ABSTRACTS

Symposium I
Systems Biology/Genomics and Proteomics

[1]

INTERDISCIPLINARY RESEARCH AT THE DONALD DANFORTH PLANT SCIENCE CENTER AND APPLICATIONS TO NASA GOALS. R.N.Beachy and K.R.Schubert, Donald Danforth Plant Science Center, St.Louis, MO.

The Donald Danforth Plant Science Center was established in 1999 as an independent, not for profit research Center in St. Louis, MO. The Center is dedicated to fundamental research in plant biology that addresses topics that benefit food production and human nutrition, and where research results are readily transferred for commercial development and ultimate use. It is also expected that the Center will provide a training ground for students, post-doctoral and other scientists to receive training and experience in cutting edge fields of research. Research at the Center will stress the integration of scientific disciplines that include computational and structural biochemistry, cellular and molecular biology and genetics. Through institutional partnerships the research activities at the Center will interface with research in agriculture and medicine.

The Center will include facilities for the physical characterization of protein structure, from which researchers will develop novel mechanisms to alter function to achieve one or more of the goals established by the NASA Advanced life support project. Among the research topics at the Center are projects that are supported by the NASA grant to the Danforth Center: (1) Development of gene switch technology for regulation of cellular metabolism and plant growth and development; (2) Metabolic channeling in complex metabolic pathways: Increasing metabolic flux through biochemical pathways; (3) IMPDH, a key biosynthetic, regulatory and assimilatory enzyme in plants: Studies of protein structure, function and gene expression; (4) Spatial and temporal compartmentalization of biosynthetic pathways; (5) Increasing sodium uptake and storage capacity of leafy vegetables; (6) Modulation of plant structure and protein accumulation through bioengineering. The relevance of these projects to the goals of NASA will be presented.

[2]

PHYLOGENOMICS: WHY EVOLUTIONARY ANALYSIS AND GENOMICS SHOULD BE COMBINED INTO A SINGLE COMPOSITE APPROACH. J.A. Eisen. Department of Microbial Genomics, The Institute for Genomic Research, Rockville, MD.

Evolutionary analysis can greatly benefit studies of genome sequences and genome analysis can reveal a great deal about evolution. There is a feedback loop between evolutionary studies and genome analysis such that it is useful to combine them into a single composite phylogenomic analysis. I discuss the reasons for using a phylogenomic approach and in particular why careful evolutionary analysis can reveal a great deal more than simple comparative studies. I will discuss specific examples of phylogenomic analyses relating to recent genome sequencing projects at TIGR including 1) prediction of gene function; 2) inferring evolutionary events such as gene loss, duplication and gene transfer 3) studying mechanisms of mutation such as inversions and deletions 4) identifying organellar derived genes in eukaryotic genomes and 5) identifying genes under strong selective pressure such as antigen sites in pathogen genomes. Finally, I discuss how genome analysis may require us to rethink our views on the origin and evolution of organisms, in particular microbes.

[3]

SIFTING SEQUENCE FOR FUNCTION: EXPLOITING THE MOUSE Eddy Rubin, Lawrence Berkeley National Laboratory, University of California, Berkeley, California

One of the major challenges in the post genomic era will be the identification of sequences participating in the regulatory circuitry controlling gene expression. For the analysis of genomic DNA, algorithms and databases are available facilitating the identification of those sequences encoding genes. While these sorts of computational aids are largely not available for the identification of noncoding sequences participating in gene regulation, cross-species sequence comparisons provide a robust means for identifying putative gene regulatory sequences. Using this approach to identify gene regulatory sequences we have examined orthologous regions of human 5q31 and mouse chromosome 11, DNA of biomedical importance due to the clustering of a several interleukin genes. The functional properties of the largest noncoding element (401 BP >85% conserved between humans and mice) located in the IL4, IL13 intergenic interval were examined in depth. To identify its function, knockout mice lacking this 401 BP element as well as YAC transgenics with and without the element were examined. Analysis of the animals revealed that this noncoding sequence plays a prominent role in the regulation of IL4, IL13 and IL5 genes that are spread over 120KB. The comparative genomic strategy used here for identifying noncoding sequence of biological import has led to the discovery of a regulatory element that acts over significant genomic distance to coordinate the expression of several genes involved in the inflammatory response. We have carried out similar studies in other regions of the genome that further illustrate the power of cross-species sequence analysis coupled with functional studies in mice to investigate the gene regulatory circuitry of mammals.

[4]

MAGNETISM AND BIOLOGY: THE MAGNETOTACTIC BACTERIA STORY. Dennis A. Bazylinski, Dept. of Microbiology, Iowa State Univ., Ames, IA.

The Earth's geomagnetic field is thought to influence the behavior of a wide range of organisms. However, the best understood example of magnetoreception and magnetonavigation is that observed in the magnetotactic bacteria (MB). Cells of this ubiquitous and diverse group of motile, mainly aquatic microbes synthesize magnetosomes which are intracellular, membrane-bounded, single-magnetic-domain crystals of a magnetic mineral, either magnetite (Fe_3O_4) or greigite (Fe_3S_4). These crystals impart a permanent magnetic dipole moment to the cell causing it to align along magnetic field lines, like a compass needle, as it swims; a phenomenon called magnetotaxis. In many MB, magnetotaxis, in conjunction with aerotaxis, appears to function as a means for cells to locate and maintain an optimal position (the oxic-anoxic interface) in vertical O_2 and/or redox gradients in natural habitats by reducing a 3-dimensional search problem to a 1-dimensional search problem.

Although little is known about the biochemistry and chemistry involved in magnetosome synthesis, the narrow size (~35–120 nm) distributions, the species-specific morphologies, and the pure chemical compositions of the mineral crystals in the MB and the fact that most MB organize their magnetosomes in chains within the cell indicate that the MB use a precise biologically-controlled mineralization process in synthesizing the magnetosome mineral phase. Some elongated Fe_3O_4 particle morphologies appear to be unique to the MB and have never been observed in abiotically synthesized Fe_3O_4 particles. Dead cells eventually release their mineral crystals in the surrounding environment (e.g., sediments) as "magnetofossils" that have been used as evidence of the past presence of MB. Such crystals have also been found in the Martian meteorite ALH84001 and their presence has been used as a line of evidence for life on ancient Mars.

(Supported by NASA: NAG 9-1115 and the NSF: CHE-9714101.)

[5]

APPROACHES TOWARDS UNDERSTANDING THE FUNCTIONAL ARCHITECTURE OF THE PLANT CELL WALL. M.C. McCann, D. Milioni, K. Sugimoto, N.J. Stacey, M. Bush, P. Dahiya, D. Fulton, P. Sado, M. Mourelatou, P. Derbyshire, J. Corsar and K. Roberts. Department of Cell Biology, John Innes Centre UK.

A paradigm shift towards systems biology, defined by a combination of high through-put analysis and data collection with information processing, is accelerating progress in all areas of plant biology. The initial impact of these new technologies will be illustrated with reference to cell wall biology. The plant cell wall constrains the final size and shape that plant cells achieve and is therefore a key determinant of the final stature and form of plants. The cell wall is a highly organized composite of many different polysaccharides, proteins, and aromatic substances that undergo dynamic changes during cell division, expansion and differentiation. However, it has been difficult to ascribe specific functions to these molecules. Advances in imaging methods have allowed direct visualisation of the molecular architecture of cell walls and the modifications that occur to polymers during growth and development. To address the structural and functional relationships of individual cell wall components, we need to identify a broad range of structural and architectural alterations in cell walls, most usefully through the generation of transgenic and mutant plant materials. Using a combination of genetic, molecular biological and cell biological approaches, we are engaged in identifying candidate genes and studying the consequences of their mis-expression in order to understand polysaccharide and protein functions in cell wall architecture and in plant development. (Supported by The Royal Society, Leverhulme Trust and the Biotechnology and Biological Sciences Research Council).

Concurrent Posters
I-A
Undergraduate Student Poster Competition

[6]

GRAPHICALLY ASSESSING THE EFFECTS OF ARTERIAL LOADING ON THE CAROTID-CARDIAC BAROREFLEX. [1]J.M. Wang, [2]D. Ratliff, [2]D. Doerr, and [3]V.A. Convertino. [1]Cornell University, Ithaca, NY, [2]Biomedical Office, Kennedy Space Center, and [3]U.S. Army Institute of Surgical Research, Fort Sam Houston, TX.

We hypothesized that a training regimen of straining maneuvers (SM) would increase the isolated carotid-cardiac baroreflex response. Measurements of heart rate and pressure exerted on the carotid artery were graphed to provide a sensitivity index. Contrary to our hypothesis, SM had no effect on improving the carotid-cardiac baroreflex response. However, while the main objective was not fully reached, a range of effectiveness from SM training on baroreflex function could provide additional insight into developing a countermeasure.

(Supported by NASA Spaceflight and Life Sciences Training Program.)

[7]

SURVIVAL RATE OF SUPERWORMS IN SPACEFLIGHT BRIC HARDWARE. Matthew Calhoun[1], Oliver van den Ende[2]. [1]Civil Engineering, University of Alaska, Anchorage, [2]Bionetics Corporation.

This experiment tested the survival rates of superworms (*Zophobus morio*) in BRIC (Biological Research In Canisters) flight hardware. The three types of BRIC used, are the BRIC-100, 60, and 100VC. The survival rate results of the BRIC-100VC was 0% in the three experiments that the canister was used. The BRIC-100 and BRIC-60 had similar survival rates for the final experiment conducted in the Orbital Environmental Simulator.

(Supported by NASA: Spaceflight Life Science Training Program (SLSTP).)

[8]

A COMPARISON OF CTC, AO, AND LIVE/DEAD CELL COUNTS IN MICROBIAL COMMUNITIES USING EPIFLUORESCENCE MICROSCOPY. Mercedes Vieira[1], Jennifer L. Adams[2], Mary P. Hummerick[2]. [1]Fort Valley State University, Fort Valley, GA, and [2]Dynamac Corporation, Kennedy Space Center, FL

The goal of this work is to compare enumeration methods for counting bacteria in an Advanced Life Support system. In this research, three different techniques for enumeration of bacteria in a continuous culture with wheat leachate as the feed are studied. Quantitative differences of these methods were resolved by 2 treatments, a starved system and a fed (control) system. These enumeration methods are used in the field of microbial ecology, and include CTC (5-cyano-2, 3-ditolyl tetrazolium chloride) , AODC (Acridine Orange Direct Count), and Live/Dead. The Live/Dead counts were consistent with the AO counts and impacted by the starved treatment. CTC counts were noticeably lower in the starved treatment.

[9]

FINGERPRINTING OF BACTERIA BEFORE AND AFTER TREATMENT IN A PRESSURIZED CARBON REMOVAL BIOREACTOR. Nitya Nair[1], Majda Nashashibi-Rabah[2], Christos Christodoulatos[2], Lee Kerkhof[1]. [1]Institute of Marine and Coastal Sciences Rutgers University, 71 Dudley Road, New Brunswick, New Jersey 08901-8521 and [2]Stevens Inst. of Technology, Center for Environmental Engineering, Castle Point on Hudson, Hoboken, NJ 07030.

This study was conducted to assess the differences in microbial populations between the feedstock of a gray water treatment reactor and the effluent for replicate reactors. Microbial biomass from samples of both influent and effluent were collected by centrifugation. DNA was extracted and microbial populations were characterized by PCR amplification of 16S rRNA (total community) and *nifH* (nitrogen fixing) target genes. Microbial communities were fingerprinted using terminal restriction length fragment polymorphism analysis (TRFLP). The feed stock was found to contain 16 major 16S rRNA peaks (90% of total TRFLP area) while duplicate reactors effluent contained 15 and 18 peaks respectively. Nitrogen fixers accounted for 2 major *nifH* peaks in the feed with 2 and 4 peaks in the reactor effluents. Four 16S peaks in the feed (87,234,405, and 437 bp) was not found in either reactor's effluent. New 16S peaks in reactor 1 were 208, 213, 217, and 250. However, these peaks were not seen in reactor 2. The *nifH* profiles were much simpler with a 227, 321, and 361 bp TRFLP peaks present in the feed, and both reactors. Again, reactor 2 was different with a novel, major 218 bp *nifH* peak in the effluent not present in either the feed or reactor 1. These results were not expected given the reactors were installed at the same time and given the same gray water feed stock. Work is underway to assess microbial populations within each reactor and determine if differences in microbial communities correlate with reactor performance. In the future, this information may help to improve reactor design or start up procedures.

(This work was supported by NASA grant 00-HEDS-01-043 in the Advanced Life Support Program)

[10]

THE ORIENTATION OF HELICAL SECONDARY CELL WALLS OF PLANTS FROM THE NORTHERN AND SOUTHERN HEMISPHERES. Blake P. Johnson and James G. Taylor. Department of Biological Sciences, Ouachita Baptist University, Arkadelphia, Arkansas.

Long-term space travel must include the growth and maintenance of plants in space. Understanding the basic developmental characteristics of plants is essential in establishing a system that could be maintained in a weightless environment. Plant secondary cell walls are important in overall plant development and a greater understanding of this process can give better insight into plant viability. This research investigates the established helical secondary wall pattern of xylem cells in developing plants in the northern hemisphere and compares that pattern to differences that may be found in the same species native to the southern hemisphere. Differences may be found between the northern and southern hemispheres due to the Coriolis Effect. This effect is a very weak influence that exists due to the rotation of the earth and is responsible for the counter-clockwise rotation of weather patterns in the northern hemisphere and clockwise rotation of weather patterns in the southern hemisphere. Microscopic analysis of specimens from South America, South Africa, and China has demonstrated a counter-clockwise orientation of the helical cell walls in plants grown in those locations. These results indicate that the Coriolis Effect does not influence the orientation of the helical cell walls observed in these specimens.

(Supported by NASA: OBU11043 and OBU11042)

[11]

BIOCONVECTION AND GRAVITAXIS IN THE CALCIFYING ALGA *PLEUROCHRYSIS CARTERAE*. V. Chheda, D. Montufar-Solis, and P.J. Duke. Dept. of Orthodontics, Dental Branch, UT Houston Health Science Center, Houston, TX.

Bioconvection is the formation of convection patterns within a culture of swimming microorganisms due to their collective behavior. Swimming strains of the calcifying alga *Pleurochrysis carterae* exhibit bioconvection when cultured in aquaria, flasks, or Rose Chambers, affirming the presumed negatively gravitaxic behavior of the cell. These unicells are being grown in our lab for preliminary studies relating to an International Space Station experiment that will study the relationship of mineralization and morphogenesis to gravitaxis. In the present study, we used Wintrack 2000 to determine the influence of bioconvection on the direction of cell movement by analysis of videos acquired at different magnifications and using different cell concentrations. Cells were grown in a completely filled Rose chamber, eliminating the variable of an oxygen gradient.

Results showed that an evenly distributed number of cells swimming up concentrate at the top of the container and then abruptly fall, forming a convection current visualized as a thin but dense column of downflowing cells. The greatest influence on movement of individual cells is seen in or near these streams. Cells detaching from the stream move upwards at an angle at least in part influenced by the convective fluid flows. This angle determines if the cell will reenter the stream, or move into the region between downstreams, where they swim directly up. Therefore, cells in a population do not swim independently of one another, but this effect is diminished at lower densities. The videotaping plan for the experiment aboard Space Station includes taping with white light and infrared, allowing us to address the question of bioconvection in the absence of gravitaxis and phototaxis, which have not been previously studied.

(Support: NASA grant NAG2-1261 and UTHSC Summer Research Program)

[12]

DEVELOPMENT OF A SHORT-TERM BIOASSAY TO EVALUATE PHOTOXICITY OF BIOREACTOR EFFLUENT. Peter K. Cudjoe[1], and Dr. Richard F. Strayer[2]. [1]Tuskegee University, Tuskegee, Alabama, and [2]Dynamac Corporation, Kennedy Space Center, Florida.

In order for humans to inhabit space there must be an adequate supply of food. Whenever food is consumed there are waste products by means of human expulsion or inedible plant parts. The fixed film bioreactor (FFB) was used to produce a nutrient solution from plant waste, in order to feed back to plants. Our objective was to develop a short-term (22 days) plant growth bioassay to test the effluent from the FFB (hydraulic retention time = 6 hr), currently under development. The nutrient solution (effluent) was tested against a modified Hoagland's solution, a nutritionally balanced medium that is currently being used for plant growth experiments. We tested the effluent on three different plant species, each with different chemical tolerances. The effluent was expected to produce a yield similar to that of the Hoagland's. The dry weight data showed that plants treated with Hoagland's and FFB effluent had similar weights. The ranges of dry weight were 0.2g to 0.3g for wheat, 0.40g to 0.50g for radish, and 0.20g to 0.30g for lettuce. Results for some of the other plant growth parameters (plant height and leaf area) were somewhat ambiguous and unexpected, in relation to the dry weight results.

(Supported by NASA's Spaceflight and Life Sciences Training Program)

[13]

THE LAZY-2 REVERSED GRAVITROPIC RESPONSE GENE OF TOMATO: HIGH RESOLUTION MAPPING USING GENETIC AND MOLECULAR APPROACHES. J. Well, M.G. Ivanchenko, K. Oh, V.L. Slater, T.J. White and T.L. Lomax. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis OR 97331-2902

When grown under red light, shoots of the lazy-2 tomato mutant (lz-2) exhibit a reversed gravitropic response, mediated by the red-light absorbing photoreceptor phytochrome (Gaiser, J.C. and Lomax, T.L. (1993) Plant Physiol. 102:339-344). The lz-2 phenotype suggests that the Lz-2 gene product is a regulator of differential growth shared by both gravity and phytochrome signaling pathways. Therefore, isolation of the Lz-2 gene would provide unique means to investigate the interaction between these two essential plant regulative pathways. Using a map-based genetic approach we have localized the Lz-2 gene to the centromeric region of the tomato chromosome 5 (Behringer, F.J. and T.L. Lomax (1999) J. Heredity 90: 489-493). Proximity to the centromere complicates mapping due to dramatically suppressed recombination. To increase the frequency of genetic recombination, we have generated a mapping population of plants by crossing the lz-2 mutant (in the domestic tomato, L. esculentum) with the relatively closely related wild tomato species L. pimpinellifolium (Well, J. et al. (2000) Gravitational and Space Biology Bulletin 14:13). We now report an expanded map of the Lz-2 region on chromosome 5 based on this new mapping population. Our cloning strategy involves a combination of comparative microsynteny analysis between tomato and Arabidopsis, the construction of a physical map spanning the genetic interval and genome walks upstream and downstream of identified molecular marker sequences. These approaches have proven useful for increasing marker density in the centromeric regions and for overcoming the potential difficulty in genotyping due to the lack of polymorphisms between L. .esculentum. and L. pimpinellifolium.

(Supported by NASA NAG2-1341 and Howard Hughes Medical Institute Summer Research Fellowship to J.W.)

{14]

COMPARISON OF NUTRIENT DELIVERY SYSTEMS FOR GROWTH OF *ARABIDOPSIS* PLANTS IN SPACE. Swati Mohan[a], Anna-Lisa Paul[b], Howard G. Levine[c], Robert J. Ferl[b]. [a]Cornell University, [b]University of Florida, [c]Dynamac Corporation, Kennedy Space Center

Investigating the responses of plants in microgravity conditions can help us plan for long-term space missions in which plants contribute to the advanced life support system (ALS). This experiment involved the evaluation of four different types of nutrient delivery systems to determine which system maximizes growth, minimizes stress, and effectively occupies the flight hardware. Transgenic *Arabidopsis thaliana* plants coding the Adh driver and GFP reporter were grown in four support systems: agar plates, agar tubes, porous tube systems, and tubes filled with glass beads of six different sizes. The value of the system was measured by three criteria: analysis of leaf and root mass, chlorophyll content as a qualitative measure of vigor, and effective organization within the Plant Growth Facility (PGF-SP and PGF). The results of the experiment showed that each system was a viable option for ALS, although the Fibrous Ion Exchange Resin Substrate (FIERS) treatment of the porous tube system and the agar tubes performed the best. Modifications should be made to the glass beads and porous tube systems to account for errors in the procedure because the systems were being tested for the first time.

(Supported by NASA's Spaceflight and Life Sciences Training Program (SLSTP))

[15]

LOW PRESSURE INCREASES TRANSGENE EXPRESSION IN *ARABIDOPSIS* PLANTS. Prayrana Khadye[1], Anna-Lisa Paul, Ph.D.[2], and Robert Ferl, Ph.D.[2]. [1]Clemson University, Clemson, SC, [2]University of Florida, Gainesville, Florida.

Long-term space missions will require plants and hardware to adapt to new environments. A problem that arises, however, is whether plants will survive in environments with such low atmospheric pressures, as experienced on Mars. Adh/GUS and Adh/GFP engineered *Arabidopsis* plants were exposed to low pressure levels in a simulated chamber and genetic technologies were used to decipher gene expression patterns. The plants exhibited high gene expression in the root tips and light expression in the meristems when exposed to a pressure level of 10 kPA. This expression was much greater than normal hypoxic induced plants, which implies that other factors of reduced atmospheric pressures may be affecting gene expression.

[16]

CONTROLLED STRESS INDUCTIONS OF GFP TRANSGENE IN *ARABIDOPSIS*. J.N. Gelinas[1], A-L Paul[2], R.J. Ferl[2] [1]University of Alberta, [2]University of Florida

Transgenic *Arabidopsis* containing the alcohol dehydrogenase gene promoter linked to the Green Fluorescence Protein reporter gene (Adh/GFP) has been created in order to assess the potential stresses associated with spaceflight. Through the use of fluorescence imaging technology, quantitative rt-PCR, and gel electrophoresis, the effects of controlled inductions on *Arabidopsis* were observed and analyzed. Patterns of expression and tissue specificity for various inductions, such as high salt concentration and hypoxia, were not identical. Qualitative imaging was supported by the numerical data gathered from rt-PCR, indicating that intensity of fluorescence can be accurately correlated with GFP mRNA concentration. The data gathered in this investigation was part of a simulated spaceflight experiment in which hardware and science operations were integrated. Comparisons of age-matched ground controls and flight plants demonstrated that environmental conditions (excluding microgravity) on board the orbiter do not significantly alter GFP expression.

(Supported by NASA and Spaceflight and Life Sciences Training)

Concurrent Posters
I-B
Graduate Student Poster Competition

[17]

REVERSAL OF WHOLE-BODY AND SKELETAL MUSCLE INSULIN RESISTANCE AFTER THREE AND SEVEN DAYS OF HINDLIMB SUSPENSION. M.P. O'Keefe[1], F.R. Perez[1], T.R. Kinnick[1], and E.J. Henriksen[1]. [1]Dept. of Physiology, University of Arizona, Tucson.

We have previously detected the development of insulin resistance following 1-day of simulated weightlessness (hindlimb suspension (HS) model), as shown by increases in plasma glucose and reductions in insulin-mediated glucose uptake in soleus muscle. The specific aim of the present investigation was to test the hypothesis that HS for 3 or 7 days would lead to a reversal of whole-body insulin resistance. Four-week-old Sprague Dawley rats were assigned to either a weight-bearing (WB) control group, a 3-day HS group, or a 7-day HS group. Whole-body glucose tolerance was measured during a 1 g/kg oral glucose tolerance test (OGTT). Blood samples were taken from a tail vein at 15, 30, 60, and 90 min, and plasma was subsequently assayed for glucose and insulin concentrations. Glucose transport activity (assessed by 2-deoxyglucose uptake) was determined in isolated soleus muscle strips in the absence or presence of insulin (2 mU/ml). Three- and 7-day HS soleus had significantly lower total protein levels (-11% and -13%) and significantly higher hexokinase activity (+40% and +93%) when compared to WB soleus. Seven-day HS soleus also displayed significant elevations in citrate synthase activity (+29%) and total GLUT 4 protein levels (+62%) versus WB soleus. Suspension produced no significant difference in plasma glucose concentration, area under the curve for the glucose and insulin responses, or the glucose-insulin index when compared to the WB group. Insulin-mediated glucose transport was elevated in the HS soleus at 3 (+68%) and 7 days (+153%) when compared to WB soleus. Intramuscular triglycerides were significantly reduced at 3 day HS (-56%), and remained reduced (-38%) at 7 days of HS. In summary, these observations suggest that 3 and 7 days of simulated weightlessness in juvenile rats reverses whole-body insulin resistance observed at 1 day of HS, likely due to an enhanced capacity for insulin-stimulated glucose transport in unweighted skeletal muscle.

[18]

SPACE SHUTTLE FLIGHT (STS-90) ENHANCES DEGRADATION OF RAT MYOSIN HEAVY CHAIN IN ASSOCIATION WITH ACTIVATION OF UBIQUITIN-PROTEASOME PATHWAY M. Ikemoto[1], T. Nikawa[1], S. Takeda[2], C. Watanabe[1], T. Kitano[1], K. M. Baldwin[3], R. Izumi[4], I. Nonaka[5], T. Towatari[6], S. Teshima[1], K. Rokutan[1] and K. Kishi[1] [1]The University of Tokushima; [2]National Center of Neurology and Psychiatry; [3]University of California Irvine; [4]National Space Development Agency of Japan; [5]National Center of Neurology and Psychiatry; [6]University of Tokushima.

To elucidate the mechanisms of microgravity-induced muscle atrophy, we focused on fast-type myosin heavy chain (MHC) degradation and expression of proteases in atrophied gastrocnemius muscles of neonatal rats exposed to 16-day spaceflight (STS-90). The spaceflight stimulated ubiquitination of proteins, including a MHC molecule, and accumulation of MHC degradation fragments in the muscles. Semi-quantitative reverse transcriptase-polymerase chain reaction revealed that the spaceflight significantly increased mRNA levels of cathepsin L, proteasome components (RC2 and RC9), polyubiquitin and ubiquitin-conjugating enzyme in the muscles, compared with those of ground control rats. The levels of □-calpain, m-calpain, cathepsin B and cathepsin H mRNAs were not changed by the spaceflight. We also found that tail-suspension of rats for 10 days or longer caused the ubiquitination and degradation of MHC in gastrocnemius muscle, as was observed in the spaceflight rats. In the muscle of suspended rats, these changes were closely associated with activation of proteasome and up-regulation of expression of mRNA for the proteasome components and polyubiquitin. Administration of a cysteine protease inhibitor, E-64, to the suspended rats did not prevent the MHC degradation. Our results suggest that spaceflight induces the degradation of muscle contractile proteins including MHC, possibly through a ubiquitin-dependent proteolytic pathway.

[19]

EFFECTS OF HIGH-LET RADIATION ON DOPAMINE-INFLUENCED PRE-PULSE INHIBITION. C.N. Zuccarelli[1,2], P.E. Haerich[2], M.J. Pecaut[1], A.L. Smith[1], and G.A. Nelson[1]. Depts of [1]Radiation Medicine, Radiobiology Program and [2]Department of Psychology. Loma Linda University and Medical Center, Loma Linda, CA.

Radiation has consistently been shown to influence behavior. Because the long-term effects of radiation on behavioral responses may have serious implications to astronauts on extended missions in space, this pilot study examines the acoustic startle response, one of the classical measurements of behavior in rodent models. The acoustic startle reflex and associated pre-pulse inhibition have been shown to be dopamine (DA)-dependent behaviors. Apomorphine and haloperidol are known to have opposing effects on dopaminergic systems. The major goal of this study was to examine the synergistic effects of high-LET radiation and the above pharmaceutical agents upon the startle model. Thus, this study examines radiation effects on two behavioral endpoints: a) the immediate effects of radiation exposure in a perceptual learning/memory task (habituation) and b) the brainstem/midbrain level sensory gating task (pre-pulse inhibition). Female C57BL/6 mice were irradiated with iron ions (^{56}Fe, Z = 26, LET = 146 KeV/□m) at doses of 1, 2, and 3 gray (Gy) at Brookhaven National Laboratory. The animals were shipped to Loma Linda University and tested 54 days later. While the responses to the drugs were as expected, there were no significant effects of radiation. These data contrast with previous studies showing an acute decrease in startle activity after proton radiation at similar doses. This suggests that radiation effects on startle behavior may only occur within several weeks post-irradiation.

(Supported by NASA: Coop. Agreement NCC9-79.)

[20]

DEVELOPMENT OF THE NERVOUS SYSTEM AND MUSCULATURE IN THE BIVALVE LARVAE, *M. EDULIS*. J.T.Plummer and R.P.Croll. Department of Physiology and Biophysics, Dalhousie University, Halifax, N.S., Canada

Gravity normally acts as an orientation cue allowing bivalve veliger larvae to migrate through a water column. In addition, larvae from a previous spaceflight mission exhibited abnormal swimming behaviour, thus prompting our current efforts to understand the physiological mechanisms underlying locomotion in these organisms. The helical swimming pattern that is typical of bivalve larval behaviour is thought to be modulated by neuronal and muscle control. However, to date little is known about the either the nervous system or the musculature in bivalve larvae. Given that bivalve veliger larvae from a previous spaceflight mission exhibited abnormal swimming behaviour, it was necessary to investigate the possible role of the nervous system and musculature in larval behaviour. Histological studies revealed an extensive nervous system and musculature in the very earliest stages of development. Furthermore neuronal innervation of the velum, the main swimming structure, appears to coincide both spatially and temporally with muscle innervation. These findings suggest a possible role of the nervous system in regulating the behavioural changes seen in microgravity. Consequently, pharmacological studies have provided evidence which suggests possible roles for these early neuronal elements. The neurotransmitters, serotonin and catecholamines have been found in locomotory organs and also appear to act as excitatory and inhibitory agents, respectively, which alter the normal swimming pattern of bivalve veliger larvae. Together, these data provide a more complete understanding of the neuronal elements, musculature and pharmacology which may act to control larval swimming behaviour. This study provides the foundation for future work on the role of the gravity in modulating both muscle and neuronal development in molluscan systems.

(Supported by: the Canadian Space Agency)

[21]

COLLAGEN IN JUVENILE THROUGH MATURE ADULT RAT METATARSALS INCREASES LINEARLY RELATIVE TO BODY WEIGHT. M.J. Palm[1] and B. Johnson-Wint[1].[1] Department of Biological Sciences, Northern Illinois University, DeKalb, IL

Embryonic skeletal development has been studied extensively but there have been limited studies on the progression of post-partum growth through mature homeostasis of skeletal bones. Previous studies of male and female Sprauge-Dawley (SD) rats have shown a clear positive relationship between body weight and overall skeletal mass, but did not address specifically load bearing bones or collagen content. Since bone receives its tensile strength from collagen, the goal of this study was to investigate collagen content of loaded bones per age and body weight in both male and female SD rats. The metatarsal bones of SD rats were studied to determine if age, body weight and collagen content correlated. Rat body and metatarsal weight were recorded. Metatarsals two, three, and four from both hind feet of normal male and female rats were collected and protein extracted. The extracted protein and residual bone were acid hydrolyzed and collagen amount was determined using the hydroxyproline assay. Bones collected ranged from 17days-post-partum through 452 days of age. Both males and females experienced a rapid growth phase from day 17 through approximately day 60 and then body weight gain continued to increase but at a decreased rate through day 452. The body weights of the male and female rats were shown to be statistically significantly different, with males larger then females per age ($p<.001$). Overall, total metatarsal collagen per age followed the same trends but showed no difference between males and females, whereas total collagen per body weight was statistically more in females then in males ($p<.05$). Metatarsal collagen density per body weight showed a positive linear trend for both male and female rats with female being higher then male collagen density. These results suggest that the amount and density of collagen in metatarsals are positively linearly related to body weight, hence load. In addition, over the time course female metatarsals showed a higher amount and density of collagen per body weight then males.

[22]

GENDER DIFFERENCES IN ONE YEAR OLD DAHL SALT-SENSITIVE AND -RESISTANT RATS. D.P. Cappiello[1], S.R. Locklear[1], M. Thierry-Palmer[2] and S.B. Arnaud[1] [1]NASA Ames Research Center, Moffett Field, CA and [2]Morehouse School of Medicine, Atlanta, GA.

We raised male and female Dahl Salt-sensitive (SS) and -resistant (SR) rats to 12 months of age on normal sodium diets of 0.36% - 0.4%, in the course of studies using these animals as a space flight model. We noted increased mortality in males. Male and Female SS and SR groups were born in the same week. Females were sacrificed at age 349 days and males 27 days later. There were 12 males, 7 SR and 5 SS; 11 females, 6 SR and 5 SS. Average ("SD) male SR BW at time of necropsy was greater than twice SR female BW (485"39g vs. 210"5g, $p<0.001$), as well as male SS vs. female SS (447"26g vs. 254"26g, $p<0.001$). Organ weight comparisons were calculated in terms of g/100g grams of BW. Gender differences revealed female SS rats had larger hearts (0.436"0.02 vs. 0.296"0.02, $p<0.05$), and smaller livers (2.426"0.15 vs. 3.13"0.21, $p<0.05$), as compared to male SS rats. Male SS kidneys were larger than female SS kidneys (0.428"0.07 vs. 0.354"0.02, $p<0.05$). Male SR kidneys were smaller than female SR kidneys (0.277"0.02 vs. 0.352"0.04, $p<0.05$). Comparisons of SS and SR BW showed no differences in males. Kidneys were the same in females, but not in males, $p<0.05$. Male SS compared to SR rats displayed larger hearts (0.370"0.08 vs. 0.296"0.02, $p<0.05$), larger spleens (0.349"0.11 vs. 0.188"0.01, $p<0.05$), and larger kidneys (0.428"0.07 vs. 0.277"0.02, $p<0.05$). There were no differences in relative organ weights between female SS or SR groups. It is unlikely that the 27-day earlier necropsy of females than males accounted for the lack of differences in the female groups. These data suggest that male Dahl rats are more prone to aging effects associated with higher mortality in males.

(Supported by NASA NAG2-1381)

[23]

RIGHTING RESPONSE OF RATS FOLLOWING CHRONIC HINDLIMB-SUSPENSION. F. Kawano[1], T. Nomura[2], T. Wakatsuki[1], A. Ishihara[3], and Y. Ohira[1]. [1] School Health Sport Sci., Osaka Univ., Osaka, [2] Res. Ctr. Health, Phys. Fit. Sports, Nagoya Univ., Nagoya, and [3]Fac. Integrat. Human Studies, Kyoto Univ., Kyoto, Japan

Seven weeks old male Wistar rats were hindlimb-unloaded by tail-suspension for 9 wk and allowed to ambulate for 8 wk thereafter. Analyses of righting responses, as well as electromyogram (EMG) of neck and back muscles, were performed immediately (R+0-hr), 2 (R+2-wk) and 8 (R+8-wk) weeks after the termination of suspension. Righting motions were videotaped at 60 frames per sec to measure hindlimb righting time when rats were dropped from supine position at ~30 cm height. The EMG patterns were synchronized with motions. The EMG activity of back muscle during suspension at 9th wk was less than the control level (~29.6 vs. ~43.7 V·sec per hr, $p>0.05$). The righting time in unloaded rats was longer than controls (~220 vs. ~120 millisec). The unloading-related change in righting time was accompanied by lowered activities of EMG, at a specific stage of falling, in both neck and back muscles. After 8 wk of reambulation, righting time recovered toward control level but still slower than in the age-matched control. However, the EMG activity of back muscle at R+8-wk was still less than controls. In contrast, EMG of neck muscle was even increased to above the control level. It is suggested that suspension-related inhibition of righting response of rat, which could be irreversible, may be closely associated with a decreased recruitment of back muscle fibers.

[24]

A PROTEOMIC APPROACH TOWARDS IDENTIFYING PROTEINS DIFFERENTIALLY EXPRESSED IN RESPONSE TO GRAVI-STIMULATION IN ARABIDOPSIS ROOTS. L.-S. Young[1,2] and P.H. Masson[1]. [1]Lab of Genetics and [2]Plant Breeding and Plant Genetics Graduate Program, Univ of Wisconsin, Madison.

A proteomic approach was used to identify proteins that are differentially expressed in response to gravi-stimulation in *Arabidopsis thaliana* roots.

The apical 2-mm root-tip portion of 600 4-day-old seedlings grown on-agar were subjected to a 3-step sequential extraction protocol based on the differential solubility of proteins (Molloy *et al*., 1998, *Electrophoresis* 19:837-844). The root tip proteome was fractionated into suitable sizes for analysis and comparison. Over 1,000 protein spots combined from three sequential extraction procedures were visible by 2-dimensional electrophoresis (2-DE). The overall protein expression pattern was similar before gravi-stimulation and after 12 min of gravi-stimulation, a time that precedes the actual curvature response but follows the presentation time. Interestingly, 12 protein spots increased in abundance after 12 min of gravi-stimulation. These same proteins were all degraded and/or down-regulated after 30 min of gravi-stimulation when the elongation zone had initiated a curvature response.

This proteomic approach using 2-DE analysis proved highly reproducible in 3 successive experiments. MS identification of those differentially expressed proteins by MALDI-TOF and ESI MS/MS is in progress. Similar comparative 2-DE protein analysis between wild-type, *pgm*, *agr1* and *arg1* mutants are also being performed to determine which phase of the gravity signal transduction/transmission response are required for the differential expression. Further reverse genetics and molecular approaches will be used to characterize the individual roles of these proteins in root gravitropism.

(Supported by NASA: NAG2-1336)

[25]

MOLECULAR CHARACTERIZATION OF THE THIGMIC STRESS RESPONSE IN SOYBEAN. A. L. Santone, M. J. Vasconcelos, K. G. Raghothama, and C. A. Mitchell. Dept. of Horticulture & Landscape Architecture, Purdue University, West Lafayette, IN.

An experimental system based on dark-grown soybean (*Glycine max* (L.) Merr.) seedlings is being studied to better understand the genetic responses to mechanical stress. Such a system allows for rapid experiments (complete in 1 week) and, since such seedlings elongate exclusively by axial cell enlargement in a specific region of the hypocotyl (the "hypocotyl elongation zone" or HEZ), results are not confounded by light-driven growth processes. Mild mechanical stress applied to the seedling hook just above the HEZ reduces seedling growth 33% compared to unstressed control seedlings when measured 24 hours post-stress. Supplied Ca^{2+} levels have been shown to negate this stress response. An increase in Ca^{2+} concentration over two orders of magnitude reduced the effects of this stress by half. This response to touch stimulation is thought to be related to expression of touch (TCH) genes in the HEZ. A cDNA library prepared from HEZs of stressed and unstressed dark-grown soybean seedlings is being probed with clones of TCH and Expansin genes from *Arabidopsis thaliana* (L.) Heynh., as well as ESTs of soybean genes with high sequence similarity to the *Arabidopsis* TCH genes in order to find soy genes thought to play a role in thigmostress-induced growth reduction.

(Supported in part by NASA: NAG2-1389)

[26]

HAPTOGRAVITROPISM: WHEN A ROOT ENCOUNTERS A BARRIER TO DOWNWARD GROWTH. G.D. Massa and S. Gilroy. Department of Biology, Penn State University.

In nature, roots will encounter a variety of obstacles that will cause a growing root to reorient. Rocks, hard-pan soil layers, and even other roots will block downward growth, requiring the root to change direction until the obstacle is bypassed. We have designed an experimental system to examine the interaction of roots with rigid obstacles, and have tested primary roots of the model plant *Arabidopsis thaliana*. A vertically growing root appears to sense an obstacle, and rapidly initiate a growth response, possibly by integrating gravity and touch stimuli. The response is characterized by the formation of a bend in the region of the root behind the tip, allowing the root tip to maintain a set angle of 136˚ as the root tracks across the surface of an obstacle. Kinetic analysis indicates that the root growth rate remains constant before, during, and after interaction with the barrier. To dissect the relative contributions of touch and gravity, laser ablations of groups of root cap cells were performed and these indicate that all of the cells in the root cap are required for a normal haptogravitropic response. The *pgm1-1* mutant, however, does exhibit normal haptogravitropism, indicating that reduced gravisensing is sufficient for a normal response. Gravitropic reorientation after the root reaches the end of the barrier appears identical to reorientation from the same angle in rotated roots that did not interact with a barrier. Other species are being examined to determine the generality of the response, and differences between species with tap root systems versus fibrous root systems appear to occur. The haptogravitropic response of lateral roots is also examined.

[27]

FLAVONOIDS REGULATE AUXIN TRANSPORT DURING GRAVITY RESPONSE IN *ARABIDOPSIS* Avery McGuire, Aaron M. Rashotte, and Gloria K. Muday Department of Biology, Wake Forest University, Winston-Salem, North Carolina, 27109

Flavonoids have been implicated in the regulation of polar auxin transport in Arabidopsis. One class of flavonoids, the aglycone flavonols, is believed to be central in regulation of auxin transport. Arabidopsis plants with the *tt4* (2YY6) mutation in the gene encoding the first enzyme in flavonoid biosynthesis, chalcone synthase, make no flavonoids. This *tt4* (2YY6) mutant has elevated auxin transport consistent with the absence of an endogenous inhibitor of transport. In a recent series of studies, the gravitational responses of *tt4* (2YY6) seedlings were examined and compared with wild-type seedlings. Roots of *tt4* (2YY6) plants showed both a distinct lag in initiation of gravitropic bending and have a much slower rate of bending in response to gravity. These investigations will be extended to include examination of the effect of the *tt4* (2YY6) mutation on hypocotyl gravitropism and phototropism. Additionally, the effect of gravity stimulation on the location and concentration of flavonoids will be examined with a dye that becomes florescent in response to binding flavonoids. Expression of auxin-responsive promoter constructs will also be examined in relationship to the changes in flavonoid localization. As a whole, these experiments should provide insight into how flavonoids act to control auxin transport during gravitropic bending and when and why the absence of flavonoid.

[28]

SUPEROXIDE DISMUTASE ACTIVITY IN ANTARCTIC AND TEMPERATE STRAINS OF *CHROOCOCCIDIOPSIS*. A. L. Price, J. L. Liles and D. J. Thomas. Science Division, Lyon College, Batesville, AR 72501.

Superoxide dismutases (SOD's) are critical components of the antioxidant systems of aerobic (and some anaerobic) organisms. The antioxidant systems of photosynthetic organisms are particularly well-developed in order to detoxify reactive forms of oxygen that occur as a by-product of photosynthesis. The amount of reactive oxygen produced during photosynthesis increases when the organism is under environmental stress. SOD's have been shown to provide protection against moderate chilling stress in chilling-sensitive cyanobacterium, *Synechococcus*. We are currently screening for SOD's that retain their activity in cyanobacteria that grow at temperatures below 17° C. Temperate and Antarctic strains of *Chroococcidiopsis* were grown in liquid culture then passed through a French press to rupture cells without denaturing the SOD's. Total SOD activity was measured in the resulting cell homogenates using the photochemical nitro-blue tetrazolium (NBT) assay. NBT is converted to a blue-colored formazan when reactive oxygen is formed. Preliminary data indicate that the Antarctic strains grow well under chilling conditions (12°C). At least one Antarctic strain has increased SOD activity as compared to temperate strains. The results of research in progress will be presented. This research could be used to develop chilling and other stress-resistant crops for use on a space station or planetary exploration base, such as on Mars, where the average ambient temperature is much lower than that of Earth.

(Supported by an Arkansas SILO Undergraduate Research Fellowship to ALP and by the Arkansas Space Grant Consortium.)

[29]

CALCIUM MOBILIZATION AND PHOSPHOINOSITIDE PRODUCTION IN RESPONSE TO HYPOXIC STRESS. J.C. Johnson, J. Torabinejad, and D.B. DeWald. Utah State University, Logan, UT.

Hypoxia, a lack of oxygen in the plant root-zone, can be caused by several different environmental factors including flooding and microgravity. Plants have developed mechanisms to survive and acclimate to these conditions through changes in morphology, metabolic pathways, and protein synthesis. One mechanism used by plants is a signal transduction pathway involving membrane phospholipids that can induce calcium mobilization, or the movement of calcium into the cytosol from extracellular or intracellular stores. The phosphoinositide phosphatidylinositol 4,5-bisphosphate can be hydrolyzed to diacylglycerol and inositol trisphosphate ($InsP_3$). $InsP_3$ acts as a second messenger to cause calcium mobilization within the cell resulting in protein synthesis and gene expression. However, the signal transduction pathways induced by hypoxia are not fully known. We have studied the response of *Arabidopsis thaliana* to hypoxic stress through an examination of calcium mobilization and phosphoinositide production. HPLC analysis was used to separate and identify changes in membrane phosphoinositides that resulted from hypoxic stress to test the hypothesis that phosphoinositide and inositol phosphate accumulation leads to calcium mobilization. We have also directly examined calcium mobilization in hypoxia-stressed root and shoot tissues using a ratiometric imaging system in conjunction with transgenic, calcium-indicating *A. thaliana*. A better understanding of how the plant responds to hypoxia may potentially allow the development of transgenic plants that can thrive in field and spaceflight-induced hypoxic environments.

(Supported by a NASA Rocky Mountain Space Grant Consortium Fellowship, a NASA GSRP, and the U.S.D.A.)

[30]

THE EFFECT OF UNCERTAINTY IN ANALYSES OF ADVANCED LIFE SUPPORT SYSTEMS L.F. Rodriguez[1], A.J. Both[1], A.B.O. Soboyejo[2] and K.C. Ting[2] [1]Rutgers University, New Brunswick – NJ-NSCORT, The Ohio State University, Columbus

During the development of complex, novel systems, such as Advanced Life Support (ALS) Systems, research and technology development is a necessity to provide knowledge bases for achieving system and mission requirements. When considering technologies still in the developmental phase, it is essential to consider the uncertainty of the observed and reported data. When the data is utilized in subsequent analysis, results will be expected to carry an inherited amount of uncertainty. One example is an ALS metric such as ESM, which is considered a valuable piece of decision support information, that will likely carry some degree of uncertainty, and decision makers should be aware of the extent of uncertainty.

By considering the variability of the data utilized to evaluate a model, it is possible to determine the amount of variability in the result by propagating the error in the data throughout the calculation. An ongoing ALS System top-level modeling effort, supported by the NJ-NSCORT at Rutgers University, provides an ideal platform for demonstration of this error propagation technique. Limiting this effort is information describing the uncertainty of data reported by ALS System researchers. Another source of uncertainty may originate from the need of making estimates for incomplete information during analyses. Therefore, reasonable assumptions regarding the uncertainty in data generated by the ALS community have been made and non-trivial uncertainty in ESM calculations has been found based on the assumptions underlying this study. To mitigate the effect of uncertainty on the decision process, it is necessary to initiate data collection of information describing the uncertainty of a process at the fundamental research level. This will enable researchers and decision makers to have a better understanding of the uncertainty in their analyses used in the ALS decision making process.

Concurrent Posters
II-A
Undergraduate Student Poster Competition

[31]

EFFECTS OF ARTERIAL BLOOD PRESSURE LOADING ON THE BUFFERING CAPACITY OF THE CAROTID-CARDIAC BAROREFLEX RESPONSE. C. B. Nguyen[1], V. A. Convertino[2], D.F. Doerr[3], D.A. Ratliff[4]. [1]University of California, Davis, [2]United States Army Institute of Surgical Research, Fort Sam Houston, TX, [3]NASA Biomedical Office, Kennedy Space Center, FL, and [4]Bionetics Corporation, Kennedy Space Center, FL.

Long-term exposure to microgravity has been shown to desensitize the carotid-cardiac baroreceptor response. Reduced baroreceptor sensitivity often leads to hypotension among astronauts. We tested a straining maneuver (SM) designed to load baroreceptors with blood in the search for an effective countermeasure for preventing orthostatic intolerance. We measured the buffering capacity of the baroreflex response as represented by the operational point. Results showed no significant change in buffering capacity when the SM training was used.

(Supported by NASA and the Spaceflight and Life Sciences Training Program (SLSTP))

[32]

THE EFFECTS OF ARTERIAL BLOOD PRESSURE LOADING ON THE CAROTID-CARDIAC BAROREFLEX RESPONSE AND ITS CORRELATION TO PULSE PRESSURE. S. Koreen[1], D. Doerr[2], D. Ratliff[3], V. Convertino[4,] [1]Monmouth University, W. Long Branch, NJ; [2]NASA, Kennedy Space Center, FL; [3]Bionetics, Kennedy Space Center, FL; [4]U.S. Army Institute of Surgical Research, San Antonio, TX

A significant percentage of astronauts develop orthostatic hypotension immediately after their return from space flight, a condition that could compromise the astronauts' safety and performance. Groundbased experiments have revealed an association between orthostatic hypotension and attenuated baroreflex that occurs during exposure to microgravity. Therefore, development of a treatment designed to acutely increase baroreflex sensitivity may prove to be an effective countermeasure against post-flight orthostatic intolerance.

We hypothesized that repeated straining maneuvers (SM training) designed to load arterial baroreceptors by increasing arterial pulse pressures (arterial pressure loading) would acutely increase cardiac baroreflex responses. We tested this hypothesis by measuring cardiac baroreflex responses to carotid baroreceptor stimulation (neck pressures) and reductions in blood pressures induced by a 15-second Valsalva maneuver in 12 subjects at 1, 3, 6, and 24 hours after performing SM training. Cardiac baroreflex responses were also measured in each subject at 1, 3, 6, and 24 hours at the same time on a separate day without SM training (control) in a randomized, counterbalanced cross-over experimental design. Our results are consistent with our hypothesis since larger elevations in pulse pressure during SM training was related ($r = +0.54$) to greater increases in isolated carotid-cardiac baroreflex responses. Our data suggest that loading of arterial baroreceptors by large fluctuations in pulse pressure represents a mechanism by which baroreflex function can be acutely enhanced. We conclude that SM training similar to that conducted by high performance aircraft pilots may provide astronauts with protection from orthostatic hypotension after return from spaceflight.

(Supported by NASA- Spaceflight and Life Sciences Training Program)

[33]

LABORATORY INVESTIGATION OF SCANRDI FOR AGENCY ACCEPTANCE. Kevin Lewis[1], Alex Lugo Valle[2], Carmen Ramos Cortes[3], and Randall Sumner[3]. [1]Univeristy of Illinois, Urbana-Champaign, [2]University of Puerto Rico in Arecibo, [3]The Bionetics Corporation, Kennedy Space Center, Florida 32899

Several traditional methods are used to determine the numbers of bacteria in fluids. These approaches are time consuming and depend on culturing the bacteria. This project investigated the validity of the nascent methodology of SPLC, which combines laser technology and fluorescence to yield a rapid accurate bacterial cell count without culture lag time. The determination of a functional timescale of the SPLC technique relative to the pour plate technique was also undertaken; this had not been previously assessed. This study suggests that the SPLC method, employed by the Chemunex ChemScan RDI, is less sensitive providing a lower approximation of bacterial cell counts. Moreover, the SPLC bacterial approximations obtained through this study most closely modeled a 48-hour pour plate culture, yet were performed in three hours. Despite being subject to systematic errors giving only an approximate number of bacteria, the pour plate culture technique in this study yielded higher bacterial counts. It was expected the SPLC method would avoid culturing errors and provide a more exact bacterial enumeration.

[34]

DETECTION OF PARA-CYMENE, A MICROBIAL-GENERATED VOLATILE ORGANIC COMPOUND (MVOC), BY USE OF A BIOLUMINESCENT BIOSENSOR. E. Worthington[1], K. Daumer[2], J. Garland[2]. [1]Dept. of Chemistry Southern Oregon University Ashland OR 97502 and [2]Dynamac Corporation Mail Code DYN 3 Kennedy Space Center FL 32899

The purpose of this project was to test procedures and protocols for the development of an integrated biosensor for the detection of para-cymene using bioluminescent bioreporter bacteria suspended in a permeable bead. Research was conducted using two cultures of a strain of *Pseudomonas fluorescens* developed by the Center for Environmental Biotechnology (CEB) as bioreporters. The preponderance of the project was spent using a bioreporter strain defective for the recombinant gene of interest. The work with the defective strain served to improve contamination control. Work with the second strain provided immediate success and validated the work done with the first strain. A light tight sealed reaction chamber was used to observe the bioluminescence triggered by the metabolism of p-cymene by *P. fluorescens* Bioluminescence was determined by measuring the current generated by the emitted photons in a photo-multiplier tube. Initial bioluminescence was triggered one hour after the introduction of *para*-cymene and continued to increase despite a decrease in *para*-cymene concentration with time. Measurements were obtained at vapor phase concentrations of less than one ppm but a maximum peak emission was not observed at the concentration used Despite the lag in response and lack of correlation between concentration and bioluminescence in this one assay it is still hypothesized that the bioreporter can produce qualitative as well as quantitative detection. Further work will be done to determine the threshold of detection and to develop an accurate flow through detection system. NASA funded this research in conjunction with the Spaceflight and Life Sciences Training Program (SLSTP) at the Kennedy Space Center and in collaboration with the CEB at the University of Tennessee-Knoxville.

[35]

PHOSPHORYLATION SIGNALING AND THE REGULATION OF AUXIN TRANSPORT IN *ARABIDOPSIS THALIANA* ROOTS AND HYPOCOTYLS. N.N. Kirpalani, A.M. Rashotte and G.K. Muday. Dept. of Biology, Wake Forest University, Winston-Salem, NC 27109.

Redistribution of auxin contributes to gravitropic bending of plant tissue, however the mechanisms by which gravitropic signaling leads to altered auxin transport are not fully understood. Protein phosphorylation and dephosphorylation have been implicated in this regulation by studies of the *roots curl in NPA1* (*rcn1*) mutant, which has a mutation in a gene that encodes a protein phosphatase 2A regulatory subunit. Roots of *rcn1* respond more slowly to gravity stimulation than wild-type (Ws) roots. Gravitropic bending and gradients in auxin induced gene expression were examined in the hypocotyls of *rcn1* mutants transformed with the DR5- β-glucuronidase (GUS) reporter gene. After gravity stimulation, the auxin induced DR5-GUS expression was localized to the lower side of the hypocotyl where gravitropic bending occurs. In order to identify a kinase that may also function in the regulation of auxin transport during gravitropic bending, plants with mutations in the PINOID (*PID*) gene, which encodes a serine-threonine protein kinase were examined. Roots of the *pid-9* mutant have a slower response to gravity stimulation compared to wild-type (Columbia) as shown with high temporal resolution using a computerized image analysis program, Multi-ADAPT. Furthermore, basipetal IAA transport was measured in *pid-9* roots since a correlation has been suggested between basipetal auxin transport and gravity response. The gravitropic response alterations in these plants with either reduced phosphatase or kinase activity indicate that phosphorylation signaling plays an important role in regulation of auxin transport in Arabidopsis roots and hypocotyls. This work was supported by the National Aeronautics and Space Administration Specialized Center of Research and Training

(grant no. NAGW-4984).

[36]

AN EVALUATION OF NUTRIENT PROVISION STRATEGIES FOR SPACEFLIGHT APPLICATIONS. J.U. Okere[1], G.K. Tynes[2], R.G. Stoesz[3] and H.G. Levine[2]. [1]Dept of Biology, University of Miami, FL. [2]Gravitational Biology Laboratory, Dynamac Corp., Kennedy Space Center. [3]Valencia Comm. College, Kissimmee, FL.

The Water Offset Nutrient Delivery Experiment (WONDER) will evaluate the growth of plants within both a Porous Tube Nutrient Delivery System and a Substrate-based Nutrient Delivery System (SNDS) under three separate wetness level set-points. The base-lined SNDS utilizes *Turface*™ (a clay-based substrate) and slow dissolving fertilizer pellets (*Osmocote*™) which supply nutrients for plant growth upon the addition of water. This has proven to be effective, however, the standard *Osmocote* formulation does not contain all of the trace elements required for plant growth. It was hypothesized that the *Turface* functioned as a source of the necessary trace elements. This study was therefore designed to: (1) evaluate the nutritional contribution of the *Turface* substrate, and (2) evaluate the nutritional efficiency of a new *Osmocote Plus* formulation which includes a trace element component (in place of some of its macronutrients) in its manufacture. Five experimental treatments, each containing 24 wheat (*Triticum aestivum* cv Yecora rojo) seeds, were evaluated for their ability to support plant growth under conditions similar to those envisioned for WONDER (23° C, 75% RH, 1,000 ppm CO_2, 185 □moles m^{-2} s^{-1}). Water samples were taken from each experimental compartment on days 0, 6, 12 and 19. After 21 days of growth, all plant tissues were harvested and subjected to tissue analyses. The *Osmocote* treatment exhibited faster growth than the *Osmocote Plus* (trace element) formulation. The *Turface*-Only treatment had sufficient nutrients to support early wheat growth, but failed to provide sufficient nutrients for long-term growth. The *Turface* with Hoaglands treatment exhibited faster plant growth than the glass beads with Hoaglands treatment. Leaching of compounds from the glass beads (which had been assumed to be inert) was apparent. This research was supported by the Spaceflight Life Sciences Training Program (SLSTP) and NASA (NAS610-12180).

[37]

TEMPORARY DELAY OF COTTON FIBER INITIATION, ELONGATION, AND CELLULOSE BIOSYNTHESIS BY COLD TEMPERATURE. D.S. Johnson[1,2] and B.A. Triplett[2]. [1]McNair Scholars Intern Program, Xavier University; [2]USDA-ARS, Southern Regional Research Center, New Orleans, LA.

Cotton fiber growth is an ideal model for investigating microgravity effects on plant gene expression. Individual fiber cells in culture will attain lengths greater than 1.3 cm in less than two weeks. Deposition of a secondary cell wall composed almost exclusively of cellulose also occurs *in vitro* beginning approximately 12 days after culture initiation and continues for at least 16 more days. We have proposed to examine the effects of microgravity on gene expression during all developmental phases using this model plant system, however, flight duration on Space Shuttle missions may involve shorter intervals. For this reason we have examined during the flight definition phase of our investigation whether development can be temporarily delayed by cold temperature exposure without deleterious effects on subsequent fiber/seed development. Replicate cultures were initiated using day-of-anthesis cotton ovules. On the day of culture initiation and five or ten days after culture initiation, cultures were transferred to 4 C for 1 or 2 days. After cold temperature treatment, cultures were returned to standard culture conditions of 32 C. Ovule fresh weight, ovule dry weight and cellulose content of the fiber were measured for all treatments. Delaying or interrupting normal culture conditions of ovule cultures by 1 or 2 day exposure to cold temperature appears to have no major or lasting effect on any of the stages of fiber development. Therefore, it is feasible to examine simultaneously microgravity effects on fiber at three distinct developmental stages.

(Supported by NASA: 99-HEDS-02.)

[38]

THE EXPRESSION OF ADH-GUS IN TRANSGENIC *ARABIDOPSIS THALIANA*. Kalin C. Lee[1], Anna-Lisa Paul[2], and Robert J. Ferl[2]. [1]Purdue University, [2]University of Florida

The Adh gene becomes active when the *Arabidopsis thaliana* plant is in certain stressful environments. The GUS reporter attached to the Adh is expressed when the Adh is active, that is, under stressful environments. When Arabidopsis is placed in a certain histochemical stain this expression can be visualized as a blue coloring in the plant. This could be used to monitor plant health or environmental conditions. The objective of this study is to calibrate the Adh-GUS transgene so monitoring can be conducted. The experimental procedure was to analyze images of stained plants that show different patterns and intensities of blue coloring depending upon the type of stress they were subjected to. These would be compared with reverse transcriptase PCR (rtPCR) / gel electrophoresis, and real-time PCR. The images showed specific patterns for different stress types. The gel electrophoresis data showed that the stain intensities do indeed correspond to the amount of active Adh, and thus to the amount of stress.

(Supported by NASA's Spaceflight and Life Sciences Training Program (SLSTP))

[39]

THE STABILITY OF RNA OF THE *ARABIDOPSIS* PLANT AS A COMPONENT OF THERMAL PROFILING TECHNIQUES: A MOLECULAR BIOLOGY ASSESSMENT. Davida Robinson[1], Dr. Anna-Lisa Paul[2] and Dr. Robert Ferl[2] [1]SLSTP Trainee: Space-flight Simulation Emphasis Group, NC A&T State University Dept. of Physics, [2]University of Florida, Program in Plant Molecular and Cellular Biology, Dept. of Horticultural Sciences

Typically Ribonucleic Acid (RNA) is stored at a temperature of –80ºC to preserve integrity. This storage temperature is not always available in a Space Flight situation. Various thermal profiles for storing harvested tissue such as in liquid N_2 (–196ºC), –80ºC, -20ºC and room temperature, were used as a comparison for various Space flight simulations. The Molecular Biology Assessment evaluates the various thermal profiling techniques in their ability to sustain the stability of RNA of harvested *Arabidopsis thaliana*, in both simulated flight and ground controls. A set number of samples were stored in the new, uncharacterized stability agent, "RNA*later*". Results can be used for further investigation into the best protocols for handling plants on the International Space Station.

[40]

LOW PRESSURE STUDY FOR THE DEVELOPMENT OF A MARS GREENHOUSE. L.M. Gravatt[1], P.A. Fowler[2], V.Y. Rygalov[3], and R.M. Wheeler[4]. [1]University of Minnesota, Minneapolis; [2]Dynamac Corporation, Kennedy Space Center, FL; [3]University of Florida, Gainesville; [4]NASA, Kennedy Space Center, FL.

The goal of this study was to design, develop, and test a low pressure, closed environment that was conducive to plant growth. An environment was designed and built in a way that allowed for the control of temperature, pressure, and relative humidity in the system. The methodology of this study consisted of determining the controllable parameters, designing the environment, constructing the environment, and testing the system. System consisted of three bell jars placed into two Percival Environment chambers. Each bell jar had a cooling coil used to cool the air and collect condensation. The condensation was then collected and measured in a graduated cylinder.

The results showed that relative humidity is not dependent on total pressure, but it is dependent on the temperatures of the jar and the cooling coil. As for the water cycle, data showed that evaporation rates did not give any significant results. However, it was observed that more water was collected in the graduated cylinder at lower pressures. From this study, it was concluded that an artificial environment can be created such that temperature, pressure, and relative humidity are controlled and a water cycle is produced. In addition, it can be concluded that it is only necessary to control two temperatures (bell jar temperature and coil temperature) in order to control the system.

(Supported by NASA, SLSTP)

[41]

REMOTE SENSING OF SEAGRASS. E. Zeledon[1], C. Hall[2]. [1]Swarthmore College Swarthmore PA 19081 and [2]Dynamac Corporation Mail Code DYN-2 Kennedy Space Center FL 32899.

The purpose of this research was to perform ground field observations to determine the feasibility of using satellite spectroscopic instrumentation for seagrass observation. Seagrass is vital to maintain healthy aquatic ecosystems, including fish populations. However, aerial photography, which is currently used to map seagrass beds, often cannot distinguish between algae and seagrass. This can result in erroneous seagrass maps that in turn can lead to faulty management decisions. It was hypothesized that advanced hyperspectral imaging systems could distinguish between various seagrass species and algae. In this study, a radiometer capable of capturing detailed spectral reflectance was used to determine that various species of seagrasses have different spectral reflectance curves. This potentially can be used to identify seagrass beds using hyperspectral imagery capable of capturing detailed spectral reflectance curves for every target in the image area. The first derivative of the spectral curve was used to identify the ideal spectral region for further observation. It was found that the red region of the visible spectrum at approximately 700 nm provided the most spectral significance between different seagrass species and algae. This research indicates that the hyperspectral radiometer should be substituted for traditional aerial photography in determining seagrass bed distributions. NASA funded this research in conjunction with the Spaceflight and Life Sciences Training Program (SLSTP) at the Kennedy Space Center.

Concurrent Posters
II-B
Graduate Student Poster Competition

[42]

A STUDY ON EFFECTS OF YOGIC EXERCISES ON SIMULATED MICROGRAVITY. P.B.Dattani, A.K.Sengupta. Department of Aerospace Physiology, Institute of Aerospace Medicine, Indian Air Force, Bangalore, India.

The study was the first of its kind to investigate the effects of yogic exercises on cardiovascular changes during 2 h of 6º head down tilt (HDT) position and recovery period. Two groups of six subjects each: Group A – practicing yogic exercises for 6 mo and Group B - practicing yogic exercises for 60 mo were compared with age and sex matched controls. Changes in heart rate (HR) and mean arterial blood pressure (MAP) were studied during the 2 h of HDT and recovery in the horizontal position for 30 min.

Group B showed a significant ($P<0.01$) fall in HR and rise in MAP in response to the tilt, similar to the controls, whereas, Group A showed minimal changes to the tilt. On return to the horizontal position, however; Group A parameters returned to baseline value in 20 min, whereas Group B showed a further fall in HR and rise in MAP. Neither Group B nor the control group showed a return to the baseline values. Symptoms of nasal congestion, heaviness in the head and backache were less prominent in both Group A and B, as compared to controls.

To conclude, deconditioning was resisted after 6 mo of yogic exercises, showing a relative parasympathetic dominance. Whether this would be beneficial during space flight, is difficult to say. There is, however, a possibility that yogic exercises practiced for a short period of six months may be of some benefit for early recovery on return to ground conditions. These responses to HDT simulated microgravity changes may reflect the manner in which yogic exercises influence the cardiovascular system in space.

[43]

REMOVAL OF SALTS FROM URINE BY ELECTRODIALYSIS USING A BATCH RECIRCULATION SYSTEM. N. E. Gordils and G. Colón. Dept of Chemical Engineering, Univ of Puerto Rico, Mayagüez.

Humans need food, oxygen and water for their survival. However, in space these basic necessities are not readily available and the cost involved in supplying them from the earth is expensive and impractical for long time space missions. The National Aeronautics and Space Administration (NASA) has developed a project in which food, oxygen, water and human wastes can be recycled and then used to support a person. In the Controlled Ecological Life Support System (CELSS), urine is recycled as a fertilizer for the plants that grow under hydroponic conditions.

The scope of the research was the recovery of sodium chloride from human urine, using an electrodialysis cell coupled with ion selective membranes. Data was obtained at constant applied voltage and at constant current density. Emphasis was given to the fouling process.

From the results obtained, the initial rate of NaCl removal was correlated as a power function of the applied voltage if operated at constant voltage. If operated at constant current density, the initial rate of NaCl removal was estimated by a power function of current density. The percentage of NaCl removal was calculated with the use of ion chromatography equipment. From the acquired data, electrodialysis with batch recirculation at constant applied voltage yields higher values of NaCl removal with approximately 94 % (for E = 7.5 V and U = 0.083 m/s). On the other hand, at constant current density, the higher percentage of removal was 73 % (for i = 43 A/m^2 and U = 0.083 m/s). Membrane fouling was always observed due to the strong affinity of the urochromes of urine for the ion selective membranes. Impedance measurements showed that the anion membranes were the most affected.

(Supported by NASA: NAG10-0257.)

[44]

SODIUM CHLORIDE REMOVAL FROM URINE VIA ELECTRODIALYSIS FOR USE IN ADVANCED LIFE SUPPORT. V. M. Aponte, N. E. Gordils and G. Colón. Dept of Chemical Engineering, Univ of Puerto Rico, Mayagüez.

A six-compartment electrodialysis cell using univalent ion selective membranes was proposed to remove sodium chloride from human urine. Limiting current density (i_{lim}) data were obtained at four fluid velocities ranging from 0.015 to 0.083 m/s, and four urine concentrations ranging from 3200 to 12800 ppm, based on chloride salts concentration. The i_{lim} was found to be a power function of fluid velocity and Cl^- salts concentration as correlated by the model $i_{lim}/C^*=4105\ U^{0.90}$. Ion chromatography revealed that the highest sodium chloride percentage for continuous once-through mode of operation was of 91.0 % achieved at the most diluted solution (U=0.039 m/s and i=57 A/m^2). For batch recirculation, the highest removal, 98.5 %, was achieved at a potential of 9.0 volts and U=0.083 m/s. An average current efficiency of 50 % indicates that half of the applied current was used in sodium chloride transport.

(Supported by NASA: NAG10-0257.)

[45]

THE EFFECTS OF HYPERGRAVITY ON VESTIBULAR NEURAL FUNCTION IN POSTNATAL RATS. S. Irons-Brown[1], S. M. Jones[2], W. Avniel[2], K. Paggett[2], G. Subramanian[2], Lisa Baer[3], A. E. Ronca[3] and T. A. Jones[1&2]. [1]Dept of Physiology, [2]Dept of Surgery-ENT, Univ of Missouri-Columbia, and [3]Life Sciences Division, NASA Ames, Moffett Field, CA.

The gravitational environment can have profound influences on physiological systems. In this study, we evaluated whether vestibular functional adaptation occurs in response to chronic gravitational loading. We used linear vestibular evoked potentials (VsEPs) to characterize gravity receptor function in rats following 14 days of 2G centrifugation. VsEP response peak latencies (P1 to N2), amplitudes (P1/N1 to P2/N2), and thresholds were quantified and compared between experimental and control animals. One experimental group consisted of animals placed on the centrifuge on postnatal day 7 i.e. P7 (P21 test group). The second experimental group was placed on the centrifuge on P14 (P28 test group). The age matched control groups were at 1G (stationary) or 1.03G (rotational). There were no significant changes in the latencies and amplitudes among the experimental and control groups. However, VsEP thresholds (in dBre: 1.0 g/ms) were significantly different. For the P21 group, thresholds for the 2G animals (-8.70 ± 3.52dB p=0.006) were significantly higher than the controls (-12.19 ± 2.44dB (pooled)). For the P28 group, thresholds for the 2G animals (-8.75 ± 2.99dB p=0.04) were significantly higher than stationary controls (-12.00 ± 4.34dB). These results tend to support the hypothesis that functional adaptation to 2G occurs in the mammalian vestibular system. The results suggest that the adaptive process may include a reduction in general sensitivity of the vestibular periphery. This contrasts with previous studies in the chicken, which demonstrated adaptive changes in VsEP central response components, but no reduction in VsEP thresholds (Jones et. al. 2000). Although, the reduction in sensitivity seen here appears to be consistent it is not dramatic.

(Supported by NASA: NAG5-4607 (TAJ).)

[46]

HINDLIMB UNLOADING RESULTS IN A DECREASE IN FEMORAL OSTEOPROGENITOR CELL NUMBER IN THE YOUNG MALE RAT. N. Basso, Y. Jia, C.G. Bellows and J.N.M. Heersche. Faculty of Dentistry, University of Toronto, Toronto.

We hypothesize that the reduction in bone mineral density observed in humans and rats following space flight or mechanical unloading is primarily caused by a sustained disruption in the development of cells of the osteoblast lineage. Using the NASA model of tail suspension, we investigated changes in fibroblastic colony forming units (CFU-F), alkaline phosphatase positive CFUs (CFU-AP) and osteoprogenitors (CFU-O) in cells isolated from the proximal femur, calvarium and proximal humerus in six-week old male Fischer rats hindlimb unloaded (HU) for 14 days. The proximal tibia was used for histomorphometric and *in situ* hybridization evaluation of changes in bone formation parameters. Normally loaded control rats were pair-fed. Cells were obtained from explant cultures and cultured for either 15 or 21 days in the presence or absence of 10 nM dexamethasone. There was no significant difference in the number of CFU-F between cell populations derived from HU and control rats for any of the 3 bone sites evaluated (n=3). With respect to CFU-AP, we observed a 31% decrease (n=3, $p<0.01$) in cells isolated from femur and a 2-fold increase (n=3, $p<0.01$) in cells isolated from the calvaria of HU rats. We also observed a 61% decrease (n=3, $p<0.01$) in CFU-O in cells isolated from femur, and a 1.3 fold increase (n=3, $p<0.01$) in cells isolated from calvaria of HU rats. CFU-AP and CFU-O were not significantly different in cells isolated from humerus from either group (n=3). Preliminary histomorphometric and *in situ* hybridization analysis performed on the proximal tibia indicates that the major difference in HU rats is a decrease in trabecular bone volume, mainly reflecting a decrease in primary spongiosa, and a decrease in collagen mRNA content. In conclusion, cell culture studies illustrate that the number of osteoprogenitors and therefore osteogenic potential is decreased in cells isolated from the proximal femur and is increased in cells isolated from calvarium as a result of HU.

[47]

EFFECTS OF 9-WK HINDLIMB SUSPENSION ON FIBER CHRACTERISTICS IN RAT SOLEUS MUSCLE. T. Nomura[1,2], F. Kawano[2], T. Wakatsuki[2], and Y. Ohira[2]. [1]Res. Ctr. Health Phys. Fit. Sports, Nagoya Univ., Nagoya and [2]Sch. Health Sport Sci., Osaka Univ., Osaka, Japan.

Effects of 9-wk hindlimb suspension (HS) and 8-wk recovery on soleus muscle fiber characteristics were studied in adult rats. Forty-five male Wistar rats (7-wk old) were divided into three groups: 1) pre-suspension control (n=5); 2) cage-control (n=20); and 3) 9-wk HS (n=20). Even though the rats were hindlimb-suspended, they were able to eat food and drink water freely by using their forelimbs. The percent distribution of each type of fibers, cross-sectional area (CSA), quantitative activities of α-glycerophosphate dehydrogenase (α-GPD) and succinate dehydrogenase (SDH) were determined in soleus muscle fibers histochemically. The growth-related gain of body weight was slightly inhibited by suspension. Although body weight was gradually increased during recovery, it was still less than controls after 8 wk. The growth of soleus, however, was completely inhibited by hindlimb suspension, although atrophy, relative to the pre-suspension level, was not induced. The relative weight of soleus to body weight at the end of suspension period was also lower than that in the pre-suspension control. The growth-related gains of muscle fiber CSA, as well as fiber transformation toward slow type, were completely inhibited by HS. No significant changes were observed in the specific activities of α-GPD and SDH of any types of fibers between HS and control groups. But the total activities of α-GPD and SDH in whole CSA were significantly decreased following HS. These results suggested that 9-wk HS inhibited both fiber transformation toward slow type and growth of CSA. Further, the HS-related inhibition of the growth of soleus muscle was not recovered completely after 8-wk ambulation.

[48]

MEASURING CANOPY GAS EXCHANGE RATES IN HYDROPONICALLY GROWN PEANUT (*ARACHIS HYPOGAEA* L.) AND SWEETPOTATO (*IPOMOEA BATATAS* L.) UNDER ENHANCED AND AMBIENT CO_2 CONCENTRATIONS. J.E. Wesley[1], D.R. Hileman[2] and D.G. Mortley[1]. [1]Dept of Agricultural Sciences, and [2]Dept of Biology, Tuskegee University, Tuskegee, AL.

Peanut and sweetpotato are being evaluated for use in NASA's Advanced Life Support System (ALS) program. On extended space missions, these crops will provide food, nutrient and waste recycling, and air revitalization. In order to predict plant effects on CO_2 and O_2 levels, season-long gas exchange measurements are required. Previous measurements of single-leaf photosynthetic rates have not correlated well with final yield, indicating that single-leaf sampling does not accurately reflect the complex interactions of leaf age, leaf position and light environment within the developing canopy. The objective of this study is to design a system for measuring season-long, whole-plant gas exchange rates under ambient and enhanced CO_2 concentrations and to correlate these gas-exchange data with final yield. An open-flow system was constructed to determine canopy gas exchange rates of hydroponically-grown peanut and sweetpotato under controlled temperature and light conditions. Carbon dioxide concentrations were maintained at 400 (ambient) and 1000 $\mu mol/mol^{-1}$. Gas exchange rates were continuously determined based on CO_2 concentrations in air entering and exiting the canopy gas exchange chambers. Results are expected to show that elevated CO_2 will lead to increases in canopy photosynthetic rates and in final yield, and that final yield will correlate strongly with whole-season total photosynthesis. This data will be useful for designing ALS systems that maintain suitable atmospheric conditions for human life.

(Supported by NASA: NCC9-51.)

[49]

USE OF RNAi TO CHARACTERIZE CALCIUM SIGNALLING GENES EXPRESSED IN *CERATOPTERIS RICHARDII* DURING SPORE GERMINATION. S.C. Stout, M.L. Salmi, T.J. Bushart, G.B. Clark, S.J. Roux. Section of Molecular, Cell and Developmental Biology, The University of Texas at Austin.

Gravity directs polarity development in the single celled spore of *Ceratopteris richardii*. The vector of gravity fixes spore polarity during a limited window between 12 and 24 hours after light initiation of spore germination. Concurrent with this period of graviresponsiveness, an efflux of calcium is found from the top of the spore and a corresponding influx is found along the bottom. This bottom to top polar calcium current rapidly reorients when the spore is rotated 180º, and treatment with a calcium channel blocker reduces both the calcium current and the spore's ability to correctly respond to gravity.

As part of a cDNA microarray project, over 2,800 ESTs were sequenced from a cDNA library. Several genes involved in calcium signaling and homeostasis are represented in the EST library, including calmodulin, calcium dependent protein kinase (CDPK), calcium-ATPase, and annexin. Our work focuses on characterizing these genes using the RNAi technique.

We have cloned and sequenced a full-length calmodulin, which is expressed during the period of gravity fixation. The expected amino acid sequence has over 90% identity to other higher plant calmodulins, and anti-cauliflower calmodulin antibodies recognize the heterologously expressed protein.

Spores imbibed with dsRNA corresponding to the calmodulin show delayed germination compared to controls. However, there is no change in polarity development. We are testing whether the germination delay can be attributed to an RNAi induced decrease in calmodulin expression, and we are continuing to investigate other genes of interest with this technique.

(Supported by NASA: NAG10-0295, NAG2-1347, and NGT5-50371)

[50]

WHEAT LEACHATE AEROBIC BIODEGRADATION: DENITRIFICATION EFFECTS. R. Colón and G. Colón. Dept of Chemical Engineering, Univ of Puerto Rico, Mayagüez.

The National Aeronautics and Space Administration (NASA) are developing an Advanced Life Support System (ALS) to sustain life in space during long space missions. NASA considers the use of hydroponics growth of wheat, tomatoes, peanuts and soy. Due to the high nitrate concentration of the wheat waste effluent it was necessary to study the possibility of denitrification in ALS.

The effect of the inoculum composition and the initial nitrate concentration were studied in order to determine their influences in the denitrification effects under aerobic condition. Batch pilot plant studies were used for wheat lixiviate bioconversion.

The results clearly showed that the only factor that appears to affect the denitrification effects was the dissolved oxygen concentration. Ammonia and nitrite composition needed to be considered to avoid overestimation of the denitrification effects. The significant levels of variability are probably due to a variation in the inoculum composition.

(Supported by NASA: NAG10-0257.)

[51]

ANATOMY OF STEMS OF *BRASSICA* PLANTS DEVELOPED IN MICROGRAVITY R. I. Ruiz[1], A. Kuang[1], and M. E. Musgrave[2] [1]Department of Biology, The University of Texas – Pan American, Edinburgh, TX 78539; [2]Biology Department, University of Massachusetts, MA 01003

Plant stems play important roles in support, transport, and storage, but there is little information available regarding whether the microgravity environment has any influence on the structure and function of stems. The current study compares stems of *Brassica rapa* L., cv. CrGC#1-33 plants grown on board the MIR space station and on the ground, in an attempt to identify microgravity effects on stem structure and function. *Brassica rapa* plants (18 d-old) developed on the MIR space station and in the post-flight ground control experiment were preserved in 1% glutaraldehyde solution. Preserved plants were dissected and stem sections between the cotyledon and the first true leaf of the preserved plants were processed and prepared for microscopy. Cross-sections of stems were used for the measurements on their diameters, the areas of cortical, vascular and pith tissues, and cell numbers of each tissue. The ratio of each tissue region relative to the cross section area of the stem was calculated. Starch grains in the cortex were also counted. Data analysis indicates that stems of MIR plants have a smaller diameter, reflecting smaller areas of cortical and pith tissues. Numerous large starch grains were present in cortical cells of space plants, [while these were absent in the ground control material]. [The results suggest that in microgravity, the storage function of the stem is emphasized over the support function. This could have consequences for the nutritional value of plants that might be grown for food during spaceflight.]

(Supported by NASA grant NAG2-1375).

[52]

IMPORTANCE AND METHOD OF CROP CULTIVAR EVALUATION FOR ALSS: APPLICATION ON TOMATO CULTIVAR SELECTION. C. Moraru[1], C.M. Gregson[1], T-C Lee[1], L. Logendra[2] and H. Janes[2]. [1]Food Science Dept., [2]Dept. of Plant Sciences and [1,2]NJ-NSCORT, Rutgers University, New Brunswick, NJ.

The challenge in selecting crops for an Advanced Life Support System (ALSS) is to find cultivars able to meet nutritional and quality requirements while growing in limited space and generating low waste. The objective of this study was to establish a logical approach to assess potential plant cultivars for fresh consumption and/or processing within ALSS, and to test it using tomato as an example crop.

This approach started by choosing all pertinent quality attributes and defining for each the acceptable range and, when possible, the optimum value. An evaluation scheme was developed based on a summation of weighted ranks, but only for cultivars whose attributes fell within the acceptable ranges. Finally, experimental data was used in this scheme to evaluate the quality of each cultivar and decide on the most suitable one. In our example, 11 growth, 12 physical/chemical and 7 sensory indexes were identified for ALS tomato. 16 tomato cultivars (10 processing and 6 fresh-consumption ones) were hydroponically grown, their attributes quantified and ranked using the proposed scheme.

In developing the evaluation method, the choice of significant attributes and their weight in the overall ranking depends highly on the mission goals and design. However, once they are set, the comparison between similar or slightly different crops (eg. processing versus fresh-consumption tomato) is facilitated. In this case, the best performance was shown by the SUN 6117 variety. Some of its indexes were: soluble solids 5.33°Brix, lycopene content 12.8 mg/100g, harvest index 0.75, firmness of 10.9 N/mm and very good sensory ratings.

This method is a logical and essential approach for evaluating crop varieties based on the relevance of quality attributes for the mission design. Among its benefits, the ability to identify key quality attributes for rapid initial evaluation may also be interesting for crop developers.

(Supported by NJ-NSCORT)

[53]

WE THOUGHT WE KNEW HOW TO GROW LETTUCE: STUDIES WITH LIGHT AND TEMPERATURE J. M. Frantz, G. L. Ritchie, and B. Bugbee. Crop Physiology Lab. Dept of Plants, Soils, and Biometeorology, Utah State University, Logan, UT 84341.

The temperature optimum for lettuce is thought to be 20 to 25 C. Warmer temperatures are not used because they increase bitterness and tip burn. Temperatures above 25 C are also thought to reduce growth rates. We examined growth of lettuce canopies at five constant temperatures: 21, 25, 30, 32.5, & 35 C with two replicate chambers at each temperature. Our studies indicate that the temperature optimum for leaf expansion, light interception, and growth is 30 C, which is much higher than used in previous studies. The growth rate increased by more than 3-fold from 21 to 25 C and by 40% from 25 to 30 C; but decreased by 20 % from 30 to 32.5 C. Plants appeared chlorotic at 20 and 25 C, but chlorophyll content increased with increasing temperature, and was more than 30 times higher at 35 than at 21 C. Tip burn was not observed at or below 30 C. These results indicate that the temperature optimum for lettuce growth and appearance is 5 to 10 C above that used in previous studies. In a separate study, PPF levels were reduced by 20 to 80% in the middle of the life cycle to examine adaptation rates. Whole plant photosynthesis and respiration rates adapted to within 80% of the initial rate after a few days. These results indicate that lettuce is surprisingly tolerant of the system failures that are associated with power loss in space flight conditions.

[54]

QUANTIFYING EXTRACELLULAR MASS TRANSPORT USING DIGITAL HOLOGRAPHY. M. R. Benoit[1], D. M. Klaus[1] and R. B. Owen[2]. [1]Aerospace Engineering Sciences Department, University of Colorado, Boulder. [2]Owen Research Inc., Boulder, CO.

It has been hypothesized that many of the cellular level physiological effects observed to occur in space result, at least in part, from a reduction of extracellular mass transport in the absence of gravity-driven sedimentation and convection. Empirical data are needed to more fully elucidate this hypothesis and to calibrate computer models designed to simulate these phenomena. The objective of this current study was to evaluate a technique using double-exposure digital holographic interferometry for monitoring real-time changes in fluid density.

Initial experiments were conducted using a device called the Digital Holographic Monitor (DHM) to measure density gradients formed by adding a drop of 0.1% saline to distilled water. Relative shifts in refractive index on the order of 10's of microns resolution were observed as density gradients formed. The imaged data were then correlated to absolute saline percentages.

Based on these initial findings, experiments were designed to observe boundary layer density gradients formed by metabolically active bacterial cells growing on a tip of agar submersed in a liquid growth medium. Buoyant streaks of less dense fluid rising from the cells were imaged, but determined to originate from a combination of agar constituents and cellular metabolic byproducts. Differentiating between these two contributing factors and quantifying the absolute density changes from the phase map data remains as a challenge to be resolved. Observing density gradients surrounding growing protein crystals represents another potential application of this technology. Measurements of lysozyme/acetate buffer solutions indicated the ability to quantify concentration differences of 0.1%.

Successful demonstration of double-exposure digital holography is expected to lead to adaptation for use in space to further quantify the effects of gravity on density gradients created by submicron particles in a fluid environment.

(Supported by NASA: NAS8-99087)

[55]

ANALYTICAL METHODS FOR INVESTIGATING LIFE PROCESSES IN EXTREME ENVIRONMENTS. C. N. Jayarajah and M. Thompson, Department of Chemistry, University of Toronto, Canada

In preparing for missions to Mars, it is important to consider the effect of planetary conditions and space travel on the complex mechanisms of life. It is thus crucial to study the genomic code along with gene transcription in light of life in extreme conditions. The acoustic wave sensor is presented here as a tool to investigate the effect of space exploration factors such as gravity, light and radiation on gene transcription. Our results to date indicate that the thickness shear mode (TSM) acoustic wave sensor is an effective biosensor for distinguishing varying degrees of binding interactions with the promoter DNA and subsequent mRNA synthesis. Furthermore, in searching for signatures of life in extreme environments on earth or on extraterrestrial worlds, it is important to consider the chemical nature of the environment. Consequently, we have begun to study the chemistry of soil and water in Devon Island in Nunavut, Canada as a Mars analog site. The chemical analysis of the Haughton crater area in the Canadian High Arctic is presented along with results of DNA isolation from soil, water and rock samples. Our results show that the Arctic terrain is poor in all sources of nitrogen, phosphates, sulfur and iron. However, we find significant levels of calcium (Ca) and magnesium (Mg) in soil and water samples, a discovery compatible with the dominating presence of dolomite in the region. We hope to be able to understand the impact of the geological history of the Haughton crater on the chemistry of its environment, and consequently, on the life forms that prevail there today as well as evidence for past or present life such as nucleic acids in the area.

Symposium II
Evolutionary Biology/Evolution of Body Plans

[56]

GENE REGULATORY VIEW OF THE ORIGIN AND EVOLUTION OF ANIMAL BODY PLANS E. H. Davidson. Division of Biology, California Institute of Technology

Body plans are generated by the process of development and in all complex animals development is driven by the operation of regulatory gene networks. The central components of those networks consist of the skein of genomic regulatory linkages that connect the *cis*-regulatory DNA sequences of sets of genes which themselves produce gene regulatory proteins. The developmental control systems responsible for body plan formation are hardwired, heritable, species-specific features of animal genomes. They determine in which cells, when, and at what rate each gene is expressed during development. An example of such a developmental gene regulatory network that is now being unraveled in the sea urchin embryo will be presented. At a mechanistic level, understanding the evolution of body plans can only be achieved by determining evolutionary changes in the relevant developmental regulatory networks. To this end a new complex of scientific disciplines is required, rooted in regulation molecular biology, but relying heavily on sophisticated new computational approaches, the accessibility of comparative genomics, and the expert use of both phylogenetic and other evolutionary information together with solid developmental biology.

(Supported by NASA/Ames NAG2-1368.)

[57]

THE GREEN BODY BEAUTIFUL: APPROACHES TO UNDERSTANDING THE ORIGIN AND EARLY EVOLUTION OF PLANT BODY SYMMETRY AND DEVELOPMENTAL RESPONSES TO GRAVITY. L.E. Graham[1] and M.E. Cook[2]. [1]Dept of Botany, Univ of Wisconsin, Madison. [2]Dept of Biological Sciences, Illinois State Univ, Normal.

Plants, defined here as the monophyletic embryophytes (bryophytes and vascular plants) achieved multicellularity independently of animals and fungi, and display a variety of body forms for which symmetry modifications form a basis. Insight into the origin and early diversification of plant bodies can be obtained by comparing early-divergent groups of modern plants (bryophytes andl lycophytes) with the modern green algae closest to plant ancestry, charophyceans. Mapping structural, physiological, reproductive, and genetic traits onto increasingly robust phylogenies provides insight into the order and mechanism of early plant evolution.

Early structural innovations that arose during charophycean diversification include: cellulose-rich cell wall produced by rosette-shaped cellulose synthesizing complexes, cytokinetic phragmoplasts, intercellular communication channels (plasmodesmata), cell division in more than one direction, simple apical meristems, asymmetric cell divisions, and cell specialization. Charophyceans have been useful model systems for experimental analysis of gravity responses and cytoskeletal dynamics.

Innovations linked with divergence of earliest embryophytes include sporophytes, histogenetic meristems, and tissue differentiation. Bryophytes offer the benefit of efficient gene targeting systems, and are also used to explore gravisensing, the origins of hormonal control of plant development, and homeobox gene diversification.

[58]

SIGNALING MOLECULES IN THE *HYDRA* HEAD ORGANIZER AND THE EVOLUTION OF AXIS FORMATION. B. Hobmayer, F. Rentzsch, and T.W. Holstein. Dept. of Molecular Cell Biology, Darmstadt Univ of Technology, Darmstadt, Germany.

The molecular nature of signaling centers (organizers) plays a pivotal role in the formation of body axes in multicellular animals. Organizers secrete growth factors, which act as long range regulators in axis formation and cell differentiation. To analyze the origin and evolution of organizers, we characterized Wnt and TGF-beta signaling pathways in *Hydra*, a member of the primitive animal phylum *Cnidaria*. Molecules of the Wnt and TGF-beta signaling pathways are expressed in the *Hydra* head organizer. Wnt, Tcf, and Chordin are transcriptionally upregulated early during asexual bud formation and head regeneration. Wnt and Tcf expression domains also define head organizers created by *de novo* pattern formation in aggregates. Thus, the *Hydra* head organizer exhibits astonishing similarities to the Spemann-organizer in vertebrates. Our results demonstrate that Wnt and TGF-beta signaling acts in axis formation in *Hydra*, and support the idea that this played a key role in the evolution of axial differentiation in the earliest multicellular animals.

(Supported by the Deutsche Forschungsgemeinschaft)

[59]

THE EVOLUTION AND ALLOMETRY OF PLANT BODY PLANS. arl J. Niklas. Department of Plant Biology, Cornell University, Ithaca NY 14853.

Plants (defined as eukaryotic photoautotrophs) have evolved multiple times. Most lineages originated in deep geological history (i.e., the algae); the most recent lineage is that of the land plants (i.e., the embryophytes). Across all lineages, only three basic body plans exist (i.e., unicellular, colonial, and multicellular), although important variants exist (e.g., siphonous algae and vascular plants). Convergence and divergence is common within and across the major algal lineages. However, the multicellular body plan is the only one successful on land.

This convergence and divergence within the algal lineages is attributed to the capacity of each body plan to maximize its surface area with respect to an increase in body volume, thereby minimizing nutrient transport time and maximizing growth rates in an aquatic and essentially gravity-free environment. This 'allometric tactic' is adjusted significantly in an aerial habitat, which biophysically requires water conservation and thus a reduction in body surface area with respect to body volume. Additionally, an aerial habitat necessitates mechanically supportive/nutrient conducting tissue types as body size either ontogenetically or evolutionarily increases.

Despite their tremendous diversity in size, shape, geometry, and internal structure, all plants, regardless of phyletic affiliation or habitat preference, conform to the same general scaling (allometric) 'rules.' Thus, across and within the plant lineages, growth scales, on average, as the 3/4–power of body mass and isometrically with respect to either cell photosynthetic pigment concentration (algae) or foliage biomass (tracheophytes). These 'rules' are attributed to unavoidable biophysical constraints that have shaped all of plant evolution (e.g., Fick's law of diffusion).

(Supported by an Alexander von Humboldt Forschungspreis.)

[60]

GENOMIC AND PROTEOMIC APPROACHES TO DELINEATING EVOLUTIONARY DEVELOPMENT OF THE PLANT VASCULAR APPARATUS. Aldwin M. Anterola, Laurence B. Davin and Norman G. Lewis Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

An essential element of vascular plant development involved the evolution of the phenylpropanoid pathway, particularly those branches affording lignins, (poly)lignans and suberins. Metabolic flux and real-time PCR analyses as well as down-regulation of the various steps of the phenylpropanoid pathway and downstream coupling systems have recently revealed the presence of complex metabolic networks. These, in turn, are involved in cellular, tissue and organ specific regulation of monolignol formation and its coupling.

This paper summarizes our current knowledge of how these complex networks are organized, and how various perturbations lead to a loss of overall plant vascular integrity.

Concurrent Oral Sessions
I
Genomics, Proteomics and Systems Biology

[61]

ALTERED CYTOSKELETAL GENE EXPRESSION IN SPACE-FLOWN T CELLS (JURKAT) EVALUATED BY cDNA MICROARRAY AND RT-PCR. M.L. Lewis[1] and L.A. Cubano[2]. [1]Dept of Biological Sciences, Univ of Alabama in Huntsville and [2]Dept of Medicine, Tulane Univ, New Orleans.

Cytoskeletal anomalies and inhibition of microtubule polymerization occur in space-flown cells and gravity dependence in microtubule self-organization in cell-free systems was recently demonstrated. Our shuttle-based research with human leukemic T lymphocytes (Jurkat) consistently shows cytoskeletal disruption and though microtubules appear to reorganize, cells do not grow. Differences in cytoskeletal gene expression in flown versus ground controls and cells subjected to simulated shuttle launch vibration, can provide insight into gravity-dependent cell function and processes most sensitive to spaceflight. To test the hypothesis that cytoskeletal gene expression may be altered, we flew Jurkat cells on STS-95 and evaluated genes expressed by cDNA microarray in flown and ground controls at 24 hours (4,324 genes) and 48 hours (>20,000 genes). Space-flown cells up-regulated messages for eleven cytoskeleton-related genes including calponin, dynactin, tropomodulin, keratin 8, two myosins, an ankyrin EST, an actin-like protein, the cytoskeletal linker (plectin) and a centriole-associated protein (C-NAP1); gelsolin precursor was down-regulated. Up-regulation of message for plectin, which functions in membrane-cytoskeletal association, filament elongation, and inter-filament integrity and C-NAP1 in both space-flown and simulated launch vibrated cells implies their role in vibration damage repair. Unlike flown cells, vibrated cells resumed growth thus growth arrest during spaceflight is not a primary result of shuttle launch vibration. Microgravity *per se* or other orbit-related factors appear to affect cytoskeletal gene expression and cell growth. Based on differential expression of cytoskeletal genes, we conclude that centriole-centriole, membrane-cytoskeletal, and cytoskeletal filament associations and their related cellular functions are altered during the orbital phase of spaceflight.

(Partial support: NASA Grant NAG2-985)

[62]

HYPERGRAVITY-INDUCED ALTERATIONS OF GLIAL AND NEURONAL PROTEINS IN THE DEVELOPING RAT CEREBELLUM E. M. Sajdel-Sulkowska[1, 2] and G.-H. Li[2]. [1]Dept. Psychiatry, Harvard Medical School, [2] Dept. Psychiatry, Brigham and Women's Hospital, Boston, MA 02115.

We previously reported that the developing rat cerebellum is vulnerable to hypergravity exposure, especially during a period of both granule and glial cell proliferation and neuronal migration. We hypothesized that the changes at the molecular level affecting cell-cell interactions contribute to retarded cerebellar development. To examine this hypothesis we measured the expression of glial and neuronal proteins involved in cell-cell interactions. The cerebellar lysates were prepared from Sprague-Dawley rat neonates exposed to continuous centrifugation at 1.5 G (HG; n=35) from gestational day (G) 11 to one of six postnatal time points: (P) 6, P9, P12, P18, P21, and P30; cerebellar lysates from stationary control (SC: n=34) neonates (housed under the same conditions) were used for comparison. Cerebellar size was maximally reduced at P6 in males (18.9%) and at P9 in females (19.2%), with a significant gender difference (ANOVA, P=0.048). Western blot analyses indicated gender-specific reduction of cerebellar proteins. In males maximal reduction of CD15 (26.7%), GFAP (43.1%) and NCAM (20.6%) were observed at P6; P9, and P9, respectively; in females CD15 (26.9%), GFAP (31.4%), and NCAM (31.4%) were most reduced at P6, P9 and P6. Northern blot analyses suggest that the reduction in cerebellar proteins in hypergravity-exposed neonates may be effected at the transcriptional level. Changes in the expression of these proteins are likely to affect cell-cell interaction which is critical to cell proliferation and migration, and thus contribute to abnormal cerebellar development under altered gravity.

(Supported by NASA Grant NCC2-1042).

[63]

UPREGULATION OF NEUROTROPHINS IN HUMAN RETINAL PROGENITORS AND RETINAL PIGMENT EPITHELIUM (RPE) CELLS CO-CULTURED IN THE NASA BIOREACTOR. K Dutt and R. Kumar. Department of Pathology, Morehouse School of Medicine in Atlanta, GA

Age related Macular Degeneration (AMD) and retinitis pigmentosa (RP) remain the leading causes of blindness. Tissue transplantation is a promising approach to treatment of these diseases. Scarcity of tissues for replacement, however, is a major obstacle. We have previously reported that formation of 3D structures is promoted when retinal progenitors and RPE are co-cultured in the bioreactor. The purpose of the present work is an attempt to identify neurotrophins, which might be altered in the 3D environment of the bioreactor. Methods: Human retinal progenitors and RPE were cultured alone and as co-cultures in the bioreactor. Tissue structures generated were evaluated by scanning and transmmission electron microscopy, immunocytochemistry and Western blot analysis. RT-PCR was performed using appropriate primers; amplified products generated for different cycles were evaluated. Results: Retinal-RPE co-cultured in the bioreactor on cytodex beads or as beadless co-cultures show a higher degree of differentiation and layering. Several retinal cell types could be identified in multi-layered structures generated in the bioreactor by immunophenotyping, of which photoreceptors were most well defined and differentiated. RT-PCR data revealed that neurotrophins bFGF, TGFα, CNTF and BDNF are all upregulated in cells co-cultured in the bioreactor as compared to monolayer cultures. There was a significant correlation between higher level of BDNF, CNTF and the complexity of tissue-like structures generated in the bioreactor. Conclusion: NASA's bioreactor possibly promotes tissue formation by upregulation of neurotrophins. Our results appear significant not only for tissue generation, but also in light of findings by others that the same neurotrophins can rescue degenerating photoreceptors in animal models of RP.

(Supported by NASA grants NAG9-964 (KD) and NCC9-112 (KD).)

[64]

EFFECT OF INFLIGHT 1-G ON MICROGRAVITY INHIBITION OF GENE INDUCTION OF GROWTH FACTORS, ONCOGENES, AND COX-2. M. Hughes-Fulford Lab of Cell Growth, UCSF, NCIRE and VAMC, San Francisco, CA

It has been previously demonstrated both in vivo and in vitro that osteoblast growth is reduced in microgravity. Fetal calf sera (FCS) stimulates expression of several growth factors in normal Earth gravity. In this study, we present new data on osteoblast growth factor induction by FCS in microgravity. Early passage MC3T3-E1 cells were launched on STS-76, 81 and 84 in a quiescent state in Biorack hardware. FCS activation occurred in orbit approximately 19 hours after launch. All data are derived using semi-quantative rtPCR from triplicate samples for each gene studied. Cyclophilin or18s mRNA expression was used to normalize expression of the genes. Analyses of osteoblast gene expression show a significant diminished induction of TGF□, TGFb, c-myc, bcl2 and PCNA in microgravity. TGF□, c-myc, and bcl2 expression was recovered in flight samples grown on the onboard 1-G centrifuge. Expression levels of EGFr, 18s, and cyclophilin were constitutive and unchanged by microgravity. Although initial FCS induction of cox-2 and its product PGE_2 were relatively high in flown osteoblasts 3h after activation, cox-2 expression was significantly lower in microgravity samples after 24 hours. In conclusion, FCS mediated induction of six growth related genes was inhibited in microgravity, expression of four of these genes was recovered by artificial gravity in orbit. These data suggest that much of the bone loss observed in astronauts during long-term spaceflight may be ameliorated by the presence of an onboard gravity facility on the Mars Vehicle.

(Supported by NASA grants: NAG-2-1086; NAG-2-1286)

[65]

IGF-I SIGNALING IN RAT OSTEOBLAST DURING SPACE FLIGHT. Y. Kumei[1], H. Akiyama[2], M. Hirano[2], H. Nakamura[3], S. Morita[4], K. Ohya[1], K. Shinomiya[3] and H. Shimokawa[1]. [1]Dept of Hard Tissue Engineering, and [3]Orthopaedic and Spinal Surgery, [4]Division of Rehabilitation Medicine, Graduate School of Tokyo Medical and Dental Univ., Tokyo, Japan. [2]Toray Research Center, Kamakura, Japan.

We examined microgravity effects on the mRNA levels of insulin-like growth factor I (IGF-I), IGF-I receptor (IGF-IR), and insulin receptor substrate-1 (IRS-1), a signaling molecule of IGF-I, in rat osteoblasts. Quadruplicate culture of cells were treated on board with 1□, 25 $(OH)_2D_3$ for 22 hrs, and fixed by guanidine isothiocyanate solution on the 5th day of space shuttle mission. The entire sequence of the procedure was repeated and fixed on the 6th day of the mission. After return to the Earth, the mRNA levels of IGF-IR, IGF-I and IRS-1 were examined by the quantitative reverse transcription-polymerase chain reaction. The mRNA levels of IGF-IR in the flight cultures were significantly increased to 173 % ($p<0.05$) and 186% ($p<0.01$) of the ground controls on the 5th and 6th days of the mission, respectively. However, the gene transcripts of IGF-I and IRS-1 were not at all detected in the flight cultures, which was clearly contrasted to the ground control cultures. All the data were reproduced by the repeated flight experiments. Microgravity increased the IGF-IR mRNA levels but decreased the IGF-I and IRS-1 mRNA levels. Disturbance in IGF-I signaling in osteoblasts might be involved in reduced bone formation in microgravity.

(Supported by National Space Development Agency and Institute of Space and Astronautical Science, Japan)

[66]

WOUND HEALING RESPONSE OF THE MCL DURING HINDLIMB UNWEIGHTING IN RODENTS. D.A. Martinez[1], R. Vanderby, Jr.[2], R.E. Grindeland[3], K. Dave[1], A.K. Lee[1], H.Y. Hoang[1], P. Provenzano[2], T. Wang[3], and A.C. Vailas[1]. [1]Connective Tissue Physiol. Lab, Univ. of Houston, TX, [2]Dept. of Surgery, Univ. of Wisconsin, Madison, WI, [3]NASA-Ames, Moffett Field, CA.

It is well known that exposure to microgravity or simulated microgravity causes bone and muscle atrophy. It is less well known whether or not microgravity has a great impact on the strength and composition of dense fibrous connective tissues. In simulated microgravity, the extracellular matrix of ligament and tendon lose mass, strength and decrease their adhesion to bone at the insertion site. The little data from spaceflight indicate that muscle and bone do not repair well after injuries, strongly suggesting that ligaments may not repair optimally. Thus, the objective of this study is to investigate the molecular, cellular and biomechanical properties of the collagen extracellular matrix in response to medial collateral ligament (MCL) injury repair in hindlimb unweighted (HLS) rodents. Two studies were performed comparing MCL wound repair during 3 weeks and 7 weeks of HLS. Each study consisted of 3 groups: hindlimb uweighted+lesion (HLS+lesion, n=24), ambulatory+lesion (Amb+lesion, n=24) and sham animals (Sham, n=24). Following suspension, rats were euthanized and the hindlimbs were frozen at –85˚ C. Bone-MCL-bone preparations were tested biomechanically for ligament strength. All other MCLs were isolated and prepared for molecular or biochemical analyses. Ultimate stress and elastic modulus tests demonstrated that the 3 week Amb+lesion MCLs were significantly stronger ($P<0.05$) compared HLS+lesion animals. Hydroxyproline concentration was substantially reduced in both the 3 and 7 week HLS+lesion groups versus the other groups. MCL collagen gene expression of type I (Col1A2), type III (Col3A1) and type V (Col5A1), measured by real time Q-PCR, showed significant differences in the HLS+lesion versus Amb+lesion groups. In conclusion, wound repair in the rodent MCL is signficantly impacted by hindlimb unweighting.

(Supported by NASA Grant: NAG9-1152)

Concurrent Oral Sessions
II
Genomics, Proteomics and Systems Biology

[67]

EFFECTS OF SIMULATED MICROGRAVITY AND HYPERGRAVITY ON THE EXPRESSION OF *ARABIDOPSIS* GENES ENCODING CALMODULINS AND CALMODULIN-BINDING PROTEINS. B.W. Poovaiah[1], T. Yang[1] and J.J.W.A. van Loon[2]. [1]Department of Horticulture, Washington State University, Pullman, WA. [2]DESC, OCB-ACTA-VU, Amsterdam, Netherlands.

Calcium and calmodulin (CaM) play an important role in plant gravity signal transduction. However, the molecular and biochemical mechanisms involved are not clearly understood. To study the effects of gravity-induced changes on the expression of genes involved in Ca^{2+}/CaM-mediated signaling, two week old *Arabidopsis* seedlings were subjected to simulated microgravity using the Random Positioning Machine, and hypergravity (10 *g*) using the MidiCAR centrifuge (ranging from 5 hrs to 5 days). The changes in mRNA levels of 11 CaM /CaM-like genes, and 20 genes encoding CaM-binding proteins were studied by RT-PCR using gene-specific primers. *Actin 8* was used as a positive internal control. Selective and significant differences were observed between controls and simulated microgravity and hypergravity treated samples. Three CaM genes were induced by simulated microgravity and one was induced by both hypergravity and simulated microgravity. Similarly, we have identified several genes encoding CaM-binding proteins that are differentially expressed in response to gravi-stimulation. Our results suggest that these genes may play an important role in gravity signal transduction.

(Supported by NASA grant NAG5-4841 to Poovaiah and SRON / NIVR combined grant # MG-051 to van Loon)

[68]

CORTICAL ACTIN FILAMENTS IN PLANT CELLS: INVOLVEMENT IN ROOT GROWTH REGULATION. E. B. Blancaflor and D. Mohamalawari. Plant Biology Division, The Samuel Roberts Noble Foundation Inc., 2510 Sam Noble Parkway, Ardmore, Oklahoma, USA 73401

Cortical microtubules have been implicated in the directional control of cell expansion. However, the role of actin filaments in this process is still uncertain. To determine the involvement of actin in cell elongation, the organization of actin filaments in primary roots was examined using an improved fluorochrome-conjugated phalloidin labeling method for fixed cells. Using this method, fine transversely oriented cortical actin was observed in cells of the elongation zone including the epidermis, cortex and vascular tissues. The orientation of cortical actin shifted from a predominantly transverse orientation to oblique, longitudinal and/or random arrangements as the cells matured. This behavior was similar to the behavior of cortical microtubules reported in previous studies. Roots treated with the microtubule stabilizing drug taxol improved the quality of actin preservation as evidenced by the thicker bundles of cortical actin suggesting that taxol was also capable of stabilizing the cortical actin networks. The elongation of roots exposed to 1 μM Latrunculin B (LB) was inhibited and after 24 hours roots exhibited moderate swelling along the transition and elongation zone. LB also caused the premature reorientation of microtubules from transverse to oblique arrays. These results indicate that cortical microtubules and actin filaments potentially interact in regulating cellular expansion. We are currently using green (GFP) and red (RFP) fluorescent-talin constructs and GFP-MAP4 to image the dynamics and possible interaction between actin and microtubules in living cells during root growth (Supported by NASA and the Noble Foundation).

[69]

EXTRACELLULAR ATP INHIBITS ROOT GRAVITROPISM AT CONCENTRATIONS THAT INHIBIT POLAR AUXIN TRANSPORT . S. J. Roux, W.-Q. Tang, and Y. Sun. Section of Molecular Cell & Developmental Biology, Univ. Texas, Austin.

There is normally a steep ATP gradient cross the plasma membrane, with cytoplasmic concentrations typically reaching several mM and concentrations in the bulk fluid of the extracellular matrix typically below nM. Previous results from this lab showed that plants might use this transmembrane ATP gradient to help power the efflux of various compounds across transporters in the ATP-binding cassette family. Here we present data showing that decreasing the gradient with exogenous ATP can inhibit the gravitropism of Arabidopsis roots, and that this effect can be correlated with the inhibition of auxin export. Raising the level of extracellular ATP [xATP] to the mM levels found intracellularly can block gravitropism in roots of Arabidopsis thaliana. When plants are grown in a medium supplied with 1 mM ATP, their roots grow horizontally instead of growing straight down. Two mM ATP induces root curling, and 3 mM ATP stimulates lateral root growth. Gravity stimulation experiments show that 5 mM ATP is able to reduce or, in some cases, completely block the angle of root bending. These effects, which cannot be mimicked by equivalent concentrations of ADP or inorganic phosphate, may be due to the disturbance of auxin distribution in roots, for xATP can inhibit the extent of auxin (IAA) distribution in Arabidopsis roots, as judged indirectly by DR5-GUS transgenic plants, and it can increase the response sensitivity of plant roots to exogenously added NAA. Additionally, in corn root segments, 30% less ^{3}H-IAA is polarly transported from the apical end to basal end when 5 mM ATP is supplied in receiver agar block. Taken together, these results suggest that the inhibitory effects of xATP on IAA distribution may happen at the level of IAA export and that there is a potential role for ATP gradients in auxin export and plant root gravity sensing.

(Supported by NASA: NAG2-1347.)

[70]

ROOT GRAVITROPISM IN RESPONSE TO A SIGNAL ORIGINATING OUTSIDE OF THE CAP. C. Wolverton[1], J.L. Mullen[2], H. Ishikawa[1], and M.L. Evans[1] [1]Dept of Plant Biology, The Ohio State University, Columbus and [2]Department of Biology, Indiana University, Bloomington.

We recently developed a feedback device for studying root gravitropism. The device uses video digitizer analysis of root growth and shape in conjunction with a rotating specimen stage to maintain any selected subsection of a root at any specified angle with respect to gravity (Plant Physiol 123: 665). Using this device we allowed the cap of a graviresponding maize (Zea mays L.) root to reach vertical while maintaining a selected region within the elongation zone at a gravistimulated angle. Under these conditions, gravitropic curvature continued long after the root cap reached vertical. The sustained gravitropic response with the cap oriented parallel to the gravity vector can not be attributed to slow damping of the initial gravitropic response since re-orientation of roots to vertical early in the response results in rapid cessation of curvature. The results indicate that a signal from outside of the cap can contribute to the curvature response.

(Supported by NASA grants NAG5-6385, NAG2-1190 and NAG2-1411)

[71]

CHARACTERIZATION OF NEGATIVE PHOTOTROPISM IN PRIMARY ROOTS OF MAIZE. M.L. Evans[1], C. Wolverton[1], J.L. Mullen[2], H. Ishikawa[1], R. Hangarter[2] [1]Dept of Plant Biology, The Ohio State University, Columbus and [2]Department of Biology, Indiana University, Bloomington.

Using customized video analysis software, we measured the kinetics and spatial distribution of the negative phototropic response of primary roots of maize (Zea mays, L. cv Merit). We also scanned the root for zones of phototropic sensing using fiber optics for localized application of light. Application of broad beam unilateral white light to the root resulted in negative phototropism following a latent period of 39 ± 8 min. The zone of differential growth driving curvature was located approximately 4-6 mm from the tip, within the central elongation zone (CEZ). Application of continuous unilateral blue (450 nm) light also caused a negative phototropic curvature in the CEZ. The threshold for blue-light-induced negative phototropism was approximately 0.01 μmol m^{-2} s^{-1}, with the response saturating near 10 μmol m^{-2} s^{-1}. Roots from which the cap had been surgically removed continued to grow vigorously but failed to show a phototropic response. Localized unilateral illumination of the cap of intact roots induced negative phototropism while localized illumination of either the distal elongation zone (2 mm behind the root tip) or the CEZ (4 mm behind the root tip) did not induce curvature. Our findings indicate that the root cap is the site of photoperception for negative root phototropism and that the responding cells are located remote from this site, within the central elongation zone.

(Supported by NASA grants NAG5-6385 and NAG2-1411)

[72]

NON-RANDOM ORIENTATION OF DARK-GROWN MOSS PROTONEMATA IN MICROGRAVITY (STS-87). V.D. Kern[1], and F.D. Sack[2]. [1]Lockheed Martin Space Operations, NASA Ames Research Center, Moffett Field, CA, and [2]Department of Plant Biology, Ohio State University, Columbus, OH.

In darkness protonemata of the moss *Ceratodon purpureus* express negative gravitropism (they grow up) with high fidelity. When grown under microgravity conditions during spaceflight (STS-87, Nov./Dec. 1997), an essentially random orientation was observed during the first 7 days of cultivation. However, in cultures grown in space for 14 days, the protonemata grew in arcs and overall formed clockwise spirals. Cultures grown on a slow-rotating clinostat on earth for 14 days also grew in clockwise spirals. These spirals formed regardless of the orientation with respect to the acceleration force or to the direction of clinostat rotation (clock- vs. counter-clockwise). Clinostat-grown cultures appeared to show less dramatic spirals than spaceflight cultures. The presence of spirals in 14 d but not 7 d cultures could be due to culture age, stage, or size and/or to the duration of exposure to microgravity or clinostat rotation. The phenomenon of protonemal phototropism allowed us to distinguish between these possibilities. When irradiated for 7 days, cultures displayed negative and positive phototropism while gravitropism was absent. When these cultures were then kept in the dark for an additional 7 days, clockwise arcs and spirals formed. This suggests that cultures may need to reach a certain stage before microgravity can induce spirals. There are only a few reports of gravitropic organs or cells orienting non-randomly in space. The presence of coordinated clockwise spiral growth in microgravity suggests that there is an endogenous growth polarity in *Ceratodon* that normally is suppressed by gravitropism. A working hypothesis is that the spirals represent a residual spacing mechanism for controlling colony growth and the distribution of branches under some conditions and in some mosses. An upcoming flight experiment on STS-107 scheduled for summer 2002 should allow us to learn more about the parameters that regulate clockwise spiral growth.

(Supported by NASA: NAG10-017).

Concurrent Posters
III-C
Genomics, Proteomics and Systems Biology

[73]

DECODING Ca^{2+} SIGNALS BY CHIMERIC Ca^{2+}/CALMODULIN DEPENDENT PROTEIN KINASE. P.V. Sathyanarayanan and B.W. Poovaiah, Department of Horticulture, Washington State University, Pullman, WA.

Chimeric Ca^{2+}/dependent protein kinase (CCaMK) is characterized by a serine-threonine kinase domain, an autoinhibitory domain overlapping with the calmodulin (CaM) binding domain and a C-terminal visinin-like domain. Visinin-like proteins are high affinity Ca^{2+} binding proteins and function as Ca^{2+} sensors in neurons. We have shown that the visinin-like domain of CCaMK functions as Ca^{2+} sensitive switch regulating autophosphorylation (*J. Biol. Chem*. 275:30417-30422, 2000). The Ca^{2+} stimulated autophosphorylation of Thr^{267} occurs by an intermolecular mechanism in the oligomeric complex (*J. Biol. Chem*. 2001, *in press*). The autophosphorylation leads to activation of kinase by significantly increasing the affinity for CaM. CaM itself acts as receptor for intracellular Ca^{2+} signals. The CaM-binding leads to removal of autoinhibition making the kinase maximally active. CCaMK is able to decode Ca^{2+} signals in a two step process. In the first step mediated by the visinin-like domain, the Ca^{2+} signals are decoded in to autophosphorylation of the oligomeric kinase. This activation enables the kinase to further decode the signals into substrate phosphorylation. CCaMK is expressed in the root tips and pollen mother cells. These results suggest that CCaMK is involved in decoding tissue and development specific Ca^{2+} signals.

(Supported by NASA grant NAG5-4841 and NSF grant MCB 0082256)

[74]

Ca^{2+}/CALMODULIN-MEDIATED SIGNALING: ACTIVATION OF PLANT CATALASE BY CALCIUM/CALMODULIN. T. Yang and B.W. Poovaiah. Department of Horticulture, Washington State University, Pullman, WA.

Environmental stimuli can induce rapid changes in hydrogen peroxide (H_2O_2) levels leading to a variety of physiological responses in plants. Catalase, which is involved in the degradation of H_2O_2 into water and oxygen, is the major H_2O_2-scavenging enzyme in all aerobic organisms. A close interaction exists between intracellular H_2O_2 and cytosolic calcium in response to environmental changes. Studies indicate that an increase in cytosolic calcium boosts the generation of H_2O_2. Here we report that calmodulin (CaM), a ubiquitous calcium-binding protein, binds to and activates plant catalases in the presence of calcium, but calcium/CaM does not have any effect on bacterial, fungal, bovine, or human catalase. These results document that calcium/CaM can down-regulate H_2O_2 levels in plants by stimulating the catalytic activity of plant catalase. Furthermore, these results provide the first evidence indicating that calcium has dual functions in regulating H_2O_2 homeostasis, which in turn influences redox signaling in response to environmental signals in plants.

(Supported by NASA grant NAG5-4841 and NSF grant MCB 0082256)

[75]

EVALUATION OF RED-LIGHT REGULATION OF ETHYLENE BIOSYNTHETIC GENES BY RELATIVE RT-PCR. M.A. Harrison and A.J. Porter. Dept. of Biological Sciences, Marshall University, Huntington, WV.

Gravitropism is controlled by the interaction of growth-regulating substances and light. While auxin is the primary plant hormone involved in regulating differential growth during stem gravitropism, ethylene modulates the dynamic cellular growth changes associated with upward curvature. Red-light treatment of etiolated seedlings alters the kinetics and curvature pattern along the stem. This red-light effect may result from its inhibition of ethylene biosynthesis, and is supported by studies showing a similar response in stems treated with inhibitors of ethylene biosynthesis or response. The major goal of this project is to evaluate gene expression changes for members of the ethylene biosynthetic gene families *ACO* (1-aminocyclopropane-1-carboxylic acid [ACC] oxidase) and *ACS* (ACC synthase) after red-light treatment of dark-grown *Arabidopsis* seedlings. Relative RT-PCR was used to study the expression of *ASC2, 4, 5, 6 and EAT1* (which encodes an ACO), in total-RNA extracts of etiolated *Arabidopsis* seedlings. Seedlings were handled under a dim green light and red-treated seedlings were given a 6 min pulse with 11 $\mu mol\ m^{-2}\ s^{-1}$ red light 18 hours prior to RNA extraction to be consistent with previous studies on the timeline of red-light inhibition of ethylene biosynthesis. 18S rRNA was used as an internal standard. RT-PCR products confirmed low levels of expression for all genes. Preliminary evidence suggests inhibition of *ACS4* after red-light treatment. Continued studies will identify and evaluate the changes in key ethylene biosynthetic enzyme association with gravitropism and after light-pulse treatment.

[76]

MICROGRAVITY INDUCED CHANGES OF GENE EXPRESSION IN THE DEVELOPING ZEBRAFISH HEART. I. Gillette-Ferguson, D.G. Ferguson and S.J. Moorman. Department of Anatomy, Case Western Reserve University, Cleveland, OH.

Microgravity induced changes in gene expression have been documented for cultured cells flown on the Space Shuttle and in cells grown in bioreactors that NASA designed to simulate microgravity for cells in culture on Earth. However, little is understood about the mechanisms that underlie these effects and it is not known whether similar changes in gene expression might be seen in animals. Using transgenic zebrafish that express the *gfp* gene under the influence of a ß-actin promoter, we examined the affect of simulated microgravity on GFP expression in the developing heart. Using one of the NASA-designed bioreactors, we exposed zebrafish embryos to simulated microgravity for 24 hours beginning at 24 hours after fertilization. The amount of GFP fluorescence associated with the heart was then determined using fluorescence microscopy of live embryos. Video images of the heart were acquired using an intensified CCD camera. Average intensity of fluorescence of the heart was measured from the video images using NIH Image software. On the average, there was a 3-9% increase in GFP-associated fluorescence in the heart of the experimental animals compared to the controls. Preliminary data from images of the caudal end of the embryo did not show significant changes in GFP fluorescence. This suggests that these changes are specific to the heart although other specific organs have not yet been analyzed. These studies indicate that microgravity can induce changes in gene expression *in vivo*. They also demonstrate the usefulness of transgenic zebrafish to determine the effects of microgravity on gene expression, *in vivo*.

(Supported by NIH: DC03531 and NASA: NAG2-1356 and NCC2-1204)

[77]

MOBILITY OF GFP-XPC FUSION PROTEIN IN LIVING CELL NUCLEUS OBSERVED BY TIME-LAPSE FLUORESCENCE MICROSCOPY AND FLUORESCENCE CORRELATION SPECTROSCOPY. H. Okumura[1], A. Tanaka[1,2], Y. Kodama[3], F. Ishidate[3], K. Sugasawa[4,5], and F. Hanaoka[4,5,6]. [1]NASDA, [2]GSC, RIKEN, [3]Carl Zeiss, [4]RIKEN and [5]CREST, [6]Osaka Univ Japan.

Nucleotide excision repair (NER) is a principal pathway by which a large variety of DNA damage is eliminated from the genome. We have interest in how does the NER system monitor the lesions in huge genome DNA molecules. To study nuclear localization and dynamics of a DNA damage-binding factor XPC-hHR23B, the initiator of global genome NER, we observed time-lapse fluorescence microscopy and fluorescence correlation spectroscopy (FCS) of the GFP-XPC fusion protein in living cells.

Localization and mobility of the GFP-XPC fusion protein were studied in XPC-deficient human primary fibroblasts (XP4KA) that had been transfected with a plasmid transiently expressing the fusion protein. In most transfected cells, GFP-XPC was observed within nucleus, whereas control GFP localized both in nucleus and cytoplasm. We found two sorts of fluorescent components, small particles and larger speckles, in the GFP-XPC transfected cells. Following by time-lapse microscopy, the small particles moved quickly and the speckles moved slowly.

Using FCS, we have determined diffusion coefficients of the fusion protein in living cells. Although the obtained values exhibited insufficient reproducibility, automated analysis suggested the presence of two types of fluorescent components in the nucleus. One had a diffusion time corresponding to the molecular weight of the fusion protein, and while the other had a 100 times larger diffusion time. These results suggest that in living cell nucleus GFP-XPC may behave in a large cluster which is susceptible to be effected by gravity.

[78]

THE CORRELATION BETWEEN MUSCLE MASS, PROTEIN CONTENT AND POLYAMINE LEVELS UNDER UNLOADING CONDITIONS. D.A. von Deutsch[1,2], I.K. Abukhalaf[1,2,3], S.W. Sahlu[1,2], S.A. Abera[1,2], N.A. Silvestrov[2,3], L.E. Wineski[4], S.A. Pitts[4], D.E. Potter[1,2], R.R.Roper[1,2]. [1]Space Medicine and Life Sciences Research Center, [2]Department of Pharmacology & Toxicology, and the [3]Clinical Research Center, [4]Department of Anatomy and Neurobiology, Morehouse School of Medicine, Atlanta, GA 30310

Anabolic agents such as ß2-adrenoceptor agonists are useful tools for probing the mechanisms by which muscles respond to disuse. One such agent, clenbuterol (Cb), was examined under different loading conditions with respect to its effects on skeletal muscle mass, protein (total and myofibrillar) content, and polyamines in mature male rats. The hindlimb muscles used in these studies were the predominately slow-twitch (type I) adductor longus [ADL] and fast-twitch (type II) extensor digitorum longus [EDL]. Pair-fed rats were divided into four experimental groups: vehicle- and Cb-treated non-suspended, vehicle- and Cb-treated (1 mg/kg) hindlimb suspended. Unloading caused significant decreases in ADL mass, myofibrillar and cytosolic protein content of such magnitude that treatment with Cb could not fully overcome the loss. On the other hand, Cb did significantly increase unloaded ADL spermidine levels. In the EDL, unloading had little effect on muscle mass or total protein content. However, a significant decrease in EDL's myofibrillar protein content was observed along with significant increases in cytosolic proteins and spermidine. Treatment with Cb significantly increased all parameters in the EDL. Conclusion: Unloading results in a general decrease in both myofibrillar and cytosolic protein content in the ADL as well as a significant decrease in spermidine content. In contrast, the EDL presents a more complex response to unloading. Understanding why EDL's cytosolic protein content increased under unloading conditions may help provide greater insight into the nature of this muscle's resistance to unloading. This work was supported, in part, by NASA grant NCC9-112 (I.K.A.) and NIH grant RCRII 2P20 RR11104-07 (I.K.A.).

[79]

CULTURE IN 3D-CLINOSTAT INHIBITS TGF-beta, p38 MAPK IN DIFFERENTIATION OF HUMAN OSTEOBLASTS. L.Yuge[1], Y. Kumei[2], M. Kanno[1], Y. Ikuta[1], K.Kataoka[1] and H.Kajihara[1] [1]Faculty of Medicine, Hiroshima University, Hiroshima, Japan. [2]Faculty of Dentistry, Tokyo Medical and Dental University, Tokyo, Japan.

A 3D-clinostat is a multi-directional gravity device for simulating microgravity (10^{-4}G). We cultured osteoblasts in the 3D-clinostat, examining differentiation of osteoblasts in terms of cytokines, MAPK-cascade, and morphology including histological detection of minerals. The results obtained are as follows: 1) Formation of bone nodules, alkaline phosphatase (AlPase) activity, and calcification were detected after 21 days of culture in a normal environment but not in the 3D-clinostat. 2) Osteocalcin production is increased in the control, while it is decreased in the 3D-clinostat. 3) Phosphorylation of p38 MAPK was repressed in the 3D-clinostat, while total p38 MAPK was not changed. 4) Formation of bone nodules, AlPase activity and calcium deposits were inhibited completely by a p38 MAPK inhibitor, SB 203580, but partially by a MAPK/ERK 1/2 inhibitor, U-0126. 5) TGF-beta and IL-1 were repressed in the 3D-clinostat. It is concluded by these results that (1) the osteogenesis is inhibited in culture in the 3D-clinostat, and (2) this inhibition is largely attributed to the suppression of p38 MAPK phosphorylation. (3) Repression of TGF-beta and IL-1 relates to the inhibition of osteogenesis.

(Supported by Japan Space Forum, Space Utilization Research and the Japanese Ministry of Education)

[80]

EFFECT OF VECTOR-AVERAGED GRAVITY ON SUB- CELLULAR LOCALIZATION OF MITOGEN-ACTIVATED PROTEIN KINASE IN MOUSE OSTEOBLAST-LIKE MC3T3-E1 CELLS. A. Sato[1], M. Fujita[1], M. Kanematsu[1], S. Kamigaichi[2], M. Takaoki[2], M. Narato[3] , H. Kumagai[3], and Y. Taniguchi[4]. [1]Space Utilization Research Programme, [2]Space Utilization Research Center, NASDA, [3]Advanced Engineering Services Co. Ltd., and [4]Toray Research Center., Japan.

We previously reported that microgravity inhibited EGF-induced c-fos gene expression but unaffected the phosphorylation of mitogen-activated protein kinase (MAPK) in MC3T3-E1 cells. To probe the mechanism, we investigated effects of vector-averaged gravity on redistribution of phosphorylated MAPK (phospho-MAPK) from the cytosol to nucleus in cells. The cells were cultured in α-MEM supplemented with 1% FCS and 10 mM HEPES for 24hr, then treated with 100 ng/ml EGF for 10 min under clinostat (rotation speed, 30 rpm) or stationary culture condition. Western blot analysis of MAPK and phospho-MAPK revealed that no significant difference in phosphorylation level of p44/42 MAPK of the cytosolic and nuclear fractions of the EGF-treated cells was observed between cells grown in simulated microgravity and those grown in stationary control. Immunofluoresence data indicated that p44/42 MAPK localized predominantly in the cytoplasm, particularly the perinuclear region in cells, and phospho-p44/42 MAPK detected in nucleus. Simulated microgravity slightly increased in the cytosolic and nuclear p44/42 MAPK and decreased in nuclear phospho-p44/42 MAPK. These results raise the possibility that gravity may affect translocation of cytosolic phospho-p44/42 MAPK into nucleus in MC3T3-E1 cells.

[81]

IDENTIFICATION OF PROMOTER SEQUENCES INVOLVED IN THE RESPONSE TO ROTATING WALL VESSEL GROWTH IN SACCHAROMYCES CEREVISIAE. Kelly Johanson[1], Patricia L Allen[2], Luis A Cubano[2], R. Bryan Klassen[3], Fawn Lewis[1], Ratan M Joshi[4], Terry Stoming[4], Linda Hyman[1] and Timothy G Hammond[2] [1]Departments of Biochemistry, Surgery, & Medicine, [2]Tulane/VA Environmental Astrobiology Center, [3]Tulane/Xavier Center for BioEnvironmental Research, New Orleans, LA 70112 and [4]Molecular Biology Core Facility Institute of Molecular Medicine and Genetics Department of Biochemistry and Molecular Biology Medical College of Georgia, Augusta, GA 30912.

Although engineering optimization of suspension culture was undertaken to model culture conditions in space flight, it has found its greatest utility in commercial ground-based applications. The suspension culture system optimized to provide minimal shear with laminar flow is the rotating wall vessel (RWV), a horizontally rotating cylinder with a co-axial oxygenator. We use genome-wide DNA microarrays and samples of mRNA from *Saccharomyces cerevisiae* cultures to determine gene expression changes that occur during RWV culture. Using periodicity and correlation algorithms (Gibbs alignment procedure) we assay clusters of rotating wall vessel responsive genes for known and new promoter elements to define the mechanisms mediating these genetic changes. Overall 446 genes were up-regulated at one or more time points in the rotating wall vessel while 282 were down-regulated. Candidate binding motifs similar to the Rap1 and Buf binding motifs were identified in the promoter regions of genes whose expression levels were influenced by growth in the rotating wall vessel. Furthermore, the identification of a significant amount of rotating wall vessel responsive genes with no known function may open the possibility of genes with functions related to changes in flow conditions.

This worked was supported by NASA NRA NAG-8-1362, NASA Cooperative agreement NCC 2-1177 and DOD DTRA to the Tulane/Xavier Center for Bioenvironmental Research. Affymetrix system was purchased by the Georgia Research Alliance.

[82]

U-51605, A PROSTACYCLIN SYNTHASE INHIBITOR, BLOCKS POST-SUSPENSION HYPOTENSION IN SPRAGUE-DAWLEY RATS. M.A. Bayorh, D. Eatman, M. Walton, R.R. Socci and N. Emmett. Morehouse School of Medicine, Atlanta, GA.

Orthostatic hypotension is frequently manifested in astronauts during standing postflight. To evaluate the role of the prostacyclin synthase inhibitor U-51605 as a countermeasure against post-suspension hypotension, we examined the cardiovascular responses to 7 day 30^{o} tail-suspension and a subsequent 6 hr post-suspension period in conscious male Sprague-Dawley rats. U-51605 (0.3 mg/kg, i.v.) or saline were injected prior to release from suspension and at 2 and 4 hrs post-suspension. During suspension, MAP did not change, in contrast, at 6 hrs post-suspension, it decreased compared to parallel tethered (control) animals. U-51605 attenuated the observed post-suspension hypotension without alteration in heart rate. Both plasma prostacyclin and nitric oxide were elevated post-suspension, but only prostacyclin was reduced by U-51605. Plasma levels of thromboxane and prostaglandin E_2 were not altered post-suspension. Baroreflex sensitivity for heart rate was modified by U-51605. Thus, simulated microgravity and the subsequent post-suspension recovery are associated with excessive production of endothelium-dependent relaxing factors (i.e., prostacyclin and nitric oxide). These observations may lead to the development of more effective countermeasures for astronauts.

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Concurrent Posters
III-D
Plant and Microbial Growth Systems

[83]

PLANT RESEARCH UNIT FUNCTIONALITY AND CAPABILITY

M.C. Lee, R.C. Morrow, J.A Bernard, T.M. Crabb, Orbital Technologies Corporation, Madison, WI.

The Plant Research Unit (PRU) is one of six life science habitats in the suite of experimental hardware being developed for the International Space Station. The plant habitat is designed for experiments in near-zero gravity or it can be rotated by the ISS Centrifuge for experiments at any gravity level from microgravity to twice Earth's gravity. Plant experimentation will be possible in up to eight Plant Research Units at one time in multiple gravity environments, isolating the effect of gravity on the biological specimens. The PRU will provide and control all aspects of a plant's needs in a nearly closed system. In other words, the shoot and root environments will not be open to the astronaut's environment except for experiment maintenance such as planting, harvesting and plant sampling. This also means that all lighting, temperature and humidity control, "watering," and air filtering and cleaning must be done in a very small volume, with very little mass and power usage and with minimal crew time. The PRU will be about the size of a large bread box weighing around 150 pounds but will consume less power than five 100 watt light bulbs. Within these strict limitations of volume, weight and power usage, the PRU must maintain "happy" plants in a very accurately controlled growth environment. The PRU will be modular and will allow scientists to select various lighting systems and nutrient delivery systems. The nutrient delivery systems may range from a soil-type matrix to hydroponics to aeroponic sprays. The PRU will also provide continuous data logging of the environment including video images of the plants. The system will be robust enough for 10 years of life and dozens of trips to and from orbit. The Plant Research Unit will provide a significant new science capability that could accelerate biotechnology and controlled agriculture applications on Earth. The paper will review the functions and capabilities for science accommodations.

[84]

IMPACT OF ELEVATED ROOT ZONE TEMPERATURE ON *BRASSICA RAPA* CV. ASTROPLANT GERMINATION AND DEVELOPMENT.

K.M. Stolp and R.C.Morrow. Orbital Technologies Corporation, Madison, WI.

The Biomass Production System (BPS) was developed to support plant growth and experimentation onboard shuttle and the International Space Station (ISS). The BPS is a four chambered environmental control unit that allows the user to control humidity, light levels, nutrient supply, CO_2, and temperature separately in each of the chambers. Currently BPS experiments are supporting the growth of *Brassica rapa* cv. ASTROPLANT and *Triticum aestivum* cv. Apogee in anticipation of delivery to the ISS in 2002. During a 24-Day Science Verification Test poor germination of *B. rapa* was observed. After investigating possible causes for the poor germination, including nutrient delivery fluid pressure control, root-zone temperature and chamber CO_2 levels, it was determined that germination and development were adversely affected by excessive evaporation at the soil/air interface. The increased evaporation resulted from the elevated temperature differential between root and shoot temperatures when the BPS was operating in a hot ambient environment. Further testing showed that raising the relative humidity of the chamber to 90% offset poor germination and development. Following the SVT, a BPS Muffler Performance Test yielded healthy plants that exceeded success criteria. However, during the performance of a subsequent Mission Verification Test (MVT), poor development of *B. rapa* was observed. This was linked to the height of the BPS root/shoot barrier air diffuser being used. Tests are being performed to investigate the performance of B. *rapa* with 1.2cm and 2.1cm high air diffusers. Seedlings rise above the barrier and are exposed to airflow from under their leaves. Preliminary data shows that percent germination is increased by 20% and mean height is increased by 1.5cm when higher air diffusers are used.

[85]

LADA, A JOINT RUSSIAN – US ISS PLANT GREENHOUSE: CONTINUING THE SVET SCIENCE AND TECHNOLOGY DEVELOPMENT TRADITION.

G.E. Bingham[1] I.G. Podolsky[2], M.A. Levinskikh[2], and V.N. Sychev[2]. [1]Space Dynamics Laboratory, Utah State University, Logan, UT. [2]Institute for Biomedical Problems, RAS, Moscow, Russia.

A new greenhouse of the *Svet* style is being prepared for deployment in the Russian section of the International Space Station. *Svet* was used for seven experiments on Mir between 1990 and 2000. LADA is being developed under a joint cooperative agreement between the Institute of Bio-Medical Problems, RAS, Moscow, Russia and Space Dynamics Laboratory / Utah State University. LADA is currently completing qualification and biotechnology testing and is expected to launch to the ISS in mid 2002. LADA is being developed to allow continuation of the Russian National Program on Microgravity Plant Technology. International cooperative experiments are being planned for LADA, structured like those conducted in the *Svet* greenhouse on Mir.

The initial (2002) experiment is expected to involve Russian and US participation (additional participation is in negotiation) and will grow Muzina (*B. rapa* var. nipposinica), using a single greenhouse for power considerations (LADA can support two). LADA will also carry a full root zone environment characterization package similar to *Svet*, but expanded to include additional sensor types. The root module is 15 x 18 x 10 cm deep with a leaf chamber 26 cm tall. The leaf chamber provides light and air temperature profile measurements as well as humidity and IR leaf temperature. The light profile varies from 200 – 400 μm m^{-2} s^{-1} through the canopy area. The standard arrangement is for two 16 cm long plant rows, with a sliding window for crew interaction.

LADA is expected to remain on the ISS, and is designed to allow crew interaction studies of the psychological, recreational and nutritional supplement that can result from an onboard greenhouse.

(LADA has been developed using SDL, IBMP, RKK Energia and Russian Space Agency support.)

[86]

SEED TO SEED GROWTH OF *ARABIDOPSIS THALIANA* ON THE INTERNATIONAL SPACE STATION.

B Link[1], B Stankovic[1], T Theno[2], G French[2], W Zhou[1], [1]Wisconsin Center for Space Automation and Robotics, University of Wisconsin-Madison, [2] Space Explorers Inc., De Pere, WI.

Living in space is difficult and requires a carefully controlled environment to reduce the stresses of the microgravity environment. This is true for plants just as it is for humans. The Advanced Astroculture (ADVASC) flight hardware was designed to provide an ideal plant growth environment in microgravity. It provides an isolated growth chamber with controlled temperature, humidity, soil moisture level, and CO_2 concentration. Here we report on the first seed to seed growth of *Arabidopsis thaliana* on board the International Space Station.

In the current experiment (in collaboration with Space Explores Inc.) dry seeds were positioned inside ADVASC prior to launch. The unit was activated on May 10th after it was installed on the ISS. ADVASC automatically watered the dry seed and began controlling the soil moisture level once it was powered on. The plants were grown at 22° C and 70% relative humidity under 18 h days 6 h nights. Set points were changed remotely from the University of Wisconsin at the end of the plants life cycle to encourage seed maturation. Some of this seed will be saved for a future mission so that plants can be started in space from space produced seed.

Preliminary data including video images, transpiration rates and CO_2 consumption indicates that ADVASC provides a good environment for growing plants in space. Furthermore, its versatility- allowing commanding from the ground without requiring crew time- makes it ideal for long-term space flight experiments.

[87]

USEFULNESS AND LIMITATIONS OF CLINOSTATS. M.Takaoki, K.Yoshimura, K.Yasuzawa-Tanaka. Space Utilization Research Center, National Space Development Agency Of Japan

The physical status on a clinostat is entirely different from microgravity and from Earth gravity. But why should clinostats simulate microgravity? Clinostats are effective for gravitational biology research despite the fact that they do not mimic microgravity.

Culturing cells or tissues on a clinostat, however, needs carefully constructed strategy. We have to control and monitor culture conditions watchfully, because they deviate significantly from standard culture techniques. We observed continuous cell growth even after dissolved oxygen level decreased in a filled-up and sealed culture flask, for example. The cells might change their metabolic pathways according to oxygen concentration rather than gravity. Fluid flow generated by rotation is another problem. The flow on a single-axis rotation was minimum even at high rate of revolution. On the other hand, double-axis clinostat caused intolerable turbulences even at slow rotation.

We have thoroughly re-examined those technical limitations on clinostats, and will discuss how to draw the best out of them.

[88]

LIGHT INTERCEPTION AND CANOPY COVERAGE OF LETTUCE AND RADISH GROWN UNDER DIFFERENT WAVELENGTHS OF RED LIGHT-EMITTING DIODES (LEDs). G.D. Goins[1], O.M. Monje[1], R.M. Wheeler[2], and J.C. Sager[2]. [1]Controlled Biological Systems, Dynamac Corporation, and [2] NASA Kennedy Space Center Spaceport Engineering and Technology, Florida, USA.

Light-emitting diodes (LEDs) represent an innovative artificial lighting source with several appealing features specific for supporting plants, whether on space-based transit vehicles or planetary life support systems. Appropriate combinations of red and blue LEDs have great potential for use as a light source to drive photosynthesis due to the ability to tailor irradiance output near the peak absorption regions of chlorophyll. This paper describes the importance of far-red radiation and blue light associated with narrow-spectrum LED light emission. In instances where radish and lettuce were grown under lighting sources in which the ratio of blue light (400-500 nm) relative to far-red light (700-800 nm) was low, there was a distinct leaf stretching or broadening response. This photomorphogenic response sanctioned those canopies as a whole to reach earlier critical leaf area indexes (LAI) as opposed to plants grown under lighting regimes with higher blue:far-red ratios. In many instances, the salad crops grown under LEDs were just as productive as crops grown under broad-spectrum light, largely as a consequence of more efficient light interception during early growth.

(Supported by NASA: NRA98-HEDS-01-67)

[89]

INFLUENCE OF SUPRAOPTIMAL CO_2 ON YIELD, STOMATAL CONDUCTANCE AND PROXIMATE COMPOSITION OF HYDROPONIC SWEETPOTATO PLANTS. D.G. Mortley[1], J.H. Hill[1], H. Aglan[2], D.R. Hileman[3], C.K. Bonsi[1], W.A. Hill[1], and C.E. Morris[1]. [1]Center for Food and Environmental Systems for Human Exploration of Space and G.W. Carver Agricultural Experiment Station, [2]Dept of Mechanical Engineering, [3]Dept. of Biology, Tuskegee University, Tuskegee AL 36088, USA.

Sweetpotato *[Ipomoea batatas (L) Lam]* cv 'TU-82-155' stem cuttings were grown in reach-in growth chambers under a 18/6 h photoperiod, a matching 28/22ºC thermoperiod, and 70% relative humidity to evaluate the effects of very high CO_2 on storage root yield and plant nutrition. Three stem cuttings were grown in each of four rectangular PVC-1 growth channels (0.15x0.15x0.6m) in two reach-in controlled environment chambers, one ambient (400 μmol mol^{-1}) and the other at 3000 μmol mol^{-1} CO_2). Each growth channel was filled to a 14.5 cm depth with Turface (fritted calcine clay) as the growing medium. Plants were supplied with a modified half-Hoagland nutrient solution with a 1:2.4 N:K ratio, by way of a microporous tube embedded in the medium. The nutrient solution was circulated under suction pressure from the reservoir through the microporous tube back to the reservoir. Leaf samples were collected at four-week intervals for elemental analyses and leaf stomatal conductance and transpiration measurements were taken weekly. Plants were harvested 120 days after planting and storage root yield data taken. Storage root fresh mass was three times greater under the very high CO_2 treatment when compared to that for plants grown under ambient levels. Foliage fresh and dry mass were similar regardless of enrichment. Stomatal conductance and leaf transpiration measurements were higher among plants grown under the very high CO_2 concentration compared to ambient grown plants. Storage roots from plants grown under very high carbon dioxide enrichment had a higher content of protein, ash, and starch and marginally lower total sugars (fructose, glucose, sucrose, maltose) than storage roots from plants grown under ambient conditions.

(Supported by NASA:NAG100209 and USDA/CSREES ALX-PS-1)

[90]

LOW PRESSURE GREENHOUSE CONCEPTS FOR PLANT PRODUCTION ON MARS. P.A. Fowler[1] , V.Ye. Rygalov[2], R M. Wheeler[1], and R.A. Bucklin[2]. Kennedy Space Center, FL and the University of Florida, Gainesville.

Plant production systems might be used for generating food and oxygen for human life support on future space missions. In nearly all cases, light will be a limiting factor. Because electric lighting systems are power intensive, use of incident solar lighting may be desirable. Thus one might consider transparent structures (e.g., greenhouses) for cultivating plants in space, but finding transparent materials that withstand large pressure and temperature differences could be difficult. By using lower internal pressures, one can reduce the structural mass, gas leakage, and increase the potential for finding suitable transparent materials. Unfortunately, little is known about managing low-pressure environments for plants and the plant responses to these environments. Our studies have shown that water saturation pressure for a given temperature is not affected by total pressure, and that a range of humidity sensors is useful in low-pressure environments (capacitance type, wet-dry bulb, and chilled mirror). Evaporation tests (with pans of water) and enclosing plants in low-pressure environments indicate that evapotranspiration increases with decreasing pressure, which is likely due to increased gas diffusion rates under low pressures. A "minimal atmosphere" for growing plants at low pressure would include O_2 (for respiration), CO_2 (for photosynthesis), and H_2O (as an unavoidable result of transpiration and closed water cycling), but the tolerable partial pressures of each need further study, particular O_2. Management of thermal issues will also present substantial challenges, and insulating covers may be required for dark periods to maintain acceptable temperatures in a greenhouse. Opportunities to implement low-pressure greenhouses might be possible on early Mars missions, either as a concept test or as a deployment prior to crew arrival. In addition, deployable greenhouses could be tied in with in-situ resource utilization systems that might be used to collect local CO_2 and / or water.

[91]

MEASUREMENT OF EVAPOTRANSPIRATION IN THE BIOMASS PRODUCTION SYSTEM O. Monje, G.W. Stutte, G.D. Goins, and D.K. Chapman. Dynamac Corporation, Kennedy Space Center, FL

Evapotranspiration (ET) of Apogee and SuperDwarf wheat was measured in the Biomass Production System (BPS) flight hardware. ET was determined using two different methods: 1) water addition rates to the Nutrient Delivery System (NDS additions) and 2) water removal rates by the Humidity Control System (HCS removal). These measurements were made as part of ground tests to determine the feasibility of obtaining ET rates for the Photosynthesis Experiment Subsystem Testing and Operation (PESTO) experiment manifested for the International Space Station. The response of ET to changes in chamber Vapor Pressure Deficit (VPD) was also characterized at 3 stages of the life cycle (8, 15, and 22 days after planting). VPD was altered by changing setpoints in chamber humidity and air temperature. We found that ET in the BPS estimated from NDS additions was linearly related to ET estimated from HCS removal. Both estimates of ET appear to differ by a constant, however, the exact causes for these differences are currently under study. The results also indicate that ET increased linearly with VPD. NDS addition is expected to lag behind the actual rates of Et, and the HCS removal rates may be less accurate if humidity control is lost. Several hardware specific issues were identified which must be accounted for before ascribing physiological significance to these measurements of ET.

[92]

DEVELOPMENT OF ASSAYS TO DETERMINE MICROGRAVITY EFFECTS ON THE VIRULENCE AND PHYSIOLOGY OF *PSEUDOMONAS AERUGINOSA*. B.H. Pyle[1], M.A. Juergensmeyer[1], S.C. Broadaway[1] and J.T. Lisle[2]. [1]Dept. of Microbiology, Montana State Univ., Bozeman, MT, and [2]Lockheed Martin, Houston, TX.

In preparation for an experiment to be flown on Shuttle Columbia Mission STS-107, culture systems have been developed to permit growth of *P. aeruginosa* ATCC 29260 in European Space Agency Phorbol Experiment Cassettes housed in Type I containers. Washed cultures of *P. aeruginosa* are resuspended in 0.06 ml water at ca. 10^6 CFU/ml in activation chambers. A sterile medium for bacterial growth and production of Exotoxin A without aeration is contained in 0.6 ml volumes in the culture chambers. Fixative chambers are filled with 0.06 ml of formalin for cell and toxin fixation, Rhodamine 123 (Rh 123) for assessment of cell membrane function, iodonitrotetrazolium chloride (INT) for enumeration of respiring cells, or water which allows the cells to remain viable. Viable samples are enumerated by agar plate counts and assessment of metabolic activity using an intracellular esterase substrate (ChemChrome). Cytotoxicity of bacteria and supernatant for eucaryotic cells is to be assessed to determine virulence. Formalin fixed samples are assayed for Exotoxin A (ETA) using an ELISA procedure. Total cell counts are determined by staining, filtration and epifluorescent microscopy on all samples. Results show that the cultures can survive storage at 5°C for 8 days before incubation, and incubated cultures are stable at 5°C for 8 days, as required by the flight timeline. During incubation for 24 hours at 37° C, consistent growth to ca. 10^9 CFU/ml along with copious ETA has been observed in preliminary ground-based studies. In addition, the in-flight physiological assays for membrane function and respiration and post-flight have been successfully demonstrated. It is anticipated that results from the flight experiment will provide new insights into bacterial physiology and virulence in microgravity.

(Supported by NASA: NAS2-14263, Ames Research Center, and ESA.)

Concurrent Posters
III-E
Systems Biology and Advanced Life Support

[93]

PARTNERS AND DELIVERABLES OF THE NASA FOOD TECHNOLOGY COMMERCIAL SPACE CENTER AT IOWA STATE UNIVERSITY: AN UPDATE. A. L. Pometto III. Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa.

The mission of NASA FTCSC is to engage the food industry and academia to develop food products and processes for NASA and the public. Currently, NASA FTCSC has nine commercial partners committing $2.225 million over five years to this effort. NASA FTCSC also has 25 affiliate faculty members at five universities and one federal research lab providing more than $600,000 from non-NASA funding sources in research over the past year. Information describing the deliverable for each commercial partner and each affiliate faculty member will be presented. The space food challenges being addressed by our commercial partners and affiliate faculty members include developing new food products, developing new food-processing equipment, extending the shelf life of foods, improving and monitoring food safety, developing new packaging methods and materials, developing new food waste management systems, and developing disinfection systems.

[94]

EVALUATION OF THE APOGEE WHEAT VARIETY FOR ITS UTILIZATION IN BAKED PRODUCTS AND PASTA. P.V.Veillard, J.L.Kokini. Dept. of Food Science and NJ-NSCORT, Rutgers University, New Brunswick NJ 08901.

The objectives of this study were to characterize this variety of wheat in terms of chemical, rheological properties and to evaluate the end quality of cookie, cake, bread and pasta products through textural and sensory evaluation tests.

Chemical, rheological and baking characteristics of Apogee were compared to those of flours commercially used for cookies, cake, bread and pasta making. Basic chemical composition and small-scale predictive chemical tests were performed. Dough rheological properties were assessed by resistograph and modified alveograph procedures. Final products were made using standard small-scale methods and characterized by texture analyzer TAXT2 and hedonic sensory evaluation tests.

Chemical analysis shows that this flour has high proteins, free lipids, ash, pentosans and starch damage contents. Mixing characteristics are a high water absorption (70%), medium dough development time and stability (4.4 and 8 min respectively). Alveograph values - overpressure P, extensibility L and area W - are intermediates (respectively 55, 43 and 99) between those of bread and cookie flours. SDS protein-gel, particle size analysis, solvent retention capacity tests (SRC) and alkaline water retention capacity tests (AWRC) further demonstrate that this variety is a high absorption, medium strength wheat flour. Baking tests show that Apogee is relatively well adapted to bread making, but produces small diameter and hard cookies, poor volume cakes and poor cooking quality pastas.

Improvement of these textural characteristics will require the use of specific additives for each end application, such as surfactants for cake and cookie making.

(Supported by NASA – NJ-NSCORT.)

[95]

MEASURING CANOPY GAS EXCHANGE RATES IN HYDROPONICALLY GROWN PEANUT (*ARACHIS HYPOGAEA* L.) AND SWEETPOTATO (*IPOMOEA BATATAS* L.) UNDER ENHANCED AND AMBIENT CO_2 CONCENTRATIONS**.** J.E. Wesley[1], D.R. Hileman[2] and D.G. Mortley[1]. [1]Dept of Agricultural Sciences, and [2]Dept of Biology, Tuskegee University, Tuskegee, AL.

Peanut and sweetpotato are being evaluated for use in NASA's Advanced Life Support System (ALS) program. On extended space missions, these crops will provide food, nutrient and waste recycling, and air revitalization. In order to predict plant effects on CO_2 and O_2 levels, season-long gas exchange measurements are required. Previous measurements of single-leaf photosynthetic rates have not correlated well with final yield, indicating that single-leaf sampling does not accurately reflect the complex interactions of leaf age, leaf position and light environment within the developing canopy. The objective of this study is to design a system for measuring season-long, whole-plant gas exchange rates under ambient and enhanced CO_2 concentrations and to correlate these gas-exchange data with final yield. An open-flow system was constructed to determine canopy gas exchange rates of hydroponically-grown peanut and sweetpotato under controlled temperature and light conditions. Carbon dioxide concentrations were maintained at 400 (ambient) and 1000 $\mu mol/mol^{-1}$. Gas exchange rates were continuously determined based on CO_2 concentrations in air entering and exiting the canopy gas exchange chambers. Results are expected to show that elevated CO_2 will lead to increases in canopy photosynthetic rates and in final yield, and that final yield will correlate strongly with whole-season total photosynthesis. This data will be useful for designing ALS systems that maintain suitable atmospheric conditions for human life.

(Supported by NASA:NAG100209 and USDA/CSREES ALX-PS-1)

[96]

EFFECTS OF HYPOBARIC PRESSURE ON PHOTOSYNTHESIS AND STOMATAL CONDUCTANCE IN LEAF LETTUCE. R.E. Lacey[1] and M.C. Drew[2]. [1]Dept. of Agricultural Engineering and [2]Dept. of Horticultural Sciences, Texas A&M University, College Station.

The effects of hypobaric pressure at 70, 50, and 30 kPa on photosynthesis and stomatal conductance rates of leaf lettuce were determined following a 24-hour period at hypobaric pressures. Photosynthesis and stomatal conductance rates were measured using a Li-Cor LI-6400 portable photosynthesis system. Measurements were taken immediately before placing the plants in the Texas A&M Low Pressure Plant Growth (LPPG) system and immediately after removal of the plants following a 24-hour residence. Instrument readings were acquired over a 25-minute period in order to determine steady state values. Gas composition was maintained at ambient ratios (i.e. 21% O_2, 79% N_2, and 350 ppm CO_2) but partial pressures of O_2 and CO_2 were reduced proportionally with total pressure reduction.

Initially after removal from the LPPG, the lettuce plants exhibited higher photosynthesis and stomatal conductance rates, but both decreased with time. After approximately 25 minutes, both rates had reached a stable value. Pre and post-test measurements were compared after 25 minutes of testing in the Li-Cor L-6400 using a paired t statistic. The null hypothesis that the pre and post-test values were equal (H_0: $\Delta_D = 0$) could not be rejected. Increased condensation was noted in the LPPG following the tests suggesting that transpiration was increased at lower pressures.

Short-term (24 hour) exposure of lettuce to hypobaric pressures of 30, 50, and 70 kPa did not appear to have any detrimental effects on the plants once returned to ambient pressure. Stomatal conductance, photosynthesis, and possibly transpiration were increased at hypobaric pressures but returned to pre-test values upon removal from the LPPG. A series of longer-term tests (7 to 21 days) at 30 and 50 kPa are planned to determine gas exchange data and biomass production over extended periods.

This work was supported by a grant from NASA's Advanced Human Support Technologies Program (98-HEDS-01).

[97]

SHORT AND LONG-TERM EFFECTS OF SPACE STATION PROTOTYPE CAGE AND FOOD TYPE ON LIVER LIPIDS AND ENZYMES IN MALE RATS . A. Lau [1], J.L. Ramirez [1], S. Pruitt [1], G. Sun [1], E.L. Hill [1], B. Girten[2]. [1] Lockheed Martin, Moffett Field, CA and [2] NASA Ames Research Center, Moffett Field, CA.

This 15-week study examined liver lipids and enzymes of rats housed in the early prototype caging for the rodent Advanced Animal Habitat (P-AAH) for the International Space Station (ISS). The effects of the wire-bottom P-AAH cages and specialized NASA rodent Foodbars (FB) were compared to standard vivarium cages (VIV) with corn-cob, litter-filled bottoms, and standard Purina rat chow (CH). Two hundred eighty-eight male Sprague-Dawley rats were divided into four treatment groups (72 rats/treatment): Group 1) VIV+CH, Group 2) P-AAH+CH, Group 3) VIV+FB, and Group 4) P-AAH+FB. Each VIV and P-AAH cage initially housed three and six rats, respectively. At five weeks (Interim), one VIV and two P-AAH rats were removed from each cage (n=96, 24 rats/treatment). Interim and end-of-study results showed statistical significance ($p<0.05$) in lower liver/body weight (LIV/BW) ratio and higher fat pad weights and fat pads/body weight (FAT/BW) ratio in FB fed rats. At both time periods, BW and LIV/BW ratios were significantly lower for P-AAH groups. Interim results indicated significantly lower lipid peroxidation (LPO)/protein ratio (19-24%) in FB fed rats and no significant change in superoxide dismutase (SOD)/protein ratio in liver tissue. End-of-study results showed statistically higher LPO/protein ratio (36-41%) and liver triacylglycerol (TAG) (36-57%) in FB fed rats, but no significant changes in SOD/protein ratio and hepatic total and esterified cholesterol levels. These results indicate that although there are statistically significant differences in LIV/BW, fat pads and FAT/BW after five weeks in FB fed animals, elevated lipid peroxidation in the liver did not occur until after long-term (>15 weeks) use of FB. The significantly increased lipid peroxidation and TAG in liver suggests that the current Foodbar formulation may need to be optimized for long-term use with rats. This study provides valuable information for optimization of the ISS rodent habitat and NASA rodent Foodbars.

[98]

SELECTION OF CARROT CULTIVARS FOR PRODUCTION IN SOLID SUBSTRATES FOR BIOREGENERATIVE LIFE SUPPORT. K.E. Henderson[1], D.J. Barta[1], C. Galindo, Jr.[2], S. L. Steinberg[3],.J.E. Gruener[2],. and D.W. Ming[1], [1]NASA Johnson Space Center, [2]Hernandez Engineering, Inc., and [3]Liberated, Houston, Texas.

Carrot is one of fifteen "baseline" crops identified by NASA's Advanced Life Support Project for development of technologies using higher plants for space vehicle and planetary surface applications. Carrots could potentially be grown within a vegetable production unit (e.g. salad machine) on a space vehicle for augmentation of the crew's food system. In an initial experiment, eight cultivars of carrot were grown in a controlled environment chamber to harvest maturity at 56 days from seeding. The environmental conditions were set as follows: 23 *C air temperature, 70% relative humidity, 300 mmol m-2 s-1 PPF, 16h/8h photoperiod, HPS lighting, 1200 ml l-1 carbon dioxide. The carrots were grown in 0.093 m2 trays (30 cm width, 30 cm length, 15 cm depth), 20 plants tray-1, filled with peat-vermiculite growing mix. The trays were irrigated with a modified half-strength Hoagland's nutrient solution based on soil tensiometer readings. The cultivars included "Baby Spike", "Oxheart", "Parmex", "Mignon", "Kinko", "Minicore", "Mokum" and "Nelson". Mean edible fresh mass per plant was19.1, 20.0, 22.2, 22.8, 29.2, 36.3, 43.4 and 48.2 grams, respectively. Harvest index, based on fresh mass, was 0.66, 0.37, 0.40, 0.67, 0.45, 0.59, 0.69, and 0.70, respectively. Additional information including dry mass, carotene content, and root length and diameter will be presented.

[99]

PRODUCTION OF FRESH VEGETABLE CROPS IN ZEOPONIC SUBSTRATES: PRELIMINARY RESULTS. D.W. Ming[1], J.E. Gruener[2], K.E. Henderson[1], D.J. Barta[1], and C. Galindo, Jr.[2]. [1]NASA Johnson Space Center and [2]Hernandez Engineering, Inc., Houston, Texas.

A synthetic substrate (zeoponic substrate) is under development for use in advanced life support system testbeds and microgravity experiments that will slowly release into the soil solution all of the essential nutrients for plant growth. The substrate consists of NH4- and K-exchanged clinoptilolite (a natural zeolite), synthetic apatite (calcium phosphate mineral with incorporated micronutrients and sulfur) and dolomite (a natural calcium-magnesium carbonate). The objective of this study was to conduct a preliminary assessment of the production of several fresh vegetable crops in zeoponic substrates. Radish (cv. Cherry Belle), lettuce (cv. Waldmann's Green), pak choi (cv. Joi Choi), spinach (cv. Tyee), carrot (cv. Mokum, cv. Baby Spike), green onion (cv. Evergreen Hardy White), and tomato (cv. Micro-Tom, cv. Red Robin) were grown in zeoponic substrates (irrigated with deionized water) and control substrates (Jiffy Mix potting soil irrigated with nutrient solution) in a controlled environmental growth chamber. In these preliminary tests, radish, pak choi, spinach, and carrot (cv. Mokum) grown in zeoponic substrates had higher edible fresh mass than plants grown in control substrates. Lettuce, carrot (cv. Baby Spike), green onion, and tomato (cv. Micro-Tom) grown in control substrates had higher edible fresh mass than plants grown in zeoponic substrates. Several of the crops were replanted in the same zeoponic substrate for successive crops, i.e., radish (4 crops), lettuce (3 crops), and spinach (2 crops). These preliminary results suggest that fresh vegetable crops can be grown using zeoponic substrates and that the growing media can be re-used.

[100]

A MULTIFUNCTIONAL SANITATION METHOD FOR FOOD PROCESSING. D. B. Elrod, G. D. Hitchens, W. White, S. Carroll. Lynntech, Inc., College Station, Texas.

Long-duration space habitation missions, either to the moon or to Mars, will require food provided from plants grown in life-support bioregenerative chambers. These systems will integrate food production with atmosphere regeneration and water recycling, increasing self-sufficiency and decreasing the need for expensive resupply from earth. Since maintaining crew health is of the utmost importance, food must be produced and processed under highly sanitary conditions to minimize the risk from food-borne diseases. New sanitation methods and procedures are urgently needed to meet this challenge. This technology concerns a new and innovative sanitation method that will meet the challenge. The method is unique because it operates at low temperature, uses minimal consumables, does not affect the quality of the food, and provides many safety features. The method employs vapor phase disinfectants, which are generated by the introduction of a miniscule quantity of a concentrated disinfectant into a sealed chamber followed by application of a vacuum. The Phase I study demonstrated that the method could reduce the levels of microbial contaminants to acceptable levels on the main ALLS crop vegetables. The disinfection method can also be applied to utensils, food preparation surfaces, and processing equipment and there are no harmful wastes produced that could impact the bioregenerative system. The method eliminates many of the difficulties of conventional liquid germicides. The aim of Phase II is to provide sanitation hardware for multiple uses within bioregenerative habitats.

(Supported by NASA: NAS 9 00018 and NAS 9 01083.)

Concurrent Posters
III-F
Advanced Life Support: Recycling and Regeneration

[101]

SODIUM CHLORIDE REMOVAL FROM URINE VIA A SIX-COMPARTMENT ELECTRODIALYSIS CELL. G. Colon, V.M.. Aponte, and N.E. Gordils. Department of Chemical Engineering, University of Puerto Rico, Mayaguez Campus.

A six-compartment electrodialysis (ED) cell using univalent ions selective membranes was used to remove sodium chloride from human urine for Advanced Life Support applications that will be used in long-term space missions. To study the feasibility of this system , the first variable under study was the limiting current density (i_{lim}). Working at current densities lower than i_{lim} allows to obtain better efficiency of sodium chloride removal and extends the membrane's useful life. The i_{lim} data were obtained at four fluid velocities ranging from 0.015 to .083 m/s and four urine dilution ratios ranging from 0.25 to 1.00 (3,200 to 12,800 Cl^{-1} ppm). The i_{lim} was found to be a power function of fluid velocity and a linear function of Cl^{-1} ion concentration by the model: $i_{lim} = 4105\ U^{0.9}C$. An average current efficiency of 50% based on Na^{+} ion removal indicated that half of the applied current was used for sodium chloride transport. Higher and more stable current efficiencies were obtained operating the system at batch-recirculation mode of operation at constant applied voltage than at a continual flow mode of operation. Ion chromatography revealed that the highest sodium chloride removal for continual once-through mode of operation was 91.0%, achieved for at the lowest salt dilution ratio at a fluid velocity of 0.039 m/s and applied current density of 57 A/m^2. For batch-recirculation mode of operation, the operational voltages were 5.0, 7.5 and 9.0 volts at a constant fluid velocity of 0.083 m/s. Under this mode of operation the highest sodium chloride removal was 98.5%, achieved at an electrical potential of 9.0 volts.

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[102]

HYBRID WATER POLISHING SYSTEM. J. Kim, L. Rutherford, and A. Gonzalez-Martin. Lynntech, Inc., College Station, Texas.

Water reclamation is one of the basic functions of a regenerative life-support system. An efficient process for post-treatment water polishing is imperative for the production of potable water in a closed regenerative system. Only a few processes have the necessary attributes such as safe operability in micro- and partial gravity, high reliability, minimal use of expendables, ease of maintenance, and low system volume, mass, and power. Two promising post-treatment methods are (i) electrochemical water processing utilizing electricity and (ii) photocatalytic water processing using UV light. However, use of one technique alone is only partially effective as a post-treatment method. Lynntech has carried out an innovative hybridization of the two techniques without losing any of attributes both techniques have to offer. Advantages of the hybrid system include oxidation enhancement, oxygen demand reduction, and efficient use of light. The hybrid system was evaluated in the need to reduce organic carbon impurities to a level below 0.25 part per million and to eliminate microbial contaminants.

(Supported by NASA: NAS9-01019)

[103]

ROTATING ELECTROLYTIC CELL FOR *IN-SITU* WATER SYSTEM STERILIZATION. Patrick I. James (PI), Wayne E. Buschmann, Anthony F. Sammells. Eltron Research Inc., Boulder, CO

This program's objective was to develop an automated electrolytic system for sterilizing the potable water generated by the WRS. This technology employs a microgravity compatible a self-pumping rotating electrolytic cell to create improved hydrodynamics and performance at reduced energy costs (about 80 W *vs*. 400 W for a standard planar cell). The cell reduces oxygen at the cathode to produce hydrogen peroxide (H_2O_2) containing solutions to be used for disinfection or sterilization. The anolyte solution becomes acidic while the catholyte solution becomes alkaline thus allowing neutralization of the treated solution to potability after treatment while leaving a H_2O_2 residual to hinder recontamination by biological organisms. This program explored the cell's operation hydrodynamics and protocols to determine the preferred effective, robust, and efficient cell design and operation. It also determined the target pH, H_2O_2 concentration, and treatment time required to effect the desired level of sterilization through bioassays using synthetic and actual product and established that solutions with pH's 10.8 and containing 10 ppm of H_2O_2 reliably eliminated *Pseudomonas cepacia* to 7 log kills and prevented regrowth. Solutions of pH 11.3 to 11.5 plus 20-75 ppm H_2O_2 were effective against a variety of potentially problematic microorganisms previously identified in NASA studies. Acceptable cell performance was verified with simulated spacecraft waste water and yielded product (pH 11, 20 ppm H_2O_2 in 225 lb. H_2O per 24 h) while maintaining a sufficient neutralization ratio to yield a potable product. Also, the electrolytic cell may be run at higher current densities ("turbo" mode) to enhance the pH shifts without destroying H_2O_2 previously generated. Additionally an automated control system incorporating the rotating electrolytic cell was designed, developed, tested, and was delivered to NASA-JSC at the completion of the program along with a simple operation manual detailing construction, use, and maintenance to complete the system package.

(Supported by NASA: SBIR PHASE II NAS9-99141)

[104]

DEVELOPMENT OF BIOLOGICAL TECHNOLOGY FOR USE IN TRACE CONTAMINANT CONTROL: BIOFILTRATION OF AMMONIA, ETHYLENE AND AN ERSATZ ISS ATMOSPHERE. R.M. Cowan, J.A. Hogan, P.F. Strom, F. Qiao, J.A. Tambwekar, NJ-NSCORT and Department of Environmental Sciences, Cook College, Rutgers, The State University of New Jersey.

For the past several years we have been working on the development of biological air treatment technologies for use in the control of trace air contaminants. Initial work focused on the removal of single compounds (ammonia and ethylene) in order to identify inherent limits that different compounds place on this treatment technology. Recently we initiated work on a more complex multi-component mixture of volatile organic compounds (acetone, benzaldehyde, benzene, 1-butanol, carbon monoxide, ethanol, ethyl acetate, ethylene, isoprene, methane, methanol, methylene chloride, methyl ethyl ketone, phenol, toluene) and ammonia. This mixture is designed to represent an ersatz version of the atmosphere that might be present in an advanced life support system like the International Space Station with the contaminants used being those with the highest expected loads and those representative of each major class of volatile contaminants. Experiments to date have led to enrichment cultures capable of degrading each of the selected contaminants and provided quantitative estimates of the biodegradation kinetics. Current efforts are focused on the operation of air treatment bioreactors fed this multi-component mixture. Additionally, further development of the mathematical models is being performed. It is anticipated that this effort will provide a tool for performing numerical optimization studies and confirming a mechanistic understanding of the observed bioreactor performance. The poster will contain information on reactor design and operation and a summary of experimental and modeling results obtained through the end of October 2001.

(Supported by NASA through the NJ-NSCORT.)

[105]

SIZE REDUCTION OF SOLID WASTE AND INEDIBLE BIOMASS RESIDUE. G.M. Savage and L.F. Diaz, CalRecovery, Inc., Concord, California.

NASA is developing sustainable advanced life support systems that will utilize solid waste and inedible biomass residue (IBR) as resources. In many cases, these conversion systems will require size reduction of the waste in order to obtain optimum conversion system efficiency. This paper describes the process of size reduction and methods of achieving the range of feedstock particle sizes required by the conversion technologies. Data with regard to resultant particle size distributions and energy requirements are presented for a variety of compositions of solid waste, IBR, and combinations of the two.

[106]

EFFECT OF CONSTANT TEMPERATURE COMPOSTING ON DEGRADATION OF SWEETPOTATO BIOMASS. A.A. Trotman and J. L. Vaughn. Center for Food and Environmental Systems for Human Exploration of Space, Tuskegee University, Tuskegee Institute, AL.

The bioregenerative approach to solid waste management of crop residue provides an alternative and reliable method for solid waste treatment. Harnessing the biological process to modulate the product for crop production is of interest in the area of redundancy for Advanced Life Support. The channeling of excess energy to maintain composters at constant temperature may allow improved process control. The current research examined the impact of incubating composters at constant temperature on the mass reduction and nutrient recovered from composting sweetpotato biomass. The hypothesis tested was that constant temperature had no effect on mass reduction and nutrient recovery. The objective of the study was to elucidate the effects of constant temperature (55ºC and 70ºC) on composting. A completely randomized block design with three treatments (ambient, 55ºC and 70ºC) and two replications, with water baths serving as blocks was used. Two-liter glass composter vessels were incubated in a water bath to provide the treatment conditions throughout the vessel. Air was constantly passing through the system via tubing that were submerged in the water bath to ensure constant temperature was maintained. The composter incubated at ambient temperature was also placed in a water bath without temperature control; in effect this water jacket ensured the entire volume of the composter was at a uniform temperature. The mass reduction and nutrient recovery from composting increased significantly ($P < 0.005$) for incubation at a constant temperature of 55ºC (34.5%) compared to the composting products at ambient temperature, over a 21-day incubation period. The results of this study revealed that controlling the temperature of the composter may be used as a means of modulating the rate of degradation and the rate of products generated by composting.

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[107]

BIOREGNERATIVE RESEARCH AND TECHNOLOGY DEVELOPMENT TECHNICAL TASK IN SOLID WASTE PROCESSING M.S. Roberts[1], J.L. Garland[1], N.C. Yorio[1], J.L. Adams[1], M. Hummerick[1], and M. Klamer[2] [1]Dynamac Corporation, NASA Kennedy Center, Florida. [2]Biology Department, University of Central Florida, Orlando, Florida.

The major focus of the solid waste processing task during the past year was evaluation of composting technologies for potential application in ALS systems. One study evaluated the recycling of soluble nutrients from composted inedible wheat material in hydroponic systems. Either fresh or oven-dried inedible wheat biomass was composted in an in-vessel composter for 28 days, after which the compost was leached and amended with reagent-grade inorganic nutrients to simulate Hoagland's replenishment solution. No variation in growth response was noted for the fresh-feed compost treatment but oven-dried feed showed significant negative growth responses, likely as a result of increased soluble organics measured in this treatment A second study evaluated plant matter decomposition, microbial community dynamics and pathogen survival in a rotating drum composter operated under three thermal control modes: 1) short-term thermophilic, 2) long-term thermophilic, and 3) no thermal control. The ability of pathogenic bacteria to re-grow in stored compost was also assessed. Both studies indicate that failure to define microbial community structure and function within solid waste recovery systems will limit engineering efforts to optimize reactor performance for resource recovery and augment pathogen destruction.

[108]

ANAEROBIC COMPOSTING SYSTEM FOR SPACE MISSIONS. D. Chynoweth[1], P. Haley[1], and T. Townsend[2], [1]Ag. and Biol. Eng. and [2]Envir. Eng. Sci., Univ. of Florida, Gainesville

The technical feasibility of applying anaerobic composting for recycling the organic fraction of solid wastes generated during space missions is being investigated. This process has the advantages of not requiring O_2, high temperature, and pressure and it produces CH_4, CO_2, nutrients, and compost after 21 days treatment. The process is simple and reliable and only requires coarse shredding for pretreatment. It also has the potential for reforming H_2 and CO_2 and treatment of ambient air by biofiltration. This project is evaluating the biochemical methane potential to determine conversion efficiencies and kinetics for several expected solid waste fractions, including paper, crop and food residues, and feces. The process involves a solid phase fermentation employing leachate recycle between new and old reactors for inoculation, wetting, and removal of volatile organic acids during startup. After 21 days conversion is complete and the compost bed may be used for biofiltration and plant growth medium. The nutrient-rich leachate may also be use as a vehicle for nutrient recycle. Modifications for operation of this process under development include flooded operation to force leachate through the beds and centrifugal separation of biogas from leachate. We have determined that 8 kg. of organic matter (typical daily solid wastes from a 6 person crew) will produce 1 kg CH_4, 3 kg CO_2, and 4 kg of compost. Conersion could be higher if more biodegradable plants and packaging are utilized. A detailed analysis this process is being conducted leading to design of a system sized to resemble that required for a space mission with a 6 person crew. This analysis will be used to determine the ESM and technical feasibility for use of this process for space missions.

Concurrent Posters
III-G
Advanced Life Support: Systems Biology

[109]

LOCALIZATION AND PUTATIVE FUNCTION OF SPECTRIN-LIKE PROTEINS IN GRAVITROPICALLY TIP-GROWING CELLS. A. Sievers, and M. Braun. Botanisches Institut, Universität Bonn, Bonn, Germany.

Spectrin-like epitopes were immunochemically detected and immunofluorescently localized in gravitropically tip-growing rhizoids and protonemata of characean algae. The antiserum showed cross-reactivity with rhizoid proteins at molecular masses of 195 and 170 kDa. Confocal microscopy revealed a distinct spherical spectrin labeling in the apices of both cell types tightly associated with a dense actin array and a specific subdomain of endoplasmic reticulum (ER), the ER aggregate which represents the structural center of the Spitzenkörper. The presence of spectrin-like epitopes, the ER aggregate and the actin cytoskeleton are correlated with the active process of tip growth. Application of cytochalasin D and A23187 has shown that interfering with actin or with the calcium gradient, which both cause the disintegration of the ER aggregate and abolish tip growth, inhibits spectrin labeling. At the beginning of the graviresponse in rhizoids, the spectrin labeling remained in its symmetrical position at the cell tip, but was clearly displaced to the upper flank in gravistimulated protonemata. The upward shift of positively gravitropic protonemata was shown to be preceded by a statolith-induced relocalization of putative calcium channels and the tip-high calcium gradient to the upper flank (bending by bulging) that does not occur in rhizoids, in which statoliths sedimentation is followed by differential flank growth (bending by bowing). These findings support the hypothesis that an active relocalization of the growth center is required for the negative gravitropic response in protonemata, but not for the positive gravitropic response in rhizoids. It is suggested that the actin/spectrin system plays a role in establishing the functional domain of the ER aggregate and represents an essential part in the mechanism of gravitropic tip growth.

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[110]

SPECIES DIFFERENCES IN LIGAND SPECIFICITY OF AUXIN-CONTROLLED ELONGATION AND AUXIN TRANSPORT: COMPARING ZEA AND VIGNA. H. Zhao[1], R. Hertel[2], H. Ishikawa[1], M.L. Evans[1] [1]Dept of Plant Biology, The Ohio State University, Columbus and [2]Institut für Biologie III, Albert-Ludwigs-Universität, Schänzlestr. 1, D-79104 Freiburg, Germany

Elongation of roots and shoots of the grass Zea mays L. and the legume *Vigna mungo* L. in response to selected auxin derivatives was examined and compared. In addition, polar transport, through segments from Vigna hypocotyls and maize coleoptiles, of one of the auxin derivatives (2-naphthoxyacetic acid) was assayed fluorimetrically. For all three assays (root elongation, shoot elongation, and polar transport) the same difference in ligand specificity was observed between the dicot and the grass species. This finding supports the hypothesis that a common protein mediates auxin efflux as well as auxin action on both root and shoot elongation.

[111]

GENOTOXIC EFFECTS OF HYPO AND HYPERGRAVITY IN *ALLIUM CEPA* L. T F. Khaleel. Dept of Biological and Physical Sciences. Montana State University-Billings.

Normal plant growth is from mitotic divisions in the meristematic cells. A mitotic index suggests a rate of cell division indirectly inferring growth rate. Genotoxic effects caused by gravitational stresses encountered during KC135 parabolic flights and ground simulated hypergravitational forces may be associated with abnormal mitosis and mitotic indices that can be observed and measured. This study determines the genotoxic effects of variable gravity followed by its consequences on sporogenesis, gametophytes, and embryo in *Allium cepa* L. Dormant and rooted bulbs of three varieties were carried aboard the KC135 flight. All bulbs were subsequently planted and grown to flowering. A set of ground experiments were performed by subjecting roots and inflorescence to 2g forces for 15, 30 and 60 minutes. All tissues were allowed to recover for 24 hours. Roots were also subjected to 2g forces for 12-24 hours and harvested without allowing a recovery time. 1000 cells from each treatment and the controls were scanned to determine mitotic index and mitotic abnormalities indicative of genotoxicity. Data on sporogenesis, gametogenesis, meiosis, and embryo development was analyzed from prepared slides of flower buds. Results show that mitotic index, mitosis, sporogenesis and gametogenesis are affected. A preponderance of prophase followed by a sharp decrease in the subsequent phases in all three varieties suggests that mitotic spindle is affected. Lower and higher than normal mitotic indices, that appeared to be variety specific were observed. The red variety had a higher mitotic index following first day of gravitational stress and a considerably lower mitotic index following the second day of stress, the white variety showed lower mitotic indices both days and the yellow variety had a higher index both days. Mitotic abnormalities such as c-mitosis, anaphase bridges, and stickiness were observed. The present study suggests that variable gravitational stresses induce genotoxicity and shed light on other aspects of development that affect the capacity of higher plants to reproduce.

(Supported by Montana Space Grant Consortium NGT5-40036)

[112]

ANTHER STRUCTURE AND POLLEN DEVELOPMENT IN *BRASSICA* PLANTS GROWN DURING SPACEFLIGHT A. Kuang[1] and M. E. Musgrave[2] [1]Department of Biology, The University of Texas – Pan American, Edinburgh, TX 78539; [2]Biology Department, University of Massachusetts, MA 01003

Anthers play an important role in providing nutrients and acting as chambers for pollen development. There is little information available regarding the correlation of anther development and pollen development in the microgravity environment. The current study investigates the changes in the structure of anthers and corresponding pollen grains of *Brassica rapa* L., cv. CrGC#1-33 plants grown on board the MIR space station and on the ground. *Brassica rapa* plants (13 d-old) developed on the MIR space station and on the ground were preserved in a buffered solution containing 1% formaldehyde and 0.5% glutaraldehyde. Post-flight, flowers were dissected from the preserved plants and post-fixed in 1% Osmium. Anthers from those flowers were processed and prepared for light and electron microscope observation. The results show that numerous large starch grains are present in the cells of the anther wall and filaments in space plants, while starch grains are rarely observed in those cells of the ground plants. There is no difference on the organization of the anther wall between space and the ground control materials. However, the inner layer of anther wall, tapetum, was swollen and the tapetal plasmodium intruded into the anther chambers. The swollen tapetal layer persisted to a later developmental stage in the spaceflight material whereas it degenerated at an earlier developmental stage in the ground plants. Most pollen grains appeared normal. The results suggest that despite the storage of starch grains in the anther wall and the persistence of tapetum, the nutrients necessary for pollen development were supplied in microgravity.

(Supported by NASA grant NAG2-1375.)

[113]

HELICAL SECONDARY CELL WALL ORIENTATION OF DEVELOPING PLANTS FROM THE NORTHERN HEMISPHERE AFTER CLINOSTAT TREATMENT. J.G. Taylor and N.D. Livers. Department of Biological Sciences, Ouachita Baptist University, Arkadelphia, Arkansas.

An area of importance in future space travel research must include the growth and maintenance of plants in space. Understanding the basic developmental characteristics of plants is essential in establishing a system that could be maintained in a weightless environment. One very important anatomical feature of higher plants is the presence of secondary cell walls that give strength and rigidity to plant cells allowing a plant to attain a larger size. Understanding secondary cell wall development can give insight into plant viability. This research investigates the established helical secondary wall pattern of xylem cells in developing plants in the northern hemisphere and compares that pattern to differences that may be found in the same species grown in the southern hemisphere and in clinostat manipulated conditions. Differences may be found between the northern and southern hemispheres due to the Coriolis Effect exerted by the rotation of the earth and possible disruption of normal gravitational influences on secondary wall development by use of a clinostat. Microscopic analysis of control specimens of bean, radish, and *Arabidopsis* in collaboration with researchers in Argentina, South Africa, and China has demonstrated that the same specimens grown in those locations have no visible anatomical differences in helical secondary cell wall patterns when compared to the controls. Each specimen was found to have a counter-clockwise spiraling orientation of the helical cell walls. Specimens grown on a clinostat showed some minor variability in overall plant development, especially the roots, but the helical secondary cell wall orientation showed no obvious differences to the controls. These results indicate that the Coriolis Effect and clinostat manipulations do not induce alterations in the helical cell wall patterns established in the embryos of the seed used in these experiments.

(Supported by NASA: OBU10038 and OBU10039.)

[114]

INTERNAL ATMOSPHERE DYNAMICS IN PEPPERS: A MODEL FOR SPACEFLIGHT

M.E. Musgrave and J. Blasiak. Biology Department, University of Massachusetts, Amherst, MA 01003

Previous studies on seed production in microgravity have suggested that the gaseous composition of the seed microenvironment may impact normal development. The small fruits of *Arabidopsis* and *Brassica* that have been the objects of spaceflight studies contain captive air spaces that surround the seeds. In the absence of buoyancy-driven convection, stagnant air layers develop inside these microenvironments. Using pepper fruits as a model, we have investigated changes in the gas composition of the seed microenvironment during development. Flowers of four pepper cultivars were marked at anthesis and samples of locular gases from the developing fruits were withdrawn and analyzed by gas chromatography. The atmospheric composition of the seed microenvironment was found to vary according to developmental stage of the fruit, illumination, and pepper type. Oxygen concentrations ranged from 16 to 21%, carbon dioxide 10,000 to 40,000 ppm, and ethylene 4-260 ppb. Detachment studies showed that the pedicel is an important avenue for gas movement in the hot peppers, while the sweet peppers act as closed systems, where oxygen and carbon dioxide fluctuate due to the processes of photosynthesis and respiration. Because of this, the sweet pepper would be an excellent model system for studying the interactions of gaseous environment and seed development in microgravity. One sweet pepper variety, Triton, is sufficiently dwarf to be grown in the Biomass Production System. The fruits are large (65 mm long X 49 mm wide) and have a locular volume of 16 ml, permitting sequential withdrawal of gas samples during development. On average, 150 seeds are produced by each fruit. Modified locular atmosphere studies are underway to determine the role of proximate gas environment in seed development.

(Supported by NASA NAG2-1375).

[115]

CHANGES IN ROOT CYTOPLASMIC PH IN RESPONSE TO AUXIN. S. J. Swanson and S. Gilroy. Biology Dept., Penn State Univ., University Park, PA 16802. sjs31@psu.edu

The plant hormone auxin is involved in the regulation of numerous aspects of plant growth including cell expansion, lateral root initiation, and gravitropism. Despite these important roles, the mechanism of auxin signal transduction remains unknown. Alterations in cytoplasmic pH have been correlated with the auxin responses of cells from aerial portions of the plant. To test the hypothesis that auxin perception by root cells results in changes in cytoplasmic pH, we monitored Arabidopsis roots expressing a cytoplasmic, pH-sensitive, ratioable GFP using a vertical stage microscope to circumvent potential confounding gravitropic responses. The GFP signal was monitored prior to and following treatment with auxin analogs. Treatment with the active auxin analog naphthalene-1-acetic acid (1-NAA) caused a drop in root cell cytoplasmic pH while the non-active naphthalene-2-acetic acid (2-NAA) did not. The decrease in cytoplasmic pH was detectable within 1 min of 1-NAA application and after 10 min dropped 0.2 to 0.3 pH units lower than the initial root cell cytoplasmic pH. In addition, the pH response in terms of magnitude and kinetics was different in the various regions of the root (eg. the cap vs. the elongation zone). This research is supported by a grant from NSF.

[116]

RESPONSE OF CULTURED FETAL MOUSE LONG BONES TO RANDOM POSITIONING. J. Paul Veldhuijzen, Jolanda M.A. de Blieck-Hogervorst and Jack J.W.A. van Loon. Dept. of Oral Cell Biology, ACTA-Vrije University, Amsterdam, The Netherlands.

Recently a Random Positioning Machine (RPM) has been developed (Fokker Space, The Netherlands) which should provide simulated microgravity conditions. Effectiveness of the RPM to duplicate the results of our real microgravity experiments where bones showed a clear reduction in mineralization, was tested in cultures of 17-day old fetal mouse bones.

The bones were cultured “free floating” in double layered polyethylene culture bags (0.7 ml medium); 8 of these bags were placed in standard flight hardware in Biorack type I containers. Containers with control cultures were placed on the non-moving frame of the RPM. Initially no effects of the RPM were found. However, the “free floating “ bones went through uncontrolled movements during RPM-operation. Immobilizing the bones in small pieces of agarose gel prevented these movements of the bones and agarose did only marginally affected growth and mineralization of these bones. Experiments with these immobili-zed bones showed that overall growth of the bones on the RPM did not change. In most experiments again no differences were found in the mineralization. However in the RPM-groups in many cases the standard deviation was higher then in the controls. In some experiments we found indeed a decrease in mineralization; in another experiment we found an increase.

This leads to the conclusion that the RPM did not provide simulated microgravity to the cultured long bones. However in the culture bags always air bubbles were present. Under real microgravity these bubbles do not move and do not affect the culture but on the RPM these bubbles revealed to be very mobile. This will have changed culture conditions on the RPM, which could have obscured possible effect of the RPM. Further experiments are needed to clarify this issue.

(Supported by the Space Organization of the Netherlands (SRON): MG-045/3)

[117]

HIGH-LET RADIATION AND IMMUNE MODULATION: LATE EFFECTS OF ^{56}Fe VERSUS ^{28}Si. D.S. Gridley[1,2], M.J. Pecaut[1], G.M. Miller, M.L. Andres, R. Dutta-Roy, A.L. Smith, T.A. Jones, and G.A. Nelson[1]. Depts of [1]Radiation Medicine, Radiobiology Program and [2]Microbiology & Molecular Genetics. Loma Linda Univ and Medical Center, Loma Linda, CA

The great majority of ground-based immunological studies have been performed with low-linear energy transfer (LET) radiation such as □-rays. Data after exposure to high-LET radiation, which may have much greater biological effects, is sparse. The major goal of this study was to evaluate and compare the long-term effects of two different forms of high-LET radiation on lymphoid cells and organs after whole-body exposure. C57BL/6 mice were irradiated with either iron ions (^{56}Fe, Z = 26, LET = 146 KeV/□m) or silicon ions (^{28}Si, Z = 14, LET = 142.6) to a total dose of 2.0 gray (Gy) at Brookhaven National Laboratory. The animals were shipped to Loma Linda University and euthanized at 113 days post-exposure. The group irradiated with ^{56}Fe, had significantly increased total lymphocyte and B cell numbers and proportionally low levels of CD3+/CD8+ T cytotoxic (T_C) cells and monocytes compared to non-irradiated controls. In contrast, the ^{28}Si group had low levels of NK1.1+ natural killer cells, decreased basal DNA synthesis, and increased response to two of three tested mitogens (PHA and LPS). Overall, the aberrations were more pronounced in blood than in spleen. These findings suggest that exposure to radiations of differing quality may affect a broader range of parameters compared to exposure to a single form of radiation. It remains to be determined if immunomodulation following irradiation with multiple forms of radiation, as would most likely occur during extended space flight, results in additive or synergistic deleterious effects. Our current studies seek to determine if the observed changes translate into impaired immune defense.

(Supported by NASA: Coop. Agreement NCC9-79.)

[118]

16 DEGREES HEAD-DOWN TILT COMPENSATES –15 mm Hg LBNP FOR THORACIC IMPEDANCE BUT NOT FOR OTHER VARIABLES IN HUMANS.

H. Hinghofer-Szalkay, I. Loder. Institut f. Physiologie, Med. Fac., Karl-Franzens-University, and ASM-IAP, Graz, Austria.

Rationale: To identify combinations of head-down positioning (HDT) and lower body suction (LBNP) that compensate their effects on various physiological variables. Methods: 10 healthy females, age 19-22, underwent various combinations of HDT and LBNP for 30 min duration each, or lied supine for 30 min (control condition). We measured hemodynamic, volume-dependent, and humoral variables with no stimulus, HDT or LBNP separately, and various combinations thereof. Results: Specific dose-response patterns emerged. Thoracic electrical impedance was the same as with no stimulus when 15 mmHg LBNP was paired with 16°HDT. Other variables, displayed other, more complex 'neutralization' responses. Conclusion: Different subsystems behave differently with HDT-LBNP-stimulus combinations. Therefore, simple 'compensation points' cannot be identified without specifying which system is addressed for a certain stimulus pattern.

Supported by the Austrian Ministry for Education

[119]

IS GRAVITY A CONTINUUM?: EFFECTS OF HYPERGRAVITY ON MAMMARY METABOLISM IN PREGNANT RATS. K. Plaut[1], R. Maple[1], L. Baer[2], C. Wade[2] and A.E. Ronca[2]. [1] Department of Animal Sciences, University of Vermont, Burlington and [2] Life Sciences Division, NASA Ames Research Center, Moffett Field, CA.

We have previously shown that metabolic activity in the mammary gland of pregnant rats was significantly increased in response to spaceflight (Plaut et al., 1999). It was unclear whether this response was due to the microgravity environment or due to the acute stress of re-entry and landing. To address whether changes in metabolic activity were related to gravity load, we exposed pregnant rats to hypergravity and measured mammary metabolism. From day 11 to 20 of the rats' 22-day gestation, animals were centrifuged (20 RPM; 1.5, 1.75 or 2.0 x gravity) or were stationary controls. On gestation day 20, 5 rats from each group were removed and euthanized. The remaining dams (n=5/treatment) were housed at 1 x g until parturition. After two hours of nursing by the pups, the post-partum dams were euthanized. Glucose oxidation to CO_2 and incorporation into lipids was measured using U-^{14}C-glucose. Prolactin (Prl) binding to mammary membranes was measured using ^{125}I-hPrl competed with rPrl. Mammary glands from dams euthanized on gestation day 20 revealed a strong negative correlation between metabolic rate and increasing g-load. Approximately 94% of the variation in glucose oxidation and 98% of the variation in glucose incorporation into lipids can be accounted for by differences in g-load. There was also a small decrease in binding to prolactin receptors to mammary membranes as g-load increased. Differences in metabolic activity due to g-force disappeared in the post-partum dams that had been housed at 1-g. To determine the relationship between changes in hyper-g compared to the microgravity environment in pregnant animals, we plotted the ratio of metabolic rate to g-load. There was a significant exponential relationship (r^2 =.99) between mammary metabolic rate and g-load indicating that metabolic rate change is constant as gravity load is altered. We conclude that there is a continuum of response in mammary metabolic activity from microgravity through the hypergravity environment.

(Supported by NASA: NCC 2-8064; 121-1040)

[120]

DOES EARTH'S GRAVITY ACCOUNT FOR THE GREATER BLOOD PRESSURE OF TALLER HUMANS? C.C. Wunder. Dept of Physiology and Biophysics, Univ of Iowa, Iowa City.

Taller organisms must make greater adjustment to gravity than must shorter organisms. If arterial pressure is appropriate to lift blood against gravity to a constant perfusion pressure for the brain, then the pressure at heart level should increase with body height. This increase should amount to 0.78 mm Hg for every cm from the heart to the base of the skull or 0.11 mm Hg per cm of body height (***h***). This expectation from Pascal's Third Law of Hydrostatics has been used to explain the greater pressures for mammalian species of greater height. This effect has been obscured or even exaggerated with human cuff-pressure measurements by the greater arm-thickness:cuff-width mismatching of larger individuals. The objective of this study to determine if correction for arm size will reveal the expected increase of pressure with ***h***.

Mean arterial cuff-pressure at heart level (***p***, 1/3 systolic + 2/3 diastolic, Phase V) was measured a total of 1421times on 733 seated university students in our teaching laboratories using a 12.5-cm-cuff width. Measurements were also recorded for other variables including ***h***, arm circumference (***c***), and body mass (***m***). Simple least-squares regression of ***p*** on ***h*** yielded an exaggerated regression coefficient of 0.20+/-0.02 (SE) mm Hg/cm. Double regression against both ***h*** and ***c*** corrected the coefficient to the more reasonable value of 0.09 +/- 0.02 mm Hg/cc. Stepwise multiple regression of ***p*** against 9 different variables, however, indicated that ***p*** was more directly determined by ***m*** than by ***h***.

Arterial pressure is more closely related to the average height for given body mass than to individual heights. Although ***p*** increases with ***h*** in the manner expected to compensate for gravitational challenge, the mechanism underlying this relationship may be set by some mass-related quantity rather than by pressure or by height *per se.*

(Supported in part by the Univ of Iowa Foundation.)

Concurrent Oral Sessions
III
Advanced Life Support and Systems Biology

[121]

CROP MODELING FOR MULTIPLE CROP PRODUCTION AND CONTROL IN ADVANCED LIFE SUPPORT SYSTEMS. D.H. Fleisher[1], K.C. Ting[2], H. Baruh[1], G. Giacomelli[3], H. Janes[1], and D. Vaccari[4].
[1]Rutgers, The State University of New Jersey, [2]The Ohio State University, [3]University of Arizona, and [4]Stephens Institute of Technology

An analysis tool for systems studies of multiple crop production and control of wheat, soybean, and white potato for use in controlled environment plant production systems has been developed. The tool was constructed as a software program using Microsoft Visual Basic. The program applied mathematical models of plant growth and a model-based control algorithm to (1) simulate crop production scheduling under nominal and off-nominal environmental conditions; (2) study the effects of environmental disturbances in light intensity, air temperature and ambient carbon dioxide concentration on crop production scheduling; and (3) suggest possible compensatory actions to ensure desired production goals for each crop could be met. Four research components were conducted. Experimentation involving hydroponic production of white potato in growth chambers was completed to produce time-series values of plant dry mass. This data was utilized to aid in modification and verification of a state-of-the-art field model of white potato growth for controlled environments. Multivariate polynomial regression equations were developed and fit to simulated time-series data from the modified white potato model and similarly modified models of wheat and soybean. These models were combined with a model-based predictive control algorithm and incorporated into a Visual Basic program for demonstration and system studies application.

(Supported by the NJ-NSCORT and NASA #NGT5-50229)

[122]

MODELING CARBON DIOXIDE UPTAKE IN SOYBEAN PLANTS USING MULTIVARIATE POLYNOMIAL REGRESSION. David A. Vaccari[1], Zhao Yan Wang[1], James Cavazzoni[2], and Konomi Kumasaka[2].
[1]NJ-NSCORT, Stevens Institute of Technology, Hoboken, NJ; [2]NJ-NSCORT, Rutgers University, New Brunswick, NJ.

Models of plant response to environment are needed for the design, operation and control of advanced life support systems incorporating biomass production. Mechanistic models are preferable, but empirical methods may also play an important role. They may be useful early on before mechanistic models are sufficiently robust. They may aid mechanistic model development by identifying significant variables, interactions, and qualitative behaviors. They may be contained as submodels within mechanistic models. Here, we develop completely empirical models of plant response to demonstrate the novel ability of multivariate polynomial regression (MPR) to describe complex plant growth behaviors. MPR models are capable of identifying complex multivariate nonlinear interactions in data. Data were collected using four plant growth chambers operated in duplicate pairs.

Two crops of soybeans were grown in the chambers, at two CO_2 levels, and two light intensity levels. At three stages of each crop cycle (vegetative, early reproductive, and late reproductive) a 48-hour short-term experiment was conducted with a factorial combination of two levels of light intensity, two CO_2 levels, and three temperatures. CO_2 mass balance data were collected at intervals ranging from 1 to 20 minutes. Only data from steady-state conditions were used. One pair of chambers was used for MPR model development; the other for final validation.

Stepwise regression is used to identify only those terms that contribute to the predictive power of the model. A cross-validation technique helps to prevent overfitting or spurious correlation. MPR produces parsimonious models that are easy to use and analyze.

(Supported by NASA through the New Jersey NASA Specialized Center of Research and Teaching.)

[123]

FORMULATION OF ALS CROP-LIST DIETS USING SIMPLEX OPTIMIZATION. C.M. Gregson[1], L. Rodriguez[2] and T-C. Lee[1].
[1]Department of Food Science, [1]CAFT, [2]Department of Bioresource Engineering and [1,2]NJ-NSCORT, Rutgers, the State University of New Jersey, New Brunswick, N.J.

Food within an Advanced Life Support (ALS) System will be obtained primarily from a small number of crops. However, nutritional requirements for ALS and long-term missions have not yet been clearly established. Due to these limitations, optimization of crew diets is difficult. The objective of this work is to provide a methodology to formulate diets that meet requirements for nutritional content, system cost and variety, and to show how the resulting diet is influenced by the objective function.

Previous diets were evaluated using published data based on Earth-grown crops as input information. Optimization of diets based on the BVAD crop-list was performed using the Simplex algorithm. Results show that diets can be readily optimized for macronutrients through the use of the sum of squared differences approach. Optimization for all nutrients (macro and micro) proved more challenging and showed that at least a small number of micronutrients must be provided through alternative sources. Optimization for growth efficiency of crops alone resulted in a diet based on a single crop unless stringent constraints were included. Optimization for multiple objectives, using novel objective functions such as combinations of growth efficiency and macronutrients, provided interesting information.

The work shows a powerful methodology, using linear programming, for optimizing crew diets. Results can be utilized for reconsideration of crop selection lists and biomass production schemes. Once more information is available regarding crew nutritional requirements, crop nutritional value and plant growth performance, more accurate optimizations can be performed.

(Supported by NJ-NSCORT)

[124]

AQUATIC HABITAT (AQH) DEVELOPING PROJECT BY NATIONAL SPACE AGENCY OF JAPAN (NASDA). M.Masukawa[1],S.Uchida[2],T.Sakimura[2],M.Takaoki[1]. [1]National Space Development Agency of Japan, [2]Mitsubishi Heavy Industries, LTD.

NASDA has flown aquatic animal experiments on four space shuttle missions; VFEU (Vestibular Function Experiment Unit) for STS-47/90/95, and AAEU (Aquatic Animal Experiment Unit) for STS-65.

We started new study on the next-generation aquatic animal experiment facility or the Aquatic Habitat (AQH), taking advantages of these experiences. The new AQH will fit both into space shuttle middeck and the Centrifuge Accommodation Module of the International Space Station (ISS) and will have following major functions: Closed fresh water loops, fish tanks with feeding system and sampling mechanism, O_2 supply/CO_2 removal by artificial lung, water quality control by nitrifying bacteria, water temperature control, light control, video observation, life support duration up to 90 days on the ISS. We will present our study status, the hardware concept, and some results of component level tests conducted.

[125]

ENZYME BASED MEMBRANE REACTOR FOR CO_2 CAPTURE.

M.C. Trachtenberg[1], J.-J. Ge[1], Yinjie Qin[1], M.L. McGregor[1], R.M. Cowan[2], [1]Sapient's Institute, New Brunswick, NJ, and [2]NJ-NSCORT, Rutgers, The State University of New Jersey.

Carbon dioxide (CO_2) control is crucial for all crew inhabited space-flight missions. Therefore, all space life support systems have included safe and reliable CO_2 extraction systems. Unfortunately these systems have been relatively costly to operate due to high consumable materials usage rates (*e.g.*, LiOH), high mass and/or volume, and/or high energy costs associated with regeneration of CO_2 adsorption capacity (*e.g.*, metal oxide). With the need for more economical operation of the ISS and with the anticipated move toward longer duration missions (*e.g.*, Mars), there continues to be a need for improved technologies for CO_2 capture. To be useful, any new technology must provide a reactor or sub-system that is not only safe and reliable, but improves on existing technology through a combination of having a small volume, low mass, low rate of energy use, minimal use of consumables, and needs little or no crew time for operation and maintenance. In recognition of these needs we are working to complete the development of a carbonic anhydrase (CA) based liquid membrane biomimetic reactor.

The major area of progress discussed here is the incorporation of electrodiffusion into the reactor design and operation. Electrodiffusion acts to improve reactor performance by increasing the effective transport rate of CO_2 without increasing that for O_2 and N_2. This occurs because electrodiffusion speeds the movement of bicarbonate and carbonate ions across the liquid membrane. Details covered in the presentation include: design of the lab scale reactor; selection of the voltage and wave form; and, results of parametric studies used to determine the preferred operating conditions (*e.g.*, CA and buffer concentration, pH, liquid membrane thickness). The results showed that use of electrodiffusion provides improvements in reactor performance including increased CO_2 permeance and increased selectivity of CO_2 over N_2 and O_2. These lead to the need for a smaller reactor and the production of a more highly concentrated CO_2 permeate stream (lower rates of N_2 and O_2 loss).

(Supported by NASA NAG9-1021 and NAG9-1923.)

[126]

ROTATING REVERSE OSMOSIS FOR WASTEWATER RECOVERY IN MANNED SPACE MISSIONS.

R.M. Lueptow[1] and S. Lee[1]. [1]Dept. of Mechanical Engineering, Northwestern Univ., Evanston

The recovery of potable water from wastewater is critical for life support of crew members during long-term space missions. Reverse Osmosis (RO) is a compact process for the removal of ionic and organic pollutants from space mission water. However, flux decline and rejection deterioration due to concentration polarization and membrane fouling hinders the application of RO technology. In this study, a rotating cylindrical RO membrane is theoretically investigated as a novel method to reduce concentration polarization and fouling. The dynamic model is based on the solution-diffusion equation with the film theory to account for concentration polarization. For a given geometry, a rotational speed sufficient to generate Taylor vortices in the annulus is essential to maintain high flux as well as high rejection. These vortices apparently reduce concentration polarization near the membrane and mix the solutes with the crossflow fluid so they can be carried out of the device. The radius ratio (r_i/r_o) of the device plays a role in the effectiveness of rotating RO with a smaller radius ratios resulting in better flux and rejection. However, the aspect ratio (r_i/L) shows only negligible effect on the flux and rejection when the rotational speed is kept constant. Hydrodynamic operating conditions including transmembrane pressure and rotational speed greatly affect the flux and rejection. The best flux and rejection occur at high rotational speeds and high transmembrane pressures. Operating in a vortical flow regime is most important to enhance the filtration performance.

(Supported by NASA: NAG9-1053)

[127]

VOLATILE ORGANIC COMPOUND PRODUCTION IN A SMALL-SCALE, IN-VESSEL COMPOSTER.

C.M. Frazier[1], M. Klamer[2], B.V. Peterson[3], A. Huff[3], and M. Hummerick[3]. [1]Dynamac Corp., Mail Code DYN-8; [2]Univ. of Central Florida, Dept. of Biology, Mail Code YA-D3; & [3]Dynamac Corp., Mail Code DYN-3, Kennedy Space Center, FL 32899 USA.

If biological waste processing is to be a part of long-duration space missions, the safety of each component must be evaluated. In the case of a composter, exit air quality may be of special concern because of the potential for compounds to build up over time in the cabin air. To address this issue, a series of experiments were designed in which the composition of the exit air stream of a small-scale (28-L), in-vessel composter was monitored. A mixture of alfalfa and wood shavings was com-posted and air samples were collected from the exit air stream. The air samples were analyzed on a gas chromatograph for their content of volatile organic compounds (VOCs). The influences of initial C:N ratio and temperature regimes were investigated by composting the same starting materials in different ratios, giving initial C:N ratios ranging from 15 to 31. Temperature in the composter was regulated to generate three thermal profiles: short thermophilic (kept at 55°C for three days), constant thermophilic (kept at 55°C for 9 days), and no thermal control (allowed to self-heat). The results indicate that when the initial C:N ratio was above 20, the total amount of VOCs produced increased with increasing initial C:N ratio. At initial C:N ratios below 20 no clear trend was observed. Thus an initial C:N ratio about 20 seems to produce the smallest amount of VOCs. Although the total amount of VOCs in-creased with increasing initial C:N ratio, the composition of VOCs was unchanged. No compound exceeded Spacecraft Maximum Allowable Concentration (SMAC) limits. The amount and composition of VOCs produced were not affected by the different temperature regimes. The amount of odor producing compounds, such as dimethyl disulfide and dimethyl sulfide, was not affected in any simple way by the changes in composting conditions.

This research was funded in part by NASA Space Act Agreement KSC-1623 (CF) and by a NASA Technology Development Grant from Kennedy Space Center through the University of Central Florida (MK).

[128]

PIONEERING SPACE EXPERIMENTS AIMED AT OBTAINING PLANT BIOMASS TO SUPPLEMENT CREW FOOD RATIONS.

M. A. Levinskikh[1], V.N. Sychev[1], I.G. Podolsky[1], and G.E. Bingham[2]. [1]Institute for Biomedical Problems, RAS, Moscow, Russia, and [2]Space Dynamics Laboratory, Utah State University, Logan, UT.

Seven experiments with various plant species were performed in greenhouse *Svet* aboard the Mir station in the period from 1990 to 2000. Vegetables were objects of investigation in the first and last experiments. In the 1990 experiment, failure to sustain an appropriate plant environment (root zone water stress) under space flight conditions reduced the productivity of Chinese cabbage to between $1/5^{th}$ and $1/8^{th}$ of the ground controls.

In the 2000 experiment, four leaf vegetables were raised for a 21-d period, including Chinese cabbage (B. rapa var. pekinensis), Muzina (B. rapa var. nipposinica), broccoli raab (B. rapa var. utilis), and mustard var. Red Giant (B. juncea). Growth and development characteristics of space-grown plants did not differ from their ground analogs. Comparison of dry mass of the Chinese cabbage crop between the space experiments of 1990 and 2000 demonstrated that productivity in the latter was 5-fold higher.

In the 2000 experiment, Cosmonauts conducted a taste test of the leaf vegetables and concluded that any of the four varieties would be a significant enhancement to their diet, and a worthy crop for a space production greenhouse, though they gave preference to Mizuna and Red Giant mustard (a video of the test will be shown).

We suggest that the plant experiment series flown in greenhouse *Svet* aboard the space station Mir (1990-2000) resulted in technology design improvements and plant cultivation procedure verifications. These improvements allow for physical condition adjustments due to microgravity, and facilitate normal growth and development of plants.

(Support for these investigations was provided by the Russian Space Agency and the NASA - Mir program.)

[129]

TECHNIQUES TO CHARACTERIZE CO_2 AND LIGHT EFFECTS ON CANOPY PHOTOSYNTHESIS OF WHEAT IN MICROGRAVITY. G.W. Stutte, O. A. Monje, G.D. Goins, and D.K. Chapman. Dynamac Corporation, Kennedy Space Center, FL

The effects of light intensity and CO_2 concentration on net canopy photosynthesis (Pn) of Super Dwarf and Apogee wheat cultivars were determined in the Biomass Production System (BPS) flight hardware. These measures were made in conjunction with ground tests to determine the feasibility of obtaining valid measurements of Pn for the Photosynthesis Experiment Subsystem Testing and Operation (PESTO) experiment manifested for the International Space Station. Photosynthesis was measured using a semi-closed system technique daily during the 24-day experimental cycle, and stand gas exchange measurements were characterized at 10, 14 and 21 days after planting using a closed system technique. The results indicated that the light response of Pn was linear during all the measurements for both cultivars. The maximum Pn rate of Apogee was higher than Super Dwarf. These differences reflected the earlier germination and faster growth of Apogee. Increases in apparent maximum Pn rate also occurred with time, which were reflected in higher apparent CO_2 compensation point for Apogee than Super Dwarf. The CO_2 increased compensation point change reflected differences in canopy coverage between the two cultivars, increased canopy biomass, and higher root respiration. Hardware specific issues regarding leak rate, pump volumes, and root zone temperature were identified which must be accounted for before ascribing physiological or developmental significance to these results.

[130]

DOES CRITICAL INSTABILITY PLAY A ROLE IN THE RESPONSES OF LIVING THINGS TO MECHANICAL LOAD? P.M. Lintilhac[1] and C.F.Wei[1,2]. [1]Dept. of Botany and Agbiochem, The University of Vermont, and [2]Dept. Of Physics, Guangxi University, P.R.C.

It is generally assumed that the effect of mechanical loading on living structural materials is mediated by specific biochemical inputs acting at the molecular level. Such phenomenological explanations of stress-mechanical responses in growing structures may have overlooked inherent mechanical necessities that underlie much of the behavior of biological materials under load. We have attempted to rigorously demonstrate the inherent critical instability of elastic biopolymers, and the role that this instability must play in many biomechanical processes. In particular we deal with the example of plant cells whose walls are subject to turgor-driven stress relaxation. Briefly, the instability shown by many structures under compression, where one equilibrium configuration may shift suddenly to another, such as a bicycle wheel which may shift from a plane circle configuration to a potato-chip configuration, can also apply to elastic materials under tension, where no major change of configuration results. These tensile instablities can be shown mathematically to be an inevitable result of many elastic materials being subject to critical stress. In plants, this tensile instability may underlie all primary wall growth and serve as a basic mechanism of stress relaxation that is tunable by biochemical means. Critical instability, coupled with passive re-alignment, may also provide the basis for many other examples in which structural molecules are optimized to a high stress environment. It may also provide insights into problems inherent in the transition between unit gravity and microgravity and vice versa. The work presented here will be supported by a mathematically detailed poster session.

[131]

MASS APPLICATION OF MECHANO-DWARFING STIMULI TO *ARABIDOPSIS* FOR MUTANT SCREEN DEVELOPMENT. C. A. Mitchell, J. A. Montgomery, A. L. Santone, A. M. Garrett, and R. A. Bressan. Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN.

Various forms of mechano-stimulation have been applied to flats containing hundreds of vegetative *Arabidopsis thaliana* (L.) Heynh. seedlings of the C-24 ecotype with the goal of obtaining uniform growth reduction relative to undisturbed controls. Candidate mechano-stimuli investigated include static impedance (perforated plates), dynamic compression and flexing (roller), and dynamic frictional contact and flexing (brushes). Mechanical treatments were applied alone or in combination, while varying the intensity, duration, and time of daily stress application. Simultaneous treatments comparing plant mechano-responses between greenhouses and controlled environments indicated inconsistent results in the greenhouse. Impedance treatments enhanced rosette size, productivity, and water status of seedlings grown in a standardized plant-growth environment. Brushing tended to damage younger seedlings, but substantially reduced leaf area and dry weight of older seedlings without causing damage. Rolling also reduced growth in leaf area but tended to damage leaves to an extent related to the weight of the roller. Combinations of treatments tended to negate individual effects or to have no synergistic effects. Additional treatments under investigation include periodic static impedance with carborundum paper on the plate undersurface, or brief periodic vibration of entire flats. The successful mechano-stress regime will be used to screen T-DNA insertional mutants for TCH-gene knockouts.

(Supported in part by NASA: NAG2-1389.)

[132]

ARABIDOPSIS ROCKET SCIENCE. B. Stankovic, B. Link, W. Zhou. Wisconsin Center for Space Automation and Robotics, University of Wisconsin-Madison.

The Wisconsin Center for Space Automation and Robotics (WCSAR) is a NASA-cosponsored Commercial Space Center that designs and commercializes new products and technologies for plant research in microgravity. WCSAR recently developed a microgravity-based plant research facility, Advanced Astroculture (ADVASC), which in its premiering spaceflight was used for the first successful seed-to-seed growth of (*Arabidopsis thaliana*) plants on the International Space Station (ISS).

Here we report on both WCSAR's progress in developing technologies for growing plants in space, and on utilizing those technologies to understand how *Arabidopsis* plants grow in microgravity. Performed in cooperation with Space Explorers, Inc., our first *Arabidopsis* growth experiment was conducted in May-July 2001 on board the ISS. This experiment was also used as a basis for creating an educational curriculum, involving students from over 600 schools nationwide.

Data showing ADVASC's performance on the ISS will be presented. Trials, tribulations and successes of *Arabidopsis* growth in microgravity will be discussed, based on the postflight-obtained morphometric and cell biology data. Our second *Arabidopsis* spaceflight experiment, to be sent to the ISS in November 2001, will utilize DNA microarrays to analyze the gene expression patterns of plants grown and developed in microgravity.

[133]

AUTONOMOUS BIOLOGICAL SYSTEM SPACEFLIGHT RESULTS AND APPLICATIONS. J. E. Poynter, T. K. MacCallum and G. A. Anderson, Paragon Space Development Corporation, Tucson, Arizona.

Materially-closed, passively controlled, aquatic life support systems containing vascular plants, invertebrate animals, algae and microbes were tested in four space flight experiments with ground controls. Modifications to the system are under development for the support of developing embryo and fry of the Japanese Medaka Fish, *Oryzias latipes,* scheduled for launch in May 2002 on STS-107. Termed Autonomous Biological Systems (ABS), the 0.9 liter systems were completely isolated from spacecraft life support systems and cabin atmosphere contaminants, and needed minimal intervention from astronauts. The first experiment, aboard the Space Shuttle in 1996 for 10 days, was the first time that aquatic angiosperms were successfully grown in space. The second and third experiments aboard the Mir space station had 4-month durations, in 1996-97 and 1997-98, and were the first time that higher organisms (*Daphnia pulex*) completed their life cycles in space. The fourth experiment was launched to the International Space Station on February 26, 2001, and is ongoing at time of writing, more than four months after launch. ABS units from the Shuttle and Mir experiments contained the macrophytes *Ceratophyllum demersum*, *Lemna minor* and *Wolffia* sp, and the invertebrate species *Hyallela azteca* (amphipod*)*, *Daphnia pulex*, cyclopoid copapods, ostracods, *Physa* sp. (snail), and planaria, and returned with all species. The system on ISS contains *Halocaridina rubra* (shrimp*)*, *H. azteca*, *D. pulex*, copepods, ostracods, *Helisoma planorbis* (snail)*,* chlorophyta and nitrifying bacteria. The ABS are the first completely bioregenerative, closed ecological life support systems to thrive in space, demonstrating their efficacy for research in space biology and gravitational ecology, while utilizing minimal valuable resources such as power and crew time.

Concurrent Oral Sessions
IV
Systems Biology: Mammalian

[134]

DEVELOPMENT OF THE MOUSE VESTIBULAR SYSTEM IN THE ABSENCE OF GRAVITY STIMULATION BY OTOCONIA. D.J. Wolgemuth[1], X.Y. Wang[1], M. Smith[2], and A.M. Murashov[2], [1]Columbia University, NY and [2]E Carolina University.

The hypothesis we are testing is that the absence of gravity perception, such as would be encountered in animals developing in space, will affect the postnatal differentiation and function of the vestibular system. We are using molecular genetic approaches to determine the hierarchy of gene function during vestibular development. The *tilted* mutant mouse is an excellent model for the study of vestibular function since the primary defect is limited to the receptor part of the vestibular system. Comparing wild type (WT) mice versus mutants (mut) showed dramatic differences in vestibular ganglion morphology. Morphometric analysis included calculation of ganglion volume, total cell number, total cellular volume and average cell size. Total ganglion volume in the WT mouse rose from $1.4x10^7\mu M^3\pm0.04$ on Day 0 (d0) to $2.9x10^7\mu M^3\pm0.04$ on d12, remained constant at $2.5x10^7\mu M^3\pm0.05$ on d15-28, finally declining to $2.2x10^7\mu M^3\pm0.05$ on d56. In contrast, in the mut mouse, ganglion volume remained constant at $1.3x10^7\mu M^3\pm0.1$ for d0-6, then rose sharply to $2.1x10^7\mu M^3\pm0.02$ and $2.4x10^7\mu M^3\pm0.1$ on d10 and 12, respectively, returning to $2.1x10^7\mu M^3\pm0.03$ on d15-56. In WT, cell number increases from ~1800 on d0 to 2200 on d10, then decreases to 1500 on d56. In the mutant, cell number decreases from ~1700 on d0 to 1200 on d6, then returns to 1700 on d10, remaining constant through d22, followed by an increase to 2200 on d28, falling to 1400 on d56. In WT, cell number increases from ~1800 on d0 to 2200 on d10, then decreases to 1500 on d56. In the mutant, cell number decreases from ~1700 on d0 to 1200 on d6, then returns to 1700 on d10, remaining constant through d22, followed by an increase to 2200 on d28, falling to 1400 on d56. The observed differences between mut and WT animals may be a result of early deprivation from the perception of gravity in mutant animals lacking otoconia in the inner ear. Our data suggests a critical period during early postnatal development and maturation of the vestibular system, which depends on gravitational stimulation.

(Supported by: NASA: NAG 2-1345.)

[135]

RETINOIC ACID IN THE DEVELOPMENT OF CENTRAL COMPONENTS OF THE VESTIBULAR SYSTEM. P. McCaffery, D. Smith and J. Zhang. Dep't Cell Biology, Univ. Massachusetts, E.K. Shriver Center, Waltham, MA.

Vitamin A (retinol) is required for normal embryonic development and its metabolic product, retinoic acid (RA), mediates the majority of its actions by binding to specific nuclear receptors and regulating gene transcription. New data points to a role of RA in the development of central components of the vestibular system. The vestibular organs consist of three semicircular canals and two otolith organs. Signals from the otolith organs are relayed via the vestibular nuclei, medullary reticular formation, inferior olive, and lateral reticular nucleus to sagittal zones in the caudal cerebellar vermis. The vestibular organs also send information to the cerebellum relayed via the pontine nuclei. We have evidence that RA signaling is a component in the development of several of these nuclei. We have used a mouse transgenic for a lacZ reporter gene driven by a RA response element (RAREhsplacZ). Embryonic brain regions in which RA activated gene transcription takes place are labeled by beta-galactosidase expression. RA signaling occurs in three components of the vestibular system i.e. the cerebellum, inferior olive and the pontine nuceli. In the cerebellum expression is localized to sagittal stripes in the caudal vermis, the cerebellar zone that receives vestibular input. This discovery of RA signaling in the developing central components of the vestibular system identifies a new regulatory factor in the generation of these nuclei.

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[136]

CLINOSTAT ROTATION INDUCES CYTOSKELETAL COLLAPSE AND MITOCHONDRIAL INACTIVATION IN LUTEAL CELLS OF THE PREGNANT RAT. R. Sridaran, H. Yang, G.K. Bhat, U.P. Singh, and A.P. Shaw. Department of Physiology, Morehouse School of Medicine, Atlanta, Georgia.

We have demonstrated that microgravity conditions generated by the clinostat rotation induce apoptosis and suppress the production of progesterone by luteal cells of the pregnant rat. A few recent studies have shown that microgravity conditions also induce changes at the cellular level including alteration in the cytoskeletal organization and apoptosis. Therefore, the purpose of this study was to determine if the cytoskeletal collapse lead to formation of pores in the mitochondrial membrane thereby releasing cytochrome *C* from the mitochondria results in the induction of apoptosis under microgravity conditions. The luteal cells were isolated from the corpora lutea of day 8 pregnant rats and placed in equal numbers in slide flasks. One slide was placed in a clinostat and the other served as a stationary control. As has been shown by us previously, at 72 h, progesterone levels were suppressed (4.52 vs 9.29 ng/ml in control) and the number of apoptotic cells were increased (5 fold) as seen by TUNEL by the clinostat rotation. Clinostat rotation induced the cytoskeletal collapse as shown by confocal microscopy with the use of antibody for α-tubulin and the cytochrome C exit from the mitochondria as shown by fluorescent microscopy. After separating the mitochondria from the cytoplasm, both fractions were examined under immunoblotting for cytochrome *C*. Clinostat rotation increased the cytoplasm/mitochondria ratio of cytochrome *C* to 3-7 fold. These results suggest that clinostat rotation induces cytoskeletal collapse and the formation of pores in the mitochondrial membrane thereby releasing cytochrome *C* from the mitochondria and thus inducing apoptosis in luteal cells of the pregnant rat. However the cause and effect relationship between the cytoskeletal collapse and mitochondrial release of cytochrome *C* must be investigated further prior to drawing conclusions.

(Supported by NASA, NAG9-963 and NCC953, and NIH, S06-GM08248).

[137]

EVIDENCE FOR PKA REGULATION OF EARLY T-CELL ACTIVATION. E. Sugano, M. Hughes-Fulford , and G. Cogoli, Lab of Cell Growth, UCSF, NCIRE and VAMC, San Francisco, CA and Space Biology, ETH Zürich, Switzerland

It has been recognized from the early days of spaceflight that microgravity blunts the immune response in humans. Studies by Cogoli have demonstrated that the most likely source of inhibition occurs early in T-cell activation. In preparation for STS-107 experiment, we have conducted ground studies of signal transduction of activation. Since expression of interleukin-2 and its receptor subunits, a, b, g is essential for T lymphocyte proliferation, we investigated early induction of these genes. IL-2, IL-2Rg and IFNg were significantly induced at 2h, and IL-2Ra at 4h after activation; IL-2Rb expression was constitutive. The induced gene expression of IL-2, IL-2Ra and IFNg was significantly inhibited by H-89 to levels equal to or greater than 76% inhibition. PKC inhibition significantly reduced IFNg, IL-2 and IL-2Ra gene expression by approximately 40%, but did not affect IL-2Rb or g subunits. In order to further study regulation of IFNg, IL-2 and IL-2Ra, PMA-stimulated Jurkat cells were analyzed. We found that H-89 inhibited IFNg, IL-2 and IL-2Ra gene expression, with no effect on 18S. Analysis of proteins showed that H-89 inhibited synthesis of IFNg, IL-2 and IL-2Ra. Finally, H-89 inhibited gene expression of IL-2Ra and IFNg in the S49 wild type cells; IL-2Ra was constitutive. In contrast, IFNg and IL-2Ra were not induced in untreated S49 kin- cells. As expected, forskolin could not induce gene expression of IL-2Ra or IFNg. Taken together, these results show for the first time that the PKA signaling pathway plays a major role in early T-cell activation via a PKA induction of IL-2, IL-2Ra and IFNg.

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[138]

PROLIFERATION OF OSTEOBLASTS IS MODULATED BY VOLTAGE-SENSITIVE CALCIUM CHANNELS. R.L. Duncan[1], K.D. Brubaker[2], C. LaFollette[1], B. Mayhugh[3] and M.S. Mendonca[3]. Dept. of [1]Orthopaedics, [2]Anatomy and [3]Radiation Biology, Indiana University Medical Center, Indianapolis, Indiana.

Studies on COSMOS 1887 and 2044 suggest that the reduction in bone formation during spaceflight results from a decrease in proliferation of preosteoblastic cells, thereby reducing the number of bone forming osteoblasts. Since progression of the cell though the cell cycle requires fluctuations in intracellular calcium, we postulated that inhibition of mechanosensitive channels (MSCC) or L-type voltage-sensitive Ca^{2+} channels (VSCC) would alter the proliferation of osteoblasts. Using two different methods to measure proliferation, we found that the VSCC blocker, nifedipine (NIFE), significantly reduced proliferation in MC3T3-E1 and UMR106.01 osteoblastic cells, increasing the doubling time of MC3T3-E1 cells from 15 $\pm$ 1 hr to 38 $\pm$ 2 hr. Interestingly, NIFE had no effect on ROS 17/2.8 cells. The MSCC blocker, Gd^{3+}, had no effect on proliferation in any of the osteoblastic cell types studied. Using flow cytometry, we found no variation in the cell cycle profile between control and NIFE-treated cells. Furthermore, when MC3T3-E1 cells were released from NIFE inhibition, the cells returned to normal proliferation rates. However, NIFE significantly increased alkaline phosphatase activity, a marker for osteoblast differentiation, suggesting that block of Ca^{2+} entry via the VSCC may "push" proliferating preosteoblasts into differentiation. Western analysis demonstrated that increased proliferation corresponds to the appearance of the α_{1C} subtype of the L-type VSCC in the membrane. These data indicate that the L-type VSCC, and not the MSCC plays a role in the control of proliferation of osteoblasts and suggests that, in microgravity, these channels are inactivated, thus reducing the proliferation of preosteoblasts.

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[139]

SKELETAL UNLOADING INDUCES RESISTANCE TO INSULIN LIKE GROWTH FACTOR-I ON BONE FORMATION T. Sakata[1], B. P. Halloran[1], C. J. Rosen[2], H. Z. ElAlieh[1], S. J. Munson[1], L. Rudner[1], L. Venton[1], D. D. Bikle[1]. [1]Medicine, University of California, [2]Maine Center for Osteoporosis Research.

Skeletal unloading results in an inhibition of bone formation associated with a decrease in osteoblast number, impaired mineralization of bone, and altered proliferation and differentiation of osteoprogenitor cells. The resistance to the growth promoting action of insulin-like growth factor I (IGF-I) has been hypothesized to play an important role. To determine whether skeletal unloading induces resistance to IGF-I on bone formation, we examined the response of unloaded (hindlimb elevation) and normally loaded tibia and femur to IGF-I treatment. To eliminate the variable of endogenous growth hormone production and secretion during exogenous IGF-I treatment, we used growth hormone-deficient dwarf rats (dw-4). These rats were given IGF-I (2.5 mg/kg/day) or vehicle via osmotic minipumps during 7 and 14 days of unloading or normal loading. This significantly increased the serum level of IGF-I in both the normally loaded and unloaded rats. Unloading did not affect the serum level of IGF-I in the vehicle-treated rats. IGF-I markedly increased the periosteal bone formation at the tibiofibular junction of loaded rats. Unloading decreased the bone formation at the tibiofibular junction in the vehicle treated rats, and blocked the ability of IGF-I to increase the bone formation. IGF-I increased the periosteal bone formation at the mid point of the humerus (normally loaded in this model) in both hindlimb-elevated and normally loaded rats. IGF-I significantly increased the colony number, the total ALP activity, and the total mineralization in the bone marrow osteoprogenitor (BMOp) cells of normally loaded rats. Unloading reduced these parameters in the vehicle treated rats, and blocked the ability of IGF-I to increase these parameters. IGF-I (10 ng/ml) treatment in vitro significantly increased the bromodeoxyuridine incorporation of the BMOp cells isolated from normally loaded bone, but not from unloaded bone. These results indicate that skeletal unloading induces resistance to IGF-I on bone formation.

[140]

LONG TERM HIND-LIMB SUSPENSION INHIBITS SPERMATOGENESIS IN ADULT MALE RATS IN THE ABSENCE OF CRYPTORCHIDISM. J.S. Tash[1], D.C. Johnson[1], S. McDonald[1], and G.C. Enders[2]. [1]Dept of Molecular & Integrative Physiology, and [2]Dept of Anatomy Univ of Kansas Medical Center, Kansas City, KS.

The International Space Station will allow long-term habitation in space and thus long-term exposure to microgravity (μG). A question of concern is the impact of long-term μG exposure on the ability of species to reproduce. The model system often used to simulate μG is rat hindlimb suspension (HLS) in which the hindlimbs are elevated off the cage bottom with a tail harness. Previous HLS studies using young male rats over short time periods (7-16 days) found declines in serum testosterone levels and minor negative effects on spermatogenesis. In this 6-wk study, we used sexually mature male rats (12 month old) in 3 groups: free roaming (controls); tail-only (TO) suspended (tail harnessed but the hindlimbs still had full contact with the cage floor); and HLS animals. The inguinal canals were partially ligated in both the TO and HLS groups to prevent cryptorchidism. Results: Testicular weights were significantly reduced in HLS rats compared to both control and TO rats. Nuclear counts of testicular sperm and maturing spermatids were also significantly reduced in HLS. Histology of HLS testes showed seminiferous tubules that lacked spermatids or were Sertoli cell only. These changes were not observed in the control or TO rats. Testosterone levels were not reduced or elevated in the HLS rats. LH and FSH levels were also not elevated in the HLS rats. Seminal vesicle weights were reduced in the HLS rats, suggesting that testosterone levels had at some point during the 6-wk period been reduced enough to cause loss in this highly androgen-dependent organ. Epididymal weights were unchanged. Cortisone levels were not elevated in the HLS group, thus stress does not appear to be a factor. These results suggest the spermatogenesis is particularly susceptible to perturbations by HLS, while testicular androgen functions appear less susceptible. If this model holds true, then extended μG exposure could impair male fertility. This study highlights the need to examine the long-term effects of μG on spermatogenesis in scrotal mammals. Supported by NIH: HD-33994 and NASA: N01-639.

[141]

HINDLIMB SUSPENSION DECREASES RESISTANCE OF MICE INFECTED WITH *KLEBSIELLA PNEUMONIAE*. T. Belay, M. Vance, K. Fountain, and G. Sonnenfeld. Dept. of Microbiology, Biochemistry, & Immunology, Morehouse School of Medicine, Atlanta, GA.

Research focusing on effects of stress on immune function and infectious disease has received considerable attention over the past several years. Studies have shown alteration of immune responses during stressful conditions, including during spaceflight. Multiple immune responses have been shown to be altered after spaceflight, including leukocyte blastogenesis, cytokine production, leukocyte subset distribution, and response of bone marrow cells to colony stimulating factors. The mechanism(s) for these changes remain(s) to be established. Alterations in these immune parameters could be a host at increased risk of infection; however, the effect of space flight on actual resistance to infection remains to be established. In order to evaluate effects of spaceflight conditions on resistance to infection, we have used the hindlimb (antiorthostatic) suspension of mice model that creates conditions similar to those occurring during spaceflight. These include: muscle and bone unloading and fluid shift. The purpose of the current study was to examine whether hindlimb suspension of mice resulted in altered resistance to infection in mice. The organism utilized was *Klebsiella pneumoniae,* a pathogen found in the intestinal tract that has been shown to cause infectious difficulties in stressful situations such as very serious trauma. Mice were hindlimb-suspended using the tail-suspension system for 2 days and then were challenged intraperitoneally with 1 LD_{50} of *K. pneumoniae*. By 5 days post-infection, 60% more mice were dead of infection in the suspended mouse group than were in the control group. These results suggest that hindlimb suspension of mice, a model of some aspects of the spaceflight environment, results in decreased resistance of mice to a bacterial pathogen.

(Supported by a grant from the NSBRI: NASA Cooperative Agreement NCC 9-58.)

[142]

THIN FILAMENT PLASTICITY IN FAST AND SLOW FIBERS OF ASTRONAUT GASTROCNEMIUS AND SOLEUS MUSCLES D.A. Riley[1], J.L.W. Bain[1], J.L. Thompson[1], R.H. Fitts[2], J.J. Widrick[2], S.W. Trappe[3], T.A. Trappe[3], and D.L. Costill[3]. [1]Depart of Cell Biology, Neurobiology & Anatomy, Medical College of Wisconsin, Milwaukee, WI, [2]Depart of Biology, Marquette University, Milwaukee, WI, [3]Human Performance Lab, Ball State University, Muncie, IN.

Structural and physiological changes induced by spaceflight and a variety of ground-based unloading models can involve skeletal muscles in general, affect specific fast and slow muscles or selectively target fast and slow muscle fiber types. The present study examined myofilament and contractile properties of fiber types before and after spaceflight. Slow type I fibers in soleus and fast white IIa/IIx, fast red IIa, and slow red I fiber types in gastrocnemius muscles were examined electron microscopically (aldehyde-fixed, relaxed fibers) and physiologically from pre- and post-flight biopsies of 4 astronauts orbited 17 days during the Life and Microgravity Sciences Spacelab STS-78 mission. At 2.5 μm sarcomere length, thick filament density (~1012 filaments/μm²) is similar in all fiber types and unchanged by spaceflight. Pre-flight, all of the gastrocnemius fiber types possessed higher percentages (~23%) of short thin filaments compared to soleus (13%). In type I fibers, spaceflight increased short thin filament occurrence from 13% to 22% in soleus and from 26% to 31% in gastrocnemius. At short sarcomere lengths, thick and thin filaments separate further transversely. The Z band lattice also expands, except in soleus type I fibers with presumably stiffer Z bands. Thin filament packing density correlates directly with specific tension for gastrocnemius fibers but not soleus. Thin filament density is inversely related to shortening velocity in all fibers. Thin filament structural diversity contributes to the functional diversity and plasticity of normal and spaceflight unloaded muscles of humans.

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[143]

EFFECTS OF CHRONIC HYPERGRAVITY ON IMMUNE PARAMETERS. M.J. Pecaut[1], D.S. Gridley[1,2], G.M. Miller[2], M.L. Andres[1], R. Dutta-Roy[1], A.L. Smith[1], T.A. Jones[1], and G.A. Nelson[1]. Depts of [1]Radiation Medicine, Radiobiology Program and [2]Microbiology & Molecular Genetics. Loma Linda Univ and Medical Center, Loma Linda, CA

The microgravity environment has consistently been shown to alter immune parameters. A better understanding of the long-term effects of changes in inertial conditions is required to develop realistic risk assessment models for astronauts on extended missions in space. However, due to the nature of spaceflight research, ground based alternatives must be considered.

This is the first of a series of experiments involving chronic exposure to hypergravity. Young adult female C57BL/6 mice (n = 105) were exposed to hypergravity at 2 & 3 G for up to 3 weeks. Animals were euthanized after 1, 4, 7, 10, or 21 days of centrifugation and series of assays were performed.

There were gravity dependent decreases in body, spleen, thymus, and liver masses ($p<0.001$). There was also a gravity effect on splenocyte spontaneous DNA synthesis. Response of splenic T and B lymphocytes to mitogenic stimulation was decreased by gravity ($p<0.001$). Hematological analysis indicated total WBC, lymphocytes, monocytes, and granulocytes, and hemoglobin concentration were significantly affected by acceleration ($p<0.05$), whereas the numbers of RBC and platelets and hematocrit, were not until day 21. Similar analysis of spleen leukocytes, also showed that WBC counts and the three major leukocyte types were significantly affected by gravity ($p<0.001$). Flow cytometry indicated that peripheral CD3+ T, CD4+ T helper (Th), CD8+ T cytotoxic (Tc), CD19+ B, and NK1.1+ NK cells were gravity dependent ($p<0.001$). Splenic T, Th, Tc, B, and NK cell counts were similarly influenced by gravity ($p<0.005$).

(Supported by NASA: Coop. Agreement NCC9-79.)

[144]

HEMATOPOIETIC STEM CELL THERAPY OF THE MOUSE MODEL OF β-THALASSEMIA: TOWARD DEVELOPING COUNTERMEASURES AGAINST HEMATOLOGICAL ABNORMALITIES IN SPACE. S. Ohi, P.S. Jiang, A.A. Aguilar, B.C. Kim. Depts. of Biochemistry & Molecular Biology, Genetics & Human Genetics, Pediatrics & Child Health, Center for Sickle Cell Disease, Col. of Medicine and The Graduate School, Howard University, Washington, DC.

It has been shown that extended spaceflight adversely affects hematopoiesis, resulting in anemia, hematological abnormalities, and immunodeficiency. In addition, radiation-induced cancer, *e.g.* leukemia, is a major concern in long-duration space exploration. Thus, our interest lies in developing countermeasures against these hematological disorders, utilizing hematopoietic stem cell (HSC) therapy and gene therapy. As the model system on earth, we are developing the recombinant adeno-associated virus (rAAV)-mediated gene therapy protocols for the hemoglobinopathies, β-thalassemia (Cooley's anemia) and sickle cell disease, which we reported last year (Ohi, S., et al. *Grav Space Biol Bull* **14**: 43, 2000). Since HSC-transplantation is a critical step in the *ex vivo* gene therapy, we established methods for purification of hematopoietic stem cells (HSCs) from both mouse and human, using density gradient centrifugation (Histopaque 1077, Sigma; Density = 1.077 g/c.c.) and immunomagnetic selection. Furthermore, we developed an easily harvestable, long-term liquid suspension culture system, which lasts more than 12 months, for growing/expanding HSCs without stromal cells. The human globin cDNAs/gene were efficiently expressed from the rAAVs in the mouse HSCs in culture. In this presentation, characteristics of the HSCs in the culture system, including a successful HSC-transplantation in β-thalassemic mice (C57BL/6- Hbb^{th}/Hbb^{th}, $Hb^{d\text{-}minor}$) will be reported.

(Supported in part by grant from The Armstead-Barnhill Foundation)

[145]

THE CATECHOLAMINE RESPONSE TO SPACE FLIGHT: ROLE OF DIET AND GENDER. T.P. Stein[1] and C.E. Wade[2].
Department of Surgery, University of Medicine and Dentistry of New Jersey-SOM, Stratford, NJ, 08084 and (2) Life Sciences Division, NASA – Ames Research Center, Moffett Field, CA 94035.

Compared to men, women appear to have a decreased sympathetic nervous system (SNS) response to stress. The two manifestations where the sexual dimorphism has been the most pronounced involve the response of the SNS to fluid shifts and fuel metabolism during exercise. The objectives of this study were to investigate whether a similar sexual dimorphism was found in the response to space flight. To do so we compared catecholamine excretion by male and female astronauts from two similar shuttle missions, Spacelab Life Sciences 1 (SLS1) and 2 (SLS2) for evidence of sexual dimorphism. To evaluate the variability of the catecholamine response in men, we compared catecholamine excretion from the two SLS missions against the 1996 Life and Microgravity Sciences Mission (LMS) and the 1973 Skylab missions. Results: No gender or mission dependent changes were found with epinephrine. Separating out the SLS1/2 data by gender shows that norepinephrine (NE) excretion was essentially unchanged with space flight in women ($98 \pm 10\%$ n=3) and substantially decreased with the men ($41 \pm 9\%$, n=4, $p<0.05$). Data are % of mean preflight value $\pm$ SEM. Comparisons between males demonstrated significant mission effects on norepinephrine excretion. After flight, there was a transient increase in NE, but no evidence of any gender specific effects. Conclusions: NE excretion during space flight is both mission and gender dependent. Men show the greater response with at least three factors being involved, a response to microgravity, energy balance and the ratio of carbohydrate to fat in the diet.

[146]

CHANGE IN FUNCTIONAL AND STRUCTURAL PROPERTIES OF TUMOR-DERIVED EPITHELIAL CALLES IN 3-D SIMULATED MICROGRAVITY CULTURE ENVIRONMENT. V. Chopra, T. V. Dinh, and E. V. Hannigan. Division of Gynecologic-oncology, Department of Obstetrics and Gynecology, UTMB, Galveston, Texas 77555-0587.

We have shown that the tumor-derived epithelial cells undergo a switch in characteristics when grown in an in vitro 3-D simulated microgravity environment as compared with conventional 2-D monolayer cultures. We investigated the production of angiogenic factors, adhesion molecules, and extracellular matrix (ECM) components by tumor-derived epithelial cells (n=6), when grown as 3-D monocultures and cocultures with human umbilical vein-derived endothelial cells (HUVEC) in the 3-D rotating wall vessels. The growth rate was enhanced 10-15 folds by 3-D monocultures of patient-derived cells as compared with 2-D monolayer cultures. The production of interleukin-2, interleukin-6, interleukin-8, vascular endothelial cell growth factor, basic fibroblast growth factor, and angiogenin was studied by using ELISA. HUVEC were also used to study the mitogenic response of the conditioned medium collected from 3-D monocultures during proliferation and migration assays. The conditioned medium collected from 3-D cocultures of cancer cells with endothelial cells also 1) increased the expression of message levels of VEGF and its receptor *flt*-1 and *KDR* by HUVEC, and 2) increased the expression of ICAM-1 and VCAM adhesion molecules on the surface of HUVEC, when measured by using Live cell ELISA assays and immunofluorescence staining as compared with 3-D monocultures of normal epithelial cells. There was an increase in production of 1) enzymatic activity that could generate bioactive angiostatin from purified human plasminogen, and 2) fibrin (red), mucin (blue), and elastic fiber (black) by cell aggregates of 3-D monocultures of patient-derived cells as compared with 3-D monocultures of normal epithelial cells. These results were also confirmed by using gene array analysis. Thus it can be concluded that this 3-D culture system which generates simulated microgravity closely mimics the host in vivo environment and can be used to study the role of endothelial cells and also the interaction of multiple cell types during physiological and pathological angiogenesis.

Concurrent Posters
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[147]

REGULATION OF ADP-GLUCOSE PYROPHOSPHORYLASE SUBUNIT EXPRESSION —A KEY ENZYME FOR STARCH BIOSYNTHESIS IN PLANTS. T.J. Gianfagna, X. Li, J. Xing, Y. Luo and H.W. Janes. Plant Science Department, Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901

Starch is the predominant carbon sink of storage organs and leaves of many plants. Substantial evidence from the analysis of starch-deficient mutants, transgenic plants, and enzyme kinetics suggest that ADP-glucose pyrophosphorylase (AGPase) catalyzes a key step for starch biosynthesis in both photosynthetic and non-photosynthetic tissues. The ability to synthesize starch shortly after fruit set may be critical for successful organ development. Increased starch biosynthesis in tomato fruit may also lead to increased assimilate partitioning into fruit, and a decrease in non-edible biomass, reducing waste processing needs in a bioregenerative life support system. Starch is also a key component of the gravity sensing mechanisms of plants. The enzyme is a heterotetramer containing two *S* and two *B* subunits. We have identified three distinct genes for the *S* subunit and one gene for the *B* subunit (Chen et al., Plant Science 136:59-676, 1998). Complete sequences for three *S* subunit cDNAs (*agp S1, agp S2,* and *agp S3*; genebank accession numbers U81033, U81034, and U85497) and one for the *B* subunit (*agp B*) were identified. Northern analysis revealed some major differences in the expression profiles of the isoforms between different tissues. In the fruit, the expression of *agp B* and *agp S1* was very strong, *agp S2* was expressed more weakly, whereas *agp S3* was very low or absent. In the leaves, *agp B* and *agp S3* were the highly expressed isoforms, whereas expression of *agp S1* was moderate and *agp S2* very low. In roots, neither agp *B* nor *agp S3* were detected, although there was moderate expression of *agp S1* and *S2*. When excised leaves were incubated in sucrose there was a significant induction of transcription for *agp B*, *agp S1*, and *agp S2* within 8 h after incubation. Transcript levels remained elevated for at least 16 h. In contrast, *agp S3* was not stimulated by sucrose, in fact, transcript levels declined by 16 h. Expression of the *S* subunit genes is therefore differentially regulated in plant organs and by sucrose.

(Supported by NASA: NAG5-6011.)

[148]

TRANSIENT DISSOCIATION OF POLYRIBOSOMES AND CONCURRENT RECRUITMENT OF SPECIFIC TRANSCRIPTS IN GRAVISTIMULATED MAIZE PULVINI. I. Heilmann[1], J. Shin[2], I. Y. Perera[3], J. Huang[3], and E. Davies[3]. [1]Biology Department, Brookhaven National Laboratory, Upton, NY 11973, [2]Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA 94720, and [3]Department of Botany, North Carolina State University, Raleigh, NC 27695.

The dynamics of polyribosome abundance were studied in gravistimulated maize stem pulvini. During the initial 15 min of gravistimulation, the amount of large polyribosomes transiently decreased. The transient decrease in polyribosome levels was accompanied by a transient decrease in polyribosome-associated mRNA. After 30 min of gravistimulation, the levels of polyribosomes and the amount of polyribosome-associated mRNA gradually increased over 24 h up to 3 to 4-fold of the initial value. Within 30 min of gravistimulation, total levels of transcripts coding for calmodulin and calreticulin were elevated 8-fold and 5-fold, respectively, in maize pulvinus total RNA. Transcripts coding for both calmodulin and calreticulin were recruited into polyribosomes within 15 min of gravistimulation. Over 4 h of gravistimulation, a gradual increase in the association of calmodulin and calreticulin transcripts with polyribosomes was seen predominantly in the lower half of the maize pulvinus; the association of transcripts for vacuolar invertase with polyribosomes did not change over this period.

Our results suggest that within 15 min of gravistimulation the translation of the majority of transcripts associated with polyribosomes decreased, resembling a general stress response. Recruitment of calmodulin and calreticulin transcripts into polyribosomes occurred predominantly in the lower pulvinus half during the first 4 h when the presentation time for gravistimulation in the maize pulvinus is not yet met.

(Supported by the NASA Specialized Center of Research and Training at North Carolina State University: NAGW–4984.)

[149]

A MOLECULAR APPROACH TO STUDY THE ROLE OF PHOSPHOINOSITIDE SIGNALING IN PLANT GRAVITROPISM. I.Y. Perera[1], J. Love[1], I. Heilmann[2], W.F. Thompson[1], W. F. Boss[1], [1]Dept. of Botany, North Carolina State University, Raleigh, NC 27695 and [2]Biology Department, Brookhaven National Laboratory, Upton NY 11973

Plants exhibit differential growth in response to a change in their spatial orientation relative to the gravity vector. To date, the signaling processes linking sensing of a gravistimulus to the initiation of a differential growth response are poorly understood. Using the pulvinus of cereal grasses as a model system, we have shown previously, that both rapid and long term increases in inositol 1,4,5-trisphosphate ($InsP_3$) correlate positively with the gravitropic bending response of both oat and maize stems (Perera *et al*., 1999, *Proc Nat Acad Sci* 96:5838-5843; Perera *et al*., 2001, *Plant Physiol* 125:1499-1507). Our work has revealed that $InsP_3$ is a major player in the signal transduction cascade mediating the gravitropic response and suggests that the PI pathway is an important target for regulating cellular responses to gravity.

We are taking a molecular approach to affect $InsP_3$ metabolism in plants with the intent of abolishing the long-term differential increase in $InsP_3$, which appears to be critical for gravitropic bending. As a first step towards this goal we have generated transgenic tobacco cells expressing the human type I inositol polyphosphate 5'phosphatase (InsP 5'ptase), an enzyme that specifically hydrolyzes $InsP_3$. We report here that the heterologous gene is expressed and the protein active in tobacco cells. Transgene expression resulted in drastically reduced basal levels of $InsP_3$, altered phosphoinositide metabolism and an attenuation of stimulus-induced $InsP_3$ changes.

(Supported by NSCORT: NAGW-4984)

[150]

DIFFERENTIAL EFFECTS OF MICROGRAVITY ON ACTIVATION OF INDIVIDUAL PROTEIN KINASE C ISOFORMS IN LEUKOCYTES Jason P Hatton, NCIRE & VAMC San Francisco, CA

Protein kinase C (PKC) isoforms are a family of Ser/Thr kinases which individually fulfil important, distinct roles in the regulation of gene expression in mammalian cells. The translocation of PKC isoforms to the membrane fraction of the cell is a critical step in signal transduction through this pathway. In two previous experiments flown aboard Space Shuttle missions we observed modifications in the distribution & translocation of total PKC in the human monocytic cell line U937. Therefore, we investigated whether individual PKC isoforms showed different patterns of sensitivity to microgravity in an experiment flown aboard STS-81. In U937 agonist induced translocation of PKC βii, δ & ε was inhibited in microgravity, but enhanced in hypergravity. Conversely in T-cells PKC βii & δ showed unique patterns of sensitivity to g-level. The relationship between g-level & PKC isoform translocation, as well as functional effects of changes in PKC signalling was further explored in ground based hypergravity experiments. Given the role of individual PKC isoforms in the regulation of important cell functions including differentiation & apoptosis, gravity dependent modifications in PKC isoform activation could result in alterations in cellular responses to agonists & cell fate following differentiation.

(Supported by CNES grants 95/270, 96/241, 97/71/6571)

[151]

HEAT STRESS FACILITATES STRETCH-INDUCED HYPERTROPHY OF CULTURED MUSCLE CELLS. K. Goto[1], R. Okuyama[1], H. Sugiyama[1], M. Honda[1], T. Sugiura[2], S. Yamada[3], T. Akema[1], and T. Yoshioka[1,4]. [1]Dept of Physiol, St. Marianna Univ Sch of Med, Kawasaki; [2]Faculty of Edu, Yamaguchi Univ, Yamaguchi; [3]Dept of Life Sci, Graduate Sch of Arts and Sci, Univ of Tokyo, Tokyo; [4]Aomori Univ of Health and Welfare, Aomori, Japan

The countermeasure for muscular atrophy is not still established. Heat shock proteins (HSPs) are an important family of endogenous, protective proteins. HSP90 and HSP70 families show elevated levels under heat stress and cell cycle-dependent expression. Mechanical stress, such as stretching and physical exercise, is known to induce not only muscular hypertrophy but also the elevation of HSPs expression in skeletal muscle. The purpose of this study was to determine whether heat stress facilitates the stretch-induced hypertrophy of skeletal muscle cells. Cultured rat myotubes (L6) were plated on collagenized Silastic membranes and incubated at 41˚C for 60 minutes (heat shock). Following the incubation, the cells were subjected to two-second stretching and four-second releasing for 4 days at 37˚C. Protein concentrations in the homogenates and pellets of the cultured skeletal muscle cells increased under heat shock and/or mechanical stretching. The protein concentration of cells following mechanical stretching following heat shock was significantly higher than that following either heat shock or mechanical stretching alone. Changes in HSPs and cellular protein concentrations in stressed cells suggest that the expression of HSPs may be closely related with muscular hypertrophy.

This study was partially supported by Matsushita Electronic Works Ltd., Osaka, Japan.

[152]

EFFECTS OF ACTIVITY INHIBITION ON THE CHARACTERISTICS OF NEWT HINDLIMB MUSCLE FIBERS. Y. Ohira, Y. Yamagishi, M., and T. Yoshinaga. Sch. Health Sport Sci., Osaka Univ., Osaka, Japan.

Effects of reduced muscular activity on the characteristics of hindlimb muscles were studied in 8 adult newts. The right femur was broken and the left limb was kept intact. The normal movement of right hindlimb was inhibited. However, the newts were able to move and eat food voluntarily. After one month, the newts were decapitated and both hindlimbs were sampled. The cross-sections of knee extensor and flexor were analyzed immunohistochemically. Fast and slow myosin heavy chain expression were stained using mouse monoclonal antibodies and Western blotting for fiber phenotype classification. Fiber cross-sectional area (CSA), mean diameter, and mean perimeter were analyzed using an image processing system. Although the weight of whole limb with reduced activity tended to be reduced, the CSA of whole knee extensor and its muscle fibers were increased. On the contrary, the CSA of whole knee flexor tended to be decreased due to the atrophy of fast-twitch fibers. The responses of mean fiber diameter and perimeter were similar to those of CSA. The increase of fiber CSA of knee extensor due to hypodynamia tended to be greater in slow and intermediate fibers than fast fibers. It is speculated that knee extension movement may be stimulated, because the activity pattern was altered due to broken bone. The distribution of slow fibers in knee extensor tended to decrease and that of fast fibers increased following reduced activity. As for the knee flexor, % intermediate fibers were increased and % fast fibers were decreased significantly ($p<0.01$). Although the precise mechanism is unclear, it suggested that the responses of newt muscles to reduced activity is different from those in human and rats.

[153]

NOS II INHIBITON ATTENUATES POST-SUSPENSION HYPOTENSION IN SPRAGUE-DAWLEY RATS. D. Eatman, M. Walton, R.R. Socci, N. Emmett and M.A. Bayorh. Morehouse School of Medicine, Atlanta, GA.

The reduction in mean arterial pressure observed in astronauts may be related to the impairment of autonomic function and/or excessive production of relaxing factors. In the present study, we examined the cardiovascular responses to 7-day 30^{o} tail-suspension and a subsequent 6-hr post-suspension period in conscious male Sprague-Dawley rats, to determine the role of a nitric oxide synthase II (NOS II) inhibitor AMT (2-amino-dihydro-6-methyl-4H-1,3-thiazine) against the post-suspension reduction in mean arterial pressure (MAP). The specific NOS II inhibitor AMT (0.1 mg/kg), or saline, was administered intravenously prior to release from suspension and at 2 and 4 hrs post-suspension. Direct MAP and heart rate were determined prior to suspension, daily and at 6 hrs post-suspension. During suspension, MAP did not change, in contrast, during post-suspension; it decreased compared to parallel non-suspended, untreated animals. There were no significant changes in heart rate. The reduction in MAP post-suspension was associated with a significant increase in plasma nitric oxide. AMT reduced plasma nitric oxide levels, attenuated the observed post-suspension reduction in MAP at 6 hrs post-suspension and modified the baroreflex sensitivity for heart rate. In conclusion, the post suspension reduction in mean arterial pressure may be due, in part, to overproduction of nitric oxide, via the NOS II pathway, and alterations in baroreflex activity.

(Supported, in part, by NASA grant NCC-9-112 and NIH grant S06GM08248-12.)

[154]

THE ROLE OF THE ACTIN CYTOSKELETON IN GRAVITY SENSITIVITY OF T-CELL ACTIVATION.

B.B. Hashemi, J.E. McClure, M.L. Marquette, T.D. Fitting, E.A. Hess, E. Salas, E. Stenseng, and D.L. Pierson. Life Science Research Laboratories/SD3, NASA - Johnson Space Center, Houston, TX. Harlingen High School, Harlingen, TX.

Studies of lymphocyte (T-cell) activation in microgravity culture during space flight indicate a dramatic reduction of proliferative response to activation. In earlier Biorack flight investigations we determined that this inhibition of T-cell activation is accompanied by alterations in the tubulin cytoskeleton. Specifically, activation of human peripheral T-cells by bead-immobilized anti-TCR failed to induce polarization of the microtubule cytoskeletal system and the re-orientation of the Microtubule Organizing Center (MTOC) towards the cell-bead contact site in microgravity culture compared to 1-g control.

In an effort to elucidate the mechanism of gravity sensitivity in cellular responses, in the current study the adaptation and response of the actin cytoskeleton was characterized immediately upon exposure to microgravity and hypergravity culture. Recent experiments on the KC-135 indicate that exposure of cells to as little as 10 seconds of microgravity results in alterations in the polymerization state of actin. Furthermore, while activation of T-cells during the hypergravity phase of flight (1.8 g) induces rapid polymerization of F-actin, little or no polymerization is detected during the microgravity phase of flight. In view of the fact that the actin cytoskeleton plays a crucial role in signal transduction and activation response of T-cells, as well as orchestration of mechanical forces inside cells, these data are providing exciting insights into the mechanism of gravity sensitivity in T-cells.

[155]

STREPTOCOCCUS PNEUMONIAE ALTERS PROTEIN PROFILES AND VIRULENCE POTENTIAL IN MODELED MICROGRAVITY. C.J. Orihuela[1], J.G. Knecht[1], D.A. Watson[1,2], D.W. Niesel[1]. [1]Dept of Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX. [2]InDyne Inc., Houston, TX.

The effects of modeled microgravity (MMG) on *Streptococcus pneumoinae* cultures were assessed after growth of the pneumococcus in a high aspect ratio vessel versus that in non-rotating control vessels (CC). Analysis of growth rates between MMG and CC cultures found no difference in growth curves and bacterial chain length. Characterization of silver stained two-dimensional PAGs found distinct changes in whole cell protein profiles. Specifically, expression of a 19-kDa protein was enhanced during MMG. This was common to all three pneumococcal isolates examined: DW4.1, WU2, R6. In addition, a 46-kDa protein was found up regulated in WU2 and DW4.1 during CCs versus MMG cultures. Assays measuring bacterial adhesion to A549 lung epithelial cells *in vitro* were also performed. These studies determined that pneumococci from CCs of DW4.1 adhered at levels four-fold higher than those isolated from MMG. No adhesion was observed for WU2 in either MMG or CCs. To determine if MMG affected bacterial virulence, the LD_{50} of pneumococci grown under MMG and CC was determined. LD_{50} assays in mice challenged i.p. with DW4.1 showed that the LD_{50} of MMGs and CCs were similar with 10 pneumococci killing 50% of challenged mice. In contrast, LD_{50} assays with WU2 found that control cultures were 10-fold less virulent than those cultured under MMG. Northern dot blot analyses of pneumococcal virulence genes were also assessed. Virulence-related genes included *ply,* the gene encoding pneumolysin, *capA3*, a gene encoding an enzyme necessary for the synthesis of type 3 capsules, *pspA*, and *lytA*. Overall, no differences in gene expression were observed between the culturing conditions. Finally, analysis of capsular polysaccharide production during MMG and CC was also evaluated.

(Supported by NASA grant 98-HEDSO2-91, and Texas Advanced Research Program Grant, 004952-0090P)

[156]

A CYTESINE PROTEASE INHIBITOR PREVENTS SUSPENSION-INDUCED DECLINES IN BONE WEIGHT AND STRENGTH OF RATS Takeshi Nikawa[1], Madoka Ikemoto[1], Chiho Watanabe[1], Takako Kitano[1], Mihoko Kano[1], Makoto Yoshimoto[2], Takae Towatari[3], Nobuhiko Katunuma[4], Fujiko Shizuka[5], and Kyoichi Kishi[1] [1]The University of Tokushima, Tokushima; [2]Medical Research Laboratories, Research Center, Taisho Phermaceutical Co., [3]The University of Tokushima, Tokushima 770-8503, Japan; [4]Institute for Health Science, Tokushima Bunri University, Tokushima; [5]Nagano Prefectural College.

In this study, we examined effects of a potent cysteine protease inhibitor, *N*-(L-3-*trans*-carboxyoxirane-2-cabonyl)-L-leucine-4-aminobutylamide (E-64a), on bone weight and strength in tail-suspended rats. We first administered vehicle, 4 and 8 mg/rat of E-64a to rats fed a low calcium diet for 3 wk to determine effective doses of E-64a on bone resorption *in vivo*. Femoral cathepsin K-like activity and serum hydroxyproline level in rats fed a low calcium diet were significantly higher than those in rats fed a standard diet. The intraperitoneal injection of 8 mg/rat of E-64a to rats decreased their serum calcium and hydroxyproline concentrations after 3 to 6 hr in parallel with changes in the femoral cathepsin K-like activity, while 4 mg/rat of E-64a had the weaker effects on these parameters. Based on these results, we next injected 8 mg/rat of E-64a to tail-suspended rats twice per day for 2 wk and compared with twice treatment per wk of 1 mg/rat of etidronate, a bisphosphonate. In tail-suspended rats, femoral weight significantly decreased from day 5 to 21. Unlike rats fed a low calcium diet, femoral cathepsin K-like activity and serum calcium and hydroxyproline concentrations did not change in tail-suspended rats. Three-point bending test at the femoral middiaphysis revealed that maximal force for breaking the femur of tail-suspended rats significantly decreased by 20% of that of control rats on day 14 and 21. E-64a as well as etidronate significantly prevented the suspension-induced declines in bone weight and strength. E-64a inhibited femoral cathepsin K-like activity, but etidronate did not. Our results suggest that a cysteine protease inhibitor could recruit microgravity-induced osteopetrosis by the distinct mechanism from a bisphosphonate.

Concurrent Posters
IV-D
Plant and Microbial Growth Systems

[157]

TOBACCO BY2 CELL SUSPENSIONS IN PERFUSED CHAMBERS: EFFECTS OF FLOW AND MIXING ON CELL GROWTH, VIABILITY AND MORPHOLOGY. I. Berzin[1], H. Park[1], J. Lagaz[2], E. Guerra[2], J. de Luis[2], N. Searby[3], G. Vunjak-Novakovic[1]. [1]Massachusetts Institute of Technology, Cambridge MA, [2]Payload System Inc., Cambridge MA, [3]NASA Ames Research Center, Moffett Field CA.

A Cell Culture Unit (CCU) is being developed for controlled studies of cells and tissues, in space and on earth. CCU consists of up to 24 perfusion loops each containing one Cell Specimen Chamber (CSC), a gas exchanger and a media delivery system. We report the developmental testing of these single loops using suspensions of tobacco BY2 cells, in order to define the cultivation conditions yielding cell growth patterns comparable to those observed under standard conditions, in shake flasks. The main requirements of plant cell cultivation in closed systems with perfusion of culture medium include cell containment, normal viability, growth kinetics, and morphology. Cells were cultured in 10 mL volume CSCs containing a cell separation membrane and operated using a forward-reverse flow algorithm and magnetic stirring. Cell concentration, viability, growth rate and morphology, as well as the pressure on the membrane, and oxygen concentration in culture medium were determined during serial subcultivations of cell suspensions, as a function of medium flow and mixing. Oxygen concentration in medium and the rate of cell growth were comparable to those measured in control shake flasks. In particular, the kinetic rate of cell growth was the same in both systems, and cell viability and the uniformity of suspension in CSC exceeded 80% of shake-flask controls. Forward-reverse flow in conjunction with magnetic stirring maintained suspension uniformity without membrane blockage. The use of a low-shear impeller was essential for maintaining normal morphology of cell aggregates. Supported by NASA Ames.

[158]

OPTIMIZATION OF ROOT ZONE SUBSTRATES (ORZS): MEASURING AND MODELING GAS DIFFUSION IN MICROGRAVITY (μg). S.B. Jones[1], G.E. Bingham[1], D. Or[1], R.C. Morrow[2] and I.G. Podolsky[3]. [1]Space Dynamics Laboratory, Utah State University, Logan, UT. [2]ORBITEC, Inc. 1212 Fourier Drive, Madison, WI, [2]Institute for Biomedical Problems, RAS, Moscow, Russia.

The ORZS experiment measures and models root zone gas exchange through plant growth substrates at varying water contents in μg. This information is critical for proper water management and prevention of root zone hypoxia during plant growth and ALS biomass production in μg. Data suggest mechanisms such as enhanced hysteresis in water retention could alter the optimal set point between 1g and μg conditions. Measuring gas diffusion in μg will provide insights into the optimal water content at which plants can be grown without root zone oxygen stress, and will allow development of gas diffusion models for improved management protocols. A few space borne experiments have investigated water movement and control in μg, but none have investigated the region where oxygen diffusion becomes limiting for plant growth. Ground studies, using prototype flight hardware, show non-typical O_2 diffusion characteristics resulting from the coarse - dual pore-sized nature of substrates used in μg.

This flight experiment was selected for definition studies in FY 00 and effort to date has concentrated on demonstrating the proposed measurement technique and defining potential flight opportunities. We have identified three hosts for the experiment, which requires experiment specific measurement chamber hardware. Our studies indicate that the ORZS experiment can be hosted by either the ORBITEC, Inc. FHAME controller on an STS flight, or the LADA controller on ISS.

(ORZS is funded under NASA contract NAG 9-1284)

[159]

SCIENCE AND TECHNOLOGY OVERVIEW OF THE BIOMASS PRODUCTION SYSTEM MISSION VERIFICATION TEST.

R.C. Morrow, J.G. Frank, K.M. Stolp, S.M. Guetschow and. T.M. Crabb. Orbital Technologies Corporation, Madison, WI.

The Biomass Production System (BPS) was developed as a plant research facility for use aboard the Orbiter and the International Space Station (ISS). The first BPS mission is scheduled for delivery to the ISS on flight 8A. This mission consists of two experiments, a Technology Validation Test (TVT) to evaluate the function and performance of BPS hardware systems, and the PESTO experiment to investigate wheat photosynthesis in microgravity. As part of the preparation for the BPS mission, a Mission Verification Test (MVT) was required. The BPS MVT took place at Kennedy Space Center (KSC), and ran for a total of 58 days beginning in April 2001. The MVT was designed to test the flight readiness, technology validation, and science objectives of the BPS. A 51 day mission timeline with associated operations was simulated, including automated activities (e.g. CO_2 drawdowns, video framegrabs) and crew activities (e.g. pollination, chamber gas sampling, plant harvest). Two cycles of wheat and *Brassica rapa* were grown and all pre-flight, in-flight, and post-flight operations were simulated and demonstrated. Preliminary results indicate that all TVT and science (PESTO) objectives were successfully met in a simulated (for temperature, humidity, and CO_2) spacecraft environment. TVT evaluation objectives met included wheat and *Brassica* germination, growth, and development, environmental control subsystem performance, data acquisition, sample acquisition, plant manipulation, resupply, and debris characterization. Performance data for temperature and humidity control, atmospheric control, lighting, fluid delivery (NDS, HCS, and reservoirs), video, system power, and data acquisition, command and data handling were all within previously specified limits. A full data set was obtained for vapor deficit tests at three growth stages for one growth cycle of wheat, and recovery and analysis operations for tissue, fluid and gas samples successfully supported TVT and science objectives.

[160]

MYOBLAST DIFFERENTIATION UNDER FLOW CONDITIONS. Lj. Kundakovic[1], S. Pretorius[2], J. Vinning[2], C. Preda[1], L. Yang[1], F. Donovan[3], M. Springer[4], J. de Luis[2], G. Vunjak-Novakovic[1]. [1]Massachusetts Institute of Technology, Cambridge MA, [2]Payload Systems Inc., Cambridge MA, [3]NASA Ames Research Center, Moffett Field CA, [4] Stanford University, Stanford CA.

A Cell Culture Unit (CCU) is being developed to study the fundamental roles of gravity in biological systems aboard the International Space Station. The CCU consists of multiple perfusion loops, each containing one Cell Specimen Chamber (CSC), with an optical glass window for on-line microscopy. For cultivation of adherent cells, the glass surface is coated with extracellular matrix proteins. The C2C12 muscle cell line was studied as a model system for animal cell monolayers. The culture conditions were selected to support cell attachment and differentiation, as follows. Flow conditions were defined to maintain physiological levels of oxygen , carbon dioxide and pH at a minimal hydrodynamic shear acting on the cells (e.g. periodic flow, 5 min/hr @ 1 mL/min in a 10 mL CSC). Medium volume and the regime of exchange were defined to replenish essential nutrients and regulatory factors while maintaining a certain level of cell self-conditioning. Cell attachment and proliferation were comparable to static controls, whereas cell differentiation under flow conditions remains a challenge. The expression of molecular markers (e.g. tropomyosin) and cell fusion (myotube density, morphology, and kinetics of formation) strongly depended on medium volume, flow rate, and regime of exchange. Overall, low medium volume per unit cell mass, intermittent flow, periodic and partial exchange increased the rate of cell fusion, as well as the density, length and thickness of myotubes formed in flow CSCs. Supported by NASA Ames.

[161]

INVOLVEMENT OF NITRIC OXIDE ON GERMINATION AND SEEDLING GROWTH IN SLOWLY ROTATING CLINOSTATS. M.C. Pedroso[1,2], C. Ubach[2] and D.J. Durzan[2]. [1]Center for Plant Biotechnology, Lisbon, Portugal, [2]Dept Environmental Horticulture, Univ of California, Davis.

The purpose of this study was to determine if nitric oxide (NO) was contributing for some of the plant development alterations observed in clinorotated cultures. Seeds of *Arabidopsis thaliana*, cultured on basal media containing NO donors, scavengers, or inhibitors (of NO-synthase and/or nitrate reductase), were kept in slowly rotating clinostats (1 rpm) and in 1-*g* (controls), under light and in darkness, for 2 weeks. NO donors increased germination in clinorotated cultures and in control-cultures in darkness. Germination decreased on medium with carboxy-PTIO (NO scavenger) and was inhibited on media with NO-synthase and nitrate reductase inhibitors. For all physical conditions tested, addition of a NO-synthase inhibitor alone significantly reduced ($P < 0.01$) root and hypocotyl growth. In contrast, the addition of NO donors (SNP or SNAP) had different effects on root and hypocotyl growth, depending on the type of NO donor used, light and gravity conditions. In clinorotated cultures, addition of NO donors did not significantly affect root elongation in darkness, but reduced it under light. Moreover, SNP and SNAP had opposite effects on hypocotyl elongation. In clinorotated cultures under light, SNAP stimulated while SNP inhibited hypocotyl elongation; however, in darkness, neither of them significantly affected hypocotyl elongation ($P < 0.05$). In controls (1-*g*), under light and in darkness, addition of SNP and SNAP did not significantly affect hypocotyl growth, compared to cultures on medium without NO donors. These results will be discussed in relation to light effect on putative NO-synthase and nitrate reductase activities and to their contribution for the overall production of nitric oxide.

(Supported by Fundação para a Ciência e a Tecnologia, Portugal)

[162]

EVALUATION OF A FIXED FEED WATER INPUT MODE FOR SPACE-BASED PLANT CULTURE APPLICATIONS. H.G. Levine, G.K. Tynes, J.H. Norikane, C.M. Frasier and T.W. Dreschel. Dynamac Corp., Kennedy Space Center, FL.

The Water Offset Nutrient Delivery Experiment (WONDER) will evaluate both a Porous Tube Nutrient Delivery System (PTNDS) and a Substrate Nutrient Delivery System (SNDS) under three separate wetness level setpoints. The primary objective is to document the offset (relative to the 1g condition) in optimum wetness level setpoints for plant cultivation in space. We report here on ground studies in which dry seeds were automatically imbibed, and germinated within the Porous Tube Insert Module (PTIM) prototype apparatus which approximates the unit that will fly in space. Results are presented from two separate experimental runs conducted under anticipated flight conditions of 23° C, 75% RH, 1000 ppm CO_2 and constant light (185 □moles m^{-2} s^{-1}). In the first PTIM study, experimental treatments were as follows: 20 seeds were germinated on each of six separate porous tubes receiving fixed feed nutrient solution input rates of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL/h. In addition, 24 seeds were germinated within each of three substrate compartments receiving fixed feed water input rates of 0.5, 1.5 and 3.0 mL/h. Increased levels of water use efficiency were evident within the PTNDS component of the study. In the second PTIM study, heat pulse moisture sensors (Orbitec™) were embedded at 1-, 3-, 5- and 7 cm depths within three SNDS compartments which received fixed feed water input rates of 1.0, 2.0 and 3.0 mL/h. The vertical moisture distribution patterns obtained over a 23 day grow-out interval are presented.

(Supported by NASA: NAS610-12180.)

[163]

ENVIRONMENTAL SYSTEMS TEST STAND. D.J. Barta[1], J.M. Castillo[2], M.K. Ewert[1], J.S. Young[2], M.T. Monk[2] and C. Bernard[1], [1]NASA Johnson Space Center, [2]Lockheed Martin Space Operations, Houston, Texas.

A test stand has been developed for the evaluation of prototype environmental control and crop cultivation technologies for plant production within an advanced life support system. Design of the test stand was based on preliminary designs of the center growth bay within the Biomass Production Chamber of the Bioregenerative Planetary Life Support Systems Test Complex (BIO-Plex). It consists of two controlled-environment shelves, each with 4.7 m2 of area for crop growth (150 cm width, 315 cm length). After stage 1 of construction (1st quarter, FY02), the test stand will be capable of evaluating candidate lighting and thermal control technologies. During stage 2 of construction (FY03), nutrient delivery and materials handling technologies will be integrated. There are two chilled water loops, one at conventional temperatures (5-10 *C) for air temperature and humidity control and one at high temperature (45-50 *C) for waste heat acquisition and utilization. The lighting systems will be modular to allow for easy replacement with new technologies. Data acquisition and control hardware will be localized, interconnected by ethernet, and will make use of software under development at NASA Ames Research Center.

[164]

CO_2 EXCHANGE RATES OF *ARABIDOPSIS THALIANA* UNDER LOW-PRESSURE ENVIRONMENTS: CO_2 SENSOR SENSITIVITY TO LOW-PRESSURE AFFECTS INTERPRETION OF RESULTS. J.T.Richards[1], A.C. Schuerger[1], and K. Corey[2]. [1]Dynamac Corporation, Mail Code:DYN-3, Kennedy Space Center, FL, and [2]32 Highland St., Miller Falls, MA.

A low-pressure plant growth chamber (LPGC) was constructed with the ability to maintain low-pressure environments capable of supporting plant physiological studies. The LPGC was configured with CO_2, temperature, RH, and pressure sensors. Various tests with other instruments confirmed that temperature, RH and pressure data were correct at different pressures within the LPGC. However, CO_2 sensor values were strongly influenced by the pressure within the LPGC. CO_2 calibration curves were subsequently generated for a 1% and a 2000 ppm sensor in the LPGC at set levels of 101, 75, 50, 25, and 10 kPa to correct for this pressure sensitivity. Slopes of the curves increased with increasing pressures, and all curves had a linear response. These results clearly indicated the requirement to properly calibrate any CO_2 sensor prior to initiating low-pressure studies.

CO_2 consumption rates were measured on five-wk-old *Arabidopsis thaliana* plants enclosed in the LPGC at 22 C, 80-85% RH, 150 $\mu mol/m^2/s^1$, and the pressure set at either 101, 75, 50, 25 or 10 kPa. Based upon the CO_2 calibration curves, CO_2 was added to a final partial pressure of 0.1 kPa. CO_2 levels were measured over time until there was no apparent net exchange, i.e., to the CO_2 compensation point. Resultant regression models indicated that as the LPGC pressure decreased from 101 kPa to 10 kPa the rate at which CO_2 was utilized by the plants increased. The first derivative of the consumption rate curves indicated increased photosynthetic rates and decreased CO_2 compensation points at lower pressures. Therefore, plants remain photosynthetically active even at atmospheric pressures approaching 10% Earth-normal.

(Supported by NASA: AO-99-HEDS-01-032)

[165]

DEVELOPMENTAL TESTING OF THE CELL CULTURE UNIT USING *SACCHAROMYCES CEREVISIAE*. L. Sun[1], S. Pretorius[2], J. de Luis[2], N.D. Searby[3], J. Vandendriesche[3], and G. Vunjak-Novakovic[1]. [1]Massachusetts Institute Technology and [2]Payload Systems Inc., Cambridge MA, [3]NASA Ames Research Center, Moffett Field, CA.

A Cell Culture Unit (CCU) is being developed to study the fundamental roles of gravity in biological systems, on earth and in space. It provides a controllable environment for cell culture, consisting of up to 24 perfusion loops each containing one Cell Specimen Chamber (CSC), a gas exchanger and a media delivery system. *Saccharomyces cerevisiae* has been selected to validate the CCU as a simple model cell system for molecular studies. Yeast cultivation in closed systems presents a unique challenge due to the rapid growth and high metabolic rate, need to maintain cells in suspension, and to remove carbon dioxide in order to avoid bubble formation. We report the developmental testing of single perfusion loops and a CCU prototype (Science Evaluation Unit, SEU) using 10 mL CSCs operated with forward-reverse flow of culture medium (net flow rates 0.83 - 2.50 mL/min) and magnetic stirring (stir bars, paddles; 60-200 rpm; two directions), and continuous gas exchange (26 - 76 mL/min of air). Cell growth rate, morphology, viability, the maintenance of cell genotype, levels of pH, oxygen and carbon dioxide in culture medium were assessed over four serial subcultivations and compared to shake flask controls. All parameters were indistinguishable for the SEU, single loops and laboratory shake-flask controls. The uniformity of cell suspension was > 90%. Replicate experiments were repeatable within 10% of each measured parameter. The promising results of yeast cell testing of CCU prototypes support the expectations that the CCU will provide scientists with the robust tools to perform cell biology studies, both in space and on earth. Supported by NASA Ames.

Concurrent Posters
IV-E
Systems Biology and Advanced Life Support

[166]

EXTRUDER SCALING AND DESIGN. J.L. Kokini[1], M. Dhanasekharan[2] and B. K. Ashokan[1]. [1]Dept. of Food Science and NJ-NSCORT, Rutgers University, NJ and [2]Fluent Inc., Lebanon, NH.

The objective of this work is to develop scale-up and design principles for the extrusion of foods in a single screw extruder using numerical methods.

Using the numerical techniques of computational fluid dynamics (CFD) it is possible to derive fundamental dependencies between screw design parameters and operating characteristics such as residence time distribution (RTD) and specific mechanical energy (SME). The viscosity model for wheat dough as described by Mackey and Ofoli (1990) is used for the analysis. A commercial CFD code Polyflow (Fluent Inc., New Hampshire) is used to solve the fundamental flow and heat transfer equations.

The impact of screw geometry on RTD and SME was studied by generating 20 screw geometries with different geometric variables such as helix angle, channel depth, screw diameter to channel depth ratio, screw length to screw diameter ratio, and the clearance between the screw flights and barrel. A trend chart for SME showing the dependencies with the screw parameters was developed. RTD curves were obtained for all the simulations. It was found that SME and RTD is maintained constant between extruders of two sizes when the smaller extruder with smaller screw diameter and channel depth, has a higher D/H ratio and helix angle compared to the larger extruder.

This CFD methodology can easily be extended to study a variety of food products by incorporating the corresponding viscosity models. Twin-screw extruders can also be designed and scaled up by the same approach.

(Supported by NASA: NJ-NSCORT)

[167]

PREDICTION OF PROCESSING PARAMETERS OF WHOLE WHEAT BREAD WITH VARYING COMPOSITIONS. M.H. Perchonok[1], J.L. Kokini[2], M. Dansby[3], I. Stevens[4], B. Swango[5]. [1]National Space Biomedical Research Institute, Houston, TX. [2]Rutgers University, New Brunswick, NJ. [3]Tuskegee University, Tuskegee, AL. [4]Lockheed Martin, [5]Houston. Spacehab, Houston.

The Advanced Food System at Johnson Space Center/NASA will be responsible for supplying food to the crew for long duration exploratory missions. Once on the planetary surface, the Advanced Food System will be responsible for processing the hydroponically grown harvested crops.

Functional properties of food ingredients and processing conditions are strongly dependent on the composition (protein, fat, and moisture) of the crops. Small variation in crop composition can cause failure in delivery of acceptable food products. For example, the protein content of field grown hard red spring wheat is approximately 10% whereas the protein content of hydroponically grown apogee wheat, grown at JSC in 1997, is approximately 16%. The higher protein affects the quality attributes of bread.

The objectives of this study is to (1) determine the fat and moisture content of the apogee wheat and the field grown wheat, (2) to characterize the protein of these two flours and (3) develop usable processing conditions (including formula) to compensate for the higher protein content of the apogee wheat. The quality of the whole wheat bread produced with the two wheat flours were evaluated for loaf volume, acceptability, texture, color, and shelf life. The formula of the whole wheat bread includes whole wheat flour, sugar, salt, water and yeast. The wheat berries were milled with a C.W. Brabender Quadramat Jr. Mill and the bread was made with Zojirushi bread makers.

It was determined that the water and yeast content had to be increased tocompensate for the lower moisture and higher protein content of the apogee wheat. Without the compensation, the bread was too dense and not as acceptable to the sensory panelists. A relationship has been determined so that if later crops of apogee wheat differ in protein or water, a bread formula can be easily developed. (Supported by the JSC Center Director Discretionary Fund Program).

[168]

NASA'S RODENT FOODBAR: LONG TERM EFFECTS IN SWISS WEBSTER MICE. D.L. Santiago[1], D.S. Yu[1], N.H. Naficy[2], P.M. Roghani[2], B.P. Dalton[3], J.E. Barrett[1]. [1]Lockheed Martin Space Operations, Moffett Field, CA; [2]De Anza College, Cupertino, CA; [3]Life Sciences Division, NASA Ames Research Center, Moffett Field, CA.

Swiss Webster male and female mice (150 of each) were fed NASA's Rodent Foodbar for more than 110 days to test the diet's nutritional adequacy for use in future long-term studies aboard the International Space Station. Mice were grouped three to a cage (one cage = one sample) and cages were assigned to either Foodbar or Purina Chow #5001 (control) diet groups. Body weights, food intake, and water intake were obtained throughout the study. There were no significant differences in body weights between male Foodbar fed and Chow fed males ($p=0.58$), and at 15 weeks into the female mouse study there appear to be no significant body weight differences. Both male and female Foodbar fed groups consumed more food and less water than their Chow controls, both factors thought to be attributable to the high moisture content of the Foodbars (26% versus 10% for Chow). All differences in gross food and water consumption had p-values of <0.01. When food and water intake were adjusted for the moisture content in the food, both male and female Foodbar fed animals consumed less food, but still had a lower water intake rate than their controls. ($p<0.01$). Preliminary analysis on blood samples from male and female halfway point dissections suggests differences in glucose and fat metabolism. In both male and female Foodbar fed animals, blood glucose values were significantly lower ($p<0.01$) but there were no significant differences in cholesterol levels ($p=0.51$). In Foodbar fed females, triglycerides were significantly higher ($p<0.01$). These data suggest that Foodbars allow for normal growth in Swiss Webster mice, but affect some blood chemistry parameters.

(Supported by NASA: UPN393-25.)

[169]

DECREASED LIVER OXIDATIVE STRESS WITH SHORT-TERM USE OF NASA RODENT FOODBARS. S. Pruitt[1], J. Ramirez[1], A. Lau[1], J. Barrett[1], D. Yu[1], B. Dalton[2], B. Girten[2]. [1]Lockheed Martin, Moffett Field, CA, [2]NASA Ames Research Center, Moffett Field, CA.

Two separate short-term rodent studies were performed at NASA Ames Research Center and both included examination of the effects of NASA's rodent Foodbars. The first study utilized 48 male Sprague-Dawley rats (SDR), half of which were fed Foodbars for 37 days with the other half being fed Purina (5012) rodent chow. The second study utilized 50 male Swiss-Webster mice (SWM), half of which were fed Foodbars for 22 days with the other half being fed Purina (5001) chow. Both studies included monitoring body weight weekly during the entire duration of the study. Neither the SDR study nor the SWM study showed significant differences in the growth rates between the chow and Foodbar groups. Both studies examined the effects of Foodbars with regard to oxidative stress in liver tissue. In the current studies, lipid peroxidation (LPO) and superoxide dismutase (SOD) were investigated to determine the oxidation/reduction status of the livers of both rats and mice. In the SDR and SWM studies, results of both LPO and LPO/Protein showed a significant difference ($p<0.05$) between the chow and Foodbar groups, with chow groups being higher than the Foodbar groups. In the SDR study, the chow group had LPO and LPO/Protein values 23% and 24% higher than Foodbar group, respectively. The SWM study showed the greatest difference, with chow showing values 105% and 101% higher than Foodbar for LPO and LPO/Protein, respectively. The chow group was significantly higher than the Foodbar group in the SWM study for SOD and SOD/Protein. In the SWM study, chow was 19% and 18% higher than the Foodbar for SOD and SOD/Protein, respectively. However, in the SDR study, SOD and SOD/Protein showed no significant difference between chow and Foodbar. In conclusion, in contrast to previous findings showing elevated oxidative stress levels in animals fed Foodbars for long-term studies (>90 days), these short duration studies showed that in both rats and mice, oxidative stress in liver tissue was significantly lower in Foodbar groups compared to chow groups.

[170]

LONG-TERM EFFECTS OF ADVANCED ANIMAL HABITAT PROTOTYPE CAGE AND NASA RODENT FOODBAR (FB) ON BODY WEIGHT, ORGAN WEIGHTS AND SERUM PARAMETERS. J.L. Ramirez[1], A. Lau[1], S. Pruitt[1], E. Melson[1], E.L. Hill[1], B. Girten[2]. [1]Lockheed Martin, Moffett Field, CA, [2]NASA Ames Research Center, Moffett Field, CA.

The Advanced Animal Habitat (AAH) is being developed to house rodents for use on the International Space Station (ISS). Early prototype AAH (P-AAH) cages with wire bottoms similar to flight design were used to evaluate long-term effects (110d) of P-AAH and NASA rodent Foodbar (FB) on the health and well being of rats to insure quality specimens for scientific research. Vivarium cages (VIV) and rat chow (CH) were compared to P-AAH and FB. 288 male SD rats (70-80g), were divided into 4 groups (72/group): 1)VIV+CH, 2)P-AAH+CH, 3)VIV+FB, and 4)P-AAH+FB. Each VIV and P-AAH cage initially housed 3 or 6 rats, respectively. Organs removed and weighed included liver (LIV), epididymal fat (EPI), and perirenal fat (PERI). Serum was analyzed for a standard clinical chemistry panel. While most serum parameters fell within the normal range for strain, age and sex, results at the end of the study indicated significant differences ($p<0.05$) with both a caging and diet effect, as well as interactions. Some parameters that showed significant differences after 110d are summarized below. For caging effect, P-AAH rats had a lower BW (~6%) and LIV/BW ratio (5-8%) than VIV rats, but slightly higher serum albumin levels (2-3%). The greatest FB effects were seen in serum cholesterol, fat pad weights and blood urea nitrogen (BUN). Cholesterol showed higher levels with 45-60% change and fat pads showed 17-36% increase with FB. BUN values were 9-24% lower when comparing FB to CH. In conclusion, although rats housed in P-AAH and/or fed FB for 110d were within normal ranges for most parameters, some significant changes were observed that warrant further investigation. This study provides a basis for evaluating higher fidelity flight-like hardware during AAH Science Evaluation Unit (SEU) testing and suggests that FB may need to be modified for long-term rodent use.

[171]

CROP PRODUCTION AND TESTING FOR ADVANCED LIFE SUPPORT AT KENNEDY SPACE CENTER. N.C. Yorio[1],G.D. Goins[1], G.W. Stutte[1], and R.M. Wheeler[2]. [1]Dynamac Corporation and [2]NASA Biomedical Operations Office, Kennedy Space Center, FL 32899

The evaluation of ALS candidate crops for FY2001 included the completion of CO_2 response tests with beans, salad crop testing with lettuce, spinach, and radish grown under red LEDs, nitrogen management tests with white potato, and a screening test of 16 radish cultivars as part of the ALS BIO-Plex salad crop objectives. Studies with a snap and dry bean cultivar at CO_2 concentrations of 8,000 and 16,000 μmol mol^{-1} have indicated no reductions in growth and yield compared to tests up to 4,000 μmol mol^{-1}. Additionally, increased stomatal conductance continued to correlate with the increasing CO_2 concentrations. Salad crops testing with red LEDs showed increased canopy growth for plants grown under the 690 nm lamps, suggesting that the additional far-red radiation caused greater leaf expansion and increased radiation capture. Two tests were performed with white potato to limit vegetative growth and increase biomass partitioning to edible yield by lowering the EC setpoint or lowering the NO_3^- concentration in the EC replenishment solution. Results showed that limiting all nutrients (via EC setpoint) or NO_3^- increased harvest index but decreased overall yield. Evaluations of radish cultivars in a large-scale test resulted in at least 4 varieties that are well-suited for hydroponic production under HPS lamps.

[172]

EVALUATION OF STRAWBERRY AS A CANDIDATE CROP FOR ADVANCED LIFE SUPPORT. E.E. Murray. Integrated Science and Technology Department, Marshall University, Huntington WV.

Strawberry is an excellent candidate crop for use in long-term space missions for several reasons: Excellent flavor and fragrance of berries provides variety in microgravity, where appetites lag. Compact plant size permits hydroponic cultivation in small spaces. Energy efficient crop, requiring lower temperatures and shorter light periods than many others. Healthful benefits of ellagitannins, which provide anticarcinogen and antimutagen activity. Strawberry horticulture offers a number of challenges for space travel, including pollination, propagation of new plants from runners, length of cold-treatment time for flower set, and optimal temperature and lighting conditions for optimal fruit yield. These issues can be addressed by selection of the optimal strawberry variety as well as manipulating the environment to improve its growth in microgravity. The ideal strawberry variety for space travel differs from commercial varieties, selected for uniform harvest time, pathogen resistence and post-harvest storage characteristics. It should be a hardy, disease resistant, everbearing or day-neutral variety that flowers several times a year and does not require lengthening days to set fruit. The variety should continuously produce flavorful strawberries with self-pollination. The plant architecture should be erect, facilitating harvest and pest control. It should produce runners capable of being rooted and transplanted directly into hydroponics with only a minimal chilling period to set flowers. We describe here our initial efforts to evaluate strawberry varieties using hydroponics systems to assess their usefulness in wastewater and nutrient recycling as well as their suitability for Advanced Life Support.

(Research support for this study was provided by Marshall University and West Virginia Space Grant Consortium)

Concurrent Posters
IV-F
Advanced Life Support: Recycling and Regeneration

[173]

ELECTROCHEMICAL REMOVAL OF AMMONIUM ION FROM BIOREACTOR EFFLUENT. A. Gonzalez-Martin, J. Kim, I. Brown, T. Uno, and C. Yalamanchili. Lynntech, Inc., College Station, Texas.

The presence of humans on extended space missions will require closed regenerative life support systems in which the supply of potable water is essential. A central step is the removal of ammonium ion, a by-product from the oxidation of nitrogen-containing substances occurring in the initial treatment steps (bioreactors) of water recovery systems. Ammonium ions are poorly rejected by the reverse osmosis unit and degrade post-treatment efficiency in the water reclamation system. Removal of ammonium ions from 1000 to less than 0.25 ppm is imperative. Lynntech's technology process is based on a cost effective, state-of-the art, electrochemical process that operates at room temperature. Five fundamental benefits of the process are: (i) nitrogen gas is the main product from the oxidation of ammonium ions, with a small production of nitrate ions (depending on operating conditions), avoiding the production of other undesirable and toxic byproducts; (ii) the required levels of ammonium removal are closely met; (iii) the oxidation of chloride ions, with the subsequent production of chlorine, a toxic gas, is completely suppressed; (iv) oxygen gas, readily available, is the only chemical that needs to be supplied; and (vi) there is no generation of a secondary waste. An update on the development and evaluation of a prototype system will be presented.

(Supported by NASA: NAS9-00120)

[174]

BIOREGENERATIVE RESEARCH AND TECHNOLOGY DEVELOPMENT TECHNICAL TASK IN GRAYWATER RECYCLING J.L. Garland, L.H. Levine, N.C. Yorio, J.L. Adams, and M.D. Hummerick, Dynamac Corporation, NASA Kennedy Center, Florida.

Non-toilet wastewater, or graywater, represents a potentially significant waste stream (i.e., estimated at over 25 L person^{-1} d^{-1}) in extended spacemissions. The graywater recycling task focused on development of analytical methods for different types of surfactants, and evaluation of the capacity of hydroponic systems to process the three types of commonly used surfactants. Analytical approaches coupling liquid chromatography (LC) with an evaporative light scattering detector (ELSD) or electrospray ion trap mass spectrometry (ESI-MS) were developed to characterize and directly quantify amphoteric and non-ionic surfactants. The LC/ELSD and ESI-MS methods are complimentary to each other and enable the investigation of chemical composition of the surfactants, as well as their biodegradation. Hydroponic plant growth studies with wheat were conducted to examine the degradation and phytotoxicity of an anionic (sodium laureth sulphates), amphoteric (cocamidopropyl betaines) and a non-ionic (polyoxyethylene 10 lauryl ether) surfactants. Surfactant loading rates as high as 2 g m^{-2} could be added to the systems without negative impact on plant growth due to the rapid degradation of the surfactants by microorganisms on the plant roots and on hardware surfaces.

[175]

APPLIED Y^2 ULTRA-FILTER TECHNOLOGY FOR A PORTABLE AIR FILTER FOR NASA SPACE STATION. Y. Yamamoto, Ph.D. Y^2 Ultra-Filter California, Inc., 1201 Via La Jolla, San Clemente, CA.

Y^2 has developed a superior air filtration technology, named "Y^2 Ultra-Filter," which can be applied for capturing a wide range of airborne particles, far beyond what the conventional methods (e.g. HEPA, ULPA, common air filter, electrostatic precipitator, etc.) can accomplish. Its capturing size range of airborne particles reaches down to 0.005 micron at 99.99915% efficiency (substantially covers all sizes of viruses, microbes, etc., and the clean air requirements for the nano-technologies) and also reaches far above the 10 micron range at 100% efficiency. Air filtration usingY^2 Ultra-Filter can accomplish dramatic reductions in energy cost, maintenance cost, and operating cost in comparison with conventional methods. Experiments have been performed with either atmospheric air or by using particulates of predetermined sizes which are injected into a totally clean (no airborne particles between 0.01 to 10 microns) air stream at the input.

The objective of the subject R&D is to build a prototype portable air filter under the NASA Phase II contract "An Advanced Air Revitalization System for Manned Space Vehicles," NAS8-00093. The initial attempt is to show, through the prototype, the capability of the technology, the ability to fulfill the need for clean air in the Space Station, and the potential for future use in air circulation systems in spacecrafts.

At the conclusion of this R&D, the final portable air filter is anticipated to be built for the Space Station (Phase III). Such filtration means shall become available to all areas of nano-technologies, including elimination and control of biological substances such as viruses, pathogens, microbes, etc.

(Supported by NASA: NAS8-00197).

[176]

ODOR TEST RESULTS: APPROVAL OF MICE TO FLY ON SHUTTLE SORTIE MISSIONS IN THE ANIMAL ENCLOSURE MODULE HARDWARE. P. Dalton[1], B.Girten[2], T.A. Bateman[3]. [1]Monell Chemical Senses Center, Philadelphia, PA, [2]NASA Ames Research Center, Moffett Field, CA, [3]BioServe Space Technologies, University of Colorado, Boulder.

For the scientific community, the ability to fly mice in the NASA Ames Animal Enclosure Module (AEM) hardware offers several advantages over the use of rats. These advantages include: the option of testing a range of transgenic animals, the ability to increase the number of animals that can be flown (8 mice vs. 6 rats per AEM), and reduced demands on shuttle resources (food, water, and animal mass) and crew time (for water refill). Mice have been flown in the AEM hardware only once (STS-90 where the mice were dissected early in the mission) whereas rats have been flown in the AEM hardware on 16 missions. This has been due in part to concerns that strong and annoying odors from mouse urine (vs. rat urine) will interfere with crew performance in the Shuttle mid-deck. In order to screen and approve mice for flight, the Monell Center, NASA Ames and BioServe Space Technologies collaborated on a study in order to determine the appropriate conditions, hardware configuration, protocol and analysis method for evaluating the abilities of AEMs to contain odors from mice. Across a 21-day test period, the odor-containment performance of AEMs housing mature female C57BL/6J mice was compared with that of AEMs housing maturing Sprague-Dawley rats, which have flown successfully on numerous prior missions. Human panel ratings were also evaluated against the odor-detection ability of an electronic nose.

Based on the results of this test, approval was given for mice to fly in the AEM hardware, for up to 17 days (total time in AEM including pre-launch and landing contingency). In addition to the odor panel data that led to flight approval, filter dissections and analysis of temperature and humidity conditions were performed as input to improved filter and hardware design for the International Space Station.

[177]

EVALUATION CRITERIA FOR SOLID WASTE PROCESSING RESEARCH AND TECHNOLOGY DEVELOPMENT. J.A. Levri[1], J.A. Hogan[2], M.P. Alazraki[3]. [1]NASA Ames Research Center, [2]New Jersey NASA Specialized Center of Research and Training, [3]Lockheed Martin Space Operations.

A preliminary list of criteria is proposed for evaluation of solid waste processing technologies for research and technology development (R&TD) in the Advanced Life Support (ALS) Program. Completion of the proposed list by current and prospective ALS technology developers, with regard to specific missions of interest, may enable identification of appropriate technologies (or lack thereof) and guide future development efforts for the ALS Program solid waste processing area. An attempt is made to include criteria that capture information about the technology of interest as well as its system-wide impacts.

Some of the criteria in the list are mission-independent, while the majority are mission-specific. In order for technology developers to respond to mission-specific criteria, critical information must be made available on the quantity, composition and state of the waste stream, the waste processing requirements, as well as top-level mission scenario information (e.g. safety, resource recovery, planetary protection issues, and ESM equivalencies). The technology readiness level (TRL) determines the degree to which a technology developer is able to accurately report on the list of criteria. Thus, a criteria-specific minimum TRL for mandatory reporting has been identified for each criterion in the list.

Although this list has been developed to define criteria that are needed to direct funding of solid waste processing technologies, this list possesses significant overlap in criteria required for technology selection for inclusion in specific tests or missions. Additionally, this approach to technology evaluation may be adapted to other ALS subsystems.

[178]

BIOLOGICAL TREATMENT OF SOLID WASTE: COMPOSTING, BIOFILTRATION OF COMPOST OFFGAS, AND NUTRIENT RECOVERY FROM COMPOST. J.A. Hogan, R.M. Cowan, J.C Ramierez-Perez, P.F. Strom, NJ-NSCORT and Department of Environmental Sciences, Cook College, Rutgers University.

Composting is a biological waste treatment technology commonly employed for the management of organic solid wastes (volume reduction, stabilization, and pathogen reduction). It may also prove useful for the recovery of water, carbon dioxide, and plant nutrients. This work was designed to study composting as a waste treatment method for inedible plant biomass and other organic solid wastes (e.g. paper, packaging, food, and potentially human solid waste), and also to address sub-system integration factors including: treatment of composting process off gases; recovery of plant nutrients from compost; and use of compost as a plant growth medium. For this work an integrated composting and biofiltration system has been designed and constructed and methods developed for analysis of compost quality, nutrient extraction from the compost, and the growth of food producing plants on the compost and/or the compost extract. The experimental system consists of a 300 L composting reactor with a thermal conduction control system, an exhaust gas collection and delivery system which allows intermittent collection of exhaust gas with continuous introduction into biofilters, and a system for the growth of wheat on mixtures of compost and Mars simulant, JSC-1. Daily samples of off gas streams from the composter and biofilter, and weekly samples of composted solids are collected for analysis and used in bioassay and compost maturity studies as well as in the growth of wheat. Previous work from this laboratory indicates composting process gases include numerous volatile organic compounds and high concentrations of ammonia. Furthermore, with appropriate design and operation this waste gas can be treated in a bioreactor. Additionally mixtures of compost and JSC-1, have been used successfully as a plant rooting medium. The poster covers details on system design and operation and a summary of results obtained through the end of October 2001.

(Supported by NASA through the NJ-NSCORT.)

[179]

RESEARCH PROGRESS: ALS SOLID WASTE BIOREGENERATIVE RESOURCE RECOVERY. R.F. Strayer, V. Krumins, M. Hummerick. Dynamac, KSC.

Inorganic nutrients can be leached from ALS crop residues and recycled to plant hydroponic growth systems. However, easily biodegraded soluble organics that reduce plant growth are also extracted. The objective of a NASA NRA funded project at KSC is to determine the optimal ALS solid waste bioprocessing system for nutrient recycling. Recent research focus: (1) Design and evaluate a two-stage separation system to remove particulates from bioreactor broth. Prefiltration compared 50, **5***, and 0.5 □m pore sizes and three rinse treatments (none, **DI*, graywater***). Membrane filtration stage compared MF **0.2*** μm vs. UF 500,000 mw cutoff and flow rates (150 vs. **450*** ml min^{-1}). [*=best treatment]; (2) Optimize a continuous stirred tank reactor (CSTR): Compared solids loading rate (160, 240, 320 gdw crop residues) and stirring rate (45, 90, 180, and 360 rpm). Vertical stratification could not be achieved; (3) Design and test a continuous flow fixed-film bioreactor (FFB) to biodegrade soluble organic constituents from crop residue leachate (HRTs compared – 24, 12, **6***, 3, 1.5 hr). BOD removal of 93 – 67%.

[180]

ADVANCED LIFE SUPPORT FLIGHT EXPERIMENTS PROGRAM OVERVIEW. K. M. Hurlbert. EC2/Thermal Systems and Engineering Support Branch, NASA Johnson Space Center, Houston, Texas.

The Advanced Life Support (ALS) Flight Experiments Program provides for the design, development and testing of flight experiments using NASA's KC-135 aircraft and on-orbit vehicles (e.g., Space Shuttle) in support of ALS technology demonstrations in the relevant mission environment. This presentation provides an overview of the planned 2001-2006 program, including the major objectives, a description of each flight experiment to be completed, and details on the implementation process. Near term program emphasis (i.e., for fiscal year 2002) is the ALS KC-135 Flight Experiment Initiative, which was developed due to a lack of manifesting opportunities for ALS payloads on-orbit. Details on previous and planned KC-135 test projects are given to allow ALS Principal Investigators to consider this type of research opportunity in developing their specific technologies.

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Concurrent Posters
IV-G
Advanced Life Support: Systems Biology

[181]

GRAVITROPISM AND PHOTOTROPISM IN PRIMARY AND LATERAL ROOTS OF *ARABIDOPSIS*. M.J. Correll, N.J. Ruppel, and J.Z. Kiss. Botany Dept., Miami Univ., Oxford, OH 45056.

Root growth and development are controlled by many environmental factors including light and gravity. The strong positive graviresponse of roots often masks the weaker phototropic responses thus making it difficult to characterize root phototropism. Although the negative blue or white light phototrophic responses in primary roots have been characterized, it was only through the use of mutants with a weakened graviresponse that a positive red-light phototropism in primary roots recently was demonstrated. The role of light in lateral root development has virtually been unexplored. To better understand root phototropism and the photosensory system involved, we studied the effects of light on lateral root development and the role of phytochromes in phototropism. As in primary roots, lateral roots displayed a negative blue-light phototropism and a positive red-light phototropism. However, lateral roots had an enhanced phototropic response relative to the response of primary roots. Mutant plants with reduced gravitropism and mutants that have deficiencies in phytochrome (A-E) are being used to determine the photoreceptor(s) involved in the phototropic responses. Our results suggest that phytochrome B may be involved in the positive red-light phototropic response in primary roots. Studies are in progress to determine which other member(s) of the phytochrome family is involved in mediating the positive red-light photoresponse.

(Supported by NASA NCC2-1200 and NIH 1R15GM57806-01)

[182]

THE MATHEMATICAL BASIS OF MECHANICAL INSTABILITY AND ITS ROLE DURING PLANT CELL GROWTH. C.Wei[1, 2] and P.M.Lintilhac[1]. [1]Dept. of Botany and Agbiochem, The University of Vermont, and [2]Dept. of Physics, Guangxi University, China.

This poster provides the detailed mathematical background for a separate platform session. Many mechanical structures under load exhibit behavior known as loss of stability. In the case of compressive load, this behavior commonly results in a sudden shift from one equilibrium configuration to another. In the case of tensile load, however, it features an irreversible deformation of the structure. We present a justification for believing that both cases may have a significant impact on our understanding of the behavior of biological structures under microgravity conditions as well as during normal growth. A straight column that is stable in microgravity may become unstable in unit gravity because the increase in the compressive load by its own weight can exceed the critical intensity. We examine, through theory and experiment, the loss of stability in plant cell walls during turgor-driven growth. Our mathematics, when supplied with experimentally derived moduli characteristic of typical primary growth cell walls, yields critical pressure values typical of maximum turgor for growing cells. When supplied with values from mature, non-growing cell walls however, the mathematics correctly predicts critical pressures in excess of typical osmotically driven turgor pressures. This confirms that loss of stability must play a role in the wall loosening process during cell growth.

[183]

SPACEFLIGHT MICROGRAVITY CAUSED AN INCREASE IN THE SENSITIVITY OF *VIGNA MUNGO* ROOTS TO ELECTRICAL STIMULATION. H. Ishikawa[1], C. Wolverton[1], J.L. Mullen[2], and M.L. Evans[1] [1]Dept of Plant Biology, The Ohio State University, Columbus and [2]Department of Biology, Indiana University, Bloomington.

Our previous studies have shown that application of transverse electric fields of 1.5 V cm^{-1} induces electrotropic curvature of *Vigna mungo* roots in ground based experiments but causes growth inhibition without curvature under the microgravity conditions of space (Wolverton et al. J Plant Research 112:493-496). We investigated the physiological basis for this difference in ground based and space flight electrotropic responses by measuring the dependence of growth and electrotropism on applied field strength in ground based experiments under 1 g. Application of higher field strength (e.g. 3 V cm-1) in ground based experiments caused growth inhibition and reduced curvature in a manner similar to that observed in flight experiments using the weaker field strength. The results indicate that the microgravity conditions of space cause an increase in the sensitivity of roots to electrical stimulation. We conclude that induction of electrotropic curvature during space flight will require reduced stimulus strength.

(Supported by the National Space Development Agency of Japan and by NASA grants NAG5-6385, NAG2-1190, and NAG2-1411)

[184]

ACTIN MICROFILAMENTS ARE NOT REQUIRED FOR GRAVITROPISM IN STEMS AND HYPOCOTYLS. K. Yamamoto, R.E. Edelmann, and J.Z. Kiss. Botany Department, Miami University, Oxford OH 45056

The sites of gravity perception are columella cells in roots and endodermal cells in stem-like organs (hypocotyls and inflorescence stems). Localization studies using Alexa Fluor-phalloidin in conjugation with confocal microscopy demonstrated both a longitudinally and transversely oriented actin microfilament (MF) network in endodermal cells of stems and hypocotyls. Latrunculin B (Lat-B) treatment of hypocotyls caused depolymerization of actin MF's in endodermal cells and a significant reduction of hypocotyl growth rates. Actin MF's in Lat-B treated inflorescence stems were also depolymerized, but growth rates were not affected. However, despite the actin MF depolymerization, Lat-B treated stems and hypocotyls exhibited a promotion of gravitropic curvature. In contrast, time course studies demonstrated that Lat-B did not affect root gravitropism but greatly reduced the growth rate. Thus, roots appear to have different mechanisms of gravitropism compared to stem-like organs. Furthermore, our results suggest that actin MF's are not a necessary component of gravitropism in inflorescence stems and hypocotyls.

(Supported by NASA NCC2-1200 and NIH 1R15GM57806-0.)

[185]

LITHIUM ION MODIFICATION OF GRAVITROPIC RESPONSES IN PRIMARY ROOTS OF MAIZE. Timothy J. Mulkey. Life Science Dept., Indiana State Univ., Terre Haute, IN 47809

Lithium has been shown to alter ethylene effects during thigmotropism, inhibit conversion of ACC ((1-aminocyclopropane-1-carboxylic acid) to ethylene, inhibit resynthesis of PIP_2, inhibit inositol-1-phosphate phosphatase. Work in our laboratory has implicated auxin-induced ethylene production and intermediaries in the second messenger system in the growth and gravitropic response of primary roots of maize. Li ions at concentrations greater than 0.1 mM promote the elongation rate of primary roots of maize. Asymmetric application of Li to graviresponding roots alters the kinetics of curvature. Application of Li ions to the lower surface of roots delays positive gravicurvature or results in negative gravicurvature. Lithium ions enhance the phosphorylation of specific protein species while suppressing the phosphorylation of other protein species. The pattern of phosphorylation that is observed as a result of lithium treatment is similar to the pattern observed upon treatment of maize roots with promotive concentrations of indole-3-acetic acid (IAA) when ethylene biosynthesis has been inhibited using AVG (aminoethoxyvinyl glycine). Lithium ions alter the dose response of root elongation to exogenously applied indole-3-acetic acid; lithium pretreatment of maize roots results in promotion of elongation by IAA concentrations that are normally inhibitory in the absence of AVG. Distinct phosphorylation patterns obtained from cytoplasmic and membrane fractions of roots which are exposed to inhibitory concentrations of indole-3-acetic acid can be suppressed by pretreatment of root tissue with 0.1 mM lithium; the resulting phosphorylation profile exhibited by these Li/inhibitory-IAA concentration treated roots are almost identical to the phosphorylation profile of IAA-stimulated root tissues. This data suggests the alteration of IAA sensitivity, directly or indirectly, via alteration of ethylene action/activity by lithium ions.

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CYANOBACTERIAL GROWTH UNDER ANOXIC AND CARBOXIC CONDITIONS. David J. Thomas, Jamie L. Liles, Amanda L. Price and Shannon L. Sullivan. Science Division, Lyon College, Batesville, AR 72501

According to current theories of early biosphere evolution, cyanobacteria and similar organisms produced most of the oxygen found in Earth's atmosphere. Early photosynthetic organisms would have adapted to an atmosphere that was rich in CO_2 and poor in O_2. However, many modern cyanobacterial species do not grow under these conditions. We are currently investigating the tolerance of several cyanobacterial species to very high (>20%) concentrations of atmospheric CO_2. Cultures of *Synechococcus*, *Synechocystis*, *Plectonema boryanum* and *Anabaena* were grown in liquid culture and bubbled with CO_2-enriched air. Culture growth was monitored by measuring optical density. None of these cyanobacteria tolerated direct transfer from ambient air CO_2 to 40% CO_2. *Plectonema* and *Anabaena* tolerated direct transfers from ambient to 20% CO_2, but *Synechococcus* PCC7942 and *Synechocystis* grew poorly under this atmosphere. Research in progress indicates that PCC7002, *Plectonema,* and *Anabaena* tolerate CO_2 concentration up to 100% when gradually increased by 10% per day. Additional research to differentiate between CO_2, O_2 and pH effects is currently in progress and will be reported. In many ways (except for the pressure), Earth's early atmosphere may have resembled Mars' current atmosphere. High CO_2 concentrations would be expected in applications such as a Martian exploratory base or greenhouse. In addition, this research provides insight into the possibilities, however remote, of forward-contamination of Mars by robotic and human exploration, and the survival of such contaminants

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EFFECTS OF VECTOR-AVERAGED GRAVITY ON ENDOTHELIAL CELLS. A. Higashibata[1], M. Imamizo[2], N. Ishioka[1] and M. Takaoki[1]. [1]Space Utilization Research Center, Space Utilization System, National Space Development Agency of Japan, [2]Space Development Division, Advanced Engineering Service Co., Ltd, Tsukuba, Ibaraki, JAPAN.

In previous studies, the effect of gravity changing on cellular events has been observed, but the gravity-sense mechanism of cells is unclear. The goal of this study is to reveal "the gravity sensor in cells". We have investigated morphologically the cytoskeletal changes of endothelial cells cultured under vector-averaged gravity condition (clinorotation). Total number of viable cells was decreased by rotation and the ratio was 80% of static cultured cells. Most of cells widely spread the size about twice of static cells. Cytoskeletal formations (F-actin and microtubules) of clinorotated bovine endothelial cells were clearly different from those of static cultured cells. Tubulin and actin stain image show that the gravity changes may affect cytoskeletal formation of cells. Two dimensional electrophoresis pattern of cytosol protein showed two characteristic spots, ranging in the area of pI 3.5 to 4.0 and of molecular weight from 40k to 60k. These proteins data have not been uploaded on protein database.

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POST SUSPENSION PLASMA CATECHOLAMINE LEVELS AND THE EFFECTS OF CLENBUTEROL. I.K. Abukhalaf[1,2,3], D.A. von Deutsch[1,2], S.A. Abera[1,2], S.W. Sahlu[1,2], N.A. Silvestrov[2,3], D.E. Potter[1,2], R.R.Roper[1,2]. [1]Space Medicine and Life Sciences Research Center, [2]Department of Pharmacology & Toxicology, and the [3]Clinical Research Center, Morehouse School of Medicine, Atlanta, GA 30310

Many of the physiological effects observed in adaptation to a microgravity environment and upon readaptation to 1 ***g*** may be associated with changes in the regulation of the autonomic nervous system. In particular, the sympathetic nervous system (SNS) is an important regulator of cardiovascular functions and intermediary metabolism, especially in times of stress. The result of SNS stimulation is the release of norepinephrine (NE) which in turn acts on adrenoceptors located on postsynaptic sites. During stress epinephrine (E), which acts equally on $ß_1$ and $ß_2$ adrenoceptors, is released from the adrenal medulla into the blood where it is transported to target tissues (e.g. heart, vasculature, and skeletal muscles). The purpose of these experiments were: 1) to determine the effect of hindlimb suspension and readaptation (30 minutes post-suspension) in the presence and absence $ß_2$-adrenergic agonist [clenbuterol (Cb), 1 mg/kg] on plasma catecholamine (NE, E) levels. Cb treatment may help lessen the impact of hindlimb suspension and the initial period of readaptation to 1 ***g*** conditions on plasma catecholamine and polyamine levels. Preliminary data suggest that after 30 minutes of recovery from 30 days of hindlimb suspension, plasma NE and E levels, and E/NE ratio were significantly higher in post-suspended rats than in non-suspended controls. In non-suspended rats, treatment with Cb had no effect on plasma NE but significantly reduced plasma E concentrations. In hindlimb suspended rats, Cb reduced catecholamine levels to nearly the level of the non-suspended controls. The relationship/interplay of catecholamines and $ß_2$-adrenergic agonist treatment may provide an insight into possible mechanisms involved in orthostatic hypotension and skeletal muscle atrophy experienced by astronauts during readaptation. This work was supported, in part, by NASA grant NCC9-112 (I.K.A) and NIH grant RCRII 2P20 RR11104-07 (I.K.A).

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EFFECT OF ACUTE MICROGRAVITY EXPOSURE ON THE EYE MOVEMENT IN HUMANS. T. Wakatsuki[1], T. Nomura[1, 2], F. Kawano[1], A. Ishihara[3], G. Mitarai[4], T. Kasai[1], and Y. Ohira[1]. [1]Sch. Health Sport Sci., Osaka Univ., Osaka, [2]Res. Ctr. Health Phys. Fit. Sports, Nagoya Univ., Nagoya, [3]Fac. Integ. Human Studies, Kyoto Univ., Kyoto, and, [4]Chyukyo Univ., Toyota, Japan.

The responses of eye movements to changes in gravity level during parabolic flight of a jet airplane (Mitsubishi MU-300, Diamond Air Service, Nagoya) were examined in 4 healthy male subjects. The parabolic flights were repeated 13~15 times during one-hour experimental period. The experiment was performed four times on different day. Various levels of gravity (Gz) between 0 and 2 were created during the ascending and descending periods. The eye movement was estimated by recording EOG. The subject remained seated keeping the seat belt fastened throughout the one-hour experimental period. Eye movements were measured during one-point gaze, right-left neck swinging, and 4-point tracking. Each trial was maintained throughout one parabolic flight. The frequency of blink during one-point gaze was gravity-dependent. The subjects blinked approximately once per second at 2-G, but the frequency at □-G was ~1 per 3 seconds. However, no significant effects of gravity level were observed in other parameters.

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EFECTS OF HYPERGRAVITY DURING PREGNANCY ON OVARIAN-HYPOPHYSEAL FUNCTION. H.W. Burden[1], J.T. Zary[1], C.A. Hodson[2], H. Gregory[2] and A.E. Ronca[3]. [1]Dept. of Anatomy & Cell Biology, [2]Obstetrics & Gynecology, Brody School of Med., East Carolina Univ., Greenville, NC and [3]NASA Ames Res. Ctr., Moffett Field, CA.

Little is known about the effects of altered gravity on ovarian function. In the present study, the effects of hypergravity, (produced by centrifugation) during gestation days (G) 11-20 on ovarian antral follicle and luteal populations and selected ovarian and hypophyseal hormones were evaluated. Pregnant rats were assigned to hypergravity (HG) 2.0-g, HG 1.75-g, HG 1.50-g, rotational control (RC) 1.03-g, and stationary control (SC) groups (N=10 each group). Five animals in each group were euthanized on G20 and five remaining animals in each group were euthanized 3 hours postpartum. Hypergravity at all levels decreased the percent body mass gain during the interval G11-G20, however the wet weight of the pituitary and ovaries was not changed. There was no effect of hypergravity on the number of healthy or atretic antral follicles at any size at G20 or postpartum. The number of corpora lutea of pregnancy was decreased in all hypergravity groups, but the number of live fetuses (G20) or pups (term) was not altered. Plasma concentrations of LH, FSH, prolactin, and progesterone were not altered at G-20 or postpartum. Lastly, pituitary content of LH, FSH, and prolactin was not altered by hypergravity at G20, but LH content was slightly increased postpartum. Collectively these data indicate that hypergravity, initiated during the postimplantation period of pregnancy, and concluded at G20, is compatible with maintenance of pregnancy and parturition and has minimal effects on hypophyseal parameters. Ovarian follicles are not altered by hypergravity, but corpora lutea may regress at a more rapid rate.

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GENETIC AND DEVELOPMENTAL STABILITY *IN DROSOPHILA MELANOGASTER* EXPOSED TO HYPERGRAVITY, VIBRATION, AND CONSTANT RADIATION. J.N. Thompson, Jr.[1], and R.C. Woodruff [2]. [1]Dept of Zoology, University of Oklahoma, Norman; [2]Dept of Biological Sciences, Bowling Green State University, Bowling Green, OH.

Ground-based studies are being done to estimate mutation rate, aneuploidy, somatic mutation, and fluctuating asymmetry (by var|[R–L]/0.5[R+L]| for several morphological traits) in *Drosophila melanogaster* exposed to hypergravity, vibration, and long-term low levels of radiation. Hypergravity, vibration, and continuous exposure to gamma radiation (1 rad/day) test a range of stresses that organisms might be exposed to on shuttle vehicle launch or other periods of time in a space environment. *Drosophila* are treated using the 1-Foot Diameter Centrifuge (2 - 5 g) and a computer-controlled Vibration Table (20 - 1000 Hz) at NASA/Ames Research Center. Data from the *zeste* test show an increased rate of aneuploidy from nondisjunction in flies exposed to extended periods (2 to 4 hours) of 5 g (2h: 12/24,768 = 0.048%; 4h: 43/114,557 = 0.038%) and to vibration, particularly in the 150-1000 Hz range (full range: 26/79161 = 0.033%; 150-1k range: 7/11,663 = 0.060%), compared to the control frequency (18/100,163 = 0.018%). Sex-linked lethal mutation rate estimates so far indicate about a two-fold increase at 5 g (11/4599 = 0.24%) compared to 1 g controls (8/7925 = 0.10%). The 2g treatments approximate control levels. These data will be used to define ground-based controls for proposed multi-generation mutation screens on the International Space Station. To test a new genetic screen for mutation accumulation on X chromosomes balanced in females, we are measuring the long-term effect of the accumulation of spontaneous and gamma-ray-induced deleterious mutations on viability. Viability affected by spontaneous genetic damage decreases with time, and longevity decreases at an even faster rate in gamma-ray treated *Drosophila*.

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PREDICTING ANTIBIOTIC EFFICACY IN SPACE FLIGHT AND ON THE GROUND. E. B. Juergensmeyer [1], M. A. Juergensmeyer [2] and E. S. Nelson [3]. [1]Judson College, Elgin, IL, [2] Montana State University, Bozeman, MT, and [3] NASA-Glenn Research Center, Cleveland, OH

Several species of bacteria have shown an increase in antibiotic resistance after space flight. Previously, we have shown that this increase in resistance is not universal, nor is there any discernable pattern based on the mode of action of the antibiotic. The ability to choose an effective antibiotic is as vital in space as on the ground. Therefore, we have compared the antibiotic effectiveness seen in ground cultures to that seen in space flown cultures. It is possible to evaluate such effectiveness by doing disk tests, using antibiotic impregnated disks and measuring the zone of inhibition (ZOI) of bacterial growth. Comparison of ZOIs of bacteria which spent three months on the Russian Space Station MIR with those grown on the ground show that it is not possible to predict antibiotic effectiveness in space flight from ground data. Neither is there a relationship between mode of action of the antibiotic and its effectiveness.

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A Mission To Mars

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MISSION TO MARS: GETTING THERE FROM HERE. K. Olsen, C. Barnes. Office of Biological and Physical Research, NASA Headquarters, Code U, Washington, D.C. 20546

NASA has a mission to explore the universe. In spite of short-term challenges that sometimes seem to cause our progress to the stars to be measured in centimeters rather than kilometers, in the Office of Biological and Physical Research, we are keeping our focus on the long range goals of answering basic and exploration-enabling questions: 1) How do the fundamental laws of nature shape the evolution of life? 2) How can humans safely and effectively utilize the space environment for research, human habitation and commercial benefit?

Creating a safe and productive environment beyond low-Earth orbit in which future astronauts can live and work comfortably and efficiently during 1000-day class missions is a formidable challenge that will require many breakthroughs and dramatic steps forward in science and technology development. NASA's Advanced Life Support Program will be a key player in enabling such long-duration missions.

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ADVANCED LIFE SUPPORT FOR A HUMAN MISSION TO MARS. D.L. Henninger, NASA - Johnson Space Center, Houston.

Life support systems are an enabling technology and are integral to the success of human space exploration. As NASA prepares for long-duration missions it becomes imperative, for considerations of both safety and cost, to minimize consumables and increase the autonomy of the life support systems. Utilizing advanced life support technologies provide this autonomy and increase productivity of the mission by reducing mass, power, and volume, necessary for human support, thus permitting larger payloads for science and exploration. Two basic classes of life support systems must be developed, those directed toward applications on board a transit vehicle and those directed toward applications in habitats on planetary surfaces. In general, it can be viewed as those systems compatible with microgravity and those compatible with hypogravity environments. The goal of the Advanced Life Support Program is to provide life support self-sufficiency for human beings to carry out research and exploration productively in space and for benefits on Earth. To accomplish this goal, five major technical objectives have been identified:

1. Provide technologies that significantly reduce life cycle costs, improve operational performance, promote self-sufficiency, and minimize expenditure of resources for long-duration missions.
2. Develop and apply methods of systems analysis and engineering to guide investments in technology, resolve and integrate competing needs, and guide evolution of technologies.
3. Resolve issues of microgravity performance through space flight research and evaluation.
4. Ensure timely transfer of new life support technologies to missions.
5. Transfer technologies to industrial and residential sectors for national benefit.

To accomplish these objectives, the Advanced Life Support Program is conducting a focused Research and Development program to advance technology readiness of regenerative life support and thermal control components, validate regenerative life support technologies integration through long-term testing with humans, and identify terrestrial applications for life support technologies.

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FLUID PROCESSES AND THEIR SIGNIFICANCE FOR SPACE AND PLANETARY EXPLORATION. J. I. D. Alexander. Department of Mechanical and Aerospace Engineering and National Center for Microgravity Research on Fluids and Combustion, Case Western Reserve University, Cleveland, Ohio.

The prediction of the long-term behavior of fluids and fluid processes under weightless conditions is a significant part of the problem of designing reliable environmental control and life support systems. Such systems control humidity and temperature, supply and revitalization of the vehicle's atmosphere and water, waste and food management. Particularly important for long-duration space travel is the development of efficient combinations of closed and open loop systems that will enable support for prolonged periods. Mass constraints, infrequent resupply opportunities, and the ability to operate under conditions ranging from weightlessness to planetary gravity also place stringent requirements on their design. The need for reliable, durable life support systems and the limitations on fluid processes associated with sustained operation under weightless or reduced gravity conditions require the development of improved technology in the area of fluid management under low gravity conditions. In addition to life support and environmental control systems, fluid flows occur in almost every biological process ranging from molecular and sub-cellular scales to entire living systems. At the molecular and intracellular level, fluids processes are relevant to signaling pathways that transduce stimuli associated with gene activation, while on a larger scale, fluid dynamics and the transport of mass and heat play a crucial role in both macro- and micro circulatory process (e.g., cardiovascular and pulmonary systems). The effect of weightlessness on fluid behavior in these systems also needs to be better understood. In this talk, examples of fluid behavior under weightless conditions will be used to illustrate the importance of understanding and predicting fluid behavior under weightless and reduced gravity conditions and areas of fluids research that are critical to the development of advanced life support systems will be discussed.

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PLANT RESEARCH FOR SPACEFLIGHT AND PLANETARY BASES. R.M. Wheeler, NASA Biological Sciences Branch, Kennedy Space Center, FL.

Studies of biological approaches for life support have been ongoing for over 40 years. Early efforts were sponsored the US Air Force, Navy, and NASA in the late 1950s and early 1960s. These tests focused on algal systems (e.g., *Chlorella*), with several demonstrations of closed life support using the photosynthetic organisms and animals, and a spaceflight demonstration of plant photosynthesis by C.H. Ward and colleagues (1966). Activities in the US diminished in the 1970s but flourished as part of the BIOS projects at the Institute of Biophysics in Krasnoyarsk, Russia. The BIOS projects continued into the 1980s and included several demonstrations of human life support using algae and plants. NASA initiated the Controlled Ecological Life Support (CELSS) Program ca. 1980, which sponsored several university projects on controlled environment production of crops (*e.g.*, wheat, soybean, lettuce, potato, and sweetpotato). Findings from these studies showed that crop yields under high light, CO_2 enrichment, and hydroponic culture could easily surpass world record yields from field settings. The university findings were then used to conduct large scale, closed atmosphere tests in the Biomass Production Chamber (BPC) at Kennedy Space Center. BPC studies using moderate to high lighting suggested that ~40-50 m^2 of plants could provide dietary energy needs for ~one human, the O_2 for ~ two humans, and clean water for ~ four humans.

Plant testing for life support continues today at universities, NASA centers, and laboratories in Europe, Japan, and Russia. Key targets for future bioregenerative life support applications include the BIO-Plex at NASA's Johnson Space Center, the Closed Ecological Experiment Facility (CEEF) in Japan, and a proposed vegetable production system for ISS. BIO-Plex and CEEF will include human crews and provide integrated testbeds for life support technologies (biological and physico-chemical). The vegetable production unit proposed for ISS would be a relatively small system (~0.5 m^2) for producing fresh foods to supplement the crew diet.

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WASTE PROCESSING IN A LONG-DURATION SPACE MISSION

J.A. Hogan. NJ-NSCORT and Department of Environmental Sciences, Rutgers, The State University of New Jersey.

While certain requirements regarding Advanced Life Support (ALS) waste processing operations are mission-independent (e.g. wastes at a minimum must be contained), the majority will display a high degree of mission specificity. For example, waste processing and resource recovery is typically minimized for missions of short duration or those with frequent re-supply, as it is more economical to contain wastes and return them to Earth. Conversely, as mission duration and/or the need for self-sufficiency increases, waste processing requirements will become more demanding and recovery/reuse of certain fractions more economical.

Several system design drivers will determine specific waste management requirements including: treatment requirements; resource recovery; microbial and operational safety; planetary protection constraints; system integration; and equivalent system mass (ESM) minimization. Although future long-range mission architectures have been outlined in the Reference Missions Document (JSC-39502), and waste processing systems preliminarily assigned, certain waste management requirements remain in a state of uncertainty. This is due in part to undefined future waste quantity and composition, resource recovery constraints, and future technology improvements and substitutions both in waste processing and other interfacing sub-system components. Further overall system modeling and analysis is required to more accurately establish mission-dependent requirements, particularly those concerning resource recovery, consumable usage (e.g. O_2), and secondary product formation (e.g. CO_2). These analyses will also facilitate effective guidance regarding funding of waste processing research and technology development.

In this presentation, both technical and overall program challenges will be discussed, and a brief survey of candidate technologies currently undergoing development will be presented.