conditions must first be achieved in order to study seed development. The study of the plant’s entire life cycle under microgravity is the goal of a joint U.S.-Russian experiment flying on the Russian space station Mir this year. Because successful pollination of *Brassica* in microgravity has not yet been determined, proposed methods must be tested beforehand. The number of pollen grains transferred from anthers to bee sticks during pollination was quantified and compared to ground control tests. Pollen was collected with bee sticks from 19-day old plants with open flowers in either 0-g (the KC-135) or 1-g environments. Pollen collected in this manner was quantified by using a hemacytometer to count grains released from the bee sticks by a detergent solution. Total numbers of pollen grains per bee stick were compared statistically using t-tests. In microgravity, bee sticks collected an average of 27,600 grains/flower. Results concluded that no significant difference occurred in microgravity compared to control. Supported by grants from NASA (NAG10-0139; NAG2-1020), the Louisiana Space Consortium, and the LSU College of Agriculture. This undergraduate research on the KC-135 was possible because of an award from the Texas Space Grant Consortium.

[147] POLLINATION OF *BRASSICA RAPA* UNDER MICROGRAVITY CONDITIONS ON THE KC-135. C. Smith1 and M.E. Musgrave2. 1Animal Science Dept and 2Dept. of Plant Pathology & Crop Physiology, Louisiana State University, Baton Rouge, LA

Plant reproduction under spaceflight conditions has a history of mixed successes. Successful reproduction by higher plants is necessary in space if we are to support human activity during long-term missions. Plants such as *Brassica rapa* will be essential to a biological life support system in microgravity due to its growth characteristics and the ability to obtain edible oil from its seeds. Pollination is the first step in reproduction. The actual transfer of pollen grains to a weightless environment could be affected by numerous physical factors. The actual number of pollen grains transferred to the pistil in microgravity was investigated. Pre-loaded bee sticks were used to pollinate the pistils of *Brassica rapa* in either 1-g or 0-g. Microgravity conditions were achieved aboard the "Zero Gravity Trainer" aircraft, the KC-135. Immediately post-flight, pistils were transferred to a 70% ethanol solution to prevent any pollen tube growth. Pollen was subsequently eluted from the stigmatic surfaces using 70% ethanol, concentrated by centrifugation, and counted. Pollen transfer was not affected by microgravity conditions since similar numbers of pollen grains were transferred both in-flight (47 ± 9 grains/pistil) and on the ground (67 ± 20 grains/pistil) (n = 20). Supported by grants from NASA (NAG10-0139; NAG2-1020), the Louisiana Space Consortium, and the LSU College of Agriculture. This undergraduate research on the KC-135 was made possible by an award from the Texas Space Grant Consortium.

[148] EFFECTS OF MICROGRAVITY IN SPACE ON LEVELS OF ISOFLAVONOID (GENISTIN AND DAIDZEIN) IN SOYBEAN CELLS. M. Gratiot1, M. L. Lewis2 and P. B. Kaufman1. 1Dept. of Biology, Univ. of Michigan, Ann Arbor, Michigan 48109-1048, and 2Johnson Research Center, Wilson Hall, Room 360, Univ. of Alabama, Huntsville, Alabama 35899.

Soybean cells were flown aboard the NASA Space Shuttle, Columbia, during the November 1996 flight in order to determine whether or not the micro-g environment of space altered the levels of medicinally important isoflavonoid metabolites (genistin, daidzein and their glucosides, genistein and daidzin) in these cells compared with cells held under similar conditions at 1 x g on Earth. After 16 days of space flight, the soybean cells from micro-g and from 1 x g environments were analyzed for levels of these four isoflavonoids. It turns out that cells flown in space had enhanced levels of the glucoside moieties (genistin and daidzin) of these isoflavonoids, by one order of magnitude, compared with levels obtained from cells grown on Earth at 1 x g. There was no change in levels of the aglycones, genistein and daidzein in the two environments. What this tells us is that the stress conditions that prevail in a micro-g environment cause net synthesis of the storage glucosyl conjugate forms of these isoflavonoids, but not the non-storage forms. Similar to what happens during drying stress in maturing soybean seeds here on Earth. These results with soybean cells grown in space have important implications for life support in space, space medicine, and prevention of loss of bone calcium and bone mass density that occur in humans subjected to long-term missions in space.

[149] EMBRYONIC AND LARVAL DEVELOPMENT OF SEA URRCHINS IN MICROGRAVITY. H.-J. Marthy1, G. Gasset2, B. Eche1 and R. Bacchieri1. 1 Observatoire Océanologique/ CNRS, Banyuls sur mer, 2: G.S.B.M.S. - Faculté de Médecine-Rangueil, UPS, Toulouse, France

Studying embryonic and larval development of suitable animal models in the near absence of gravity (mg) is a promising approach for a better understanding of the role of gravity in ontogenesis. Using eggs, embryos and larvae of sea urchins (*Paracentrotus lividus*; *Sphaerechinus granularis*) as models, we studied the potential impact of mg on fertilization, morphogenesis and skeletogenesis as an organogenetic event. The experiments were performed during the space flights of sounding rockets (Maser 4-6 in 1990-93), the Russian satellite Photon-10 (IBIS of CNES) in 1995 and the American space shuttles STS-65 (IML-2) in 1994 and STS 76 (S/MM-03) in 1996. Our presentation illustrates the technical aspects of the experiments and gives an overview of the essential scientific results. As to the latter, it appears that fertilization, morphogenesis and skeletogenesis occur essentially normally in mg. Larvae develop normally in space and, when recovered, continue on the ground. However, gravity (and mg) are actually perceived by the cleaving eggs and the developing embryos and larvae, and this is expressed by modifications of the cell cycle length, the developmental speed and the positioning of the skeletogenic cells. Ref.: Marthy, H.-J. et al.: Adv. Space Res. 14, 1994; J. of Biotechnol. 47, 1996; ESA SP-1206, 1997, Adv. Space Res., 1997 (Supported by CNES, ESA and CNRS.)

The skeletal system of vertebrates is known to adapt to various conditions of loading or unloading such as those exerted by gravity, buoyancy, or mechanical forces. To characterize spaceflight effects on bone cells, the presence of non-collagenous proteins in osteoblast cultures derived from calvaria of normal 17 day old embryonic chicks was examined following a recent NASA shuttle mission. Cells (~7x10^6, grown in DME + 10% FBS supplemented with 8-glycerophosphate and ascorbate) were inoculated into Cellocell Cellmax Quad bioreactor cartridges (Celloc, Inc., Germantown, MD) and flown during STS-63 (February 3-11, 1995). Other cartridges, identicaly prepared, were maintained at normal gravity until launch (basal cultures) or shuttle landing (ground controls). Cartridge contents were aldehyde-fixed, dehydrated, embedded in Spurr resin, and sectioned for electron microscopy. Proteins synthesized by the cultured osteoblasts were identified and localized by immunocytochemistry using the protein A-gold method (1). Osteopontin, bone sialoprotein, and osteocalcin were found in the extracellular matrices from flight cultures but with reduced immunoreactivity compared to that in control and basal cultures. The appearance of such non-collagenous proteins, as well as type I collagen observed in other current studies, is consistent with previous work demonstrating some of these constituents in the respective cartridges by biochemical means and an apparent down-regulation of collagen and osteocalcin gene expression in spaceflight (2). The presence of the proteins here can be correlated with the progressive development of mineral also detected in STS-63 flight and control cartridges as well as in vivo under normal gravity. These results suggest that spaceflight mediates adaptation of skeletal structure and underscore the utility of this chick osteoblast culture model for assessing skeletal changes in response to gravity or environmental forces in general.


[152] EFFECTS OF SPACE FLIGHT DURING PREGNANCY ON RAT FETAL MAINTENANCE, OVARIAN FOLLICLES, AND CORPORA LUTEA POSTPARTUM. H. W. Burden, J. Zary, and J. R. Alberts. Dept. of Anatomy and Cell Biology, East Carolina University, Greenville, NC and Dept. of Psychology, Indiana University, Bloomington, IN.

The large ovarian corpora lutea formed from ovulated follicles secrete progesterone and help maintain pregnancy. Postpartum, the ratio of corpora lutea to live conceptuses (fecundity ratio) is a useful metric for evaluating the effects of various treatments during pregnancy on fetal maintenance and development. Since rats have a postpartum ovulation, during pregnancy, follicular growth (and atresia) occurs in preparation for this event. The objective of the present study was to evaluate the effects of space flight during pregnancy on the fecundity ratio and the population of healthy and atretic follicles. Pregnant rats were launched on gestation (G) day 11 and recovered on G-20 (July 22, 1995: STS-70). Six rats were allowed to go to term and approximately three hours after vaginal delivery the ovaries were removed, trimmed, weighed and fixed by immersion in Bouin’s fluid and subsequently sectioned serially and stained with hematoxylin and eosin. Each section was examined and all antral follicles, both healthy and atretic, were counted at the level of the oocyte nucleus. Corpora lutea of pregnancy were also counted. Follicles were assigned to one of four size categories. Space flight did not alter the fecundity ratio. Also, space flight did not affect the number of antral follicles in any of the four size categories or the number of healthy follicles in three of the four categories. Space flight significantly increased (p < 0.001) the number of large antral follicles 401-570 μm diameter. These data show that space flight during days 11-20 of gestation in rats has no effects on the development and maintenance of live fetuses in utero. Since only a small percentage of follicles 401-570 μm undergo atresia, the data suggest that space flight may promote the development of increased numbers of large antral follicles to move into the ovulatory pool of follicles. (Supported by NASA NCC 2-870)


The NIH-R4 payload was a collaborative experiment conducted by NASA’s Ames Research Center in conjunction with the National Institutes of Health (NIH). This middeck payload was the fourth in a series of experiments focusing on developmental biology and the effects of microgravity on mammalian systems.

The NIH-R4 payload was flown onboard STS-80, which launched 11/19/96 and landed at Kennedy Space Center on 12/7/96 and was the longest shuttle mission to date. Fourteen male Spontaneously Hypertensive rats (SHR) were flown; seven in each of two Animal Enclosure Modules (AEM) in the shuttle middeck. The flight animals were exposed to 18 days of microgravity. Two synchronous control groups were utilized for this study in addition to an asynchronous post-flight AEM control study conducted at the PI lab.

The animals were fed two different calcium diets in the NASA food bar (2.0% and 0.2%) three weeks prior to launch and inflight. Blood pressures were taken at predetermined intervals and were the basis for flight selection. Upon recovery, flight animals blood pressure was measured and a variety of tissues were collected. Project testing and data will be presented.
[154] BIORACK ON SPACEHAB MISSIONS TO MIR. R.L. Schaefer¹, J.L. Fishman², P.D. Savage³, P. Davies⁴, C. Brillouet⁵, and E. Brinckmann⁶. ¹Lockheed Martin Engineering & Sciences, SLO, NASA-Ames Research Center, Moffett Field, CA; ²ESA - European Space Research and Technology Centre, Noordwijk, the Netherlands.

The ESA-developed Biorack with 3 successful Shuttle missions behind it flew 3 more times -- integrated into Spacehab on STS-76, 81, and 84. On these missions Biorack housed two incubators each with 2 variable g centrifuges, a Glovebox, 2 Passive Thermal Conditioning Units (for temperature-controlled late access), and ambient stowage trays. The NASA LSLE refrigerator/freezer was also used by many Biorack experiments. Presently will be the management structure and techniques used for accommodating numerous experiments from many European countries and the U.S. Special emphasis will be on the challenges of performing the experiments within the constraints of the docking mission timelines.

[155] MICROGRAVITY STIMULATES CHANGES IN PROTEIN PHOSPHORYLATION DURING INITIATION OF MOTILITY IN SEA URCHIN SPERM. J.S. Tash, J.J. Fritch, M.E. Landis, G.E. Bracho. Dept. Molecular & Integrative Physiology, Univ. of Kansas Medical Center, Kansas City, KS.

Previous European Space Agency (ESA) studies demonstrated that bull sperm swim with higher velocity and greater flagellar bend angles in microgravity (µG) than at 1G. Since protein phosphorylation and sperm motility are coupled, we examined whether changes in protein phosphorylation that occur during initiation of sperm motility are altered in µG. Experiments were conducted using the ESA Biorack flown on STS-81 and 84. Completely immotile sea urchin sperm were collected and loaded into ESA 'Phorbol' hardware in identical flight and ground controls sets. Sperm were activated to swim (80-90% motility) at launch + 20h by dilution into artificial sea water (ASW). Activation was terminated after 0, 30, and 60 s with electrophoresis sample buffer. Samples were then stored at -20°C until analysis on the ground. Parallel ground controls were performed in the Biorack ground unit at KSC 2h after the flight experiment. Prior vibration and acceleration tests on sperm in flight hardware demonstrated no effect of these launch parameters on sperm viability. Ground controls during STS-81 and STS-84 included measurement of motility in parallel incubations to assess viability at the same time as the flight experiments. Immunoblot analysis of phosphorysine, phosphothreonine, and phosphotyrosine demonstrated that the changes in phosphorylation that occur during initiation of motility in µG occur 2-4 times faster than at 1G. The proteins that become phosphorylated during initiation of motility were the same at 1G and µG, including subunits of dynein. The reversal in protein phosphorylation after initiation also occurred faster in µG than at 1G. The shortened window of maximal phosphorylation during motility initiation suggests that µG may shorten the window of optimal fertility of sperm. This has important implications for efficiency of fertilization and energy utilization by motor proteins in µG. (Supported by NASA NAG2-1016 and NIH HD-33994)

[156] HEAVY WATER INHIBITS CHANGES IN PROTEIN PHOSPHORYLATION DURING INITIATION OF MOTILITY IN SEA URCHIN SPERM. G.E. Bracho J.J. Fritch, M.E. Landis, J.S. Tash. Dept. Molecular & Integrative Physiology, Univ. of Kansas Medical Center, Kansas City, KS.

Activation of sperm motility is coupled to changes in phosphorylation of flagellar proteins. Previous European Space Agency (ESA) studies demonstrated that bull sperm swim with higher velocity and greater flagellar bend angles in microgravity (µG) than at 1G. In experiments conducted on STS-81 and STS-84 using the ESA Biorack, we demonstrated that the changes in protein phosphorylation during initiation of sea urchin sperm motility occur significantly faster in microgravity (µG) than at 1G. Since motility and phosphorylation are coupled, one possible explanation for the more rapid motility and signal transduction in µG is that water has lower viscosity in µG. Lower viscosity would allow more rapid motility, hence more rapid changes in protein phosphorylation. The converse hypothesis is that higher viscosity will inhibit sperm motility and thus the associated changes in protein phosphorylation. To test this latter hypothesis, sea urchin sperm were spawned into sperm storage buffer made with H₂O (MSSB) or D₂O (HSSB). D₂O has 11% higher viscosity than H₂O at 22°C. MSSB and HSSB keep sperm in a completely immotile state until dilution with artificial sea water (ASW), whereupon up to 90% motility is achieved. However, if sperm in HSSB were diluted into ASW made with D₂O (HASW), then initiation of motility was inhibited by 60-80%. Parallel analysis of the changes in protein phosphorylation under these conditions using western immunoblotting to detect phosphoserine, phosphothreonine and phosphotyrosine demonstrated that the rate of change in phosphorylation of key flagellar proteins associated with activation of motility was also diminished in the D₂O environment. This suggests that changes in phosphorylation during activation of sperm in HASW in µG might be similar to sperm in ASW at 1G. This latter experiment was conducted on STS-84 and is currently under analysis. (Supported by NASA NAG2-1016 and NIH HD-33994)


Current models describing feeding and locomotor processes in marine zooplankton suggest that gravity plays an integral role in the development and life histories of these animals. The complex planktonic development phase and the post-larval stages of heavier plankters such as bivalve larvae represent a resolution of tradeoffs between optimising feeding and locomotor efficiencies in a three-dimensional, low Reynolds number (Re) environment. Although the amount of energy allocated to locomotion would be minimal in neutrally buoyant animals, low Re fluid dynamics models suggest that feeding efficiency should be increased if the animal is capable of creating shear between feeding appendages and surrounding fluid. In bivalve larvae, this shear is created by the rapid beating of metachronally-coordinated cilia acting to pull the relatively heavy animal against the gravity vector. This same mechanism also serves to orient the larva within the water column, which is of vital importance for a small animal without gravity- or photo-sensing organs that must stay close to the surface where its food source is located. An experiment designed to investigate the fundamental issues comprising the basis of these models was conducted using the Canadian Space Agency's Aquatic Research Facility (ARF) during STS-77 in May 1996. Larvae of the blue mussel Mytilus edulis were observed throughout the 10-day mission using a macroscopic video recording system, and samples of larvae and their algal food source were preserved on Flight Days 3, 5, and 7. Post-flight analyses revealed little evidence to support hypotheses of active buoyancy regulation; however, significant changes in behaviour and orientation were observed. These observations support a hypothesis describing a wide-animal solution to orientation mechanisms in animals without dedicated sensory organs, and help form a comprehensive model outlining the role of gravity in the basic biology of marine zooplankton.
SESSION J: CONCURRENT POSTER SESSION IV
PLANT DEVELOPMENT, GROWTH AND GENETICS II

The effects of activators and inhibitor of the second messenger signal transduction system in animals were examined to determine possible interaction with plant somatic embryo initiation and development of tissue explants taken from immature seeds of different cultivars of Brassica rapa. Non-embryogenic callus was induced in several cultivars of B. rapa cultured on media supplemented with various concentrations of 2,4-D: embryogenic callus was obtained when B. rapa cv. var was cultured for 4 weeks on MS medium containing various concentration of 2,4-D and then transferred to the same medium containing 5 mg/l of 2,4-D. After 1 week of culture, embryogenic callus of B. rapa cv. var on MS hormone-free liquid medium produces somatic embryos with a functional bipolar organization possessing a fused cotyledon and a completely differentiated radicle. Development of adventitious shoot from somatic embryos was significantly promoted by treatment with TPA. Treatment of mature somatic embryos with mTPA was effective in decreasing kinetin-induced adventitious shoot development. Treatment with TPA increased the development of adventitious roots significantly, whereas the treatment of mature somatic embryo with mTPA was effective in decreasing IAA-induced adventitious root development. The treatment with mTPA also completely eliminated phosphoproteins of 19- and 45-kDa in vitro protein phosphorylation. The treatment with mTPA, a negative control of TPA, also decreased protein kinase activities. In this study, we observed the inhibitory effects of mTPA on both the kinase activity and the regeneration of adventitious organs. These results suggest that the hormonal signal involved in the control of root and shoot initiation and/or development during somatic embryogenesis may be transduced by second messenger systems.

[158] ELECTRICAL SIGNALS AND GENE EXPRESSION IN PLANTS. E. Davies, A. Vian, and C. Vian. Botany Department, North Carolina State University, Raleigh, NC

When one tomato leaf is flame-wounded, a variation potential is generated, when one is electrically-stimulated an action potential is generated. These electrical signals are transmitted rapidly throughout the plant, evoking accumulation of protease inhibitor (PIN) mRNA (Stankovic and Davies, FEBS Lett 390:275-279).

In order to determine the array of genes turned on by the variation potential, we have constructed a subtractive library (flame minus control) and isolated over 600 putative up-regulated clones. Of these, 36 have been examined and all found to be up-regulated, and of these, 12 have been sequenced and 10 different up-regulated mRNAs identified. Some of these mRNAs increase as much as 5-fold within 15 minutes in a leaf 5 cm distant from the wounded leaf before declining to basal levels within 1 hour and remaining at that level. Others show a second increase in activity, often peaking at about 6 hours. We are using hormone (e.g., ABA, ethylene, gibberellin, auxin) mutants to decipher the comparative roles of electrical signals and hormones on synthesis and degradation of these mRNAs.

In a related series of experiments we have given a short heat pulse to one leaf of an aequorin expressing Arabidopsis plant and shown that cytoplasmic Calcium levels begin to rise throughout the plant within the first 3 seconds, are maximal at 6 seconds and then decline to close to basal levels within 12 seconds. We are currently trying to determine what role is played by these transient increases in cytoplasmic calcium on the transient accumulation (synthesis and degradation) of these mRNAs.

(Supported by the NSCORT in Gravitational Biology at North Carolina State University and by NSF grant #IBN-93-10508 to ED).


1Dynamac Corp. and NSCORT in Gravitational Biology, NC State Univ, Raleigh, 1Dynamac Corp., KSC, 3NASA Biomedical Office, KSC, 1Dept of Botany, Univ of Wisconsin, Madison, and 3Dept of Horticulture, University of Wisconsin, Madison.

Plant-based bioregenerative life support systems (BLLS) have been proposed for long-term exploration and habitation of space by humans. White potato (Solanum tuberosum L.) is one of the candidate species under investigation for a BLLS due to its high yield potential, high harvest index, ease of propagation and nutritional value. Other plant species cultured in space have exhibited altered growth and metabolic properties. Therefore, our objectives were to determine if the spacecraft environment affected 1) tuber formation on potato leaf explants and 2) tuber carbohydrate concentration and metabolism. Five potato leaf explants were flown on the Space Shuttle Columbia as part of STS-73, a 16-day mission. The Astroculture flight unit provided growth conditions conducive to tuber formation. Environmental data, including CO2 concentrations, were recorded in-flight. Plants were recovered postflight for growth and metabolic measurements. Tuber formation took place in space. Four of the five explants produced tubers which were not different from the ground controls with respect to diameter or fresh weight. The dry/fresh weight ratio was lower in the space-grown tubers, which may have been due to the accelerated leaf senescence in space. Of eight enzymes of carbohydrate metabolism measured, only the activity of the starch synthetic enzyme ADP-glucose pyrophosphorylase (AGP) was different compared to the ground controls. Although AGP was lower in the space-grown tubers, there was no difference in starch concentration when expressed as a percentage of the dry weight. Potato tuber formation and starch deposition took place in space, however there are indications that some aspects of starch metabolism may be sensitive to microgravity.

(Supported by NASA Grant NAGW 4022 to CSB, JGC and TWT and NASA Contract NAS10-12180 with the Dynamac Corporation).

[160] REPRODUCTIVE ONTOGENY OF WHEAT GROWN ON THE MIR SPACE STATION. D.L. Rubenstein and J. Stieber. NASA Ames Research Center, Space Technology Division, Moffett Field CA

The reproductive ontogeny of 'Super-Dwarf' wheat grown on the space station Mir is chronicled from the vegetative phase through flower development. Changes in the apical meristem associated with transition from the vegetative phase to floral initiation and development of the reproductive spike were all typical of 'Super Dwarf' wheat up to the point of anthesis. Filament elongation, which characteristically occurs just prior to anthesis and moves the anthers through the stigmatic branches thus facilitating pollination, did not occur in the flowers of spikes grown on Mir. While development of spikes on tillers typically occurs later than that of spikes on the main stem, all flowers appear to be arrested at the same developmental point.
SESSION J: CONCURRENT POSTER SESSION IV
PLANT PHYSIOLOGY II
[161] GRAVISTIMULATION INDUCES CHANGES IN SUCCROSE PHOSPHATE SYNTHASE ACTIVITY IN MAIZE PULVINI. J.L. Huber1, H. Winter1, and S.C. Huber2. 1 Horticultural Science and 2Botany Dept., NC State Univ., and 3USDA-ARS, Plant Science Res. Raleigh, NC

Gravistimulation of maize induces bending exclusively in the stem pulvinus. The growth response of the stem appears to be due to the differential elongation of cells on the lower side of the stem relative to the upper side. After 12 - 14 h of stimulation, Sucrose Phosphate Synthase (SPS) activity decreases on the lower side of the bending stem compared to the upper portion. The decrease in SPS activity in the elongating cells is consistent with the predicted metabolic changes which would require sucrose hydrolysis rather than synthesis for the increased demands of respiration and cell wall synthesis.

Immunological evidence suggests that the maize pulvinus SPS is distinct from the leaf enzyme. Current studies are exploring whether the changes in pulvinus SPS activity during gravirespons are due to regulatory protein phosphorylation or altered enzyme protein levels. (Supported by the NSCORT in Gravitational Biology at North Carolina State University, NASA grant NAGW 4984)


The objective of these investigations is to establish a baseline for interpreting effects of spaceflight conditions (STS-89, STS-90) on Ceratophyllum demersum L. cultivated within the C.E.B.A.S.-Mini Module. The module, which fits within a standard Space Shuttle mid-deck locker, is a self-sustaining ecosystem capable of supporting its aquatic inhabitants (fish, snails, plants and bacteria) up to 3 wk for. The biological components of C.E.B.A.S. are contained in an 8.6L aquarium tank with no surface open to the atmosphere. Physical and chemical parameters (pH, O2 concentration, temperature, etc.) are monitored continuously and living components of this closed ecosystem maintain a stable, biologically vital system. We report here on data obtained from two Payload Verification Tests (PVTs) conducted at KSC during July and August 1997 relating to the pre- and post-PVT pigment and carbohydrate composition within the aquatic angiosperm C. demersum (the "plant" component of C.E.B.A.S.). For pigments, tissues frozen at the beginning and end of experiments were extracted in acetone. Compounds were identified and quantified by reverse phase HPLC/Diode array detector. C. demersum contained all of the major pigments characteristic of higher plants (chlorophylls a & b, α- and β-carotene, neoxanthin, violaxanthin, lutein). Photosynthetic pigment composition is a key indicator of plant health status and relates directly to plant O2 production capability, which is vital for the system's performance. Soluble sugars and starch were also measured. Sugar composition provides another key measure of photosynthetic capability, and will provide the opportunity to test previous results on terrestrial angiosperms relating to a shift in carbohydrate metabolic pathways under conditions of microgravity. (Funded under DAR Grant #WS50WB9319-1, Ministry of Sci. and Res. Grant of NRW #IVA1216-00588, and NASA contract NAS10-12180)

[163] ESTIMATION OF THE ANATOMICAL, STOMATAL, AND BIOCHEMICAL COMPONENTS OF DIFFERENCES IN PHOTOSYNTHEIS AND TRANSPIRATION OF WILD AND TRANSGENIC (EXPressing YEAST DERIVED INVERTASE TARGETED TO THE VACUOLE) TOBACCO LEAVES. L.B. Pachepsky1, B. Acok1, S. Hoffman-Benning1, L. Willmitzer1, and J. Fisahn1. 1USDA-ARS, Remote Sensing & Modeling Laboratory, Beltsville, MD; 2Duke University, Durham, NC; 3Max-Plank-Institute fur molekular Physiologie, Golm, Germany.

Photosynthesis and transpiration rates of transgenic (expressing yeast derived invertase targeted to the vacuole) tobacco (Nicotiana tabacum L.) Leaves were, respectively, 50 and 70% of those of a wild type at 20°C, 350 ppm CO2 concentration, 450 mmol (photons) m^-2 s^-1 of light intensity, and 70% relative air humidity. These differences could be attributed (a) to changes in leaf anatomy and, consequently, to changes in gases diffusion between the cells' surfaces and the atmosphere, (b) to different stomatal apertures, and, for photosynthesis rate, (c) to the altered CO2 assimilation rate. Our objective was to estimate a relative contributions of these three sources of differences. Measurements on the wild type and the transgenic leaf cross-section showed that the cell area indices (CAI, cell area surface per unit leaf area surface) had values equal to 15.91 and 13.97, respectively.

The two-dimensional mode 2DLEAF for leaf gas exchange was used to quantitatively estimate anatomical, stomatal, and biochemical components of these differences. Transpiration rate was equal to 0.9 for the wild type and 0.63 mmol m^-2 s^-1 for the transgenic leaf. 24.0% of the difference (0.066 mmol m^-2 s^-1) was caused by the greater cell area surface in the wild-type leaf, and 66.0% caused by a smaller stomatal aperture in the transgenic leaf. Photosynthesis rate was 3.10 and 1.55 mmol m^-2 s^-1 for the wild-type and transgenic leaves, respectively. Only 10.3% of this difference (0.16 mmol m^-2 s^-1) was caused by the difference in CAI, and the remaining 89.7% was caused by altered CO2 assimilation rate.
SESSION J: CONCURRENT POSTER SESSION IV
PLANT GRAVITY PERCEPTION II
[164] AUTOTROPIC STRAIGHTENING AFTER GRAVITROPIC CURVATURE OF LEPIDIDIUM ROOTS B. Stankovic, D. Volkman, and F.D. Sack. Department of Plant Biology, Ohio State University, Columbus, OH; Botanisches Institut, Universität Bonn, Germany.

The term "autotropism" has been used to describe several phenomena related to gravitropism. To clarify whether autotropism straightening occurs after gravitropic curvature, curved roots of Lepidium sativum (cress) were rotated on a clinostat and individual roots were followed through time. Roots that were horizontal for 1 h curved down 60°. After 6 h of clinostat rotation, gravitropic curvature was lost from almost all roots and they straightened. However, the final angle was 24° from the prestimulus vertical indicating that some gravitropism had been retained. We tested whether roots that were completely curved gravitropically (90°, 5 h horizontal) would also straighten on a clinostat. Most such roots lost all gravitropic curvature and straightened, but their final angle (53°) was further from the prestimulus vertical than roots stimulated for 1 h. Control roots (moved from the vertical to the clinostat without horizontal stimulation) were slanted at various angles after 6 h of rotation. These results provide a clear demonstration of the phenomenon of autotropistic straightening of gravitropically-curved roots after the randomization of the g stimulus on a clinostat. They indicate that gravitropic curvature is not necessarily permanent, and that the root retains some "memory" of its orientation prior to gravitropic stimulation.

(Supported by NASA: NAG2-1023 to F.S., and by DARA: 50 9429 and MWF to D.V.)

[165] INTERACTION BETWEEN GRAVITROPIC AND PHOTOTROPISM IN PROTONEMATA OF THE MOSS CERATODON. V.D. Kern, and F.D. Sack. Dept. of Plant Biology, Ohio State Univ., Columbus, OH.

Moss protonema are among the very few cell types known that both sense and respond to gravity and light. Apical cells of Ceratodon protonema grow by oriented tip growth which is negatively gravitropic in the dark or positively phototropic in unilateral red light. Phototropism is phytochrome-mediated and limited data suggest that PFR also represses gravitropism. To determine whether any gravitropism persists in the presence of red light of different intensities, cultures were turned at various angles with respect to gravity and simultaneously illuminated so that the light and gravity vectors acted in the same or in different directions. Unilateral red light for 24 h at intensities >100 nMol s^{-1} m^{-2} caused the majority of protonemata to be oriented directly towards the light. Similarly, protonemata grew directly towards the light regardless of light position with respect to gravity indicating that at these light intensities all growth is oriented strictly by phototropism, not gravitropism. At light intensities lower than 100 nMol s^{-1} m^{-2}, no phototropism occurs and mean protonemal tip angle is above the horizontal; however, protonemata are not upright as they would be in the dark indicating that low intensity red light permits some gravitropism but also modulates the response. Protonemata of an aphototropic mutant of Ceratodon, prt-1, which lacks a functional phytochrome chromophore, exhibit gravitropism regardless of red light intensity. These data indicate that red light acts via phytochrome to modulate gravitropism at low intensities and to completely suppress gravitropism at intensities >100 nMol s^{-1} m^{-2}.

The effects of microgravity on tropsins and on development will be evaluated as part of the Collaborative Ukrainian Experiment on STS-87 (currently scheduled for Nov. 1997) using newly developed hardware that enables both unilateral illumination and chemical fixation in petri dishes. (Supported by NASA: NAG10-0179.)

[166] CHARACTERIZATION OF NUCLEAR PEA ANNEXINS. G.B. Clark, M. Dauwalder, and S.J. Roux. Department of Botany, Univ. of Texas, Austin.

Annexins are a multigene family of calcium-dependent, membrane-binding proteins which have been implicated in secretory processes, polar growth and cell wall formation in plant cells. Because calcium changes appear to be critically involved in the signalling steps leading to gravitropism, and because this growth response requires the asymmetric secretion of new wall materials, our laboratory has been interested in testing a possible role for annexins in gravitropism. While carrying out an immunolocalization study of annexin distribution in peas during gravitropism, we found that leaf and stem epidermal strips, which required a shorter fixation time than conventional tissue blocks and were not subjected to the usual dehydration and embedding procedures, showed a strong nuclear staining pattern not reported in prior immunocytochemical assays. Nuclear staining was also seen in cell layers prepared from pea plummules. The amount of nuclear stain was reduced both by increased fixation time and by treatment with organic solvents during dehydration and embedding. Observation with confocal microscopy demonstrated that the anti-p35 stain was diffusely distributed throughout the nuclear structure. The immunolocalization findings were confirmed by immunoblots. Purified pea nuclei, nuclear envelope, and chromatin fractions all showed a cross-reactive annexin protein band at 35 kDa. In animal cells, monomeric annexin II and several other annexins have been documented in nuclei. Annexin II has been suggested to function as part of the primer recognition protein (PRP) complex with alpha DNA polymerase in lagging strand DNA replication and DNA repair. Following a procedure used for animal PRP isolation we have also demonstrated that the corresponding pea protein fraction contains annexin. These data are the first to show annexins are in plant cell nuclei, and they reveal that nuclear annexins are another potential target of calcium action during calcium-mediated growth changes in plants. (Supported by NASA: NAGW 1519.)


Gravity-induced upward bending of maize stems occurs because of cell elongation on the lower side of the pulvinus, a narrow disc-shaped region of cells found in the stem immediately above each node. While it is the pulvinus that contain amyloplasts and generate bending (internodes neither bend nor contain starch), not all pulvinus will be involved in any particular graviresponse. There is an upward migration of the potential bending site that accompanies stem maturation, with each pulvinus having, in turn, maximum potential for bending during the days following the cessation of elongation in the internode immediately above it. During this time, lignin reinforcement of bundle sheath cell walls occurs immediately above and below the pulvinus while cell walls in the pulvinus remain unreinforced. Concurrent with the cessation of internodal elongation, the internodal microtubules reorient from transverse to oblique. In the pulvinus, the microtubules of parenchyma cells remain transverse while those in the unligified bundle sheath cells become oblique. As the stem matures further, the potential bending site migrates further up the stem and lignification and microtubule realignment, from transverse to oblique, occur within the now inactive pulvinus. On gravistimulation, one or several pulvinus respond over the course of several days, usually to a maximum of 35° per pulvinus. In these responding pulvinus, there is no microtubule reorganisation, and the changes eventually observed in microtubule structure 10 days after gravistimulation correlate to the natural changes in microtubules pattern associated with stem/pulvinus maturation. These results clearly demonstrate the tight developmental controls on the maize stem pulvinus. (Supported by the NSCORT in gravitational biology at the North Carolina State University.)
[168]
GRAVITACTIC BEHAVIOR OF CHLAMYDOMONAS. L.J. Feldman J. Nemson and V. Kam. Dept of Plant Biology, Univ of California, Berkeley.

We are investigating the evolution and development of the gravitactic process in plants and for this effort are using the unicellular green alga Chlamydomonas reinhardtii. We have confirmed that Chlamydomonas exhibits negative gravitaxis and have developed simple spectrophotometric methods to monitor this response. Cells in mid-log phase (2-5 x 10^6 cells/ml) are used to fill tubes of various diameters (0.9-4 mm), and lengths of 60-160 mm. The filled tubes are then placed in darkness for various times (usually 1 hr) and then individual fractions from the tubes are collected and spectrophotometrically monitored at 750 nm. At this wave length there is a linear correlation between absorbance (0-1.5 OD) and cell concentration (2x10^5-1x10^7 cells/ml). We demonstrate that a sub-population of cells responds negatively to gravity and swim upward. This ability to respond to gravity appears sensitive to a number of environmental factors, including pH and temperature of the medium. We will discuss this quantitative characterization of the gravitactic response and as well, consider some mechanistic implications.

[169]
THREE-DIMENSIONAL ANALYSIS OF NUCLEAR SIZE, SHAPE AND DISPLACEMENT IN CLOVER ROOT CAP STATOCYTES FROM SPACE AND A CLINOSTAT. J.D. Smithb, P.W. Todd, L.A. Staelinb, NASA Ames Biocomputation Center, Moffett Field, CA; Deps. of Chemical Engineering and MCD Biology, Univ. of Colorado, Boulder, CO.

Under normal (1g) conditions statocytes of root caps have characteristic polarity with the nucleus in tight association with the proximal cell wall; but, in altered gravity including microgravity (μg) and a clinostat (cg), movement of the nucleus away from the proximal cell wall is not uncommon. To further understand the cause of gravity-dependent nuclear displacement in statocytes, three-dimensional reconstruction techniques were used to precisely measure volumes, shapes, and positions of nuclei in white clover (Trifolium repens) flown in space and rotated on a clinostat. Seeds were germinated and grown 72 hours aboard Space Shuttle (STS-63) in the Fluid Processing Apparatus (BioServe Space Tech., Univ. of CO). Clinerotation experiments were performed on a two-axis clinostat (BioServe). Computer reconstruction of selected groups of statocytes were made from serial sections (0.5 μm thick) using the ROSS (Reconstruction Of Serial Sections) software package (Biocomputation Center, NASA, ARC). Nuclei were significantly displaced from the tops of cells in μg (4.2±1.0 μm) and cg (4.9±1.4 μm) compared to 1g (3.4±0.8 μm); but, nuclear volume (113±36 μm³, 127±32 μm³, and 125±28 μm³ for 1g, μg, and cg) and the ratio of nuclear to cell volume (4.3±0.7%, 4.2±1.0% and 4.9±1.4%) were not dependent on gravity treatment (ANOVA, α=0.05). Three-dimensional analysis of nuclear shape and proximity to the cell wall, however, showed that nuclei from 1g appeared ellipsoidal while those from space and clino- stat were more spherically shaped. This change in nuclear shape may be responsible for its displacement under altered gravity conditions. Since cytoskeleton affects nuclear polarity in root cap statocytes, those cytoskeletal elements could also control nuclear shape. This alteration in nuclear shape and position in μg and cg compared to 1g may lead to functional differences in gravity signaling systems of plants subjected to altered gravity environments.

[170]
THE ROLE OF BASIPETAL AUXIN TRANSPORT IN ROOT ELONGATION, GRAVITROPISM AND WAVING IN ARABIDOPSIS THALIANA. S.R. Brady, R.C. Reed, and G.K. Muday. Dept. of Biology, Wake Forest Univ., Winston-Salem, NC.

The role of polar auxin transport in root elongation, gravitropism, and waving in Arabidopsis thaliana has been examined in preparation for analysis of mutants altered in these growth processes. Growth of roots on NPA containing agar reduces elongation, gravitropism and root waving, suggesting that all three processes depend on auxin transport. Gravitropism and waving are more sensitive to inhibition by NPA with I₅₀ values (concentration where processes are inhibited by 50%) of 0.5 and 0.4 μM respectively, as compared to the I₅₀ for elongation of 4.7 μM. In roots, auxin is transported in two distinct polarities and each polar stream may be responsible for different growth and developmental events. In order to determine which polarity controls these growth processes, it was necessary to selectively block one of the two polarities and observe the effect. Transport of auxin from the shoot into the root was previously shown to control development of lateral roots. The hypothesis that auxin transport from the root tip toward the base controls these processes was tested. It was found that 5 mm root tips excised from the rest of the seedling were able to elongate, respond to gravity and wave. The inhibition of these processes by NPA yielded similar I₅₀ values as for intact plants. Application of agar containing 10 μM NPA to the root tip led to an immediate loss of gravitropism. In contrast, reorientation of apical auxin movement by excision of the shoot, NPA application to the root/shoot junction or dark growth does not immediately affect gravitropism. These results are consistent with auxin moving from the tip toward the base controlling root elongation, gravitropism, and waving. Experiments are currently in progress to examine the question of whether auxin moving from the tip arises at the tip or is auxin from the shoot that is redistributed at the tip. (Supported by NASA grant NAGW-4052 and the NSCORT in Gravitational Biology at North Carolina State University)

[171]
MATHEMATICAL MODEL OF ROOT GRAVIREACTION. B.I. Lev', A.V. Kondrachuk'. Institute of Physics, Kiev, Ukraine, NASA Ames Research Center, CA, USA.

The present mathematical model is based on prior results of the qualitative model, mathematical approaches and known experimental findings. The following chain of events resulting in the gravitropic reaction of the root has been taken into account in modeling: a) the graviperception mechanism (GPM) localized in the cap region of the root produces the signal in response to the change of the G-vector orientation relative to the root axis; this signal modulates initially uniform lateral distribution of some specific substances (S) in the cap region; b) this already nonhomogeneous lateral distribution of S is transferred (and transformed during transfer) to the zone of elongation (ZE) to initiate the change of growth rate on opposite sides of the root; c) the different rates of growth cause the bending of the root resulting in the change of the GPM signal in the cap region and then in the change of the lateral distribution of S in this region; the processes of graviperception, gravirespond and transduction of information from the GPM to the ZE are spatially separated as well as the pathways of S transportation to and from the cap.

Because the root gravireaction involves a complex chain of the spatially distributed processes of different nature, the main goals of the work were: to define and formulate the mathematical constructions corresponding to these processes; to incorporate these constructions into one model (equation(s)) which could allow for comparison the results of modeling with the observed experimental data; and to indicate and analyze the simplifications of modeling. The kinetics of the root apex bending (angle A) in response to the time(t)-dependent change of the G vector orientation was described by the integral-differential equation relative to A(t) (spatially 1-D model). The results of modeling were found to be in a good correlation with the known experimental data which allowed us to estimate and analyze the parameters of the root gravireaction model. The work was supported by NRC, NASA and Ukrainian Space Agency.
[172]
ANALYSIS OF TROPIC RESPONSE MUTANTS OF ARABIDOPSIS THALIANA M.A. Olney¹ and W. Briggs². ¹Department of Biological Sciences, Stanford University, Stanford, CA. ²Carnegie Institution of Washington, Department of Plant Biology, Stanford, CA.

Three Arabidopsis tropic-response mutants were isolated from lines mutagenized by T-DNA insertion. Mutants were selected by planting T4 seed on nutrient agar in petri dishes that were held vertically for three days in darkness. Seedlings that had hypocotyls not exhibiting a strong initial negative gravitropic response were selected. Genetic analysis indicated that the mutant phenotype of each is the result of a single-gene recessive mutation. When grown on vertical plates in darkness, WT hypocotyls show strong negative gravitropism.

Following rotation of the petri dish ninety degrees and two additional days in darkness, WT hypocotyls reorient strongly away from the new direction of gravity. Etiolated hypocotyls of the three mutants do not exhibit initial negative gravitropic responses; instead, they grow in random directions. Reorientation in response to rotation is reduced. Light-grown hypocotyls exhibit phenotypes similar to those of etiolated hypocotyls. Reorientation of the roots of light-grown seedlings and of inflorescence stems is not reduced. Two of the mutants exhibit normal phototropic responses. The third mutant is phototropically and gravitropically impaired and is likely to be tagged with a single right border T-DNA insert. (Supported by Carnegie Institution of Washington, department of Plant Biology.)
SESSION J: CONCURRENT POSTER SESSION IV
ADVANCED LIFE SUPPORT
abstracts 1997 annual meeting

[173]
PLANT LIFE SUPPORT DURING 16 DAYS IN MICROGRAVITY.
During the 4 day flight of STS-84 and the 16 day flight of STS-94, the Plant-Genetic Bioprocessing Apparatus (PGBA) supported 62 plants (10 species) for biotechnology and pharmaceutical research. PGBA provides atmospheric, thermal, and humidity control as well as lighting and nutrient supply in a 23.6 liter chamber. Atmospheric treatment includes ethylene and other hydrocarbon removal, CO₂ replenishment, and O₂ control. The plants include 12 Artemisia annua (sweet wormwood), 11 Catharanthus roseus (periwinkle, 3 radio-labeled with Ca⁶ and Fe⁶), 3 Pinus taeda (lobolly pine), 4 Arabidopsis thaliana, 8 Tetragonia tetragoniodes (New Zealand spinach), 4 Trifolium repens (white clover), 3 Trichitum aestivum (wheat), 4 Piper aduncum (pepper), 4 Lycopersicon esculentum (tomato), and 2 Echinacea (purple cone flower) plants.
The overall rates of photosynthesis, respiration and evapotranspiration have been measured for the flight and ground unit throughout the mission with on-board sensors. The plants were grown at 25°C day / 22°C night temperature, 80% relative humidity, 500 ppm day / 600 ppm night time carbon dioxide concentration. Plants were grown under a high intensity (PAR) between 220 and 330 µmol/µs² at a distance of 20 cm. The plants were grown in individual "Nutrient Pack" systems (Dr. Gerard Heyenga), using solidified agar (non-water replenished) and soil-formulated packs (water replenished). Water throughput was on the order of 150 ml H₂O / day average for the entire plant growth chamber. The recovered condensate water was returned to the individual packs from the dehumidification system.
(Research supported by NASA: NAGW-1197 and NASA-MAR:#NCC8-131).

[174]
COMPARISON OF TWO NUTRIENT APPLICATION
PROTOCOLS ON YIELD RESPONSES OF 'TU-155'
The objective was to clarify the differences between two nutrient application protocols for sweetpotato growth in NFT so as to optimize nutrient resource recycling and balanced production of storage roots and foliage. The study compared a nutrient replenishment protocol used at Kennedy Space Center (KSC) and a nutrient replacement protocol used at Tuskegee University (TU). The protocols were applied to 'TU-155' sweetpotatoes in Tu rectangular (0.15m x 0.15m x 1.2m) or 0.48m²) and KSC trapezoidal-shaped (.25 m²) growth channels. Environmental conditions in growth chambers included: 28/22°C light/dark diurnal temperature, 70% RH, 600 mmol m⁻³ s⁻¹ photosynthetic flux using high pressure sodium lighting under a 12/12 photoperiod. Mean yields at harvest (120 DAP) in repeated studies using the KSC protocol and channel were 596 g/plant of storage root fresh weight with an L/D ratio of 7.3 and fresh foliage weight of 2016 g/plants. Mean yields for the TU protocol and channel were 557 g/plant of storage root fresh weight with an L/D of 2.28 and fresh foliage weight of 423 g/plant. The KSC replenishment protocol, having a higher N/K ratio, produced excessive foliage. While the TU protocol resulted in more desirable bolting of storage roots, as a replacement protocol there may be inefficient use of nutrient resources. As a result of this comparison, recommendations will be made for subsequent studies of a nutrient composition and replenishment protocol that will produce consistently high storage yields and limited foliage.
(Supported by NASA: NAGW-2940 and USDA CSREES ALX/SP).

[175]
Recycling water used for personal hygiene will be necessary in a closed, space-based system. Incorporation of human hygiene water (gray water) into hydroponic plant production systems, and subsequent recovery of the water transpired by the plants, is one potential means for water purification and recycling. The use of plants, and the active microbial community associated with their roots, for water processing would eliminate the need for physical-chemical treatment and concomitant resupply of physical components, (i.e., filters, etc.).
Volunteers showered and washed clothes with predetermined amounts of soap (Igepon) and deionized water to provide gray water with an approximate soap concentration of 1000 ppm. Gray water was added daily (1.5 L) to 20 L hydroponic tanks supporting a mixed crop of wheat and potato or a monoculture of either wheat or soybeans. Separate tanks contained a) no gray water (control), b) soapy water additions (Igepon and water), c) filtered gray water (0.2 μm filter to remove bacteria and skin cells), (soybean study only) and d) unprocessed gray water. Both the hydroponic solutions and the rhizosphere were sampled for microbial composition to determine the survival of human-associated bacteria. The rate of soap degradation was assessed throughout the plant growth cycle. Plant material was harvested and fresh and dry mass were determined. Preliminary findings demonstrated that soap degradation rates increased as plant root mass, and associated rhizosphere microbial activity, increased. A mechanism of Igepon degradation via the hydrolysis of amide linkage and the breakdown of fatty acid components is proposed. Sodium levels increased in all gray water treatments over time. The increase was greater in the human derived gray water treatment than the soapy water treatment, which may indicate that the additional sodium is derived from the volunteers.

[176]
THE CONTROLLED ECOLOGICAL LIFE SUPPORT SYSTEM
ANTARCTIC ANALOG PROJECT: PROTOTYPE CROP
PRODUCTION AND WATER TREATMENT SYSTEM
The Controlled Ecological Life Support System (CELS) Antarctic Analog Project (CAAP), is a joint endeavor between the National Science Foundation, Office of Polar Programs (NSF-OPP) and the National Aeronautics and Space Administration (NASA). The fundamental objective is to develop, deploy and operate a testbed of advanced life support technologies at the Amundsen-Scott South Pole Station that enable the objectives of both the NSF and NASA. The functions of food production, water purification, and waste treatment, recycle and reduction provided by CAAP will improve the quality of life for the South Pole inhabitants, reduce logistics dependence, enhance safety and minimize environmental impacts associated with human presence on the polar plateau. Because of the analogous technical, scientific, and mission features with Planetary missions such as a mission to Mars, CAAP provides NASA with a method for validating technologies and overall approaches to supporting humans. Prototype systems for sewage treatment, water recycle and crop production are being evaluated at Ames Research Center. The product water from sewage treatment using a Wiped-Film Rotating Disk is suitable for input to the crop production system. The crop production system has provided an enhanced level of performance compared with projected performance for plant-based life support: an approximate 50% increase in productivity per unit area, more than a 65% decrease in power for plant lighting, and more than a 75% decrease in the total power requirement to produce an equivalent mass of edible biomass.