GENERAL INFORMATION

GRAVITATIONAL AND SPACE BIOLOGY BULLETIN (ISSN 1089-988X) is a journal devoted to research in gravitational and space biology. It is published by the American Society for Gravitational and Space Biology, a non-profit organization whose members share a common goal of furthering the understanding of the biological effects of gravity and the use of the unique environment of spaceflight for biological research. The Bulletin is overseen by a steering committee consisting of the Publications Committee, the Bulletin Editor, the President, and the Secretary-Treasurer of the ASGSB. This issue of Gravitational and Space Biology Bulletin was printed at City Press, Tucson, Arizona.

The American Society for Gravitational and Space Biology was created in 1984 to provide an avenue for scientists interested in gravitational and space biology to share information and join together to speak with a united voice in support of this field of science. The biological effects of gravity have been acknowledged since Galileo’s time, but only in this century has gravitational biology begun to attract attention. With the birth of the space age, the opportunity for experimentation over the full spectrum of gravity finally became a reality, and a new environment and research tool became available to probe biological phenomena and expand scientific knowledge. Space and spaceflight introduced new questions about space radiation and the physiological and psychological effects of the artificial environment of spacecraft.

The objectives of ASGSB are:

- To promote research, education, training, and development in the areas of gravitational and space biology and to apply the knowledge gained to a better understanding of the effect of gravity and space environmental factors on the flora and fauna of Earth.
- To disseminate information on gravitational and space biology research and the application of this research to the solution of terrestrial and space biological problems.
- To provide a forum for communication among professionals in academia, government, business, and other segments of society involved in gravitational and space biological research and application.
- To promote the study of concepts and the implementation of programs that can achieve these ends and further the advancement and welfare of humankind.

MEMBERSHIP: The American Society for Gravitational and Space Biology welcomes individual, organizational, and corporate members in all of the basic and applied fields of the space and gravitational life sciences. Members are active in the fields of space medicine, plant and animal gravitational physiology, cell and developmental biology, biophysics, and space hardware and life support system development. Membership is open to nationals of all countries. Members must have education or research or applied experience in areas related to the Society’s purposes: i.e., Doctorate, Masters with 2 years experience, Bachelors with 4 years experience (student members must be actively enrolled in an academic curriculum leading toward a career related to the Society’s purposes), or special appointment by the Board of Directors. Membership applications may be obtained by writing the American Society for Gravitational and Space Biology, P.O. Box 12247, Rosslyn, VA 22219.

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Program and Abstracts

Fourteenth Annual Meeting

AMERICAN SOCIETY
FOR GRAVITATIONAL AND SPACE BIOLOGY

October 28-31, 1998
Adam's Mark Houston Hotel
Houston, TX
SHORT PROGRAM

AMERICAN SOCIETY
FOR GRAVITATIONAL AND
SPACE BIOLOGY

FOURTEENTH ANNUAL MEETING
October 28-31
Adam's Mark Houston Hotel
Houston, TX

***** Wednesday, October 28, 1998 *****

11:00 am  Registration opens
1:00 - 6:00 pm  Tour of JSC
7:00 pm  Set up Posters
7:00 pm  Student Mixer
7:30 pm  ASGSB Governing Board Meeting I

***** Thursday, October 29, 1998 *****

7:00 - 8:30 am  ASGSB Committee Meetings
7:30 am  Registration Opens
8:30 - 8:40 am  Welcome, Jackie Duke
8:40 - 10:00 am  Session A: Biominalization Symposium
10:00 - 10:30 am  Break
10:30 - 12:30 pm  Session A: Biominalization Symposium (cont.)
12:30 - 2:00 pm  Lunch
2:00 - 3:30 pm  Session B: Concurrent Poster Session I
  Space Life Sciences Training Program
  Undergraduate Student Poster Competition
  Graduate Student Poster Competition

3:30 - 5:00 pm  Session C: Concurrent Poster Session II
  Space Life Sciences Training Program (continued)
  Undergraduate Student Poster Competition (continued)
  Graduate Student Poster Competition (continued)

7:00 - 9:00 pm  Reception
9:00 pm  Space Biology Research Associates Annual Meeting
***** Friday, October 30, 1998 *****

7:00 - 8:30 am  ASGSB Committee Meetings (Breakfast)
8:40 - 10:00 am  Session D: Biotechnology Symposium
9:50 - 10:20 am  Break
10:20 - 12:30 pm  Session D: Biotechnology Symposium (cont.)
12:30 - 2:00 pm  Lunch; ASGSB Committee Meetings
2:00 - 4:00 pm  Session E: Oral Session - Plant Biology I
4:00 - 5:30 pm  Session F: Concurrent Poster Session III
                  Animal Development and Growth I
                  Animal Gravity Sensing I
                  Animal Structural Systems I
                  Biotechnology/Instrumentation I
                  Cell Biology I
                  Collaborative Ukrainian Experiment I
                  Plant Biology II
                  Space Biomedical Research I
                  Spaceflight Experiment Results I

6:30 - 9:00 pm  Banquet/Business Meeting/Awards Presentation
9:00 pm  ASGSB Governing Board Meeting II

***** Saturday, October 31, 1998 *****

HAPPY HALLOWEEN

8:15 - 10:00 am  Session G: Oral Session - Spaceflight Experiment Results II
10:00 - 10:30 am  Break
10:30 - 12:30 pm  Session H: Oral Session - Spaceflight Experiment Results III
12:30 - 2:00 pm  Lunch
2:00 - 4:00 pm  Concurrent Oral Sessions
                  Session I: Cell Biology and Biotechnology/Instrumentation
                  Session J: Space Biomedicine and Structural Systems
4:00 - 5:30 pm  Session K: Concurrent Posters IV
                  Animal Development and Growth II
                  Animal Gravity Sensing II
                  Animal Structural Systems II
                  Biotechnology/Instrumentation II
                  Cell Biology II
                  Collaborative Ukrainian Experiment II
                  Plant Biology III
                  Space Biomedical Research II
                  Spaceflight Experiment Results IV
AMERICAN SOCIETY FOR
GRAVITATIONAL AND SPACE BIOLOGY

FOURTEENTH ANNUAL MEETING
October 28-31, 1998

Adam’s Mark Houston Hotel
Houston, TX

PROGRAM

WEDNESDAY, OCTOBER 28, 1998

11:00 am  Registration opens
1:00 - 6:00 pm  Tour of JSC
7:00 pm  Set up Posters
7:00 pm  Student Mixer
7:30 pm  ASGSB Governing Board Meeting I

THURSDAY, OCTOBER 29, 1998

7:00 - 8:30 am  ASGSB Committee Meetings
7:30 am  Registration Opens
8:30 - 8:40 am  Welcome, Jackie Duke

SESSION A: BIOMINERALIZATION
SYMPOSIUM

8:40 am to 12:30 pm  Moderator: Emily Morey-Holton

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THURSDAY AM/PM

PROGRAM 1998 ANNUAL MEETING

TIME

10:00  BREAK

10:30  AN OVERVIEW OF VERTEBRATE MINERALIZATION WITH EMPHASIS ON COLLAGEN-
MINERAL INTERACTION.  W.J. Landis [3]  4

11:10  LOCAL REGULATION OF ENDOCHONDRAL DEVELOPMENT BY STEROID HORMONES

11:50  ILLUSTRATIVE DISORDERS OF ECTOPIC SKELETAL MORPHOGENESIS:
A CHILDHOOD PARALLAX FOR STUDIES IN GRAVITATIONAL AND SPACE BIOLOGY.

12:30  LUNCH

SESSION B: CONCURRENT POSTERS I

2:00 to 3:30 pm

NOTE: Presenters are to be next to their posters the entire time.

Space Life Sciences Training Program

POSTER #

A01  THE FOURTEENTH ANNUAL SPACE LIFE SCIENCES TRAINING PROGRAM AT

A03  NASA SPACE LIFE SCIENCES TRAINING PROGRAM: STUDIES BY THE GRAVITA-
TIONAL BIOLOGY EMPHASIS GROUP.  K. Leanza, W. Piastuch, D. Stutes, Y. Hua,
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A05  NASA SPACE LIFE SCIENCES TRAINING PROGRAM: EXPERIENCING PROJECT
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EXPERIMENT.  P. Currier, D. Thomas, R. Hall, S. Hart, A. Fitch, C. Gunn, J. McIntire,
and M. Soliman.  [8]  7

Undergraduate Student Poster
Competition

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B03  GRAVITROPISM AND PHOTOTROPISM OF FLOWER STALKS IN ARABIDOPSIS.
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B05  CONCEPTUAL DESIGN FOR A MICROGRAVITY RESISTANCE TRAINING UNIT:

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<td>J.R. Ascher, O. van den Ende, and D. Thomas.</td>
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<td>SENSITIVITY OF LETTUCE AND WHEAT SEEDLINGS TO VARIOUS SURFACTANTS.</td>
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<td>THE STUDY OF LIMULUS POLYPHEMUS ALONG THE SHORELINE OF MERRITT ISLAND NATIONAL WILDLIFE REFUGE (MINWR).</td>
<td>A.S. Gavurnik, and G. Ehlinger.</td>
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<td>L.K. Barger and C.A. Fuller.</td>
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SESSION C: CONCURRENT POSTERS II

3:30 to 5:00 pm

NOTE: Presenters are to be next to their posters the entire time.

Space Life Sciences Training Program

POSTER #


Undergraduate Student Poster Competition (continued)

B02  RICE-PATHOGEN INTERACTIONS: A MODEL SYSTEM FOR SPACE-FLIGHT EXPERIMENTS. A. Chambers, M.J. Ryba-White, E. Hilaire, J.A. Guikema, and J.E. Leach [32]

B04  ALGAL AND CYANOBACTERIAL GROWTH IN 100% CO₂. R.D. Hall, P.A. Currier, and D.J. Thomas. [33]

B06  THE EFFECTIVENESS OF THREE PHARMACEUTICAL COUNTERMEASURES IN PREVENTING VESTIBULAR DISORIENTATION WHILE MAINTAINING COGNITIVE ABILITY. R.K. Bashya, D. Woodard, and D.J. Thomas. [34]

B08  EFFECT OF pH ON IGEPON*SOAP DEGRADATION UNDER DENITRIFYING CONDITIONS. V. Hsu, M. Alazraki, and R.F. Strayer [35]

B10  PSEUDOMONAS AERUGINOSA: PERSISTENCE IN THE RHIZOSPHERE. S.J. Bernick, A. Matos, and J.L. Garland [36]

B12  CREATING A SOIL ALTERNATIVE FROM CLASSROOM MATERIALS: A PROJECT DESIGN. B. Weber and M. Lewandowski [37]

B14  COMPARING THE EFFEC TIVENESS AND PRECISION OF TWO TECHNIQUES FOR SEDIMENT SAMPLING AT JOHN F. KENNEDY SPACE CENTER. T.L. Hollar, M. Mota, and D.M. Scheidt [38]
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<td>EFFECTS OF 14-DAY HINDLIMB UNLOADING ON RAT CEREBRAL, SPLENIC, AND MESENTERIC ARTERIAL MORPHOLOGY. M.K. Wilkerson, J.M. Delp, P.N. Colleran, and M.D. Delp [40]</td>
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<td>HINDLIMB SUSPENSION ALTERS ARTERIAL MORPHOLOGY IN RAT HINDLIMB SKELETAL MUSCLE. P.N. Colleran, J.M. Delp, M.K. Wilkerson, and M.D. Delp [41]</td>
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<td>GRAVITY INDUCED CALCIUM CURRENTS IN GERMINATING FERN SPORES. A. Chatterjee, M. Porterfield, P.J. Smith, and S.J. Roux [45]</td>
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<td>REGULATION OF TCH4 GENE EXPRESSION IN ARABIDOPSIS THALIANA. E.A. Iliev, W. Xu, and J.A. Braam [47]</td>
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<td>LOW OXYGEN ALTERATIONS IN ARABIDOPSIS LEAF STRUCTURE RESEMBLE BRASSINOLIDE-DEFICIENT MUTANTS. K.M. Ramonell and M.E. Musgrave [48]</td>
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<td>GROWTH IN MICROGRAVITY INCREASES SUSCEPTIBILITY OF SOYBEAN SEEDLINGS TO A FUNGAL PATHOGEN. M. Ryba-White, O. Neduksa, E. Hilaire, J.A. Guikema, E. Kordyum, and J.E. Leach [49]</td>
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7:00 - 9:00 pm  Reception

9:00 pm  Space Biology Research Associates Meeting
FRIDAY, OCTOBER 30, 1998

7:00 - 8:30 am  ASGSB Committee Meetings

SESSION D: BIOTECHNOLOGY SYMPOSIUM

8:30 am to 12:20 pm  Moderator: Paul Todd

8:30
PROTEIN CRYSTAL GROWTH AND THE INTERNATIONAL SPACE STATION.  
L.J. DeLucas, T. Bray, K. Moore, and C. Nicolet [51]

9:10
GRAVISENSING, APOPTOSIS, AND DRUG RECOVERY FROM TAXUS CELL SUSPENSIONS.  
D.J. Durzan [52]

9:50
BREAK

10:20
MICROGRAVITY TISSUE ENGINEERING: DEVELOPMENTAL AND FUNCTIONAL STUDIES.  
L.E. Freed and G. Vunjak-Novakovic [53]

11:00
ENGINEERING PLANTS FOR SPACEFLIGHT ENVIRONMENTS.  
B. Bugbee [54]

11:40
TRANSGENIC MODELS TO STUDY REPRODUCTION, ONCOGENESIS, AND DEVELOPMENT.  
M.M. Matzuk [55]

12:20 - 2:00 PM  LUNCH/ASGSB COMMITTEE MEETINGS

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2:00
ROOT PHOTOTROPISM AND GRAVITROPISM IN WILD-TYPE AND STARCHLESS MUTANTS OF ARABIDOPSIS  
S. Vitha and F.D. Sack [56]

2:15
THREE-DIMENSIONAL ULTRASTRUCTURE OF LENTIL ROOT CAP STATOCYTES GROWN IN SPACE.  
J.D. Smith, S. Burwen, N. Marinkovich, D. Driss-Ecole and G. Perbal [57]

2:30
CO-LOCALIZATION OF ACTOMYOSIN AND CALRETICULIN AT AMYLOPLASTS PERIPHERIES: POSSIBLE IMPACTS FOR GRAVIPERCEPTION.  
D. Volkmann, M. Pilger and F. Baluska [58]

2:45
AMYLOPLAST MAGNETOPHORESIS MIMICS GRAVITROPISM IN SINGLE-CELL GRAVISENSING MOSS PROTONEMATA.  
O.A. Kuznetsov, J. Schwuchow, F.D. Sack, and K.H. Hasenstein [59]

3:00
FUNCTIONAL DOMAINS AND THE GENOMIC STRUCTURE OF CHIMERIC CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE.  
B.W. Poovaiah, Z. Liu, W. Wang, and P.V. Sathyanarayanan [60]
PROGRAM 1998 ANNUAL MEETING

TIME

3:15 FUNCTION AND REGULATION OF TCH2 EXPRESSION IN ARABIDOPSIS. K.A. Johnson and J. Braam [61] 30

3:30 CELLULAR, MOLECULAR, AND ELECTROPHYSIOLOGICAL CHANGES DUE TO DEVELOPMENTAL POLARITY INDUCED BY GRAVITY IN SINGLE CELLS. S.J. Roux, A. Chatterjee, D.J. Eastburn, W.M. Hanson, M. Porterfield, and P.J. Smith [62] 30

3:45 GRAVITRESPONSE OF luzy-2 TOMATO SEEDLINGS IS NOT AFFECTED BY CURVATURE-INDUCING MAGNETIC GRADIENTS. K.H. Hasenstein and O.A. Kuznetsov [63] 30

SESSION F: CONCURRENT POSTERS III

4:00 to 5:30 pm

NOTE: Presenters are to be next to their posters the entire time.

Animal Development and Growth I

POSTER #

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D03 HYPERGRAVITY & MICROGRAVITY EFFECTS TO AVIAN INNER EAR STRUCTURES. H. Hara, T. Jones and C.D. Fermin [65] 32

D05 SKELETAL TISSUE GROWTH AND DEVELOPMENT IN THE NASA BIOREACTOR. B.J. Klement, B.J. George, and N.D. Houston [66] 32


Animal Gravity Sensing I

E02 FERROELECTRIC-LIKE PROPERTIES OF HORNET STRUCTURES OR CONSTRUCTION. J.S. Ishay and L. Litinetsky [68] 34

E04 GROWTH RATE VARIABILITY OF LAMELLAR BONE IN RATS EXPOSED TO MACROGRAVITY (2G+). T.G. Bromage, I. Smolyar, S.B. Doty, and E. Holton [69] 34

Animal Structural Systems I

F01 EFFECTS OF HINDLIMB UNLOADING ON THE VASO-CONSTRICTOR RESPONSIVENESS OF SKELETAL MUSCLE ARTERIOLES. M.D. Delp [70] 36

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TESTOSTERONE AND AN ANABOLIC STEROID ALLEVIATES WEIGHTLESSNESS INDUCED MUSCULO-SKELETAL LOSSES. S.M. Wimalawansa, D.J. Simmons, M. Quast, K. Westlund, J. Wei, and S.J. Wimalawansa [73]

GROUND BASED FACILITIES FOR LONG TERM MICROGRAVITY RESEARCH. J.P. Veldhuijzen, J.J.W.A. van Loon, J. Kiss, C. Wood, H. van Ende, and A. Guntemann [74]

POTENTIAL USE OF NASA BIOREACTORS IN PRODUCTION OF CANCER VACCINES. D. Yetman, S.P. Tomasonic, and C.A. Savary [75]

ASYMMETRIC LOCALIZATION AND REDISTRIBUTION OF ANNEXINS IN GRAVISTIMULATED PEA PLUMULES. G.B. Clark, M. Dauwalder, D.S. Rafati, and S.J. Roux [76]

GENETIC CONTROL OF OSMOREGULATION IN DROSOPHILA. X. Huang, L. Huff, Q. Huang and M. Stern [77]


EXPERIMENTAL DESIGN AND PRELIMINARY RESULTS OF A STUDY OF BONE CELLS SUBJECTED TO HYPERGRAVITY. W.J. Landis, M.A. Kacena, and K.J. Hodgens [79]

DEVELOPMENTAL REGULATION OF COLLAGENASE-3 MRNA IN NORMAL, DIFFERENTIATING OSTEOBLASTS THROUGH THE ACTIVATOR PROTEIN-1 AND THE RUNT DOMAIN BINDING SITES. N.C. Partridge, R.C. D’Alonzo, and S.K Winchester [80]

STATHMIN PHOSPHORYLATION DURING GROWTH AND GRAVITROPIC RESPONSE OF ROOTS OF ZEA MAYS L. T.J. Mulkey and D.A. Prentice [81]

THE GRAVITROPIC RESPONSE OF CHARA PROTONEMATA IS REDUCED IN MODERATE HYPERGRAVITY. A. Sievers and D. Hodick [82]

DIRIGENT PROTEINS. TISSUE SPECIFIC EXPRESSION OF GENES AND SUBCELLULAR LOCALIZATION OF ENZYMES. V. Burlat, M. Kwon, L.B. Davin and N.G. Lewis [83]

PHOTOSYNTHESIS IN SPACE: GROUND-BASED EVALUATION OF ENVIRONMENTAL FACTORS AFFECTING WHEAT DEVELOPMENT IN CLOSED ENVIRONMENTS. G.W. Stutte, G.D. Goins, and D.K. Chapman [84]
Collaborative Ukrainian Experiment I

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MICROGRAVITY EFFECTS ON FREE AMINO ACIDS CONTENT IN BRASSICA RAPA.
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SATURDAY AM

6:30 - 9:00 pm  BANQUET/BUSINESS MEETING/AWARDS PRESENTATION

9:00 pm  ASGSB GOVERNING BOARD MEETING II

SATURDAY, OCTOBER 31, 1998

SESSION G: ORAL SESSION - SPACEFLIGHT EXPERIMENT RESULTS II

8:15 am to 10:00 am Moderator: Jay Buckey

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8:15  PRODUCTION OF STATOCONIA IN THE STATOCYST AND GRAVITACTIC CRAWLING BEHAVIOR IN POND SNAILS REARED IN MICROGRAVITY. M.L. Wiederhold, J.L. Harrison, J. Griffith and C.A. Ortiz [100]  52


8:45  THE AVIAN MODEL OF INNER EAR RESEARCH FOR SPACE. C.D. Fermin, H. Hara, and T. Jones [102]  52


10:00  BREAK

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10:30 am to 12:30 pm Moderator: Karl Hasenstein

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10:45  MICROGRAVITY EXPERIMENTS WITH *ARABIDOPSIS* IN BIORACK SUPPORT A STATOLITH-BASED MODEL FOR GRAVIPERCEPTION. J.Z. Kiss, R.E. Edelmann, and P.C. Wood [108]

11:00  AUTOTROPIC STRAIGHTENING AFTER GRAVITROPIC CURVATURE OF *LEPIDIUM* ROOTS IN MICROGRAVITY. B. Stankovic, F.D. Sack, A. Johnsson, F. Antonsen, and D. Volkman [109]


11:30  EFFECTS OF MICROGRAVITY ON PATHOGENESIS AND DEFENSE RESPONSES IN SOYBEAN TISSUES. E. Hilaire, M. Ryba-White, O. Nedukha, E. Kordyum, J.A. Guikema, and J.E. Leach [111]


12:00  A COMPARISON OF SPACEFLIGHT AND GROUND CANOPY GAS EXCHANGE MEASUREMENTS. O. Monje, G.E. Bingham, B.K. Eames, W.F. Campbell, V. Sytchev, M.A. Levinshik, and I. Podolskiy [113]

12:15  WATER MANAGEMENT LESSONS FROM PLANT FULL LIFE CYCLE EXPERIMENTS ON MIR. G.E. Bingham, S.B. Jones, D. Or, I. Podolski, and V. Sytchev [114]

12:30 - 2:00 pm LUNCH

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**SESSION I: CONCURRENT ORAL SESSION - CELL BIOLOGY AND BIOTECHNOLOGY/ INSTRUMENTATION II**

2:00 to 4:00 pm Moderator: Marian Lewis

2:00  MICROGRAVITY INDUCED OSTEOPOROSIS STUDY ON STS-80 SPACE FLIGHT. K.E. Forkheim and E.B. Schenker [115]

2:15  THE ROLE OF GRAVITY IN REGULATING THE PRODUCTION OF EPIDERMAL GROWTH FACTOR. E.M. Durban and S. Das [116]

2:30  NEURAL STEM-LIKE CELLS GROWN IN A SIMULATED MICROGRAVITY ENVIRONMENT. H.P. Low, T. M. Savarese and W.J. Schwartz [117]

2:45  MECHANISMS OF LYMPHOCYTE FUNCTION INHIBITION IN MICROGRAVITY. D. Risin, D. Cooper, A. Sundaresan and N.R. Pellis [118]

3:00  A MODEL SYSTEM FOR INVESTIGATING THE EFFECTS OF CLINOSTATIC ROTATION ON T-CELL ACTIVATION AND SIGNAL TRANSDUCTION. C.L. Adams, and C.F. Sams [119]

3:15  BIORACK ON THREE SHUTTLE-TO-MIR MISSIONS (S/MM): FACILITY PERFORMANCE AND EXPERIMENT OPERATIONS. PRESENTATION OF THE NEW ESA FACILITY BIPACK. C. Brilhouet and E. Brinckmann [120]
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SPACE BIOMEDICINE AND
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2:00 to 4:00 pm Moderator: Marc Tischler

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MEASUREMENT OF ENERGY BALANCE ON FOUR LMS ASTRONAUTS DURING SPACE FLIGHT. T.P. Stein, M.J. Leskiw, M.D. Schlueter, R. Gretebeck, H.W. Lane and R.W. Hoyt [124]

2:30
REDUCTION OF THIN FILAMENT DENSITY AND LENGTH IN HUMAN SOLEUS MUSCLE AFTER 17 DAY SPACEFLIGHT. D.A. Riley, J.L.W. Bain, J.L. Thompson, R.H. Fitts, J.J. Widrick, S.W. Trappe, T.A. Trappe, and D.L. Costill [125]

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ANIMAL SELECTION PROCEDURES FOR SPACE FLIGHT AND GROUND-BASED STUDIES. M.K. Steele and J. Calabrese [126]

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3:15
EFFICACY OF INSULIN-LIKE GROWTH FACTOR-1 IS NOT ALTERED BY SPACEFLIGHT UNLOADING. T.A. Bateman, R.J. Zimmerman, R.A. Ayers, V.L. Ferguson, S.K. Chapes, and S.J. Simske [128]

3:30
PROGRAMMED ADMINISTRATION OF PARATHYROID HORMONE INCREASES BONE FORMATION IN HINDLIMB UNLOADED Ovariectomized Rats. R.T. Turner, G.L. Evans, J.M. Cavolina, B. Halloran, E. Morey-Holton [129]

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F06 EFFECTS OF UNLOADING AND FOOD INTAKE ON BONE HISTOMORPHOMETRY. S.B. Arnaud, M. Navidi, P. Milbury, T. Curren and E. Morey-Holton [139] 68

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**Space Biomedical Results II**


L06  EFFECT OF RESISTANCE TRAINING ON GLUT-4 CONTENT IN SKELETAL MUSCLE OF HUMANS SUBJECTED TO 20 DAYS OF BED REST. I. Tabata, Y. Suzuki, T. Fukunaga, and T. Yokozeki [162]  81

ABSTRACTS
SESSION A: BIOMINERALIZATION SYMPOSIUM

Life is a major geological force, and it is therefore impossible to understand the dynamism of the earth without considering the role of the biota in global models. However, a flagrant contrast exists between the limitations of sound modelisation and the dynamics of life in the real world. In the first place, models must be simple, while life is complex and diverse. And secondly, models can only operate over a limited range in time and space, while living systems characteristically operate over a wide range of temporal and spatial scales.

In this lecture, I shall briefly discuss how these problems may be solved: by creating a nested suite of hierarchical models, each concentrating on one particular level of organisation, and by concentrating the associated experimental research on model systems representing an important geological force. The exemplary role of the coccolithophore Emiliania huxleyi as a model system for the study of global dynamics will be discussed in some detail. Emiliania produces elegant scales of calcium carbonate; it is the most important carbonate mineral producing species on earth. With its gigantic blooms it influences the global carbon cycle and the earth’s climate.

[2] BIOMINERALIZATION IN COCCOLITHOPHORIDS. Mary E. Marsh. University of Texas Dental Branch, Houston, Texas, USA.

Coccolithophores are phytoplanktonic characterized by an extracellular covering of calcareous scales (cocolets) formed intracellularly in Golgi-derived vesicles. In Pleurochrorella carterae the coccolets consist of an oval organic base plate with a distal rim of interlocking calcite crystals, and a narrow flexible ribbon of organic material which tethers the mineral ring to the base plate. Each crystallite is surrounded by a thin organic coat consisting primarily of two acidic polysaccharides PSI and PS2, which have a central role in the biominalization process. The most abundant polyanion PS2 is extremely acidic (having four carboxyl groups per disaccharide repeat) and capable of sequestering massive quantities of calcium ions. PSI and PS2 are synthesized in medial Golgi cisternae where they coaggregate with calcium ions to form 20-nm particles. The particles are secreted into the coccolith-forming vesicle where they associate with the rim of the unmineralized base before the onset of calcium carbonate deposition. After mineralization ceases, the PSI/PS2-containing particles are reorganized into the amorphous organic coat which surrounds the mature crystallites. Pulse-chase studies on wild-type cells and analysis of P. carterae mutants which do not express PSI2 suggest that all calcium destined for coccolith formation is first complexed with PSI1/PS2 for transport to mineralizing foci. Further analysis of mutants may lead to identification of elements controlling crystal nucleation and morphology.

Supported by the United States Office of Naval Research.


This presentation summarizes current understanding of molecular and macromolecular relationships between collagen and mineral and their importance in the progressive growth and development of normally calcifying bone, cartilage, tendon, dentin, and cementum among vertebrate tissues. Collagen represents the principal organic component in such tissues and it strictly mediates the nucleation, growth, and development of the mineral, a calcium phosphate salt (apatite). The mineral of collagenous tissues is composed of crystals having unusually small sizes (minimally ~45 x 30 x 2-4 nm) and shapes resembling irregular platelets. The crystallographic g-axes follow the longest dimension of the crystals, corresponding to their 100 faces, and, in association with collagen, the g-axes are aligned in parallel to the collagen long axes. The 100 faces of the crystals also lie generally parallel (~20°) to each other. Thus, the size, shape, alignment, and orientation of the crystals in vertebrate tissues are highly regulated. Crystal location and distribution are also ordered. Control of all such parameters of mineral formation is a consequence of collagen assembly and aggregation at different levels of structural hierarchy. Fundamentally, the hole and overlap zones (A.J. Hodge and J.A. Petruska, in G.N. Ramachandran [Ed.], Aspects of Protein Structure, Academic Press, NY, pp.289-300, 1963) of adjacent collagen molecules appear in very close register. This arrangement in three dimensions would create spatial gaps in the form of channels through collagen molecular assemblies. The channels likely define specific stereochemical and electrostatic charge characteristics that in turn support sites for crystal nucleation and accommodate apatite growth with the crystal character described above. The interaction between collagen and mineral in this manner leads to a composite tissue having improved strength and biomechanical properties different from those of either component separately considered. Conversely, changes in collagen content, assembly, or aggregation could have profound effects on mineralization and subsequently on the nature of tissue integrity and mechanical behavior.


Growth plate development involves the synthesis, maturation, and mineralization of the extracellular matrix by chondrocytes, a process that is under both systemic and local regulation and involves extracellular organelles called matrix vesicles. Studies in our lab have shown that resting zone chondrocytes (RC) produce and maintain a proteoglycan rich matrix under regulation of 24,25-(OH)2D3 (24,25). 24,25 induces these cells to become responsive to 1,25-(OH)2D3 (1,25), which is the hallmark of growth zone chondrocytes (GC) derived from the prehypertrophic and upper hypertrophic zones of the growth plate. This effect of 24,25 is potentiated by TGF-β. GC cells produce matrix vesicles that are enriched in alkaline phosphatase and matrix metalloproteinase activity. Both cell types produce 1,25 and 24,25 locally and secrete these metabolites under growth factor and hormonal control into the extracellular matrix, resulting in differential regulation of expression and secretion of the matrix vesicles, thereby permitting regulation of events at sites distant from the cell. One consequence of the action of 24,25 and 1,25 on RC and GC cells is the differential synthesis of latent TGF-β binding protein, and therefore, the incorporation of latent TGF-β into the matrix. GC matrix vesicles treated directly with 1,25 can activate latent TGF-β. TGF-β regulates the Iα and IαR hydroxylases involved in production of 1,25 and 24,25. This results in a feed-back loop between the local production, storage and activation of the growth factor. Tetrahedral steroids are converted to testosterone which may exert direct effects on the chondrocytes and matrix vesicles via cell maturation-dependent mechanisms. 1,25, 24,25, testosterone may exert their effects via rapid membrane-mediated mechanisms involving membrane fluidity, specific membrane receptors, calcium ion fluxes, changes in lipid metabolism, and PKC, as well as by conventional steroid hormone-dependent pathways. (Supported by NIH: DE-05637 and DE-08603.)
ILLUSTRATIVE DISORDERS OF ECTOPIC SKELETAL
MORPHOGENESIS: A CHILDHOOD PARALLAX FOR STUDIES IN
GRAVITATIONAL AND SPACE BIOLOGY. Frederick S. Kaplan,
M.D.\textsuperscript{1,2} and E.M. Shore, Ph.D.\textsuperscript{1,3}. Departments of \textsuperscript{1}Orthopaedic
Surgery, \textsuperscript{2}Medicine, and \textsuperscript{3}Genetics, The University of Pennsylvania
School of Medicine, Philadelphia, PA 19104

Ever since the explosive growth of the animal phyla during the
Cambrian radiation nearly 600 million years ago, the evolution of life on
Earth has been characterized by gradual changes in body shape and form.
Among the most ancient and conserved genes in the animal kingdom are
those that regulate morphogenesis. Mutations, or changes, in the genes
that control the body plan have lead to the changes in shape and form that
we see as the evolutionary process. However, some alterations of these
important genes, rather than resulting in the diversity that fills an
ecological niche, can cause catastrophic medical problems. Genetic
disorders of tissue modeling and morphogenesis provide an important
parallax to disturbances of tissue re-modeling that are of paramount
importance to gravitational and space biologists as humans begin to
explore and live in environments beyond the planet on which they
evolved. Disorders of osteogenesis are of particular concern to space
biologists due to the dramatic changes in skeletal biology in altered
gravitational fields.

Heterotopic ossification is a key feature of at least three distinct genetic
disorders of osteogenesis in humans: fibrodysplasia ossificans
progressiva, progressive osseous heteroplasia, and Albright's hereditary
osteodystrophy. All three conditions are genetic disorders of childhood,
but the pathobiology of osteogenic induction, the histopathology of
osteogenesis, the anatomic distribution of heterotopic lesions, and the
developmental patterns of disease progression differ among the three
conditions. The phenotypic distinction of these disorders is critically
important in counselling patients and families as well as in advancing
research to define the molecular pathophysiology of heterotopic
osteogenesis in these disabling genetic disorders. In addition, these
disorders of osteogenesis provide an important parallax to disturbances of
bone remodeling that are of paramount importance to gravitational and
space biologists.
SESSION B: CONCURRENT POSTERS I
Space Life Sciences Training Program
ILLUSTRATIVE DISORDERS OF ECTOPIC SKELETAL MORPHOGENESIS: A CHILDHOOD PARALLAX FOR STUDIES IN GRAVITATIONAL AND SPACE BIOLOGY. Frederick S. Kaplan, M.D. 1,2 and E.M. Shore, Ph.D. 1,2. Departments of Orthopaedic Surgery, Medicine, and Genetics, The University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Ever since the explosive growth of the animal phyla during the Cambrian radiation nearly 600 million years ago, the evolution of life on Earth has been characterized by gradual changes in body shape and form. Among the most ancient and conserved genes in the animal kingdom are those that regulate morphogenesis. Mutations, or changes, in the genes that control the body plan have lead to the changes in shape and form that we see as the evolutionary process. However, some alterations of these important genes, rather than resulting in the diversity that fills an ecological niche, can cause catastrophic medical problems. Genetic disorders of tissue modeling and morphogenesis provide an important parallax to disturbances of tissue re-modeling that are of paramount importance to gravitational and space biologists as humans begin to explore and live in environments beyond the planet on which they evolved. Disorders of osteogenesis are of particular concern to space biologists due to the dramatic changes in skeletal biology in altered gravitational fields.

Heterotopic ossification is a key feature of at least three distinct genetic disorders of osteogenesis in humans: fibrodysplasia ossificans progressiva, progressive osseous heteroplasia, and Albright’s hereditary osteodystrophy. All three conditions are genetic disorders of childhood, but the pathobiology of osteogenic induction, the histopathology of osteogenesis, the anatomic distribution of heterotopic lesions, and the developmental patterns of disease progression differ among the three conditions. The phenotypic distinction of these disorders is critically important in counselling patients and families as well as in advancing research to define the molecular pathophysiology of heterotopic osteogenesis in these disabling genetic disorders. In addition, these disorders of osteogenesis provide an important parallax to disturbances of bone remodeling that are of paramount importance to gravitational and space biologists.
SESSION B: CONCURRENT POSTERS I
Space Life Sciences Training Program

The 1998 Space Life Sciences Training Program class was made up of forty students from across the United States. The students selected one of four research emphasis groups centered around projects related to: Ecological Programs; Flight Experiments and Occupational Medicine; Advanced Life Support research, and Geobiological Research. This year, the students spent more time during the six week program in the field or laboratory (about two-thirds of their time) with much of the rest of the time spent in lectures and tours. The lecturers included NASA personnel (including astronauts), NASA contractor personnel, and representatives from universities and other agencies. The students also utilized a local university library for literature searches and visited the Epcot Center, Brevard Community College Astronaut Memorial Planetarium and Observatory, and Sea World. The students also took tours of Kennedy Space Center, Cape Canaveral Space Museum, and U. S. Space Camp and the Astronaut Hall of Fame. The students earned five hours of college credit from Florida A & M University, who administers the program for NASA. To earn their grade, each student was required to submit and present a technical paper on their specific emphasis research project during the final week of the program. (Supported by the NASA Space Life Sciences Training Program)


Students in the six-week program were involved in science and engineering activities pertaining to gravitational and space biology research of plants. The focus of this group is to understand the individual components of spaceflight affecting plant growth and development, towards the eventual goal of food production for human sustenance in space or other planetary environments. The particular research efforts of the 1998 SLSTP students included: Testing of light emitting diodes (LEDs) as a light source for both terrestrial and aquatic plants; testing of nutrient solutions and different nutrient delivery systems for plant growth in both gravity and microgravity environments; use of hypo- and hyper-gravity to study plant growth and physiological responses to altered gravity environments; molecular and cellular investigations of plants grown under super-elevated atmospheric concentrations of CO₂; compilation of educational activities for elementary schools to demonstrate recycling for plant growth in remote habitats; and developing and testing protocols for a plant spaceflight experiment using prototype flight hardware. The students presented the results of their research efforts in a seminar at the end of the program and submitted a written report to the NASA and Florida A&M SLSTP program managers. (Supported by the NASA Space Life Sciences Training Program)

[8] NASA SPACE LIFE SCIENCES TRAINING PROGRAM: EXPERIENCING PROJECT MANAGEMENT, SCIENCE, ENGINEERING, AND INTEGRATION OF A K-12 EXPERIMENT. P. Currier1, A. Fitch2, C. Gunn1, R. Hall1, S. Hart1, J. MacIntire1, M. Soliman1, and D. Thomas2. 1The Bioetetics Corporation, 2Kennedy Space Center, FL; 3Space Life Sciences Training Program; 4Department of Biological Sciences, University of Idaho, Moscow, ID.

The Experiment Design Team was developed to provide multiple experiences for the students: to learn Project Management and integration of an experiment, to experience the roles of a manager, scientist, and engineer through hands-on training; to understand aspects of technical decisions involved in the development and implementation of a flight experiment, and to stress the importance of teamwork. In this context, the students were told to complete a fairly easy NASA-related experiment suitable for teachers to use in K-12 students. To act as Project Manager of a NASA experiment, each student was responsible for creating a schedule (Gantt chart), analyzing budget and expenditures, and reporting results. Each student also played the role of engineer and biologist by designing hardware and preparing specimens. The students designed and built hardware for their experiments, conducted verification tests, reviewed and reworked designs, and eventually conducted fully integrated biology experiments. Collaboration and teamwork within the group of scientists was required. Student majors included aerospace engineering, ceramics engineering, pharmacology, and biology, so they were able to make significant contributions to projects other than their own. Several overlapping experiments were developed. Two students analyzed hypergravity effects on development by building a centrifuge and running experiments at varying G levels. One scientist used a Mars soil analog to grow a variety of plants and another examined Martian soil toxicity on a denitrify bacteria. That student also studied the effects of Martian atmosphere, paralleling a study on bacterial and algal growth "on Mars." Students proved to be good project managers, were innovative and artistic, and gained an appreciation of the intermarriage of science and hardware. Their experiments will eventually be developed into K-12 outreach activities available on the Internet. (Supported by the NASA Space Life Sciences Training Program)
SESSION B: CONCURRENT POSTERS I
Undergraduate Student Poster Competition

The TCH2 gene of Arabidopsis thaliana is rapidly and transiently upregulated following a variety of exogenous stimuli, including touch, darkness, and temperature shifts. The TCH2 gene encodes a protein with 44% amino acid identity to Arabidopsis calmodulin proteins and has been demonstrated to bind calcium. Computer modeling of the primary TCH2 sequence (Khan et al., 1997, Proteins 27:144) predicts that the tertiary structure is similar to calmodulin, but there are significant differences, including loss of acidic residues and other highly conserved residues, to suggest that the targets of TCH2 are unique from those of calmodulin. To understand the function of TCH2 in plant development and in response to exogenous stimuli, we are using a radioactively-labeled TCH2 fusion protein to identify cDNAs from an Arabidopsis expression library which encode TCH2 target proteins. The target proteins of initial interest will be those which interact with TCH2 in a calcium-dependent manner.

(Supported by NASA Specialized Center for Research and Training, grant no. NAGW 5007.)

[10] GRAVITROPISM AND PHOTOTROPISM OF FLOWER STALKS IN ARABIDOPSIS. S.E. Weise and J.Z. Kiss. Department of Botany, Miami University, Oxford OH.

Relatively little work has been done on the tropistic responses of Arabidopsis inflorescence stems (flower stalks). Previous research in our laboratory has assayed gravitropism of both the roots and hypocotyls of a wild-type (WT) Arabidopsis and three starch-deficient strains. In the present study, the time course of curvature of the inflorescence stems of Arabidopsis WT and a starchless mutant (ACG 21), and two reduced starch mutants (ACG 20 and ACG 27) was used to study gravitropism. Short inflorescence stems (1.0 - 2.9 cm) were less graviresponsive compared to the long stems (3.0 - 6.0 cm). In both studies, the WT initially had the greatest response and the starchless mutant had the least response, while the reduced starch mutants exhibited an intermediate response. Growth rates for all four strains were approximately equal. Approximately 8 hours after reorientation, flower stalks of all four strains returned to a position parallel to the gravity vector. Therefore, it appears that statoliths play an important initial role in gravitropism, accelerating the response of the inflorescence stem. However, after longer time intervals, the three mutant strains had a full gravitropic response similar to that of the WT. In addition, results of phototropism experiments show a high degree of variability, which we attribute to a large amount of mutation in the flower stalks. Taken together, our study suggests that, in flower-stalks, the longer term response to gravity is independent of the total mass of the statoliths. (Financial support was provided by NASA grant NAG 2-1017 and the Summer Scholar & Howard Hughes programs at Miami University.)


Long-term manned space flight and human habitation in microgravity is an inevitable part of the future of the space program. Obvious physiological problems of microgravity adaptations include muscle atrophy due to disuse, and bone weakness due to calcium loss. While muscles are weakened by the lack of gravity against which to do work, such lack of physical stress also causes bone weakness due to calcium reabsorption. Therefore, it is important that a microgravity exercise resistance-training program be implemented during long-term space flights or habitation in low-gravity. A conceptual design for a constant force resistive exercise unit using either constant torque springs or constant force springs is proposed as a countermeasure for muscle atrophy and bone demineralization in microgravity. The force packs, located within the resistance unit, are designed to allow for a constant force to be applied during isometric, eccentric, and concentric muscle actions. This provides the essential loading requirements for bone and large muscle groups that are weakened in space. The force packs are designed to provide constant force, be lightweight, fully interchangeable, and allow smooth action through entire range of motion.

[12] THE DESIGN AND FUNCTION OF A CLOSED MARINE ECOSYSTEM FOR SPACE FLIGHT. J.R. Ascher, O. Van den Ende, and D. Thomas. 1Chemistry Dept., University of South Florida, Tampa, FL; 2Bionetics Corporation, Kennedy Space Center, FL; 3Dept. Of Biological Sciences, University of Idaho, Moscow, ID.

Closed systems are good models for ecosystems on the earth, a very large closed system, or for systems used for life support in a closed environment, such as in a space station.

Our first objective was to create a passive closed marine ecosystem for space flight using existing hardware. After devising a system, we used Nanochloris oculata, Gracilariia , and Artemia salina to determine the optimum Artemia density with a constant amount of both algae.

A Plant Growth Chamber, used in previous shuttle flights, was modified to support a closed marine system by lining with a sealed bag. In the first trial three densities of Artemia were used and the optimum density was 93 Artemia per liter of solution. The first trial was run with the capability of removing solution for chemical analysis. Light and temperature were controlled. Algal cell concentration, pH and ammonia levels were measured. Artemia population was determined. In the second trial the optimum density of Artemia was placed into each chamber and the bag was completely sealed and kept at 25°C to simulate space flight conditions.

In trial 1 survival rates went down as population density of Artemia went up, and the differences were statistically significant. In trial 2 the survival rate of Artemia was higher than any of the three groups in trial 1. Nanochloris oculata concentrations increased as Artemia population decreased. Variations in pH and ammonia levels were not statistically significant between groups.

The results of this experiment indicate that a closed marine ecosystem could be successfully sustained on earth in existing space flight hardware with only a light source. Further tests are indicated to assure the stability of the experimental design in micro-gravity. (Supported by the NASA Space Life Sciences Training Program.)

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The amount of water and other required resources cannot be stored on long-duration space flights due to limited space. High costs make resupplying the crew undesirable. A potential alternative is using an Advanced Life Support system to recover and recycle resources. Direct recycling of gray water through a plant growth hydroponic hydroponic system minimizes the need for a bioreactor. However, plants exhibit phytotoxic effects due to surfactants in gray water. The aim of this study was to identify a surfactant that caused minimal to no phytotoxic effects. The surfactants were selected based on ionic content: potassium (K-based mug soap), sodium (Ivory®), sulfate and sodium (Igepon®). It was believed that all the surfactants were equally toxic, but the best surfactant was sodium and/or sulfate free. The degree of lettuce and wheat sensitivity to toxicity tests was investigated. To determine which surfactant had the least effect on growth, seeds were placed on Petri dishes containing solutions of various surfactants at different concentrations. Effects on growth were compared using percent germination, average root length, and percent growth reduction. Average root length of wheat was less affected by K-based solution at 1000mg/L than lettuce. Lettuce exhibited a growth reduction of 80% in K-based solution, while wheat growth was reduced by 30%. Comparison of dosage curve trends showed that lettuce was more sensitive and that K-based mug soap was the best surfactant. (Supported by the NASA Space Life Sciences Training Program)


Plant growth and development has been shown to be affected in a microgravity environment. Studies have shown various morphological and physiological changes including inhibited growth, reduction in cell division, chromosomal damage, and disoriented growth. The growth hormone auxin is involved in plant growth and development and is shown to play a role in the gravitropic response. Recently it has been shown that corn plants grown in microgravity do not exhibit any overall changes in auxin levels. Therefore, if auxin levels are not affected in microgravity, perhaps the transport or tissue sensitivity to auxin may be affected in altered gravity environments. It is the objective of this study to measure auxin inducible gene expression and protein expression under altered gravity environments in a transgenic tomato line containing the GH3-GUS transgene system. Tomato seedlings were grown in altered gravity environment on a clinostat or centrifuge. Hypocotyls were harvested, then inverted and placed into an agar media containing auxin. The hypocotyls were placed in various gravity environments for 24 hours. The hypocotyls were removed and placed in either X-GLUC substrate or frozen in liquid nitrogen for protein extraction. Protein was isolated through phenol:chloroform extraction and separated by two-dimensional SDS PAGE for comparative analysis.

Results have shown that the staining patterns of the hypocotyls were similar between various treatment groups. However, parenchyma staining was found to be more intense than vascular bundle staining. Auxin transport measured through X-GLUC staining found no significant differences between the various altered gravity environments. Significant variation in hypocotyl staining occurred in all treatments. Protein analysis between vertically grown plants and clinorotated plants found that protein expression may be diminished in clinorotated plants. (Supported by the NASA Space Life Sciences Training Program)


A short-term study was conducted in participation with Florida A&M University’s six-week Space Life Sciences Training Program within the parameters of a more extensive study. The study quantitatively analyzed the population of L. polyphemus and hydrogen sulfide (H₂S) concentration in pore water of the habitat’s sediment within the intertidal zone, in order to create a baseline of data for future studies. Six study sites, individually consisting of 200 m of shoreline, were dispersed throughout three lagoons within MINWR. L. polyphemus were collected and tagged by hand and through seine netting along sites. Pore water was collected using syringe barrel and centrifuge tubes filled with nitrogen gas and zinc acetate. The Methylen Blue method was used to analyze the H₂S concentration. Results revealed no apparent correlation between the concentration of H₂S in the pore water and the amount of L. polyphemus inhabiting the area. The horseshoe crabs seemed to prefer the Indian River Lagoon and showed a larger number of females and overall adults at most of the sites. Much gratitude is extended to Dynamic Corporation, Florida A&M University and NASA for their infinite support and resources. (Supported by the NASA Space Life Sciences Training Program)
SESSION B: CONCURRENT POSTERS I
Graduate Student Poster Competition
[16] INITIAL RESULTS OF EXPERIMENTS WITH OSSEOBLASTS CULTURED UNDER HYPERGRAVITY CONDITIONS. M.A. Kacena1, P. Todd1, and W.J. Landis1, 1Dept. of Orthopedics, Harvard Medical School and Children’s Hospital, Boston, MA, and 2Depts. of Aerospace Engineering and Chemical Engineering, University of Colorado and BioServe Space Technologies, Boulder, CO.
To understand further the role of gravity in bone growth and loss, 17-day-old embryonic chick calvarial osteoblasts were subjected to high gravitational forces at the Hypergravity Facility for Cell Culture (HyFaCC) at NASA/Ames Research Center, Moffett Field, CA. Osteoblasts, grown in DME, 10% FBS, 12.5 μg/ml ascorbate and 10 mM β-glycerophosphate and attached to type I collagen-coated coverslips, were loaded into Fluid Processing Apparatus (FPA) units. Other osteoblasts were grown in 35 and 100 mm culture dishes. FPA units and dishes were placed in incubators at 37°C, 5% CO2 and exposed to 3.3 G or 4.0 G on a 9 foot centrifuge arm of the HyFaCC or maintained as controls at 1.0 G. Cells under the various G conditions were collected at 0 and 3 hrs and 2, 4 and 6 days (interval sampling 1 hr every 2 days), and they were processed for Northern blot analysis, immunohistochemistry and electron microscopy. Results to date indicate: (1) By 2 days, scanning and transmission microscopy of all FPA units suggest osteoblasts have spread in centrifuged samples greater than in counterpart controls and contain more vacuoles and cellular debris; and (2) immunocytochemistry of all 35 mm centrifuged and control culture dish samples at all times shows f-actin, tubulin, vinculin, fibronectin and talin but no detectable differences among experimental and control paired groups. Northern blotting analysis remains. While preliminary, these data reveal changes in osteoblast size, shape and cytoplasmic structure following hypergravity exposure. Such alteration could be correlated with possible changes in cytoskeletal elements and attachment proteins as well as in expression of genes critical to bone formation, whose complete analyses are yet to be determined.
(Supported by NASA grants: NGT-51421 and NAG5-4377.)

[17] GENDER DIFFERENCES IN THE RESPONSES OF RHESUS MONKEYS TO 2G. L.K. Baizer, and C.A. Fuller, Section of Neurobiology, Physiology and Behavior, University of California, Davis.
With increasing numbers of women participating in Space Programs, it is important to characterize the responses of females to an altered force environment. This chronic centrifugation study was compared to an identical study done in our lab using male subjects. Six female Rhesus (Macaca mulatta) were housed individually on a 6.0 m diameter centrifuge. Husbandry was performed one hour each day on a non-24 hour basis. Data were collected for: 2 weeks (1G), 2 weeks (2G), and 2 weeks (1G). Water was available ad lib through a liquid system. Drinking counts (number of contacts with the lickit) were summed and stored on a microcomputer in 10 minute bins and volume consumed was recorded daily. A pelletized diet was provided through the Psychomotor Test System (PTS), developed at Georgia State University. Performance on psychomotor and memory based tasks was monitored using the PTS. Each animal was implanted with a telemetry transmitter to measure heart rate and body temperature; these data were stored in 10 minute intervals on microcomputer. Mean, phase, and amplitude of each physiological rhythm was calculated to elucidate any effects on the circadian timing system. Urine was collected daily. Electroencephalogram and electroencephalogram conjugates were assayed and quantified to assess any variation between phases of the menstrual cycle. Analyses show there are gender differences in the responses of Rhesus monkeys to a hyperdynamic field. (Supported by NASA Grants NAG5-4320 and GSRP NFT-51417, and Zonta Int'l Amelia Earhart Fellowship)

[18] REGULATION OF THROMBIN RECEPTOR ON SMOOTH MUSCLE CELLS BY MECHANICAL FORCES. K.T. Nguyen, M. Papadaki, J. Ruel, C. Patterson, M.S. Runge, S.G. Eskin, and L.V. McIntire, Dept. of Bioceng., Rice Univ., Texas, 2Cardiology Division, Univ. Texas Medical Branch, Galveston, and 3Dept. of Cell Biology, Texas Biotechnology Corporation.
The objective of this study is to investigate the effect of mechanical forces such as shear stress and cyclic strain on the expression of protease-activated receptor-1 (PAR-1) in human aortic smooth muscle cells(HASMC). HASMC were exposed to different levels of shear stress using the parallel plate flow chamber system. Northern blot and flow cytometry analysis indicated that PAR-1 mRNA and protein were downregulated 3 fold by high shear stress, 25 dyn/cm2. mRNA half-life studies showed that the decreased mRNA was not due to instability of PAR-1 mRNA. In addition, alterations in HASMC function after exposure to shear stress such as thrombin-induced increase in cytosolic Ca2+ and proliferation were examined. Using Fura-2, Ca2+ rise after thrombin stimulation in sheared cells was 50% less than in control cells.

[19] FUNCTIONAL AND MOLECULAR EXPRESSION OF CALCIUM CHANNELS IN OSSITOBLASTS IN RESPONSE TO MECHANICAL LOADING. K.D. Ryder, J. Bergh, M.C. Farach-Carson and R.L. Duncan, Dept. of Orthopaedic Surgery, Physiology and Biophysics, Indiana University Medical Center, Indianapolis, IN and Dept. of Biological Sciences, University of Delaware, Newark, DE.
We previously characterized a mechanosensitive, cation-selective channel (MSCC) in osteoblasts that we have tentatively identified as an alternatively spliced isoform of the α2δ subunit of the L-type voltage-sensitive Ca2+ channel (VSCC). We postulate that this channel plays an important role in the response of osteoblasts to mechanical stimulation. Patch clamp studies indicate that non-loaded MC3T3-E1 cells exhibit little activation of currents when challenged with a hypotonic solution. However, when MC3T3-E1 cells are subjected to fluid forces induced by a four point bending device, a 7-fold increase in whole cell inward currents is observed. These data correlate with intracellular Ca2+ ([Ca2+]i) imaging studies that show that approx. 50% (54/106) of non-loaded MC3T3-E1 cells respond to hypotonic challenge with a peak [Ca2+]i transient of 180 nM. Mechanically loaded MC3T3-E1 cells exhibited a more vigorous response (peak [Ca2+]i=240 nM) with 88% (67/76) of the cells responding to challenge. To ascertain if the MSCC was involved in this response, 10 μM GdCl3 (a known MSCC blocker) was added prior to hypotonic challenge. Gd3+ totally abolished the [Ca2+]i, response to challenge. rt-PCR studies using primers designed to amplify all α2δ subunit isoforms of the L-type VSCC demonstrated that non-loaded MC3T3-E1 cells express low levels of this subunit. Yet when cells are mechanically loaded as described above, a 3-5 fold increase in α2δ subunit expression is observed within 4 hr after the initiation of loading. These data indicate that loading increases functional and molecular expression of this VSCC isoform which may be integral in the [Ca2+]i response to mechanical stimulation. (Supported by NASA: NAG5-4917, NASA/TMC.)

Male Sprague-Dawley rats flown for 10 days aboard the Space Shuttle Endeavor (STS-77) and compared to simultaneous vivarium ground controls exhibited decreases in splenic helper T-cell and neutrophil percentages. There was a simultaneous increase in the percentage of splenic cytotoxic/suppressor T-Cells.

Several aspects of spaceflight may be involved with these population shifts. Launch & landing loads are the primary acute stressors experienced by animals flown on the space shuttle. Cephalic fluid distribution shifts, unloading of the limbs, and exposure to a novel environment are the primary chronic stressors. Two experimental conditions can be used to model either the acute or chronic stressors: centrifugation and anti-orthostatic tail suspension. To date, no consistent immunological changes in the above splenic populations have been observed in either model. This suggests two possible explanations for the spaceflight results: 1) There is something inherently stressful about the space shuttle environment that can not be modeled on the ground; or, 2) The results are due to a combination of acute and chronic stressors.

To further investigate a possible synergistic response, the ground models were combined. 8 male Sprague-Dawley rats were placed into a centrifuge and exposed to forces similar to those experienced during a shuttle launch. After "launch," the animals were suspended to simulate the microgravity portion of a shuttle experiment. After 10 days of suspension, the animals were returned to the centrifuge and "landed." Other groups (n=8/gp) included unlaunched/non-suspended controls and 10 day suspension animals. Upon "landing," the rats were sacrificed and dissected. Splenocytes were labeled with antibodies against CD4, CD8, CD11b, and δβTCR and analyzed by flow cytometry.

There were no consistent changes in these treatment groups in the cell populations observed. This finding supports the hypothesis that there are stressors unique to the spaceflight environment that can not be fully modeled on the ground.


Mechanical stimulation elicits an elevation of calcium levels in a wide variety of cell types, ranging from maize coleoptile to mammalian epithelial cells (Gehring et al., 1990; Hansen et al., 1993). The broad evolutionary conservation of this response points to a central role for calcium in mechanical signaling.

Recently calcium and calcium/calmodulin fluorescent indicator proteins have been developed, based on engineered forms of green fluorescent protein (EGFP's; Romoser et al., 1997). These indicators consist of two different EGFP's coupled together by a region of the calcium/calmodulin target myosin light chain kinase. The overlap of the emission spectrum of one EGFP with the absorption spectrum of the second EGFP allows fluorescent energy transfer (FRET) to occur when these two moieties are close. Binding of calcium-saturated calmodulin to the linking target region moves the two EGFP's apart and FRET is disrupted. These indicators provide a novel method for analysis of calcium-based responses that avoids the requirement for introduction of indicator dyes into the cells under analysis. As a result, calcium imaging in the tissues of an intact organism is now possible. We are expressing these indicators in the organism Drosophila melanogaster so that the spatial and temporal characteristics of calcium responses can be investigated in detail. Three types of indicators are currently being introduced into flies, two calcium/calmodulin sensors and one calcium indicator. The latter contains a calmodulin coding region in addition to the two EGFP modules so that the sensor is independent of extramembranous calmodulin. Progress in calcium and calcium/calmodulin imaging in vivo will be presented. Supported by the NASA sponsored NSCRO at Rice University. Gehring et al. 1990. Nature 345, 528-530.


The magnetotactic bacterium Magnetospirillum magnetotacticum, strain MS-1, is thought to respond to magnetic fields to guide tactic movement. Owing to its internal magnetite particles ("magnetosomes"), cells can also be moved and guided by externally applied magnetic fields, which can be combined in various ways with the gravity vector. This organism therefore provides an opportunity to test our previously demonstrated hypothesis that suspended bacteria prevented from sedimenting (Escherichia coli and Bacillus subtilis), as in microgravity, grow to higher final cell concentrations than when allowed to sediment at 1 x g. M. magnetotacticum cultures were grown in defined medium ATTC-1643 at 30°C and were found to have a doubling time of about 4.5 h and a final cell concentration of about 9 x 10^6 cells/ml. Current-carrying wire coils were designed and constructed to form magnetic fields capable of imposing forces comparable in magnitude to that of gravity on suspended bacteria in vertical 9 ml tube cultures. Field mapping showed a vertical trapping region having maximum field strength of 20 mT (miliTesla) and horizontal variance of ± 5%. The objective of this design was to oppose, to the extent possible, the gravitational force on bacteria. Such a design is used as a basis for space flight experiments in which, rather than being quiescently suspended, bacteria would be subject to a gravity-like force, so that complementary experiments can be performed in which a magnetic field can be used, on one hand, to establish functional weightlessness and on the other, magnetopseudogravity.

This research was supported by NASA Grant NAGW-1197, and by SHOT, Inc., Floyd Knobs, IN.


To explore novel aspects of the mechanism regulating gravitropic signal transduction, we have used four mutants of tomato (Lycopersicon esculentum Mill.) which are altered in their response to either gravity, auxin, or ethylene. Ethylene is known to interact with auxin in regulating stem growth, yet evidence on the role of ethylene in tropic responses is contradictory. We find that we find that wild-type gravitropic response of tomato is influenced by ethylene in a concentration-dependent manner, with decreased graviresponsiveness at moderate levels which do not inhibit overall growth, and complete inhibition of curvature at growth-inhibiting high concentrations. A requirement for ethylene is indicated by the fact that ethylene inhibitors dramatically reduce wild-type gravicurvature at concentrations where overall growth is not affected. The reduced gravitropic response of the auxin-insensitive diegetropic (agt) mutant can be restored to wild-type levels by extremely low ethylene concentrations, which confirms that low levels of ethylene are necessary for a full gravitropic response. In contrast, the ethylene-insensitive never-ripe (nr) mutant shows a slightly delayed but otherwise normal gravitropic response in ambient air and no reduction in gravicurvature in an ethylene atmosphere. The ethylene-overproducing constitutive response mutant epinastic (epi) exhibits a reduced gravitropic response in both ambient air and ethylene. The reversal of gravicurvature by red light in the lazy-2 (lz-2) mutant does not appear to be affected by either ethylene or ethylene inhibitors, indicating that ethylene does not play a role in the direction of the gravitropic response. Taken together, these data indicate that while low levels of ethylene are necessary for a full gravitropic response, moderate levels of the hormone inhibit gravicurvature in a specific manner which does not inhibit overall growth. (Supported by NASA award No. NAGW-3716 to T.L.L.)

Root gravitropism involves three phases: gravity perception, signal transduction, and the growth response. Although the growth response phase has been well characterized, very little is known about the perception and transduction phases. The root cap is proposed to be the site of perception and early transduction events in gravitropism, therefore, changes in putative signaling molecules are likely to occur in this region. In plant cells, changes in H+ concentrations have been suggested to be involved in the signaling cascades linking the perception of a stimulus to the physiological response. To determine whether pH changes occur in the cap during the early stages of root gravitropism, we used fluorescent pH indicators, ratio imaging and vertical stage fluorescence microscopy. To visualize cytoplasmic pH, we used BCECF linked to a 10kDa dextran. The dye was microinjected into the cytoplasm using iontophoretic methods and remained in the cytoplasm for the duration of the experiments. Preliminary measurements showed that there was a change in cytoplasmic pH in inner columella cells during gravitostimulation and that the changes were different in the various tiers of columella.

(Supported by NASA grant # NAGW-4984).


Arabidopsis possesses a family of xyloglucan endotransglycosylases (XETs), enzymes that endolytically cleave and religate xyloglucan polymers; xyloglucan is one of the primary structural components of the plant cell wall. Therefore, XET function may affect cell shape and plant morphogenesis during growth and in response to mechanical or environmental stimuli. To gain insight into the biochemical functions of this enzyme family, we produced four of the XETs using a baculovirus/Sf9 insect cell expression system. An analysis of the structural requirements of TCH4 for optimal XET activity revealed the necessity of disulfide bonds and N-linked glycosylation for TCH4 activity. Using site-specific mutagenesis, we demonstrated that the first glutamate residue of the conserved DEIDDFPLG motif (E97) is essential for activity. A change to glutamine at this position resulted in an inactive protein, supporting the hypothesis that, in analogy to Bacillus glucanases, this region may be the active site of XET enzymes.

To identify potential unique roles for specific XETs during plant growth, we have compared the activities of four XETs (TCH4, Meri-5, EXT, and XTR9) under several different conditions, including various temperatures, pH's and substrates. (Supported by NASA Specialized Center for Research and Training grant no. NAGW 5007.)

[26] PRODUCTIVITY OF COWPEA CANOPIES WITH INTRACANOPY LIGHTING USING ELEVATED CARBON DIOXIDE AND DIFFERENT PHOTOPERIODS IN CONTROLLED ENVIRONMENTS. J.M. Frantz, R.J. Joly, and C.A. Mitchell, Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907-1165

Plant-growth lighting for long-term manned space missions will be a major source of power consumption in advanced life-support systems. Intracanopy lighting, a lighting technique that allows plants to grow through and around multiple layers of low-intensity lamps, may help reduce the energy necessary for crop production. Results with cowpea (Vigna unguiculata L. Walp 'ITR7D-941-1'), a legume species with edible leaves, pods, and/or dry beans, indicate that, at similar energy consumption totals, intracanopy lighting yields 20-25% more, have a 30-35% higher harvest index, and use light energy nearly twice as effectively as traditional overhead (above canopy) lighting strategies. Optimization of intracanopy lighting can improve the yields of crops while further increasing the efficiency with which the crops are produced. Increasing CO₂ from ambient levels to 1000 μmol·mol⁻¹ improved edible yield totals and yield rates by 20% without the use of additional light energy. Also, a reduction in photoperiod from 24 h to 18 h significantly improved energy efficiency without a significant reduction in yield. Further steps in the optimization of photoperiod and CO₂ are expected to improve upon the efficiency of crop production using intracanopy rather than traditional lighting systems. Optimization of intracanopy lighting may improve the sustainability of long-term manned space missions by reducing the energy requirements for crop production. Research supported in part by NASA grant NAGW-2329.


The photosynthetic apparatus contains a number of multisubunit protein complexes, many of which are regulated by environmental conditions. In our study (BPAW), we examined the influence of growth during spaceflight on PSI of Brassica rapa as part of the Collaborative US/Ukrainian Experiment (CUE). Brassica rapa seeds were germinated and grown for 14 days in the controlled environment of the new Plant Growth Facility aboard the space shuttle Columbia. Control plants were grown under the same conditions on Earth. Three hours after shuttle landing, cotyledon leaves were harvested and frozen. Analysis of total Chl and protein showed an increase in both Chl a/b and protein/Chl ratio in the flight samples. Thylakoid membranes from the space samples also showed an increase in protein/Chl ratio, but the change in the Chl a/b ratio was not apparent. Thylakoids contained more Chl and protein by 35% and 32%, respectively, than the ground controls. Determination of electron transport rates showed a 30% decrease in PSI activity for the flight samples. Electrophoretic analysis revealed that intact PSI was reduced in the flight samples, and these decreases were quantified by western blotting. PsAa and PsAb were decreased by 33% and 24%, respectively. In contrast, three LHCl proteins and the D2 protein of PSI were reduced by little more than 10%. This pattern of PSI reduction was also observed in our ongoing photoinduction experiments. An unidentified 54 kDa thylakoid protein was increased in the flight samples, and in prior experiments, we have observed this peptide during conditions which favor PSI photoinduction. Taken together, these results suggest that the spaceflight conditions during STS-87 favored a reduced level of PSI activity, and we are uncertain at this point if the reduction was due to a photoinduction of existing enzyme activity leading to protein turnover or a down-regulation of protein biosynthesis. (Supported by NASA grants NAG10-0142, NAGW-2328, and the Kansas NASA EPSCoR Program)
BLOCKING OF BIOTINYLATED PROTEINS PRIOR TO USE IN THE AMPLIFIED ALKALINE PHOSPHATASE IMMUN-BLOT DETECTION. M.J. Palm and B. Johnson-Wint. Department of Biological Sciences, Northern Illinois University DeKalb, IL

The analysis of protein from tissue samples using a biotin-streptavidin detection system is common place in today's cell and developmental biology lab. One analysis that uses this detection methodology is western blot detection using the Bio-Rad Amplified Alkaline Phosphatase Immun-Blot Detection. This detection format uses a biotinylated secondary antibody with the amplification of streptavidin. Then biotinylated alkaline phosphatase completes the sandwich and color developing reagent, 5-bromo-4-chloro-indoly phosphate and nitroblue tetrazolium, is added. This forms a purple precipitate at any point of binding of the biotinylated alkaline phosphatase. In analyzing total protein pellets using extremely sensitive biotin-streptavidin detection, endogenous biotinylated proteins can give erroneous results through cross reaction with the streptavidin in the detection kit. The purification of proteins, to eliminate this cross reaction, can be labor intensive and time consuming therefore any reduction in time and cost of deleting nonspecific interactions is beneficial. In the model system when total protein extracts from bone are run on the western blot detection numerous non-specific bands are encountered. We have developed a methodology to block these endogenous biotinylated proteins from being detected in the western blot. The blocking step involves the use of avidin and biotin in a pre-primary antibody step to block the biotin-streptavidin interaction, this new step is called the BLOCKO step. The addition of this step allows the specific bands of interest to be viewed without elaborate purification of the total protein. The BLOCKO step is easy, cost-effective, and requires little time. This new information about detection of biotinylated proteins could also be used to identify endogenously biotinylated proteins from total protein extracts.
SESSION C: CONCURRENT POSTERS II
Space Life Sciences Training Program

Students in the six-week program were involved in science research and engineering activities pertaining to the NASA ALS program. Research efforts included: The design, construction and evaluation of a sealed Mars simulation chamber; microbiological studies in hydroponic systems; studies of plant micronutrient disorders; investigations into grey water recycling in plant culture systems; and biological filters for air treatment. The students participated in a seminar at the end of the program and presented the results of their efforts as well as submitting a final paper.

(Supported by the NASA Space Life Sciences Training Program)


Students in the SLSTP Flight Group were divided into four small teams. The Medical Operations Team conducted an experiment to compare the effects of a placebo and three pharmaceutical countermeasures for motion sickness. Sea/Space motion sickness was induced by rotating on a tilt chair on a 15 degree angle. Testing indicated a significant difference between drugs and placebo and a commercially available medication was recommended. One student represented the Aquatics Team and created a closed marine ecosystem. The student successfully maintained brine shrimp in the small environment. The Payload Team performed characterization of space hardware and materials currently in use by NASA. Location, orientation, and freezer type indicated differences in freezing time in the Biological Research in Canisters. Another student tested an external thermometer for use in shuttle mid-deck locker experiments. A third student compared different types of cushioning foam for offgassing of organic and inorganic compounds. Each Experiment Design Team member was given the role of Project Manager of an independent experiment. Tasks included developing schedules, analyzing budget, constructing hardware, conducting hardware verification tests, integrating hardware and biology, and reporting. To understand some of the technical and scheduling difficulties of scientists and engineers working in concert, a simple experiment was conducted. These tests included hypox and hypergravity effects on root initiation, brine shrimp hatching efficiency and changes in Drosophila development time. In other studies, bacteria were cultured and plants were grown under simulated Mars conditions, including martian soil analog and a high CO₂ atmosphere. These projects will be refined and worked into K-12 outreach activities through the Internet. In addition to a submitting a final paper, all students presented their projects and lessons learned at the end of the program.

(Supported by the NASA Space Life Sciences Training Program)


Seven out of 40 undergraduate students who competed for independent research support in the 1998 SLSTP were selected for involvement in the Ecology Program. They worked under the direct supervision of principal investigators in several ongoing field projects related to the wide variety of ecosystems found in the contiguous lands of KSC, Merritt Island National Wildlife Refuge, Canaveral National Seashore, and Cape Canaveral Air Station. Research conducted by the group included: (1) Comparing the use of digital ortho quad maps to differential Global Positioning System in radio-tracking Indigo snakes (Dryamachon corais coupert); (2) conducting baseline studies of horseshoe crabs (Limulus polyphemus) including the relationship of hydrogen sulfide in sediments to local populations; (3) investigating marsh sediment sampling techniques for heavy metals analysis near launch pads; (4) evaluating scrub jay (Aphelocoma coerulescens) habitat restoration subsequent to bulldozing, drying, piling, and burning; (5) measuring volatile organic emissions from important native and exotic shrubs; (6) watershed model evaluations; and (7) fish foraging of important wading birds. The SLSTP studies continue to provide important data for long-term research efforts at KSC, some projects involve older ecological and climatological data (1945) while others have been ongoing for at least a decade and still others are new long-term studies initiated this season. (Supported by the NASA Space Life Sciences Training Program)
SESSION C: CONCURRENT POSTERS II
Undergraduate Student Poster Competition (continued)
[32] RICE-PATHOGEN INTERACTIONS: A MODEL SYSTEM FOR SPACE-FLIGHT EXPERIMENTS. A. Chambers, M.J. Ryba-White, E. Hilaire, J.A. Guikema, J.B. Leach. Department of Plant Pathology and Division of Biology, Kansas State University, Manhattan 66506-5502

Rice is not only an excellent food source for sustaining life in space, but it also offers many advantages as a model system for studying the effects of microgravity on plant growth, development, and most importantly, on plant pathogens. The rice genome is small relative to other cereal species and it is the simplest of cereal plants to transform, allowing for molecular genetic manipulation. Rice can be adapted to contained systems used for flight experiments, for example, the BRIC (Biological Research in Canisters) or PGC (Plant Growth Chambers). Finally, the physiology and molecular biology of interactions between rice and a bacterial pathogen, Xanthomonas oryzae pv. oryzae in unit gravity have been well-characterized. We have modified an experimental system for use in the analysis of the effects of microgravity on rice/bacterial interactions in BRICs. Autoclavable plastic growth pouches containing germination paper provide support for the developing rice seedlings and protection from contamination. The pouch design allows for visual or photographic monitoring of the growth and development of the rice roots. Seedlings can be easily removed from the pouches for subsequent treatments, such as chemical fixation for microscopy. Channels are sealed into the pouches and surface-sterilized seeds are positioned so that root growth is directed towards the pouch bottom, even under clinostat conditions. The rate of growth of rice roots in the pouches has been determined. Results of pathogen inoculation experiments will be presented.

(Supported by NASA: NAGW-2328 and the NASA Space Grant Consortium.)

[33] ALGAL AND CYANOBACTERIAL GROWTH IN 100% CO2. R.D. Hall, P.A. Currier, D.J. Thomas. Howard Community College, MD; The Biometrics Corporation, Kennedy Space Center, FL; Department of Biological Sciences, University of Idaho.

The effect of 100% CO2 on the growth rates of marine and freshwater algae and cyanobacteria was studied in this experiment. The specimens studied were Euglena gracilis, Prochlorococcus marinus, Synechococcus sp. PCC7942, and Synechococcus sp. PCC7902. Four asexual cultures of each strain were cultured in test tubes in a 27°C water bath and continuously sparged with filtered 100% CO2 or compressed air. For two weeks cell density was measured every 48 hours using optical density at 750 nm. Results indicate that CO2 has an adverse affect on the algae and cyanobacteria.

PCC7902 showed some growth in 100% CO2, though the growth rate was considerably less than that of the strain grown in air. PCC7942 and P. marinus also grew in 100% CO2, although the PCC7942 culture had a much lower growth rate than its counterpart grown in air while P. marinus did not grow in compressed air.

(Sponsored by NASA Space Life Sciences Training Program)

[34] THE EFFECTIVENESS OF THREE PHARMACEUTICAL COUNTERMEASURES IN PREVENTING VESTIBULAR DISORIENTATION WHILE MAINTAINING COGNITIVE ABILITY. B.K. Bhashyam, D. Woodard, D.J. Thomas. Department of Biological Sciences, University of Illinois, Urbana/Champaign, IL; The Biometrics Corporation, KSC, FL; Department of Biological Sciences, University of Idaho, Moscow, ID

The effects of three pharmaceutical motion sickness countermeasures were measured in this experiment. Specific interest in this study was directed towards occupational motion sickness. Twenty human subjects were subjected to a fifteen minute rotational exposure on a custom built tilt chair at 15 rpm once a week for four weeks. Each exposure examined the effects of a different pharmaceutical treatment: scopolamine, cinnarizine, meclizine hydrochloride, or placebo. Objective cognitive data was obtained through the administration of a digit span test and subjective symptom data was collected by questionnaire during and after exposure. Subjects under meclizine treatment were found to have much greater tolerance of the rotational exposure than any of the other experimental groups (P value < 0.05). In addition, the mean intensity of the motion sickness symptoms, measured every minute during exposure, for these subjects under meclizine treatment peaked at 0.842 (at the fourteenth minute) on a 0-4 scale, which indicated that the symptoms were below a noticeable threshold. Scopolamine and cinnarizine had average peak levels that indicated significantly higher symptom intensity than experienced under meclizine treatment. Subjects under placebo treatment had the lowest threshold among all groups with a peak of 2.316 (mean at the fourteenth minute), a level approaching a threshold that would prevent the completion of a complex task. The digit-span test indicated that no significant differences exist between any of the counter-measures or placebo in maintaining cognitive function level. Future studies with larger sample sizes and a wider variety of counter-measures will aid in determining the optimal pharmaceutical combination to combat occupational motion sickness.

(Supported by the NASA Space Life Sciences Training Program)

[35] EFFECT OF pH ON IGEPON® SOAP DEGRADATION UNDER DENITRIFYING CONDITIONS. V. Hsu, M. Alazraki, R.F. Strayer. Department of Biology, Washington University, St. Louis; Dynamic Corporation, Kennedy Space Center.

The effect of pH on Igépon® soap degradation/denitrifying bacteria was examined using anaerobic shake flask cultures. Igépon® soap was selected for use on International Space Station. Because of the sensitivity of bacteria to extremes of pH and the expected pH increase during denitrification, pH was expected to be a significant environmental factor. The high pH levels that can accompany denitrification were predicted to inhibit bacterial growth. In addition, inoculum sources for denitrifying soap degraders were examined. Enrichment cultures were inoculated with either bacteria from hydroponically-grown wheat roots or the liquid medium of an Intermediate Scale Aerobic Bioreactor (ISAB). Both cultures were subjected to anaerobic conditions in a soap and nitrate medium and were able to degrade soap using denitrification. The enrichment cultures were used to inoculate anaerobic shake flasks buffered at pH 7.2 or 9.0. Half the flask medium was replaced daily for a retention rate of two days exposing bacteria to a maximum soap concentration of 1000ppm each day. Soap degradation, turbidity (bacterial growth), and pH were assayed regularly for each flask. Cultures grew most rapidly and degraded soap more readily in pH 7.2. The root cultures at pH 7.2 achieved a steady state of soap degradation that removed all but trace amounts of soap within six days of culture initiation. ISAB cultures at pH 7.2 required 10 days to reach a steady state of degradation. The root and ISAB cultures at pH 9.0 had limited growth and minimal soap degrading activity. These results indicated that denitrifying bacteria grown at high pH have significantly reduced soap degrading ability. Either inoculum could be used for a microgravity anaerobic bioreactor to process Igépon®-containing gray water. Both would require pH control to maximize the effectiveness of gray water processing. (Supported by the NASA Space Life Sciences Training Program)
[36] PSEUDOMONAS AERUGINOSA: PERSISTENCE IN THE RHIZOSPHERE. S.J. Berneck1, A. Matos2, J.L. Garland3. 1Olympic College, Bremerton, WA, 2Department of Biology, Univ. of South Florida, 3Dynamac Corporation, Kennedy Space Center.

Advanced Life Support (ALS) systems such as the planned International Space Station will utilize bioregenerative life support systems to facilitate extended duration space missions. Plants will play an integral part of such systems. As a result of their potential role as a source of oxygen, food, and filtration, these plants may also serve as reservoirs for human-associated bacteria (H-AB). Certain opportunistic bacteria, such as Pseudomonas aeruginosa, warrant particular attention. The ability of P. aeruginosa to persist in the rhizosphere of hydroponically grown wheat was examined by enumeration of these organisms after a seven-day incubation period. The factors examined were: (1) the diversity of the rhizosphere community, (2) the density of the invading organism, and (3) the time after planting at which the invasion occurs. Pseudomonas aeruginosa was introduced into wheat plants containing rhizosphere microbial communities of high, medium, and low diversity. Thirty six plants were inoculated with P. aeruginosa, with half of the plants receiving inoculation seven days after planting and half receiving inoculation 21 days after planting. The amount of P. aeruginosa in the inoculum was manipulated to vary the invader density. This research indicates that the time after planting at which invasion occurred does not effect the persistence of the P. aeruginosa. The diversity of the microbial community at the time of invasion also has little effect. However, the density of the P. aeruginosa at the time of invasion plays a significant role in its ability to survive. This could be an important consideration when engineering biomass production or greywater recycling systems in an ALS environment. (Supported by the NASA Space Life Sciences Training Program)

[37] CREATING A SOIL ALTERNATIVE FROM CLASSROOM MATERIALS: A PROJECT DESIGN. B. Weber1 and M. Lewandowski2. 1Nebraska Wesleyan University, Lincoln, NE, 2Dynamac Corporation, DYN-3, Kennedy Space Center, FL.

With the technology of space flight becoming more advanced each day and the prospect of long duration missions becoming more of a reality, measures should be taken to inform the public, primarily children, of how the space program is reaching a new era of exploration. The primary goals of this project were to design, carry out and inform grade school students of the various recycling and waste recovery methods that might someday be implemented for use in space.

A physical experiment was carried out using different materials found only in a classroom to grow Raphanus sativus, radishes. Many different types of organic compounds were used from leftover lunch scraps for nutrients and base materials such as paper and rocks were used to create a soil-like texture. Out of the five different organic materials used, two of them showed plant growth. The plants were tested against a soil control for total height and weight. After the materials had been tested and suitable growth candidates were chosen all of the information needed to perform the experiment was written up in a project booklet to be used for further outreach project in elementary classrooms. (Supported by the NASA Space Life Sciences Training Program)

[38] COMPARING THE EFFECTIVENESS AND PRECISION OF TWO TECHNIQUES FOR SEDIMENT SAMPLING AT JOHN F. KENNEDY SPACE CENTER. T. L. Holler1, M. Motz1, and D. M. Schreider1. 1Erskine College and 2Dynamac Corporation.

Metal contamination is a major concern in the wetlands surrounding the Kennedy Space Center (KSC). This project compared the effectiveness and precision of two techniques to detect metal concentrations in sediment samples. The present technique, the use of a lexon core sampler, was compared to a newer technique, the use of modified 20 cc syringes, to decide which was more efficient in detecting metal concentrations in the top 1 cm of sediment. This was very important for the Ecology Department at KSC in order to maintain proper environmental monitoring and compliance. Metals tested included: Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sr, Ti, V, Zn, and Ag. While comparing the two different techniques of sediment sampling, the benefits and disadvantages of each technique were considered. The syringe samplers proved to be superior for collecting shallow sediment samples due to their small size, ability to create their own vacuum, and easy storage capabilities, which , unlike the lexon core sampler, kept the samples from getting homogenized before analysis. After the metals' concentrations were measured by Inductively Coupled Plasma analysis (ICP), the three syringe results from each core were tested for significant differences (a < 0.05) from their corresponding lexon core sample using Bonferroni ANOVA. Test results revealed that 53% of the time there was a significant difference between the two methods used. This indicated that, by using the present sampling method, there would be a 53% chance of not detecting proper metal concentrations. Due to the consequences of metal bioaccumulation, it is paramount to use the sediment sampling technique that provides the most accurate data. (Supported by the NASA Space Life Sciences Training Program)
SESSION C: CONCURRENT POSTERS II
Graduate Student Poster Competition (continued)
EFFECTS OF 2G ON LEAN AND OBESE ZUCKER RATS. L.E. Warren, B.A. Horwitz and C.A. Fuller. Section of Neurobiology, Physiology and Behavior, University of California, Davis.

Changes in the ambient force environment alter the regulation of food intake and body fatness. Since fat regulation underlies human survival capacity, an understanding of the related regulatory pathways and their responsiveness to the ambient force environment may be critical to long duration spaceflight. We have studied the effects of 2G on food intake and body mass of both lean (Fz/Fz) (n = 19) and obese (fa/fa) (n = 22) male Zucker rats. Rats were individually housed in metabolism cages with food and water provided ad libitum. The control rats were similarly maintained at 1G. Following a 2-week baseline period at 1G, the 2G group was subjected to centrifugation on a 1.5-m diameter centrifuge for 8 weeks. Food intake and body mass measurements were obtained weekly. All animals were sacrificed immediately post-centrifugation. The lean rats' response to 2G included an initial reduction in body mass, and a resumption of growth at a rate greater than the controls (though at a decreased mass). In addition, the lean rats exhibited a 50% reduction in percent body fat after 8 weeks of 2G. In contrast, obese rats exhibited a decrease in body mass with resumption in growth at a slower rate, and a smaller decrease in body fat than the lean rats. In both lean and obese rats, mass adjusted food intake decreased upon centrifugation and then recovered to control levels within 4-5 weeks. Percent body fat differed significantly (p < 0.05) for each of the following comparisons: 1G obese > 2G obese > 1G lean > 2G lean. As expected, obese rats had significantly higher circulating leptin levels (mg/ml) than did the lean rats. Additionally, 2G rats and 1G had significantly higher leptin levels than lean rats at 2G. While this trend persisted in the obese rats, the difference did not reach significance. There was a differential response to 2G in the obese vs. lean rats, indicating that while both genotypes responded to hypergravity, the response of obese Zucker rats was quantitatively different from that of their lean counterparts. The observed differences further investigated the effects of altered ambient force environments on regulatory pathways mediating food intake, body mass and body fatness. (Supported by NASA: NAG5-3959 to BAH and GSRP-98-009 to LEW)

HINDLIMB SUSPENSION ALTERS ARTERIAL MORPHOLOGY IN RAT HINDLIMB SKELETAL MUSCLE. P.N. Colleran, J.M. Delp, M.K. Wilkerson and M.D. Delp. Deps. of Health and Kinesiology, Texas A&M University, College Station, TX, and ²Sam Houston State University, Huntsville, TX.

Simulated microgravity diminishes the force producing capacity of large conduit vessels in rats. It has been hypothesized that this attenuation in vasocostricor force may be due to smooth muscle atrophy. Therefore, the purpose of this study was to determine if the cross sectional area (CSA) and thickness of the medial layer of rat skeletal muscle feed arteries and 1A arterioles is altered by hindlimb suspension. Vessels from control (n = 7) and hindlimb suspended (n = 6) rats were dissected free and cannulated on glass micropipets. Luminal pressure was set at 60 cm H2O, and the vessels were dilated with sodium nitroprusside (10⁻⁴ M), fixed with paraformaldehyde, and embedded in paraffin. Vessel cross sections were cut 5 mm thick and stained with hematoxylin and eosin. Medial layer CSA and thickness were measured with a BioQuant image analysis system. Hindlimb suspension (14 day) reduced medial layer CSA and thickness in gastrocnemius muscle feed arteries and 1A arterioles (P < .05), but did not alter the number of smooth muscle cell nuclei. Vessels from control (n = 7) and hindlimb suspended (n = 6) rats were dissected free and cannulated on glass micropipets. Luminal pressure was set at 60 cm H2O, and the vessels were dilated with sodium nitroprusside (10⁻⁴ M), fixed with paraformaldehyde, and embedded in paraffin. Vessel cross sections were cut 5 mm thick and stained with hematoxylin and eosin. Medial layer CSA and thickness were measured with a BioQuant image analysis system. Hindlimb suspension (14 day) reduced medial layer CSA and thickness in gastrocnemius muscle feed arteries and 1A arterioles (P < .05), but did not alter the number of smooth muscle cell nuclei. Medial layer CSA was also reduced in tibialis muscle feed arteries and 1A arterioles (P < .05), but medial thickness was unaltered. In addition, hindlimb suspension did not alter medial CSA, thickness, or the number of smooth muscle cell nuclei in triceps muscle feed arteries in the forelimb. These data suggest that the simulated microgravity-induced reduction in the vasocostricor responsiveness of the resistance vasculature in hindlimb muscle may be due to a decrease in the thickness of the medial layer that results from smooth muscle cell atrophy. These data have important implications in regard to the regulation of muscle blood flow and vascular resistance. (Supported by NASA grants NAGW-4842 and NAG5-3754 and NSBRI grant NCC-9-58.)

EFFECTS OF 14-DAY HINDLIMB UNLOADING ON RAT CEREBRAL, SPLenic, AND MESENTERIC ARTERIAL MORPHOLOGY. M.K. Wilkerson, J.M. Delp, P.N. Colleran, and M.D. Delp. Departments of Health & Kinesiology, Texas A&M University, College Station, TX, and ²Sam Houston State University, Huntsville, TX.

Hindlimb unloading (HU) of rats has been shown to cause a caudal shift in body fluids. As a result of this shift, the systemic blood pressure gradient is altered so that there is a higher arterial pressure in the head region while arterial pressure in the abdomen remains unchanged. We postulated that this caudal pressure change would alter the medial layer structure of small resistance arteries from 14-day HU male Sprague-Dawley rats. The middle cerebral artery (MCA) and mesenteric (MA) and splenic (SA) feed arteries were removed from control (C, n = 8) and HU (n = 7) animals. The vessels were cannulated on glass pipettes attached to a fluid reservoir and luminal pressure was set to 60 cm H2O. The vessels were relaxed with 10⁻⁴ M nitroprusside, fixed with paraformaldehyde, and embedded in paraffin. From vessel cross-sections (5 mm thick), cross-sectional area (CSA) and thickness of the medial layer were determined using an image analysis system. No differences in medial layer CSA or thickness from MA and SA were detected between groups. However, in the MCA, both medial CSA (HU 17893 ± 2539 μm²; C 12904 ± 1433 μm²) and thickness (HU 33.9 ± 4.06 μm; C 22.3 ± 3.19 μm) were increased in HU, while no significant changes were detected for number of nuclei and medial outer perimeter. These findings suggest that the medial layer of MCA expands with 14-day HU. We suggest that the chronic increases in arterial pressure that occur caudally with HU elevate transmural pressure in the MCA and result in the hypertrophy of smooth muscle cells in the medial layer. This hypertrophy may be involved in the reduction of MCA blood flow velocity observed in humans following prolonged bed rest and space flight. (Supported by NASA grants NAGW-4842 and NAG5-3754 and NSBRI grant NCC-9-58.)

MIGRATION OF LYMPHOCYTES INCUBATED IN CLINOSTATs AND STATIC CULTURES. A.J. Bergman and K. Zygosurakis, Departments of Chemical Engineering and Bioengineering, Rice University, Houston.

The objective of this study was to determine if different mechanical stimuli affect the migration of lymphocytes on surfaces coated with the extracellular matrix protein fibronectin. Jurkat cells (a T lymphocyte line) were incubated for 30 days either in a clinostat or in a normal static culture. The cells were then seeded on tissue culture plates that were preincubated with solutions containing different concentrations of fibronectin. Remaining binding sites were blocked using bovine serum albumin. Migrating cells were monitored for 8 hours using a digital video microscopy setup and their positions on the plate were determined at 10-minute intervals. From these measurements, the cell trajectories were reconstructed using a nearest neighbor algorithm. Cell locomotion was analyzed using the persistent random walk model for two-dimensional systems to determine the random motility coefficient for the cell population. This parameter is similar to an effective diffusion coefficient and was used to compare the locomotory behavior of different cell populations.

For tissue culture plates coated with fibronectin solutions of 0, 4.3, 21.5, and 43 μg/ml, the random motility coefficients of Jurkat cells measured 3-7 hours after cell seeding were 4.1±0.3, 11.3±1.0, 9.6±0.6 and 16.2±2.3 μm²/min respectively. Four hours after cell seeding, Jurkat cells cultured in the clinostat showed a lower random motility coefficient (5.0±2.2 μm²/min) than cells cultured statically (10.0±0.27 μm²/min). Similarly, centrifugation (166g) for 10 minutes prior to seeding decreased cell migration rates for the first 5 hours after cell seeding. These effects, however, were reversible. Seven hours after cell seeding, the random motility coefficients of the clinostated and control cells were not significantly different. (This work was supported by the NASA grant NAGW-5007)

A serious problem encountered by the orthopedic or oral surgeon is the procurement of materials to provide support and induce bone during repair of bony defects. Because many bones form by bone replacement of a cartilage model, e.g. endochondral ossification, cartilage can be used to repair bone. But the use of cartilage has been limited, in part by the amount of tissue available from conventional culture systems. A new method of growing cartilage uses a rotating bioreactor system developed by NASA, which provides homogenous distribution of nutrients, waste, and gases, and little accompanying turbulence or shear force. In previous studies, aggregates of mouse embryonic limb cells cultured in a Rotating Wall Vessel, when implanted subcutaneously, were found to hypertrophy, calcify, vascularize, and recruit additional cells. The actual stage of differentiation at time of implantation, however, was not assessed and the capability of the bioreactor to support cartilage differentiation from undifferentiated cells was not clear. In the present study, aggregates of embryonic limb bud cells to be used in repair of a skull defect were cultured in the bioreactor for three weeks. Some aggregates were fixed for histological studies and the remaining aggregates were implanted into a 2mm defect created in the skulls of C57 black mice. Control mice had defects, but no implant. Sections of nodules showed extensive cartilage differentiation and hypertrophy at time of implantation. Healing in the defects showed vascularization and mineralization in the wound site, but analyses of undecalcified and decalcified sections are still in progress.

This study demonstrates that the NASA bioreactor supports differentiation of cartilage from undifferentiated limb bud cells and that such cartilage is suitable for healing of defects in bone.

Supported in part by NIH/NIDR Training Grant T35 DE07252.

[44] LYTIC VIRUS REPLICATION IN ASTRONAUTS DURING SPACE FLIGHT. R.P. Stowe1, D.L. Pierson2, and A.D.T. Barrett3. Dept. of Pathology, University of Texas Medical Branch, Galveston, and 3Life Sciences Research Laboratories, NASA/JSC, Houston, TX.

The majority of humans are infected with Epstein-Barr virus (EBV) early in life and thereafter carry the virus in a latent form. Reactivation of latent EBV may be an important threat to crew health during extended space missions. Control of EBV replication in vivo is mediated primarily by cytotoxic T-cells, and severe clinical symptoms have been associated with reactivation of EBV in patients with defective cellular immunity. Using a space analog of prolonged isolation (Australian Antarctic expedition), we have previously shown increased antibody responses to EBV lytic proteins associated with decreased delayed-type hypersensitivity.

In this study, we investigated the effects of short-space flight on latent EBV reactivation. Peripheral blood samples were collected from 16 astronauts before Launch (L-10 and L-5) and again upon Return (R+0 and R+3). The samples were analyzed for EBV antibodies to structural (viral capsid antigen-VCA) and nonstructural (early antigen-EA) proteins, human (h) and/or viral (v) interleukin (IL)-10, and IL-6. The VCA geometric mean titer was significantly increased (p<0.05) at L-10 as compared to baseline (annual physical) samples. In two astronauts, 4-fold decreases in VCA titers were observed at R+0 followed by a 4-fold increase at R+3. Plasma levels of hIL-10/VIL-10 were generally elevated at R+0. Three astronauts exhibited high hIL-10/VIL-10 levels (11.5-15.4 pg/ml) which positively correlated with either 4-fold increases in EA titers or high VCA/EA titers. IL-6 was significantly elevated at landing indicating an acute stress response to atmospheric reentry. These results indicate that lytic replication of EBV, as reflected by rising EA/high VCA antibodies, occurs during short-space flights and may pose a significant health risk on longer Lunar/Mars expeditions.

(Supported by NASA GSRP Grant NGT-51666, Houston Advanced Research Center, and UTMB Sealy Center on Aging)

[45] GRAVITY INDUCED CALCIUM CURRENTS IN GERMINATING FERN SPORES. A. Chatterjee1, M. Porterfield2, P.J. Smith3, and S.J. Roux1. 1Dept of Botany, Univ of Texas, Austin, and 2BioCurrents Research Center, Woods Hole, MA.

Gravity fixes the developmental polarity in germinating spores of Ceratopteris richardii during the first 24 hours after germination is initiated by light. A key objective of our laboratory is to document cellular changes that occur during this period in order to understand the mechanisms by which gravity alters cellular metabolism. As part of our effort to achieve this objective, we used an ion specific microelectrode to measure calcium currents associated with polarity fixation. We found that during the period of polarity fixation, a strong influx current emerges from the top of the cell, a weaker efflux current occurs at the sides of the cell, and an even weaker influx current enters from the bottom of the cell. This same pattern is reestablished within 5 minutes after rotating the cells 180°, indicating that turning the cells upside down rapidly reverses the direction of the current. The magnitude difference between the outward current at the top and the inward current at the bottom of the cell is highest in the middle of the polarity fixation period and decreases significantly toward the end of this period. The possible role of this current in gravity-induced polarity fixation will be discussed. This is the first report of a gravity-directed calcium current in single cells.

(Supported by NASA grants NAGW 1519 & NAG10-0202.)


Integrins are a large family of integral membrane proteins that are involved in signal transduction in animal cells and tissues. Some experimental evidence suggests that plant cells also contain integrin-like proteins and that these proteins may be involved in signal transduction in gravitropism. Several researchers have attempted to localize integrin-like proteins in plants using antibodies raised in animals, despite the potential limitations of this approach. In this study, we compare results from localization of integrin-like proteins in Arabidopsis using a synthetic RGDS-ligand to localization of these proteins using a polyclonal antibody raised to a chicken β-integrin. These results demonstrate that use of the animal antibody to localize integrin-like proteins produces artificial immunoglobulin and immunofluorescence signal in plastids due to the high affinity of this antibody for polysaccharides. In addition, preliminary results suggest that use of synthetic ligands such as RGDS may provide a more efficient tool to analyze integrin-like proteins in plants and to study their role within gravitropic perception, transduction, or response.

(Financial support was supplied by NASA through grants NAG 2-1017 to JZK and grant NRT 5-50041 to LJS.)

Arabidopsis TCH4 encodes a xyloglucan endotransglycosylase. Xyloglucan polymers are thought to crosslink cellulose microfibrils in the plant cell wall. TCH4 cleaves xyloglucans internally and ligates the newly generated ends onto new xyloglucan chains; our hypothesis is that this activity is used for cell growth and/or cell wall strengthening. TCH4::GUS fusions are expressed in rapidly growing tissues as well as sites predicted to be experiencing mechanical strain such as root-shoot junctions and vascular tissue. TCH4 expression is rapidly up-regulated in response to touch, darkness, and temperature extremes, as well as auxin and brassinosteroids. Using transgenic plants harboring TCH4 upstream sequences fused to reporter genes, we are identifying potential cis-regulatory elements conferring gene expression regulation. The region between -258 and -45 of TCH4 is sufficient to confer response to all the tested stimuli. Preliminary dissection of this region indicates that despite the complexity of TCH4 regulation, expression may be controlled by a single short 54 bp region. (Supported by NASA Specialized Center for Research and Training grant no. NAGW 5007.)

[48] LOW OXYGEN ALTERATIONS IN ARABIDOPSIS LEAF STRUCTURE RESEMBLE BRASSINOLIDE-DEFICIENT MUTANTS. K.M. Ramonell and M.E. Musgrave. Dept. Plant Pathology & Crop Physiology, Louisiana State University, Baton Rouge, LA.

Plants are the foundation of the proposed Controlled Ecological Life Support System (CELSS) which NASA will utilize on future missions. It has been postulated that if plants could be grown under lower O2 concentrations, their productivity might be increased due to a repression of photosrespiration, resulting in increased yield. Previous studies in Arabidopsis thaliana grown under low O2 have shown that at 2.5% O2, plants exhibit a reduction in overall size as well as increases in stomatal density and distribution across the leaf surface. The A. thaliana mutant det2, which is defective in brassinolide synthesis, also exhibits reduced size. Since brassinolide synthesis has five O2-requiring steps, its synthesis may be inhibited under low O2. In this study, the stomatal density of the det2 mutant, its relationship to the stomatal density of A. thaliana grown under low O2, and the ability of brassinolide to rescue det2 were investigated. A. thaliana var. Columbia and det2 were grown on media alone or media supplemented with 10^{-7}, 10^{-6}, and 10^{-5} M brassinolide in magenta vessels receiving 200 \mu mol/m^{2}/s PAR at 25°C for 12 days. Leaves were examined using scanning electron microscopy and statistical analysis was performed. The leaves of det2 show similar patterns of increased stomatal density and aberrant stomatal pairing to leaves grown under 2.5% O2. When supplemented with 10^{-7} M brassinolide, the abaxial stomatal density of det2 leaves returns to wild-type levels. The leaf length of det2 also returns to wild-type levels at 10^{-6} M brassinolide. These data indicate that inhibition of plant growth and changes in leaf structure that occur under low O2 may be caused by lack of brassinolide synthesis. Supplementation of plants grown under 2.5% O2 with brassinolide may restore normal growth and development. (Supported by NASA grant NAGW-3759 and the Louisiana Space Consortium.)

[49] GROWTH IN MICROGRAVITY INCREASES SUSCEPTIBILITY OF SOYBEAN SEEDLINGS TO A FUNGAL PATHOGEN. M. Ryba-White, O. Nedukha, E. Hilaire, J.A. Gukema, E. Korodyum, J.E. Leach. 1Dept of Plant Pathology, Kansas State University, Manhattan, KS; 2Institute of Botany, Kiev, Ukraine; 3Division of Biology, KSU.

Extended life in microgravity will require adequate and continuous food production. Continuous crop production in contained systems and the negative effects of microgravity on plant growth will increase the potential for disease. The effects of microgravity on the susceptibility of soybean roots to disease caused by the fungal pathogen, Phytophthora sojae, were evaluated in Space Shuttle Mission STS-87 as part of the Collaborative US/Ukrainian Experiment (CUE). An experimental system was designed for use in the BRIC (Biological Research in Canisters) hardware. The design allowed soybean seed (cultivar Williams 82) to germinate in microgravity with growth directed toward the fungal inoculum. Seedlings were untreated or were inoculated with the pathogenic P. sojae isolate R25. Seedlings were fixed in microgravity at 4, 7, and 8 days after planting (DAP, flight days 3, 6, and 7). At all harvest times and in both gravity treatments, lengths of treated or untreated roots or numbers of lateral roots did not differ. No symptoms characteristic of infection by P. sojae were observed on fungal-treated seedlings at 4 DAP. At 8 DAP, however, the flight-grown roots infected with R25 showed significantly more disease (% brown and macerated areas) relative to the ground-grown roots infected with R25. Light and electron microscopy studies confirmed that soybean was more extensively colonized by R25 in flight than ground conditions. Although more fungal structures were present in the flight treatment, no morphological differences were observed in electron micrographs of fungal hyphae and haustoria. These data indicate that soybean grown in microgravity are more susceptible to colonization by a fungal pathogen relative to ground controls. (Supported by NASA: NAG1-0142.)


In order to comprehend the origin of life on Earth, it is essential to understand the genesis of the complex protein synthesis machinery shared by all extant organisms. A promising model envisions co-evolution of this translation machinery from a small RNA/peptide complex capable of peptide synthesis to the modern form of template-directed protein synthesis. Can such an RNA/peptide complex actually catalyze the formation of a peptide bond? We are attempting to determine the feasibility of such a scenario. To this end, we have shown that leu-leu dipeptides are formed in the presence of a specific catalytic (ala-lys), and aminoacylated tRNA with or without its anticodon domain. The mechanism of this apparent catalytic activity was analyzed using specific molecule analogues with structural similarity to ala-lys and a series of engineered RNA constructs. The next step will be to determine if the synthetic capability is maintained for RNAs of less than 25 nucleotides. RNAs derived from leucine tRNA were made by in vitro run-off transcription from specific DNA templates using T7 RNA polymerase and aminoacylated with Leu-tRNA synthetase. Aminoacylated RNAs were purified under acidic conditions and incubated with potential dipeptide catalysts in a range of acidic to basic conditions. A thin layer chromatography assay for separating and purifying the leu-leu product was developed. Product identity was established based on mobility using phosphorimage visualization and confirmed by mass spectroscopy. (Supported by NASA: NAGS-4004 and NGT5-50182.)
SESSION D: BIOTECHNOLOGY SYMPOSIUM

Protein structural information plays a key role in understanding biological structure-function relationships and in the development of new pharmaceuticals for both chronic and infectious diseases. X-ray crystallography is the predominant technique used to obtain three-dimensional structure information at atomic resolution. The Center for Macromolecular Crystallography (CMC) has devoted considerable effort studying the fundamental processes involved in macromolecular crystal growth both in a 1-g and μg environment. Results from experiments performed on more than 35 U.S. space shuttle flights have clearly indicated that microgravity can provide a beneficial environment for macromolecular crystal growth. The CMC has used crystals grown in microgravity to accelerate structure-based drug design projects. This research has led to the development of a new generation of pharmaceuticals that are currently in preclinical or clinical trials for diseases such as cutaneous T-cell lymphoma, psoriasis, rheumatoid arthritis, AIDS, influenza, stroke and other cardiovascular complications.

The International Space Station (ISS) provides an opportunity to have a complete crystallographic capability on orbit, which was previously not possible with the space shuttle orbiter. As envisioned, the X-ray Crystallography Facility (XCF) will be a complete facility for growing protein crystals; selecting, harvesting, and mounting sample crystals for x-ray diffraction; cryo-freezing mounted crystals if necessary; performing x-ray diffraction studies; and downlinking the data for use by crystallographers on the ground. Other advantages of such a facility include crystal characterization so that iterations in the crystal growth conditions can be made, thereby optimizing the final crystals produced in a three month interval on the ISS.

(Supported by NASA: NASA-40189, NCC8-126)

[52] GRAVISENSING, APOPTOSIS, AND DRUG RECOVERY FROM TAXUS CELL SUSPENSIONS. D.J. Durzan, Environmental Horticulture, University of California, One Shields Ave., Davis, CA.

Haploid and diploid cell suspensions of Taxus sp. were examined for their adaptive plasticity in response to simulated microgravity, unit gravity, and hypergravity (3 to 150 x g in a centrifuge). Microgravity was simulated in rotating wall vessels (Synthecon, TX) and in liter vessels on a clinostat having significant Coriolis mixing. Cell suspensions produced the taxane, paclitaxel (Taxol™), used in the treatment of ovarian, lung, and breast cancer.

Immunocytochemical results showed that amyloplasts contributed to taxane ring biosynthesis and to drug release at the cell wall. Drug-producing cells reacted as gravisingensing osmotic tesiometers. In stressed cells, amyloplasts docked and fused in clusters to sites on the plasmalemma before taxane release into the culture medium. In simulated microgravity and compared to all other treatments, taxane production was reduced nearly 100-fold. However, the percent paclitaxel of total taxanes was 3-to 6-fold greater, and biomass doubled. When p35-independent programmed cell death was induced, taxanes were released into the culture medium as free molecules (soluble and insoluble) or bound to membranes, nuclear fragments, hemicellulose and other particulate materials.

Unit gravity and especially hypergravity to 150 x g promoted terminal differentiation (xylogenesis) and significant drug overproduction. Low levels of bound taxanes were removed from cell walls by xylanase treatment after solvent extraction of the biomass.

A model relating the expression of families of 'touch' (TCH), nuclear cycling, and apoptosis-regulating genes to gravisingensing, cell wall modifications, and to taxane recovery accounted for most but not all of the observations.

(Supported by NASA: NAG-9-825)


A system to cultivate functional tissue equivalents using isolated cells, three dimensional polyester scaffolds, and rotating tissue culture bioreactors has been developed. Engineered cell-polymer constructs are cultivated in a state of continual free-fall, which simulates some aspects of micro-g. Our goals are to use this model system for controlled studies of tissue development and function on earth and in space, and ultimately to produce medically useful musculoskeletal and cardiovascular implants.

In engineered cartilage grown at 1g, chondrocytes produced extracellular matrix, polymer scaffolds degraded at a controlled rate, and mechanically functional, differentiated cartilaginous tissue developed. In engineered cardiac muscle grown at 1g, cardiomyocytes formed electrically conductive junctions and contracted spontaneously and synchronously. Engineered tissues grown in rotating bioreactors at 1g were morphologically, compositionally and functionally superior to those grown in conventional in vitro culture systems.

The cartilage tissue engineering system was selected for the first long term study of tissue culture in space. Bovine chondrocytes were grown on polymer scaffolds in rotating vessels, first for 3 months on earth and then for an additional 4 months either on the Mir Space Station or on earth. Final constructs from both groups were 8-10 mm in diameter x 5-8 mm thick, weighed 330-440 mg, and contained viable, metabolically active cells. Mir-grown constructs became more spherical while earth-grown constructs tended to maintain their initial discoid shapes. Constructs grown on Mir were compositionally and mechanically inferior to those grown on earth, which were in turn comparable to natural cartilage.

In summary: 1. ground studies: functional, engineered cartilage and cardiac tissues were grown using cells, polymers, and rotating bioreactors, and 2. space studies: engineered cartilage grown for 4 months on Mir was metabolically active, and structurally and functionally different from that grown on earth. We hope to extend the experience gained on Mir to future ISS studies using both the rotating bioreactor system and a new cell culture unit now under development that will be compatible with the space centrifuge, in order to determine the specific effects of gravity on tissue development and function. (Support: NAG8-836, CCU-97001)

[54] ENGINEERING PLANTS FOR SPACEFLIGHT ENVIRONMENTS. B. Bugbee, Crop Physiology Laboratory, Utah State Univ., Logan UT.

The terrestrial conversion efficiency of photosynthetic radiation into biomass and yield has steadily increased for centuries because of continuous improvements in both plant genetics and environment. To date, we have primarily manipulated the environment to improve plant growth in space. We have tremendous potential to manipulate plant genetics to improve our ability to understand gravitational effects and to improve the efficiency of regenerative life support systems. There are thousands of cultivars of each of our major crop plants, each specifically adapted to a unique environment on our planet. Matching genetics with the environment alleviates stress and can dramatically improve productivity. We cannot fully characterize higher plant response to the spaceflight environment without understanding and manipulating the genetics of our plants.

For example, continuous light is often used in space to improve plant growth but it induces calcium deficiencies in wheat and other plants. We were not able to eliminate this deficiency through environmental manipulation and we finally solved it through genetic selection of wheat lines that did not develop the deficiency. Examination of the genetic / environmental interaction was crucial to our understanding the nature of the calcium deficiency.

(Research supported by the Advanced Life Support Program, administered by the NASA Johnson Space Center).
TRANSGENIC MODELS TO STUDY REPRODUCTION, ONCOGENESIS, AND DEVELOPMENT. M.M. Matzuk, Departments of Pathology, Molecular and Human Genetics, and Cell Biology, Baylor College of Medicine, Houston, TX.

In mammals, there are approximately 100,000 genes which govern the development of an organism. For development to proceed normally, there must be coordinate interaction of thousands of these gene products in any given cell of the being. Beginning with fertilization, precise expression of these gene products is required during embryonic, fetal, post-natal, and adult development. Aberrant synthesis of even one of these gene products can be disastrous - birth defects, cancer, infertility, and even death are all possible when this developmental program is altered. To fully understand these processes in humans, it is necessary to have physiological models that closely mimic developmental events which occur during the creation of a human being.

It is now possible to manipulate the mammalian germline to generate transgenic mice that either overexpress a wild-type or mutant gene or lack a functional copy of an endogenous gene. Studies in my laboratory have been directed at elucidating some of the critical gene products involved in both normal and abnormal mammalian development. Using ES cell technology, we have created several mouse models which have reproductive or developmental defects. Female mice deficient in growth differentiation factor 9 (GDF-9), follicle stimulating hormone (FSH), and activin receptor type II (ActRII) are infertile due to blocks at specific stages of folliculogenesis leading to infertility. Furthermore, mice lacking the α inhibin gene develop ovarian and testicular tumors in the adolescent stage which resemble juvenile granulosa cell tumors which arise in young girls. Lastly, we have also created a number of models for birth defects. For example, mice with mutations in the activin βA and follistatin genes have cleft palate, a common birth defect in human infants. These and newly created transgenic mice will continue to be useful in vivo models to study reproduction, oncogenesis, and development.
SESSION E: ORAL SESSION - PLANT BIOLOGY I
ABSTRACTS 1998 ANNUAL MEETING
FRIDAY PM

[56] ROOT PHOTOTROPISM AND GRAVITROPISM IN WILD-TYPE AND STARCHLESS MUTANTS OF ARABIDOPSIS S. Vitha1, 2 and F.D. Sack1. 1Department of Plant Biology, Ohio State University, Columbus, and 2Dept Biology, Univ. Nevada, Reno (current address). Arabidopsis roots are negatively phototropic (grow away from the light), and gravitropism-impaired mutant roots (aux1) displayed stronger phototropism than the wild-type (WT; Okaeda & Shimura 1992 Aust J Pl Physiol 19: 439). In many gravitropism experiments, the light came from above, a configuration that could exaggerate the effect of gravitropism if phototropism were not taken into account.

Many data indicate that starch-filled amyloplasts trigger gravitropic sensing. However, roots of the starchless mutant, pgm-1 (TCP75), have been reported to be gravitropic, especially when grown in the light. But studies at threshold g-doses have determined that pgm-1 roots are about twelve times less sensitive than WT roots (Kiss et al. 1989 Planta 177: 198-206). These results demonstrate that starch is not necessary for some gravitropism but is required for full sensitivity. However, these data were obtained with light from above and thus it is possible that the contribution of root phototropism led to an overestimate of the gravitropic sensitivity of either or both genotypes.

To determine the contribution of phototropism to the measurement of apparent root gravitropism, various measures of gravitropism were performed so that the responses with light from above or below were compared in the WT and in the starchless mutants pgm-1 and adg1-1 (TL255) of Arabidopsis. The position of the light significantly influenced the measurement of virtually every gravitropic parameter tested, and in all cases the root angles of the starchless mutants were affected more by phototropism than the WT. By accounting for the contribution of root phototropism, the pgm-1 mutant was found to be significantly less sensitive to gravity than the WT compared to previous estimates. These results provide additional support for the importance of amyloplasts in gravitropic sensing and also point to the need for consideration of light position in the design of gravitropism experiments. (Supported by NASA grants NAGW-4472 and NAGS-3774)

[57] THREE-DIMENSIONAL ULTRASTRUCTURE OF LENTIL ROOT CAP STATOCYTES GROWN IN SPACE. J.D. Smith1, S. Burwen1, N. Marinkovich1, D. Driss-Ecole2 and G. Perbal2. 1NASA Ames Research Center, Moffett Field, CA, 2Univ. Pierre et Marie Curie, Paris, France.

The gravity-dependent sedimentation of amyloplasts in the root caps of higher plants is thought to initiate gravity signaling events that direct plant growth. In a g environment amyloplasts typically sediment atop a complex of Endoplasmic Reticulum (ER) located at the distal cell pole. When plants are gravistimulated (turned on their sides) amyloplasts sediment away from the distal ER complex, but as plant curvature progresses the plastids return to their positions atop the distal ER. Amyloplast sedimentation back onto the ER after gravicurvature may induce a signal to stop gravicurvature [Perbal and Driss-Ecole (1993) Acta Bot. Gallica 140:615-632]. Additionally, for seedlings raised in microgravity, amyloplasts are grouped together near the centers of root cap statocytes [Lorenzi and Perbal (1990) Physiol. Plant. 78:532-537; Smith et al. (1997) Plant J. 12:1361-1373]. The objective of this research is to determine if amyloplasts are closely associated with ER in a microgravity environment, even when plastids are grouped and/or located near the cell center, away from the distal ER complex. Lentil root caps from seedlings grown in 1-g on earth, in microgravity and in an onboard 1-g centrifuge in space (Spacelab LML 2 mission, 1994) were embedded in Araldite, serially sectioned and imaged by transmission electron microscopy. Three-dimensional reconstructions of whole amyloplasts and ER complexes were made using Reconstruction Of Serial Sections (ROSS), a software package developed by the Biocomputation Center at NASA Ames Research Center. With 3-D reconstructions, the absolute positions and proximities of amyloplasts to ER were visible, thus allowing for complete measurement of plastid/ER contacts. Quantitative analysis of the plastid/ER connections are underway to determine if gravity-dependent sedimentation of amyloplasts causes these organelles to physically interact. If plastid/ER contact is involved in the stop of the gravity-signal pathway then, in a microgravity environment where fewer contacts between these organelles are apparent, normal signaling events are disrupted which could explain that graviresponsiveness of the lentil root is greater in microgravity (Perbal and Driss-Ecole, S/MM-05 mission, 1997). (Supported by NASA Ames Research Center.)

[58] CO-LOCALIZATION OF ACTOMYOSIN AND CALRETICULIN AT AMYLOPLASTS PERIPHERIES: POSSIBLE IMPACTS FOR GRAVIVERCEPTION. D. Volkmann, M. Pilger and F. Baluska. Botany Institute, University Bonn, Kirchsteele 1, D-53115 BONN, Germany.

Actin, myosin II and calreticulin have been localized at the peripheries of amyloplasts in different cell types of roots and shoots from maize by immunofluorescence microscopy. Sedimentable amyloplasts from the root cap statocytes showed prominent labelling for myosin whereas weaker labelling was observed for calreticulin, and actin. In non-sedimentable root cortex amyloplasts, labelling was prominent both for myosin as well as calreticulin, less so for actin. After osmotic stress, the labelling pattern had changed. In non-sedimentable amyloplasts of cortex cells, calreticulin decreased at the peripheries of amyloplasts and it was observed at pit fields located mainly at the longitudinal cell walls. The same situation was true to lesser extent for myosin. Actin labelling remained associated with the retracting proplastid of plasmolysed cells.

These results indicate that the localization of prominent molecules related to the field of cytoskeleton and calcium is influenced by mechanical stress factors.

[59] AMYLOPLAST MAGNETOPHORESIS MIMICS GRAVITROPISM IN SINGLE-CELL GRAVISING SENSING MOSS PROTONEMATA. O.A. Kuznetsov1, J. Schwuchow2, F.D. Sack1, K.H. Hasenstein1. 1Botany Dept., University of SW Louisiana, Lafayette, LA 70504-2455 & Dept. of Plant Biology, Ohio State University, Columbus, OH 43210.

After gravistimulation of Ceratodon purpureus protonemata in the dark, amyloplast sedimentation is followed by upward curvature in the wild-type (WT) and downward curvature in the wrong way response (wwr) mutant. Both gravity sensing and the response take place in the same apical cell. We used high gradient magnetic fields (HGMF) to displace the amyloplasts inside protonemata and to simulate the effect of gravity. The field was applied by placing protonemata either between two permanent magnets at the edge of the gap, or close to the edge of a magnetized ferromagnetic wedge, or close to a small (< 1 mm) permanent magnet. Continuous application of an HGMF in all three configurations resulted in plastid displacement and induced curvature in tip cells of WT and wwr protonemata. Generally, WT cells curved toward the HGMF, and wwr cells away from the HGMF, comparable to gravitropism. Plastids isolated from protonemal cultures had densities ranging from 1.24 to 1.38 g/cm3. Plastid density was similar for both genotypes, but the mutant contained larger plastids (diameter ±SE = 1.97±0.03 μm) than the WT (1.74±0.03 μm), possibly explaining the stronger response of the wwr protonemata to HGMFs. These data support the plastid-based theory of gravitropic sensing and suggest that HGMF-induced ponderomotive forces can provide a gravity-like stimulus (supported by NASA grants NAG10-0179 [FS] & NAG10-0190 [KHH]).

Biochemical characterization and structure/function studies of chimeric Ca<sup>2+</sup>/calmodulin-dependent protein kinase (CaCMK) have revealed that it has dual modes of regulation by Ca<sup>2+</sup> and Ca<sup>(Ca<sup>2</sup>+</sup>/calmodulin (Patil et al., Proc. Natl. Acad. Sci. 92: 4897-4901, 1995; Takezawa et al., J. Biol. Chem. 271: 8126-8132, 1996). CaCMK has unique structural features, including a catalytic domain, a calmodulin-binding domain, and a neural visinin-like Ca<sup>2+</sup>-binding domain. The existence of these three features in a single polypeptide distinguishes it from other kinases. The calcium-binding C-terminal region of CaCMK is similar to mammalian neural visinin-like calcium-binding proteins. Calcium binding to the EF hands in the visinin-like domain controls autophosphorylation of CaCMK. The autophosphorylated CaCMK shows maximal kinase activity in the presence of calmodulin (Takezawa et al., J. Biol. Chem. 271: 8126-8132, 1996). The use of deletion mutants of CaCMK lacking EF hands I, II, and III indicated that these calcium-binding sites were crucial for full calcium/calmodulin-dependent kinase activity (Ramachandran et al., J. Biochem. 121: 984-990, 1997). Site-directed mutants were produced to alter the calcium-binding to each EF hand in the visinin-like domain. The calcium-binding properties of these mutants as well as auto- and substrate phosphorylation are being investigated. The genomic clone of CaCMK was isolated and characterized. It contains 6 introns and the putative promoter region (1.7 kb) of CaCMK has a cAMP regulatory element (CRE, nucleotides 1696 to 1702) and the consensus sequences for activator protein 2 (AP-2) element (nucleotides 1565 to 1574). The functional significance of these elements and the two regulatory domains (calmodulin-binding domain and the Ca<sup>2+</sup>-binding visinin-like domain) will be discussed. (Supported by NASA grant NAG-10-0061 and NSF grant MCB 96-30337.)


Plants are capable of adapting to diverse environments. What proteins function to alter morphogenesis in response to stimuli? An early response to a variety of environmental stimuli, like touch, darkness, and temperature shocks, is the induction of expression of TCH2 in Arabidopsis. TCH2 encodes a Ca<sup>2+</sup> binding protein with 44% amino acid identity to calmodulin. Transgenic plants have been generated which express the uida gene encoding8-glucuronidase (GUS) under control of -1 kb TCH2 upstream sequences. This upstream sequence is sufficient for induction of TCH2-driven GUS expression following touch, darkness, and temperature shifts. Major sites of basal level TCH2-driven GUS activity are root tips and branch points. GUS activity is also observed within the floral tissues, with staining observed during stigmatic papillae and pollen maturation, and stigmatic staining disappearing after fertilization. TCH2-driven GUS activity is also observed in guard cells following stimuli known to result in stomatal closure. We are investigating the role of TCH2 in closure of the stomata. Another prominent site of TCH2-GUS expression is the hydathode. Hydathodes are passive pores on the leaf margin of many plants that are involved in guttation (water release in response to root pressure). TCH2 expression at this site may indicate a role in biogenesis and/or function of these complex structures in Arabidopsis. (Supported by NASA Specialized Center for Research and Training, grant no. NAGW 5007.)

[62] CELLULAR, MOLECULAR, AND ELECTROPHYSIOLOGICAL CHANGES DUE TO DEVELOPMENTAL POLARITY INDUCED BY GRAVITY IN SINGLE CELLS. S.J. Roux, A. Chattoraj, D.J. Eastburn, W.M. Hanson, M. Porterfield, and P.J. Smith. Dept. of Botany, Univ of Texas, Austin, and BioCurrents Research Center, Woods Hole, MA.

Ceratopteris richardii is a tropical fern which has become a useful model system for studies of plant growth and development due to the ease with which genetic studies can be carried out on it. We recently reported that in single cell spores of Ceratopteris there is a defined period during which gravity sets a developmental polarity that orients the direction of nuclear migration and subsequent rhizoid emergence. We have established the cellular, molecular, and electrophysiological basis for this gravity response. We employed Differential Display Reverse Transcriptase-PCR to find differentially expressed cDNAs during the critical period of gravity responsiveness. We have identified 17 cDNAs by this method, and independently confirmed the differential expression of the mRNAs they encode by Northern analysis. We are currently attempting to clone the full length cDNAs using PCR based methods. In order to assess actin cytoskeletal changes that may occur during polarity fixation, spores were treated with rhodamine-phalloidin and their actin cytoskeleton visualized by confocal laser microscopy at various points during the gravity response window. We are testing whether cytoskeletal changes precede and predict the direction of nuclear migration or occur as a result of that migration. We demonstrated that calcium currents are associated with this process, using a calcium selective electrode to record the net movement of calcium across the membrane of the spore at different locations with respect to the vector of gravity. Results from these cellular, molecular, and electrophysiological analyses will be discussed. (Supported by NASA grants NAGW 1519 & NAGW 0202.)


Shoots of the lazy-2 mutant of tomato (Lycopersicum esculentum Mill., cv. Alisa Craig) exhibit a normal negative gravitropic response in the dark, but respond positively gravitropic in (red) light. In order to test whether high gradient magnetic fields (HGMF) exert only ponderomotive effects on amyloplasts or affect other physiological processes, we induced magnetophoretic curvature in wildtype (WT) and lazy-2 mutant seedlings. Straight hypocotyls of four day old plants were selected and the tips of their hooks were placed in a HGMF near the edge of a magnetized ferromagnetic wedge [grad (H<sup>2</sup>/2) = 10<sup>-6</sup>-10<sup>9</sup> Oe<sup>2</sup>/cm] and mounted on a 1-mp clinostat. After 4 h in the dark 85% of WT hypocotyls and 67% of the mutant curved toward the wedge. When the seedlings were exposed to red light for 1 h prior to and during the application of the HGMF, 78% of the WT seedlings curved toward the magnetic gradient, but the majority of the lazy-2 seedlings (75%) curved away from the stronger field area. Intracellular amyloplast displacement in HGMF was similar for both varieties and resembled the displacement due to horizontal reorientation. The WT showed distinct graviresponse depending on the orientation of the hook, even after excision of the hook. Application of HGMF to decapitated hypocotyls resulted in curvature consistent with that obtained after reorientation. The data imply that the lazy-2 mutants perceive the displacement of amyloplasts similar to the WT and that the HGMF does not affect the graviresponse mechanism. The study demonstrates that ponderomotive forces due to HGMF are useful for the analysis of the gravity sensing mechanism in plants. (Supported by NASA grant NAG10-0190.)
SESSION F: CONCURRENT POSTERS III
Animal Development and Growth I
[64] EFFECTS OF MICROGRAVITY ON EMBRYONIC QUAIL EYE DEVELOPMENT. J.E. Barret1, D.C. Wells2, A.Q. Paulson2, and G.W. Conrad3. 1NASA-Ames Research Center, Moffett Field, CA; 2Division of Biology, Kansas State University, Manhattan, KS 66506-4901.

Fertilized Japanese quail embryos were incubated aboard Mir for selected periods of development. Eyes from Embryonic Days 14 and 16 (E14 and E16) Flight embryos were compared with eyes from control embryos incubated on Earth. Measurements were recorded for eye weights, for eye, corneal and scleral ring diameters, and for numbers of bones in scleral ossicle rings. Transparency of E16 corneas was documented, immunohistochemical staining was performed to observe corneal innervation, and corneal substructure was observed at the electron microscope level. Except for corneal diameter of Flight embryos compared with that of one of the sets of controls, results indicate that eye development occurred normally in microgravity. Quality of tissue fixation in the Flight and various matching control groups was unsatisfactory, and precluded ultrastructure and neural immunohistochemical analyses. (Supported by NASA grants NAG 2-1005 to G.W.C and NAGW-2328 to B. Spooner.)


Because the avian inner ear - completes its embryonic development in less than 21 days in the absence of parental care, -much information of avian embryology is available, and has containment for waste and feces during development, made possible by semi-permeable membranes and a mineral shell, fertilized eggs are ideal for space research. Continuous exposure of embryos to 2xG affected the otolithic organs which are responsible for detection of linear acceleration. The most prominent morphological alterations of the macular epithelium which aids in the transduction of physical to neural energy, were • displacement of capillaries, • remodeling of the basement membrane • alteration in the ratio of type I to type II afferent terminal endings and • otocyst shape and size modifications.

Supporting and secretory cells may be affected by altered gravity less than sensory cells and/or changes induced by altered gravity to the non-sensory cells may be less persistent than sensory cells. Microgravity also affects neurons connecting the inner ear to the brain, but effects that microgravity may have on the nonsensory cells is unknown. (Supported by NASA NAGW2-999, Tulane Pathology & Yamaguchi Univ., Japan)

[66] SKELETAL TISSUE GROWTH AND DEVELOPMENT IN THE NASA BIOREACTOR. B.J. Klement, B.J. George, and N. D. Houston. Space Medicine and Life Sciences Research Center, Morehouse School of Medicine, Atlanta, GA.

In standard organ culture dishes, embryonic mouse pre-metatarsal mesenchyme explants differentiate to form cartilage tissue, undergo terminal chondrocyte differentiation, form a mineralized matrix, and increase significantly in length. Studies were conducted to analyze pre-metatarsal development during culture in the NASA High Aspect Ratio Bioreactor Vessel. Culture for 5, 7, and 14 days in the bioreactor resulted in pre-metatarsals that were 472μm, 98μm, and 364μm shorter than pre-metatarsals cultured in standard culture dishes, respectively. Pre-metatarsals cultured for the first 2, 5, or 7 days in the bioreactor followed by culture in standard dishes for a total culture time of 14 days were 5%, 19%, and 24% shorter than pre-metatarsals cultured for the entire 14 days in standard dishes, and showed little terminal chondrocyte differentiation or mineralization. In an additional experiment, a bioreactor apparatus was suspended in the incubator so that the rotating vessel was turned 90° from the normal bioreactor position. The modified bioreactor was rotated at a very low rpm. Pre-metatarsal development in the modified bioreactor was compared to development in the standard bioreactor and in standard culture dishes. After 5 days of culture the pre-metatarsals in the modified bioreactor were 214μm longer in length than the pre-metatarsals in the normal bioreactor, but were 307μm shorter than the pre-metatarsals in standard culture dishes. The difference in length between the 2 bioreactor cultures decreased after 9 days of rotation, but were 30% shorter than the pre-metatarsals in standard culture dishes, and demonstrated no terminal chondrocyte differentiation. (Supported by NIH GM08248, RR 03034, NASA NCCW-0083 and NASA NAG2-1215).


Results from the Jellyfish-in-Space Experiment from the SLS-1 mission indicated that the hormone (JF-T4) utilized by the jellyfish for metamorphosis induction may be synthesized or utilized differently in micro-g than on Earth. Therefore, research was begun to develop methods for the measurement of the hormone and its receptors in jellyfish flown in micro-g and their ground controls. An experiment for this purpose was proposed and accepted for a flight experiment utilizing ARF hardware.

The hormone measurement techniques (although constrained by a maximum sample of 30 polyps per group) were developed accordingly. Polyps are homogenized and the resulting homogenate is extracted with ethanolic-NaOH. The ethanolic-NaOH mixture is homogenized at 4°C and the solution centrifuged at 10,000g for 10 minutes. The supernatant is removed and the pellet is re-extracted two more times. The pooled supernatant is then diluted with aqueous HCl. To extract hormone from artificial sea water (ASW), the ASW is adjusted to an identical ethanol-HCl concentration. The ethanol-HCl mixture is run through columns of Sephadex LH-20 resin. The columns are washed consecutively with HCL, H2O, ethanol: NH4OH, and NH4OH in ethanol. The first portion of the NH4OH in ethanol contains the iodinated compounds of interest. These fractions are combined and dried under nitrogen and then separated by HPLC. The JF-T4 was eluted from HPLC as a single peak as detected by both radiochemical and UV detectors. This peak did not coincide identically with T4 (thyroxine) nor did isolated JF-T4 cross react with several different sources of T4 antibodies, so it is likely that the compound is different from T4. Of particular significance is the fact that the parent compound and metabolites can be measured by this method using mass spectrometry. This method can be used to obtain the information proposed in the ARF-2 experiment through another future Jellyfish-in-Space experiment. Sponsored by NASA grant NAG10-0178 and an EVMS Institutional grant.
SESSION F: CONCURRENT POSTERS III
Animal Gravity Sensing I
FERROELECTRIC-LIKE PROPERTIES OF HORNET STRUCTURES OR CONSTRUCTION. J.S. Ishay and L. Litinetsky.

Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, Tel-Aviv, Israel.

Various structures or constructions of the Oriental hornet Vespa orientalis (Hymenoptera, Vespinae) such as the cuticle, the spun silk and the comb cell walls discharge an electric current. In the dark, at a temperature range of 5-30°C, this current increases with rise in the temperature and decreases as the temperature drops. Between the ascending and descending "lines" of the current, a broad hysteresis is formed. The created current may attain a level of up to 700 nano Amperes (nA). Upon exposure to light of the hornets or its constructions, the electric current diminishes within minutes to its minimal values, no hysteresis is formed between the warming and cooling lines and the voltage increases. The event described suggest that the structures or constructs in question contain polar materials that undergo change in conformation and polarized, becoming a capacitor with layers of opposite polarization. This explains why the voltage rises and the current decreases during exposure to light, whereas in the dark and at suitable temperature, the mentioned materials revert to a state of spontaneous polarization and gradually release, as electric current the charge that was picked up under illumination. The observed phenomena are characteristic of materials that are semiconductors endowed with ferroelectric properties. The influence of these properties on the gravity perception is discussed.


We have previously reported on the circadian growth rate variability of bone lamellae formed on exposure to microgravity (0G), pointing to a reduction in bone formation of the midshaft humerus from ca. 300 gm male Harlan (Sprague/Dawley) rats flown on the 9-day SLS-1 Space Shuttle mission (STS-40). Here, the midshafts tibiae of ca. 300 gm male Wistar rats were examined for variability of bone growth rate and relative bone density owing to 1G (control), 2G, and 3G exposure for 14 days: a protocol following that of the 14 day Cosmos 2044 biosatellite mission. Embedded polished 60-80 micron thick sections were imaged by both polarizing light microscopy (PLM) and backscattered electron microscopy in the SEM (BSE-SEM). PLM images of endosteal lamellae were obtained and subjected to image processing. Resulting binary images were passed to a quantification program for measurements of the widths between adjacent lamellae. Same fields of view were also imaged in BSE-SEM imaging mode at 20 kV, 500 pa, and a 15 mm working distance for grey level analysis of relative bone density.

Quantitative analyses indicate increased daily growth rates in rats exposed to 2G+. The average lamellar formation rate, in microns per day, was as follows: Vivarium Control, 4.7; On Center Axism Control, 4.6; 2G, 6.1; 3G, 6.6. BSE imaging revealed density dependent differences owing to macrogravity. The grey level histogram of lamellar bone formed during exposure to 2G contains a greater proportion of less dense than the 1G control. The peak bone density is shifted slightly toward lower bone density and the overall average density is 5-10% less than for 1G controls. Further research will be required to determine whether the combination of increased growth rate and lowered bone density is a result of a mineral balance or bone matrix perturbation in the presence of increased loading, or a normal adaptation to elevated strain combined with a lag in bone mineralization/maturation owing to the rapidity of matrix formation.

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SESSION F: CONCURRENT POSTERS III
Animal Structural Systems I
[70] EFFECTS OF HINDLIMB UNLOADING ON THE VASOCONSTRICTOR RESPONSIVENESS OF SKELETAL MUSCLE ARTERIOLES. M.D. Delp. Departments of Health & Kinesiology and Medical Physiology, Texas A&M University, College Station.

Hindlimb unloading of rats results in elevations in blood flow at rest and during moderate intensity exercise in muscle composed primarily of type IIB fibers, and reductions in flow to muscle composed primarily of type I fibers during intense exercise. The purpose of this study was to determine whether the intrinsic responsiveness of arterioles from muscles composed of type IIB fibers (superficial portion of gastrocnemius muscle) and type I fibers (soleus muscle) to vasoconstrictor agonists is altered by hindlimb unloading. First order (1A) arterioles from the superficial gastrocnemius and soleus muscles of control (C, n = 9) and 14 day hindlimb unloaded (HU, n = 8) rats were isolated and cannulated with glass micropipettes in vitro. Intraluminal pressure was set at 60 mmHg. Spontaneous tone developed in all arterioles, but tone was greater in arterioles from the gastrocnemius muscle of C (55 ± 8%) and HU (46 ± 10%) animals. There was a decrease in the sensitivity (EC50) of IA arterioles from gastrocnemius muscle of HU rats to the vasoconstrictor agonists norepinephrine (10-4·10-6 M) and KCl (10-100 mM), but no decrease in sensitivity of arterioles from soleus muscle of C and HU rats to these vasocostrictors. These data indicate that HU diminishes spontaneous and agonist-induced constriction of IA arterioles from muscle composed predominantly of type IIB fibers, but has no effect on contractile function of IA arterioles from muscle composed primarily of type I fibers. This adaptation is consistent with the HU-induced elevations in blood flow observed in muscle composed of type IIB in rats and the diminished ability of astronauts to elevate peripheral resistance following spaceflight.

(Supported by NASA grants NAGW-4842 and NAG5-3754 and NSBRI grant NCC 9-58.)


The β-adrenergic agonist, clenbuterol (Cb), produces skeletal muscle hypertrophy and diminishes muscle atrophy caused by a variety of conditions. Most studies in rats have been carried out in subadult animals. In this study, we further characterize the effects of this potential countermeasure to microgravity-induced muscle atrophy on the muscles of mature rats, in which fiber type and myosin heavy chain (MHC) expression are stable. Mature, male, Sprague Dawley rats (6 months old; > 400 g) were subjected to standard hindlimb suspension for two weeks and treated in a 2-days-on-2-days-off regimen of subcutaneous Cb injections (1 mg Cb/kg body weight). Pair-fed controls included vehicle treated suspended rats, vehicle and Cb treated non-suspended rats, and vehicle and Cb treated “tethered” rats which were fitted with the hindlimb-suspension apparatus but not suspended. The predominantly slow-twitch soleus, and the predominantly fast-twitch plantaris muscles, from each group of animals, were analyzed for both muscle wet weight and protein density (µg protein/mg muscle wet weight). Cb induced a significant increase in the wet weight of both muscles in the nonsuspended and tethered animals. In the suspended animals there was a significant Cb-induced increase in wet weight in the plantaris, but not in the soleus. Conversely, Cb treatment significantly increased muscle protein density in the soleus, but not the plantaris. These findings suggest that Cb increases muscle wet weight, but not protein density, in predominantly fast muscles and increases protein density, but not wet weight, in predominantly slow muscles. This suggests that mechanisms for regulating β-agonist-induced muscle growth and atrophy differ in slow and fast fiber types. Analyses of MHC expression and protein content in these and several other hindlimb muscles are underway to test this conclusion. (Supported by NASA 1NAA4438 & NAG9-971 and by NIH GM08248 & RR03034).

[72] TEMPERATURE INCREASE MAY MEDIATE VIBRATION STIMULATION OF COLLAGEN GEL CONTRACTION BY RAT TENDON FIBROBLASTS AND BONE FORMING CELLS. B. Johnson-Wint1 and M. Cobb1. 1Biological Sciences, Northern Illinois University, DeKalb, 1Life Sciences, NASA Ames Research Center, Lockheed Martin Engineering and Sciences Company, Moffett Field, CA

Collagen Type I is a major load bearing structural component of both bone and tendon, and muscle is attached to bone by tendon. Coordination of the collagen I based structural strength of tendons and bones in response to load is essential for musculoskeletal development and function. We investigated the possibility that muscle vibration influences the collagen I packing and strength of attached tendon and bone.

Foot tendon fibroblasts and metatarsal osteoblasts/osteocytes from young adult rats were grown for 4-6 passages in culture and assayed for their ability to organize collagen I fibers in a contracting collagen gel assay. Cell embedded floating Type I collagen gels were cultured stationary, with vibration or at different temperatures. A vibrating table was used to deliver known vibration frequencies and amplitudes to the cultures. Cell mediated physical remodeling of the collagen gels in the gel (contraction) was followed by measuring the area of the collagen gel at appropriate time intervals.

Both cell types contracted collagen up to 30% faster than controls over the frequency range 8.5-65 Hz. At 82-97 Hz the difference in contraction rate from controls was minimal. Collagen contraction at 35-45 Hz increased with amplitude of vibration up to 0.95 mm/s2 (0.09 g) to maximum increase over controls at 1.81 mm/s2 (0.18 g). The temperature of culture medium vibrated at 35-45 Hz increased linearly with amplitude from no change at 0.74 mm/s2 (0.07 g) to 3°C elevation of temperature at 2.25 mm/s2 (0.23 g). The direct effect of temperature alone on collagen contraction by these cells was examined over the temperature range of 33°C (37° ± 4°C) and was found to increase above and decrease below 37°C. These results demonstrate that tendon fibroblasts and osteoblasts/osteocytes are sensitive to certain frequency/amplitude vibration in their organization of collagen and that part of the vibration effect may be due to elevated temperature.

(Supported by NASA: NASA-ASEE-Stanford Summer Faculty Fellowship Program.)

[73] TESTOSTERONE AND ANABOLIC STEROID ALLEVIATES WEIGHTLESSNESS INDUCED MUSCULO-SKELETAL LOSSES. S.M. Wimalawansa, D.J. Simmons, M. Quast, K. Westlund, J. Wei, and S.J. Wimalawansa. Departments of Internal Medicine, Anatomy and Neurosciences, Orthopedic Surgery, University of Texas Medical Branch, Galveston, Texas, 77555.

Simulation of microgravity is known to cause rapid bone and muscle loss. Because, serum testosterone level become negligible in the tail-suspended (TS) rat, we hypothesized that the replacement of testosterone during TS could prevent those musculo-skeletal losses. 10-week old male Wistar rats were randomly allocated to received single injection of a placebo (control), or 16 mg/kg testosterone, or the anabolic steroid nandrolone decanoate (ND) (n = 10/group) and were tail-suspended for 12 days. An additional 10 rats were injected with the vehicle and kept as ground controls. Bone and muscle changes were quantitated using nuclear magnetic resonance imaging, and bone mineral density (BMD) with DXA. The muscle mass of the ground control rats was significantly greater than the placebo-treated TS rats (56.5 ± 1.8 vs. 42.9 ± 3.0 density units, p < 0.01). Testosterone and ND prevented the reduction of muscle loss (51.5 ± 2.2, 51.6 ± 1.2; 63% improvement; p < 0.05). The BMD of the placebo-treated TS rats were significantly lower than that of ground control rats (0.416 ± 0.011 vs. 0.354 ± 0.014, p < 0.05), and testosterone and ND prevented this bone loss (0.404 ± 0.013 vs. 0.409 ± 0.011, respectively). These data suggest that both testosterone and ND therapy can prevent the musculo-skeletal losses associated with exposure to simulated weightlessness.
SESSION F: CONCURRENT POSTERS III
Biotechnology/Instrumentation I

In the last decades many experiments using different biological systems studied effects of microgravity and find the cellular mechanisms leading to the biological response. Unfortunately many experiments could not be repeated due to limited flight opportunities and associated high costs for the actual space experiment. Also in view of reduced flight opportunities prior to the ISS-utilization, the development and utilization of cheap and readily available ground based experiment tools for simulated (or real) microgravity and hypergravity will be necessary.

Recently a new Random Positioning Machine (RPM), that generates microgravity, became available. Preliminary experiments with developing Arabidopsis showed comparable effects as under space flight conditions (3). Experiments with isolated fetal mouse long bones are just started (1). In the future the RPM will be prepared for threshold studies between 0 and 1xg. The Free Fall Machine (FFM), which generates short but constantly repeated periods of real microgravity, is in use for some time already. Experiments with Chlamydomonas showed comparable results in cell cycle changes and cell morphology as found during BION-missions (4). To broaden the scope for acceleration studies a Tissue Culture Centrifuge (McBi-CAR) is available which can generate accelerations up to 100xg and allows culture in tissue culture plates and standard flight HW. The centrifuge was successfully used to study launch effects (TEXUS g-profiles) on cultures of bone forming osteoblasts (5). These ground based facilities may help efficiently to clarify the cellular mechanisms leading to the biological response to microgravity.

[75] POTENTIAL USE OF NASA BIOREACTORS IN PRODUCTION OF CANCER VACCINES. D. Yetman¹, S. P. Tomasovic¹, and C.A. Savary². Deps. of Tumor Biology and Surgical Oncology, Univ. of Texas M.D. Anderson Cancer Center, Houston.

Previous studies have shown that tumor-associated antigens are better expressed when malignant cells are grown in 3-dimensional (3D) environments, as opposed to standard monolayer conditions. This suggested to us that human carcinomas grown in 3D NASA bioreactors might be a better source of antigen for preparation of tumor vaccines. Recent attention has been drawn to the use of heat shock proteins (Hsp) for vaccination against cancer. In their role as molecular chaperones, Hsp bind to many proteins, including tumor-associated peptides. This could explain why Hsp-peptide complexes extracted from murine tumors were found to have both therapeutic and protective potential in mice challenged with the same tumor. Neither Hsp nor peptide alone was effective, suggesting that Hsp are acting as a natural adjuvant. Early clinical trials of Hsp-peptides in treatment of cancer patients have shown promising effects. However, the amount of complexes available for vaccination and subsequent boosting is expected to be limited to the size and viability of each patient's tumor at surgery. In certain cancers, e.g. breast cancer, the tumor volume may be quite low, so that alternate approaches will be needed to generate enough Hsp-peptide for vaccination. We have found that the NASA bioreactors support the anchorage-independent growth of the HER-2/neu gene-transfected E8.1 breast carcinoma line. Moreover, these cells were found to express a higher level of Hsp70 compared to 2D monolayer controls as evaluated by Western blotting and flow cytometry. Using the HER-2/neu-encoded p185 protein as a surrogate tumor-associated marker, we are currently evaluating whether Hsp70-p185 complexes are increased in parallel in the 3D cultured E8.1 cells. If this is found to be the case, our data could suggest that tumors expanded in NASA bioreactors might allow for continuous, upscaled production of Hsp-peptides for therapeutic and/or vaccination purposes, and might allow for treatment of patients who would not otherwise qualify for this promising new therapy. (Supported by NASA: NAG8-1347.)
SESSION F: CONCURRENT POSTERS III
Cell Biology I
[76] ASYMMETRIC LOCALIZATION AND REDISTRIBUTION OF ANNEXINS IN GRAVISTIMULATED PEA PLUMULES. G.B. Clark, M. Dauwalder, D.S. Rafati, and S.J. Roux. Department of Botany, Univ. of Texas, Austin.

Annexins are a multigene family of calcium-dependent, membrane-binding proteins which have been implicated in secretory processes, polar growth, and cell wall formation in plant cells. One of the signalling steps leading to gravitropism appears to require calcium and involve a redistribution of calcium across the region of tropistic bending. The gravitropic growth response is also known to involve the asymmetric secretion of new wall materials. An objective of our laboratory is to test a possible role for annexins in gravitropism. Using immunocytochemical techniques we investigated the effects of gravistimulation on annexin localization in etiolated pea shoots. In both longitudinal and cross-sections an asymmetric annexin immunostaining pattern was observed in groups of cells located in and close to a zone of cell differentiation just below the growing apex. This area has been traditionally referred to as the leaf gap. Some of the cells in this region show high levels of PAS staining in their cell wall. Changes in annexin localization were able to be detected within 15 minutes of gravistimulation. These data are the first to show annexins may be an early target of calcium action during the gravitropic response in plant shoots. (Supported by NASA: NAGW 1519).

[77] GENETIC CONTROL OF OSMOREGULATION IN DROSOPHILA. X. Huang, L. Huff, Q. Huang and M. Stern. Dept. of Biochemistry, Rice University, Houston TX 77251.

One mechanical stress that can be sensed by all cells is osmotic stress, which is applied when a cell is exposed to a non-isotonic extracellular environment. Exposure to such an environment causes major water and ion fluxes across the cell membrane, changes in cell volume, and thus causes forces to be applied to the cell membrane. Knowledge of the molecular mechanisms by which cells respond to changes in osmolarity would not only be useful in understanding osmotic stress response, but also cellular responses to other mechanical forces on the cell. We are currently studying the molecular mechanisms underlying osmotic stress response in Drosophila. Our starting point for these studies is the inebriated (ine) gene, which encodes a member of the Na+/Cl- dependent family of neurotransmitter/osmolyte transporters. Among other functions, members of this family transport osmolytes such as betaine across cell membranes; this osmolyte accumulation enables cells to withstand a hypertonic environment. By in situ hybridization to developing Drosophila embryos, we found that this transporter is transcribed in two cell types that perform fluid reabsorption in insects: the Malpighian tubule and the hindgut. These tissues together comprise the invertebrate analogue of the mammalian kidney. This observation raises the possibility that the ine-encoded transporter might perform osmolyte transport and enable the fly to survive a hypertonic environment. This possibility is supported by our observation that ine mutants are much less viable than wildtype when grown on high [NaCl] or [sorbitol]. We are currently using the lethality of ine mutants on salt to isolate mutations that suppress this phenotype. The genes identified by such suppressors might encode additional molecules controlling the osmotic stress response in Drosophila. (Supported by NASA grant NAGW-5007)

[78] CHARACTERIZATION OF 3-DIMENSIONAL VASCULAR CELL CO-CULTURES MAINTAINED IN THE ROTATING BIOREACTOR. D. Ellerson, G.L. Sanford, S.A. Harris-Hooker and C.D. Melhado, Space Medicine & Life Sciences Research Center, Morehouse School of Medicine, Atlanta, GA.

Vascular remodeling is a complex set of events involving endothelial cell injury and/or dysfunction that results in intimal/medial thickening. Although this area has received significant attention, the cellular and molecular mechanisms of vascular remodeling are not completely understood. The development of 3-D co-culture models of the blood vessel will provide a unique opportunity to conduct mechanistic studies into vascular remodeling. We characterized the 3-D growth of endothelial (EC) and smooth muscle (SMC) cells, and in co-culture, using the NASA horizontally rotating bioreactor (HRB). Cells were continuously cultured on cytoxen-3 microcarriers for up to 30 days (HRB and SF) and were processed for scanning electron microscopy examination, immunocytochemical assessment of phenotypic marker. Controls were maintained in spinner flasks (SF) over the same period. In both systems, microcarriers and cells remain uniformly suspended in the fluid. We found that both EC and SMC grew at a slower rate in the HRB than in the SF. All cultures grew as 3-D aggregates after 14 days. These cultures were positive for the von Willebrand factor (EC) and alpha actin (SMC). The glucose consumption were monitored as an index of cell growth. The cross section for Transmission Electron Micrographs demonstrate the ultrastructural characteristics of SM and EC. With the large aggregates formed by co-cultures, the surface EC appear to be invaginating, and after 30 days, tube-like structures can be seen in the interior of aggregates. These results suggest that in the HRB, vascular cells spontaneously form 3-D capillary like structures. Hence such cultures may provide a unique model for mechanistic studies of vascular remodeling and angiogenesis. (Supported by grants from NASA: NAG9-852 & NCCW-0083 and NIH/RCMI (3G12 RR03034).

[79] EXPERIMENTAL DESIGN AND PRELIMINARY RESULTS OF A STUDY OF BONE CELLS SUBJECTED TO HYPERGRAVITY. W.J. Landis, M.A. Kacena*, and K.J. Hodgens. Department of Orthopedic Surgery, Children's Hospital and Harvard Medical School, Boston, MA 02115 and *Department of Aerospace Engineering, University of Colorado/Bioserve Space Technologies, Boulder, CO 80309.

To support a more complete understanding of the potential effects of hypergravity on bone development, a set of experiments has been designed utilizing calvarial osteoblasts obtained from normal 17-day old embryonic chicks and subjected to high gravitational (G) forces at the Hypergravity Facility for Cell Culture (HyFACC) at NASA/Ames Research Center, Moffett Field, CA. Cellmax Quad modules (Celico, Inc., Germantown, MD) containing 4 bioreactor cartridges, each inoculated with 7x10⁶ cells growing in DME supplemented with 10% FBS, 12.5 μg/ml ascorbate and 10 mM β-glucuronate, were placed in an incubator (37°C, 5% CO₂) mounted at the end of a 9 ft centrifuge arm at the HyFACC. Cartridges were exposed for 2 wks to either 3.3 G or 4.0 G on centrifugation with sampling and medium changes occurring (~1 hr duration) every 2 days. A Quad module prepared and treated identically was maintained at normal gravity (1.0 G) as a control. Centrifuged and control cells are being analyzed in terms of their metabolism of glucose and lactate; type I collagen, osteocalcin, and osteopontin gene expression; and cellular and extracellular matrix ultrastructure.

Results from the first cartridges examined indicated that, over the 2 wk experimental period, glucose levels of control cells decreased from ~90 to ~20 mg/dL and lactate levels increased from ~1.8 to ~8.0 mmol/L (n=5 cartridges); centrifuged cells followed the same trends for both glucose and lactate as those in controls, but measurements were more variable (total of n=6). Scanning electron microscopy showed numerous cells and extensive development of matrix in centrifuged (4.0 G) samples, qualitatively comparable to the cells and matrix observed in controls. These initial data do not yet permit unequivocal conclusions regarding possible effects of hypergravity on osteoblasts and more comprehensive analyses are continuing in this study.

This work was supported by NASA grant NAG5-4377.
DEVELOPMENTAL REGULATION OF COLLAGENASE-3 MRNA IN NORMAL, DIFFERENTIATING OSTEOSTALS THROUGH THE ACTIVATOR PROTEIN-1 AND THE RUNT DOMAIN BINDING SITES. N.C. Partridge, R.C. D'Alonzo, and S.K. Winchester. Saint Louis University School of Medicine, St. Louis, MO.

Previously, we have shown that collagenase-3 mRNA is developmentally expressed in normal, differentiating rat osteoblasts. Collagenase-3 mRNA is not detectable during osteoblast proliferation (day 5), but expression increases as the osteoblasts begin to differentiate and mineralize an extracellular matrix (days 14 and 21). We demonstrated by nuclear run-on analysis that this increase in expression is due to an increase in transcription of the collagenase-3 gene. Through transient transfection of deletion and mutated collagenase-3 promoter constructs, we demonstrated that the activator protein-1 (AP-1) and runt domain (RD) binding sites are responsible for transcription of the collagenase-3 gene. Mutation of either the AP-1 or the RD binding sites resulted in a loss of collagenase-3 promoter activity. The AP-1 and RD binding sites have been shown to bind members of the activator-protein-1 family of transcription factors and Acute Myelogenous Leukemia (AML) family of transcription factors, respectively. Overexpression of both c-Fos and c-Jun into proliferating osteoblasts or overexpression of osteoblast-specific factor 2 (Osf2) resulted in an increase in collagenase-3 promoter activity. Furthermore, overexpression of c-Fos, c-Jun and Osf2 into osteoblasts resulted in a synergistic increase in collagenase-3 promoter activity. However, mutation of either the AP-1 or the RD binding site resulted in the inability of c-Fos and c-Jun or Osf2 to increase collagenase-3 promoter activity suggesting that both the AP-1 and RD binding sites and proteins are required for expression of collagenase-3 in differentiating osteoblasts. (Supported by NASA: NAG-4538, NCC2-884, and GSRP-98-087).
SESSION F: CONCURRENT POSTERS III
Plant Biology II
[81] 
STATHMIN PHOSPHORYLATION DURING GROWTH AND GRAVITROPIC RESPONSE OF ROOTS OF ZEA MAYS. L. T.J. Mulkey and D.A. Prentice. Life Science Dept., Indiana State Univ., Terre Haute, IN.

Stathmin (also known as pp17, prosolin, Op18, p17, P19, pp20, pp21, pp23, and 19-K) is a 19-kDa cytosolic protein which has been implicated as a relay phosphoprotein in multiple signal transduction systems. Numerous roles in signal transduction in animal systems have been identified for the twelve phosphorylated and unphosphorylated forms of stathmin. These roles are associated with key events during growth, development and differentiation. Stathmin appears to be highly conserved proteins in animals and has been identified in plants (BBRC 196:589; Plant Physiol. Biochem 36(6) 449). Our data indicates that the phosphorylation status of proteins from maize roots that are in the molecular-weight range of stathmin, and identified by Northern blots, can be alter by treatment with EDTA, calcium, indole-3-acetic acid (IAA), tetraedrion phospholipid:choline:TDP, and staurosporine. TPA induces similar phosphorylation patterns of putative stathmin proteins and elongation responses in maize root tissue as promotive concentrations of IAA. Staurosporine inhibits the phosphorylation of the putative stathmin proteins in both TPA and IAA treated roots. Additionally, staurosporine inhibits the elongation response that can be induced by TPA or IAA. Further characterization of the role of stathmin as a possible regulator of signal transduction events during root gravitropic response and elongation will be presented.

[82] 
THE GRAVITROPIC RESPONSE OF CHARA PROTONEMATA IS REDUCED IN MODERATE HYPERGRAVITY. A. Sievers and D. Hodick. Botanisches Institut, Universitats Bonn, Venusbergweg 22, D-53115 Bonn, Germany.

The interaction between the position of the statoliths and the direction and rate of tip growth in the negatively gravitropic protonemata of the *Chara globularis* was studied with the centrifuge-video microscope NIZEMI. Protonemata were regenerated from isolated thallus-nodes, which were embedded in agar and kept in darkness for approx. 2 weeks. Protonemata placed perpendicular to the acceleration vector (stimulation angle 90°) showed a gradual reduction of the gravitropic curvature with increasing accelerations from 1 g to 8 g despite a complete sedimentation of all statoliths on the centrifugal cell flank. It is argued that the increased weight of the statoliths in hypergravity impairs their acropetal transport which is induced when the cell axis deviates from the normal upright orientation. When the statoliths were centrifuged deep into the apical dome at 6 g and a stimulation angle of 170° the gravitropic curvature measured after 1 h was identical to the one determined for the same cells at 1 g and the same stimulation angle. This indicates that the gravitropism in *Chara* protonemata either is independent of the pressure exerted by the statoliths on an underlying structure or that it is already saturated at 1 g. When the statoliths were moved along the apical cell wall in a centrifugation at 8 g and a gradual increase of the stimulation angle from 170° to 220° the gravitropic curvature sharply reverted when the cluster of statoliths passed over the cell pole. The experiments support the hypothesis that in *Chara* protonemata statoliths distributed asymmetrically inside the apical dome displace the Spitzenkörper and thus the center of growth, which results in gravitropic bending.

(Supported by DLR - The AGRAVIS Project-)

[83] 

The dirigent protein controlling outcome of stereoselective coupling of E-menthol alcohol gives pinenoxin (as its (+)- or (−)-antipode, according to the species). Its genes for each of these proteins/enzymes have been cloned, and the recombinant proteins expressed in functional form. This provided the opportunity to clearly establish whether monolignol coupling and subsequent metabolic events involved in lignin and lignan formation differed biochemically, temporally and spatially. Accordingly, a combination of tissue printing, *in situ* hybridization and immunolabeling studies were carried out in order to define the tissue specific expression of these genes as well as the subcellular localization of the corresponding proteins/zymes. Results were compared with those obtained with immunoproteins directed against different synthetic lignin polymers, concerning the specific ultrastructural localization of different types of lignins.

REFERENCES


(Supported by NASA: NAG100164)

[84] 

Ground-based studies to develop protocols for direct determination of photosynthesis of wheat in microgravity are being conducted in preparation for a space flight experiment selected during NASA NRA 96-OLMSA-01A. This space flight experiment will be conducted in the Biomass Production System (BPS), a double middeck-sized plant growth chamber being developed by Orbital Technologies Corporation (Madison, WI). A series of tests to determine the effect of rooting media conditions on shoot growth, photosynthesis, and transpiration of wheat cv. USU Super Dwarf have been conducted at Kennedy Space Center using rooting modules designed for the BPS. Arcillate particle sizes of 1-2 mm appear sufficient to maintain adequate media water potential to support plant growth for 20 days at a water tension of -0.1 kPa, but not at -0.5 kPa. Transpiration rates have averaged 2.6 L m⁻² day⁻¹ over a 20 day experiment, with a maximum rate of 4.4 L m⁻² day⁻¹ at full canopy coverage. Maximum photosynthetic rates were 7 μmol m⁻² s⁻¹ at 310 μmol m⁻² s⁻¹ PAR at 15 DAF. Photosynthetic rates declined slightly as plants increased in height after day 15. This is likely due to decreased light interception by the wheat canopy due to mutual shading in the plant growth chambers.

(Supported by NASA: NCC-027)
SESSION F: CONCURRENT POSTERS III
Collaborative Ukrainian Experiment I
[85] COLLABORATIVE UKRAINIAN EXPERIMENTS (CUE) 
CONDUCTED ON STS-87 MISSION NOV 1997. DK Chapman1, C. Johnson2, G. Godin1, D. Johnson2 and G. Stute1. 1Dynamac Corporation, Kennedy Space Center, FL, 2Cornell Univ., Ithaca, NY 
The Collaborative Ukrainian Experiment (CUE) was a payload of ten plant space-biology experiments conducted in the middeck of the shuttle Columbia during a 16 day mission which was launched on 19 November and landed on 5 Dec 1997. It was a collaborative effort consisting of 14 United States scientists and 20 Ukrainian scientists. Col. Leonid Kadenyuk, a Ukrainian Payload Specialist, conducted the CUE experiments during the 16-day mission. He was assisted by Commander Kevin Kregel and Mission Specialist Kalpana Chawla. 
The CUE experiments examined the effects of microgravity on (1) the reproductive process (2) development of the photosynthetic apparatus (3) biomass partitioning (3) susceptibility of plant to fungal pathogens (4) differentiation and phototropic responses and (5) gene expression. 
The flight hardware used to conduct the CUE experiments were developed at the Kennedy Space Center. The hardware consisted of the PLant Growth Facility (PF), Biological Research in Cansisters (BRIC) and BRIC-LED. The PF was used for culture of Brassica rapa seedlings. The BRIC canisters were used for three soybean experiments, while a modified BRIC canister provided red Light-Emitting Diode (LED) lighting to individual petri dishes was used for experiments on two species of moss. The Kennedy Fixation Tubes (KFT) were used fix plant material and a gaseous nitrogen (N2) freezer was used to freeze plant material for the soybean and Brassica rapa experiments.

As part of the Cooperative Us/Ukrainian Experiment (CUE), an experiment was designed using the Plant Growth Facility to monitor the effects of spaceflight on the photosynthetic apparatus of Brassica rapa. Pre-flight operations included modifying the chamber environment to support optimal B. rapa growth. This included studies on plant nutrition and the rooting environment, as well as studies on appropriate lighting and temperature conditions. During the STS-87 mission, germination was initiated in microgravity by applying nutrient solution to positioned seeds, and growth was monitored throughout by photography. Ground controls were similarly activated, delayed 48 hr such that identical conditions of temperature, humidity, and gas concentrations could be programmed in the Orbiter Environmental Simulator chamber. At two time points during the flight, a subset of the plants were harvested and were fixed on orbit for subsequent microscopic examination. Landing occurred in the 16th day of plant growth, and fresh tissue was harvested beginning 2 hr following landing. Cotyledon leaves were frozen at the Kennedy Space Center, for subsequent biochemical examination at Kansas State University, while the first tier of true leaves were examined immediately for a suite of photosynthetic characteristics. Preliminary data obtained from cotyledon tissue supports a reduction of PSI activity caused by growth on STS-87, and this reduced activity corresponds with a reduction in PSI proteins observed by western blot analysis. 
(supported by NASA grants NAGW-2328, NAG10-0142, and the Kansas NASA EPSCoR program.)

[87] ALTERATIONS IN THE ROOT TIP CELLS OF SOYBEAN SEEDLINGS GROWN UNDER MICROGRAVITY. D.O. Klyuchnik1, C. S. Brown1, W.C. Piatuch1 and E.L. Kordyum1. 1Institute of Botany, Nat. Acad. Sci. of Ukraine, Kiev, Ukraine, 2NC State University and Dynamac Corp., Raleigh, USA and 3Dynamac Corp., KSC, FL, USA. 
Soybean seedlings in the presence of Purafil (to remove ethylene) and in the absence of Purafil were grown in microgravity during the STS-87 flight of the Space Shuttle. Dry seeds were launched and then activated by hydration. Root tips were taken postflight and examined using transmission electron and light microscopy. Columella cells, as well as secretory and meristematic cells, of root tips, developed in space were larger and were more vacuolated compared with the ground controls. More essential differences were observed in samples without Purafil treatment. It is suggested that microgravity-induced changes in root tip cells are accompanied by alterations of metabolic pathways that regulate the cell growth by expansion. Among of other effects, space flight samples have exhibited the changes in stamectra ultrastructure, and the localization of amyloplasts and nucleus in the stamectras. The data indicates that the microgravity environment impacts root formation and supports the idea that ethylene is involved. (Financial support was provided by Ukrainian Space Agency.)

[88] PHYTOHORMONES IN ASTROPLANTS BRASSICA RAPA. L. L. Musateno1, V. Generalova1, V. Negretsky1, N. Venedicheva1, L. L. Kadenyuk1, and K. Symik1. 1Dept. of Phytohormonology, Inst. of Botany NASU, Kiev, Ukraine. 
Studies on the effects of space flight factors on integral processes of plant growth and development require an investigation of phytohormones - compounds that affect cellular, tissue-specific and organinal interactions which are necessary for triggering and controlling the physiological and morphological processes. Research object - AstroPlants Brassica rapa L. grown in soil culture on earth for 9 and 15 days and for 9 days in microgravity condition (on the space shuttle Columbia STS-87). The phytohormones were investigated by HPLC, bioassay and MS/MS spectrometry. During studies of phytohormones at the various ontogenesis stages of B. rapa grown in earth conditions, we identified cytokinins - zeatin (Z), zeatin riboside (ZR), dihydrozeatin (DHZ); IAA, ABA and indoleacetonitril (IAN), typical of Cruciferae, whose characteristics were obtained by qualitative analysis. As a result of studies on IAA, ABA and cytokinins in plants grown on orbit, it was shown that plants contain the hormonal complex typical of all higher plants. As far as chromaticographic characteristics are concerned, substances extracted from B. rapa are similar to standard solutions of corresponding phytohormones ( Sigma, USA). The phytohormones qualitative analysis give evidence for the presence of IAA, ABA, Z, ZR, zeatin glucoside (ZG), dihydrozeatin riboside (DHZ), isopenentenilin (IP) and isopenentenilinos (IPA). Thus, similarity in qualitative composition of identified components of phytohormone complex in B. rapa grown on earth and in space was shown. The obtained results testify to the promising prospects of conducting the studies on the regulation of plant growth and development in conditions of space flight. (Supported by NSAU)
[90] MICROGRAVITY EFFECTS ON FREE AMINO ACIDS CONTENT IN BRASSICA RAPA. T. Cherevchenko, and N. Zaimenko, Central Botanical Garden of National Academy of Sciences of Ukraine

As a result of our experiments, we determined that total content of amino acids in plants at 9, 15 and 28 days of development during spaceflight increased 1.4-2.1 times. Important changes in amino acid content were observed at 15 and 28 days of Brassica rapa development. Following growth in microgravity, the average concentration of asparagine in leaves of 15-day-old plants increased 21 times, while threonine, serine, glutamic acid, alanine and valine increased 1.2-1.8 times. In stems of tested plants, proline content increased 4 times, arginine - 7%, aspartic acid - 1.8%, and threonine - 2.1 times. We observed a sharp increase in free arginine in leaves of Brassica rapa 5.5 times, in stems - 25.6 times, and this suggests the lack of phosphate. Accumulation of free amino acids in these plants suggests water stress. We found a negative dependence between intensity of growth processes and increasing of amino acid concentrations in tissues. It must be noted a critical decrease of asparaginic acid concentration in stems of Brassica rapa at 28 days of plant development indicates nitrogen deprivation. Since Brassica rapa is a C3 plant, AMINO ACIDS production in leaves is higher when these plants are cultivated on ammonium compounds. Glutamic acid concentration after 15-day growth in indicates increasing biosynthesis activity of photosynthetic pigments. We observed a 1.2-1.9 times increase of photosynthetic pigments content in Brassica rapa at 15 day of development and decreasing of their number 2.3-3.7 times at 28 days. Obtained results allow us to improve the technology of plant growth during spaceflight by improving physical and chemical features of soil substitutes and using of balanced fertilizers of prolonged action in response to biological peculiarities of plants.


Previous spaceflight experiments have shown that starch production is depressed in microgravity in a variety of plant types. To confirm these results, etiolated soybean seedlings (Glycine max cv. McCall) were imibed and germinated in NASA’s Biological Research In Canister (BRIC) hardware several days into space shuttle mission STS-87. The seedlings grew for 6 days in the spaceflight environment. Upon recovery, selected seedlings were either fixed and processed for Schiff/PAS staining to determine starch deposition patterns, or harvested and frozen for enzymatic carbohydrate assay for total starch content per tissue type. Surprisingly, rather than supporting previous findings, starch concentrations were higher in spaceflight grown tissues than in their corresponding ground controls. Deposition patterns of the starch appear altered as well. In flight grown tissues, starch deposits are clustered around the vascular structures in the hypocotyl, whereas few such deposits are evident in the ground controls. These deposits extend through the mesophyll of the flight tissue as well, while there are no such deposits in the mesophyll of the ground control plants. One possible explanation for the changes in results between our experiment and past experiments involving soybean seedling growth in BRIC canisterns, is that significant levels of ethylene have built up within the canisters during these previous studies. In our study, a new design was devised for the plant growth hardware which resulted in significantly lower ethylene levels. Perhaps it is this difference in hardware design that will allow us to observe the true reactions plants have to the space flight environment. (This work supported by NASA contract NAS10-12180, Dynamac Corp.)
SESSION F: CONCURRENT POSTERS III
Spaceflight Experiment Results I
[92] EARLY DEVELOPMENT OF MICE EMBRYO IN MICROGRAVITY ENVIRONMENT ON STS-80 SPACE FLIGHT. E.B. Schenker1,2 and K.E. Forkheim1. 1Israeli Aerospace Medicine Institute, POB 4572 Jerusalem, ISRAEL and 2University of Manitoba, Faculty of Medicine, Manitoba, Canada.

During the STS-80 Columbia space flight missions, forty-nine mice embryos at the two cell stage were launched on the CMIX-5 Payload of ITA Inc. The Mammalian mice embryos were allowed to develop in microgravity in a Liquid Mixing Apparatus as part of CMIX-5 Payload. They developed for four days at 37 C and were compared to 2-cell stage embryos exposed to 1g on Earth treated in a similar manner.

Thirty-seven of the fifty ground control 2-cell embryos divided to 4- and 8-cell stages. Eleven of the fifty ground control 2-cell embryos reached the hatching stage. In the space flight group none of the forty-nine, space flight embryos at the 2-cell stage showed any sign of development and all of the embryos degenerated.

Conclusion: At this stage we can not conclude that mammalian mice embryos can not develop due to microgravity alone. Future studies will have to be conducted to evaluate why the 2-cell mice embryos did not develop. Other possible contributing factors may include: cosmic radiation, vibration during space flight, and other space environmental factors.

[93] CALCIUM UPTAKE BY QUIL EMBRYOS INCUBATED IN SPACE. J.I. Orban and P.Y. Hester. Dept. of Animal Sciences, Purdue University, West Lafayette, IN.

The objective of the present study was to determine the effect of space flight on mineral uptake (mineral utilization) from eggshells by Japanese quail embryos. Comparisons were made with results obtained from laboratory (LAB-1) and synchronous (SYN-1) controls conducted on earth corresponding to flight study period. The LAB-1 control eggs were incubated in a Lyon RX2 incubator at 37.5 C with egg rotation occurring hourly. The SYN-1 eggs were also incubated in a Lyon RX2 incubator with hourly rotation, but the temperature was maintained at 39 degrees C to 40 degrees C to simulate the temperature of the Slovakian incubator used during space flight. Both LAB-1 and SYN-1 eggs were incubated in a horizontal position. Neither of the two groups of eggs was subjected to launch dynamics (acoustics, vibrations or g-load). Eggs incubated in microgravity were not turned, but were free floating; therefore, random movement of eggs may have occurred. Embryos from flight, LAB-1, and SYN-1 were fixed in 4% paraformaldehyde at 3, 7, 10, 14 and 16 days of incubation. Eggshells from the treatments were analyzed for calcium (Ca) content. Space flight embryos used less Ca (P < .02) from eggshells than LAB-1 and SYN-1 controls. Calcium uptake by embryos increased with age of incubation with the most increase occurring by the 16th day of incubation. Calcium uptake by flight and SYN-1 embryos at day 16 was not different but was significantly less than the uptake from LAB-1 controls. Results showed that space flight impaired Ca uptake by quail embryos. However, it was not clear whether the impairment was due to factors other than microgravity since Ca uptake by synchronous (flight simulation) embryos did not differ from space flight embryos at day 16 of incubation.

(Supported by NASA (ARC): NAG 2-1001.)

[94] SPACEFLIGHT HARDWARE ALLOWING UNILATERAL IRRADIATION AND CHEMICAL FIXATION IN SITU IN PETRI DISHES. F.D. Sack1, V.D. Kern1, N.J. White1, K. Anderson2, W. Wells3, C. Martin2. 1Department of Plant Biology, Ohio State University, 1735 Neil Ave., Columbus, OH, 2Bionetics Corporation, Mail Code BIO-3, Kennedy Space Center, FL, and 3NASA, KSC, Mail Code CG, FL.

To accommodate a spaceflight experiment with moss (SPM), purpose-built flight hardware was developed by engineers at Kennedy Space Center. The hardware allows sterile culture for an extended period of time in commercial petri dishes, lateral illumination of each culture with light of a specific wavelength (660 nm) and a range of intensities (0.05 to 5 Pmol m^-2 s^-1), incubation in complete darkness, and chemical fixation to terminate the experiment under conditions of microgravity. The use of a fixative required triple containment to protect the astronaut crew. An external panel on the experiment container allowed the timing of illumination and fixation to be controlled by the crew. Light quality is provided by LEDs that are located in the lid of the outer container, the BRIC/LED canister. Each canister accommodates 6 Petri Dish Fixation Units (PDFUs), each of them housing one standard 6 cm petri dish. All components of the PDFU are autoclavable. LED illumination is piped through a transparent glass rod. Each PDFU contains fixative in a reservoir that is released by the depression of an actuator. This hardware performed perfectly during its first flight, the 16-day STS-87 mission in Nov./Dec., 1997 as part of the Collaborative US and Ukrainian Experiment (CUE). It supported strong and sterile moss growth, cells were maintained in position and were well-fixed, and there was a vigorous and consistent response to light. Although here used for moss, in future flight experiments this unique new hardware can be used for many types of organisms normally grown in petri dishes, with or without a requirement for illumination. (Supported by NASA: NAG10-0179).


Tuskegee University has leased three Liquid Mixing Apparatus vials from Instrumentation Technology Associates (Exton, PA), which has manifested commercial space on the STS-95 Space Shuttle Flight planned for October 29 through November 7, 1998, a 10-day flight. Each vial will contain two TU-82-155 sweetpotato cuttings (pieces of stems) in order to note the effects of microgravity on root initiation/emergence/growth, thus examining their potential for regeneration. Each cutting will have two or three nodes, where rooting occurs. Prior to the incidence of microgravity in orbit, the vials will be held without light at 6°C. Once in orbit, their environment will be at 20°C, still without light, for the duration of the flight. Cuttings aboard Shuttle will be compared, postflight, with those grown simultaneously under the same conditions but in a ground-based unit at Tuskegee University. A modified Karnovsky's fixative will be added to two of the three vials, one on day 5 and the other on day 10 of the flight. Simultaneously, the same fixative will be applied to two of the ground-based vials. Electron microscopy (Phillips 201 transmission electron microscope) will be used to study the effects of the two gravitational forces on the roots with a primary focus on amylloplast development in the root caps. The two cuttings in the third, unfixed vial will be returned to Tuskegee University after the flight and grown to harvest hydroponically (using the nutrient film technique) along with the two cuttings in the unfixed vial in the ground-based unit. Physical measurements taken and the results of EM studies will be used to compare root growth and plant development in flight and on the ground as described above.

(Supported by NASA: NAG10-0209 and USDA/SCREES ALX-PS-1)
SESSION F: CONCURRENT POSTERS III
Space Biomedical Results I
[96] MONITORING BACTERIA IN SPACE ENVIRONMENTS. J.A. Wibbenmeyer1, M. Larrie-San2, K. Maillard3, D. Pierson2, R.C. Wissi, and G.E. Fox.1 1Department of Biology and Biochemistry, University of Houston, Houston, TX; 2Department of Aerospace and Mechanical Engineering, University of Texas, Austin, TX; 3Department of Biological Sciences, University of Houston, TX. 2

Crew health is a dominant issue in space travel, and, as the trend towards longer-duration space missions continues, potential microbial problems are of increasing concern. Methodology to measure and differentiate bacteria during flight is highly desirable. We are developing an assay based on the sequence of the small subunit ribosomal RNA (16S rRNA) that will be compatible with DNA hybridization array technology. The highly conserved, and the interspecies conservation and variability make 16S rRNA an attractive molecule to use as a probe target. 16S rRNA targeted probes are designed using two computer programs, find probes and check probes. One probe is chemically attached to a matrix such as a glass microscope slide, and is used to capture 16S rRNA from targeted organisms. A second probe is labeled with biotin and is used to “detect” the RNA bound to the capture probe. Streptavidin-conjugated to alkaline phosphatase binds to the biotin and a fluorescent signal is obtained by adding the substrate methylumbelliferone phosphate. Probe designs and experimental evaluations will be presented for a variety of bacteria that need to be detected in a water monitoring system. These include Escherichia coli, Burkholderia, Neisseria, Pseudomonas, Acinetobacter, and Staphylococcus. (Supported by NASA: NBSR-NCC9-59; 10-6-20)

[97] SPACE FLIGHT ASSOCIATED CHANGES IN THE FUNCTIONS OF NK CELLS. I. Kaur1, S.K. Mehta1, E.A. Grimm3, C. Smid4, D.L. Fieeback, and D.L. Pierson1. 1Enterprise Advisory Services Inc., Houston, TX; 2Department of Tumor Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX; 3Life Sciences Research Laboratories, National Aeronautics and Space Administration, Johnson Space Center, Houston, TX.

The cytolytic activity of NK cells and phenotypic expression of PBMCs isolated from 11 U.S. astronauts were determined before and after 9 or 10 days of space flight aboard the space shuttle. Blood samples were collected 10 and 3 days before launch, within 3 hours after landing, and 3 days after landing. All PBMC preparations were cryopreserved and analyzed simultaneously in a 4-hour cytotoxicity 51Cr-release assay using NK-sensitive K-562 target cells. Cytotoxicity was calculated as absolute 51Cr released and 51Cr released per 1000 lymphocytes. Lymphopenia was observed after landing. When corrected for this lymphopenia, we observed a decrease in the cytolytic activity at landing as compared to the preflight values (p<0.0001). Three days after landing, there was an apparent recovery in the cytolytic activity of NK cells. Expression of major lymphocyte surface markers (CD3, CD4, CD8, CD14, CD16, CD56), determined by flow cytometric analysis, revealed no consistent phenotypic changes in relative percent of NK or other lymphoid cells after 10 days of space flight. Significant increases in plasma adrenocorticotropic hormone were observed at landing compared to preflight values. Russian studies have shown a decrease in NK cell cytotoxicity during long-term space flights (lasting from 60 to 366 days). Our studies show that the cytotoxic functions of NK cells decrease even after short-term missions (9-11 day missions). (Supported by NASA: 106-50-10-10)

[98] DEVELOPMENT OF MEMBRANE PROBES FOR THE IN VIVO STUDY OF BONE MINERALS. E.M. Janke1, and J. Sjo1. 1Bioanalytical Systems, Inc. West Lafayette, IN; 2Dept of Veterinary Clinical Sciences, School of Veterinary Medicine, Purdue Univ.

Understanding of the mechanisms involved in bone loss due to prolonged microgravity and development of would be facilitated by the ability to monitor changes in bone minerals at the tissue level. Ultrafiltration and microdialysis membrane probes have been developed which can be used to sample directly from the interstitial fluid of bone, muscle and subcutaneous tissue. These probes consist of semipermeable membrane fibers attached to non-porous microbore, polymer tubing. The fibers are implanted in the tissue to be sampled and the polymer tubing is externalized for sample collection. Microdialysis probes were perfused with normal saline solution and analytes diffused across the membrane driven by concentration gradients. Ultrafiltration probes have a needle hub attached to the polymer tubing. Interstitial fluid was removed by a negative pressure gradient generated by insertion of the hub into a Vacutainer. Using the sheep as a model for probe development, both microdialysis and ultrafiltration probes were implanted in the marrow cavity of the femur, quadriceps muscle and subcutaneous tissue. Calcium, magnesium and phosphate were measured in the probe samples. Under baseline conditions muscle had the highest concentration of ultrafiltrable calcium (3.50 mg/dL) and bone (2.97 mg/dL) the lowest. For magnesium, the concentration gradients were reversed with bone at 1.73 mg/dL and muscle at 1.57 mg/dL. In all tissues, the concentration of ultrafiltrable calcium was significantly higher than the ionized calcium indicating the existence of calcium in multiple forms in interstitial fluid as in blood. To demonstrate the ability of the probes to follow changes in analyte concentrations, a calcium borogluconate solution was infused IV and changes in the tissues were monitored and compared to blood changes. (Supported by NASA: NAS9-97020)


NASA Johnson Space Center is designing and building a habitat (Advanced Life Support Systems Integration Testbed, ALSSIT) intended for evaluating advanced life support systems developed for long duration missions to the Moon or Mars where all consumables will be recycled and reused. ALSSIT is baselined to support a food system in which designated crops (such as soybeans) will provide a significant part of the diet. These higher plants will have the dual role of providing oxygen (removing carbon dioxide) and, after processing, food. The impact of processing soybeans into soy milk and soy bread on other systems such as water recovery, waste processing and other equipment was determined Soy milk was obtained using a small-scale prototype machine. Results showed less than 2% solid waste generated from edible biomass and wash water with a composition of 36.5 mg/l inorganic carbon, 70.5 mg/l of total organic carbon, and 452.5 mg/l total solids. Additionally, the air volatiles Spacecraft Maximum Allowable Concentrations (SMAC) for 180 days were exceeded for ethanol, acetaldehyde, methanol, hexanal, propional, acetone and carbon disulfide. Nutritional, various essential amino acids were low in the soymilk. A Hazard Analysis Critical Control Point plan was developed for the manufacture of soy milk and soy bread to identify and reduce food safety risks for crew members. Soy bread was prepared in an automated bread machine using chamber grown crops. Waste consisted mainly of inedible biomass and SMAC values exceeded for ethanol and acetaldehyde. Both soy milk and soy bread were found to comply with the ALSSIT as long as various precautions were incorporated into the system.

SESSION G: ORAL SESSION -
SPACEFLIGHT EXPERIMENT RESULTS II

Previous studies in the marine mollusk, Aplysia californica, demonstrated that statoconia production was reduced, in a graded manner, with increasing g in embryos and larvae reared on a centrifuge. Here we have tested the hypothesis that a larger mass of statocoria would be produced in the statocysts of mollusks reared in microgravity. Adult pond snails ( Biomphalaria glabrata) were maintained in the Closed Equilibrated Biological Aquatic System (CEBAS) on STS-89 (9-day flight) and STS-90 (16-day flight, Neurolab). These snails are hermaphroditic, so each animal will lay a spawn pack, containing 20 - 30 eggs, almost every day. Statocoria are first formed 4 days after spawning and the young hatch at about one week. Thus, functional statocysts can be formed in snails conceived and developed during either the 9- or 16-day flight. Several hundred neonatal snails were retrieved from both the flight and ground-control units.

The total volume of statocoria in 1-mm snails retrieved from the flight unit on STS-89 was 30% larger than in animals of the same size reared in the ground-control unit. For 2-mm snails, the total volume was 50% larger in the flight-reared animals.

This snail normally shows a preference for downward crawling on a vertical plate, beginning at hatching. Small (1-3 mm) flight-reared snails tested within 5 hours of shuttle landing crawled in random directions after STS-89, whereas normal downward crawling was seen more than one day after landing. Preliminary data from STS-90 indicate that most animals showed no, or minimal, crawling on landing day and gradually obtained a gravitactic directional preference by day 4 after landing.

Thus, the production of statocoria is enhanced in snails reared in microgravity and the normal gravitactic behavior was not displayed until they had been in a 1-g environment for several days. (Supported by NASA: NAG2-952, NAG10-0180 and NSF: IBN-9529136.)


Adult gravid females and juveniles (less than one week old) swordtails (Xiphophorus helleri) were flown in the CEBAS (Closed Equilibrated Biological Aquatic System) Minimodule on the STS-89 (launched on January 22, 1998; 9 days) and the STS-90 (launched on April 17, 1998; 16 days) shuttle flight missions. In a sample-sharing agreement with Ruhr University and DLR, we examined the distribution of neuropeptides (neurotensin, FMRFamide, neurokinin, dynorphin) and steroid receptors in the brain and pituitary gland of adults and juveniles utilizing immunocytocchemical techniques. These data were compared to that gathered in animals maintained in laboratory modules identical to the flown minimodules. Data will be presented on the differences between animals maintained in the laboratory and flight modules regarding the distribution of neuropeptides and steroid receptors. The rate of post-flight growth and sexual development has also been charted. There was no statistical difference in the post-flight growth (standard length and weight) nor in the rate of post-flight sexual development when animals in the flight and laboratory modules were compared (STS-89).

Supported by NASA (NCC 2-963).


The avian model of inner ear research for short and long term duration space flights is attractive: 1) chickens and quail hatch in less than 21 days, 2) both species are bipedal, able to walk and balance immediately after hatching, and 3) much information on avian embryology exists. In addition, fertilized eggs and/or embryos are self contained necessitating no crew maintenance if housed in a dependable apparatus in orbit. These properties of the avian model were demonstrated by both STS-29 & STS-47 flights. Experiments in both flights showed a preponderance of embryos younger than 72 hours of pre-incubation at 1.0G to stop development in microgravity around or after 96 hours. Embryos pre-incubated at 1.0G longer than 96 hours seem to better withstand the effects of microgravity for at least 5 days and are able to continue development upon return to 1.0G, and go on to hatch normally. Exposure of chicks continuously during development to 2xG in ground experiments also affected young and older embryos differentially, suggesting that gravity constitutes an important variable whose effect must be understood. Data from the avian inner ear model developing at 1.0G strengthened the notion that the morphological differences between avian and mammalian inner ear organs facilitate rather than hinder in flight tissue processing. (Supported by NASA NAG2-998 & Tulane Pathology & the Japanese Ministry of Education)


Our finding of a microgravity-related increase in the soluble form of the cell death factor, sFas/APO-1, (Lewis, et al. Gravitational and Space Biology Bulletin, Vol 11: 1997 and The FASEB J., Vol 12: 1998), provides a new basis for understanding the mechanisms for blunted growth responsiveness of human lymphocytes during spaceflight. Levels of sFas/APO-1 protein were significantly increased in microgravity but not in the in-flight centrifuged or ground controls in Jurkat cells flown in the Biocorack facility on the SMM-03 mission. The cells were growth stimulated by increasing serum concentration in flight and ground controls. Four, 24, and 48 hours later, cells were filtered from medium and fixed with formalin. Medium was frozen until evaluated for sFas/APO-1 post flight. Nuclei of cells fixed at 4 hours and stained with Hoechst 33258 post-flight showed more flight than ground cells with nuclear condensation characteristic of apoptosis. Thus, fewer cells were available for growth stimulation early in the flight. By 48 hours, frequency of morphologically detectable apoptosis was the same for flight and ground cells. The role of soluble sFas/APO-1 is not completely understood and it may be an inhibitor of Fas-mediated apoptosis in lymphocytes by competitive binding to the Fas ligand. If so, sFas/APO-1 may have functioned to block apoptosis and facilitate recovery from apoptosis observed earlier in the mission. In any case, apoptosis in human lymphocytes (Jurkat) in microgravity appears to be regulated through a Fas/APO-1 mechanism. The time dependent increase in soluble Fas/APO-1 identifies it as a cellular control mechanism responsive to the microgravity environment per se. The Fas/APO-1 model provides a means to investigate mechanisms of lymphocyte population dynamics in normal and malignant cells during spaceflight. (Supported by NASA NAG2-985.)
[104] THE TRANSLLOCATION OF DIFFERENT PKC ISOFORMS IN U937 CELLS AND T-CELLS IS ALTERED DURING SPACEFLIGHT. J.P. Hatton¹, D.A. Schmit², F. Gauthier³, S. Roquefeuil⁴, B.B. Hashemi⁵, J.-P. Cazenave¹.Institut de Transfusion Sanguine, Strasbourg 67085, FRANCE; ²ESA-ESTEC, Postbus 299, 2200AG Noordwijk, Netherlands; ³INSERM U151, HU Rangueil, Toulouse 31054, FRANCE. ¹Life Science Research Laboratories, NASA Johnson Space Center, TX 77058.

Protein Kinase C isoforms are an important family of kinases which mediate intracellular signal transduction associated with the regulation of cellular proliferation & differentiation. We have previously shown in the "Phorbol" experiments flown aboard STS-65 and STS-76 that the total quantity of PKC in U937 cells (a human monocytic cell line) was increased in microgravity & the translocation of PKC from cytosol to membrane in response to phorbol ester stimulation was modified in microgravity (FASSEB J. 10, 1627-1634). However, in these experiments it was not feasible to examine the behaviour of individual PKC isoforms, which are believed to fulfill distinct signalling functions. Therefore, in the "Isozyme" experiment flown in the ESA Biocor facility during STS-81 we examined the translocation of individual PKC isoforms in U937 cells & T-cells response to agonist stimulation (phorbol ester or stimulatory ligand). Cells were stimulated in-flight & the permeabilised at different times after activation with a digitonin / inhibitor mix. Post flight samples were fractionated to yield cytosolic and particulate fractions. Western blot was used to determine the relative quantity of different PKC isoforms in each cellular fraction. In U937 cells the translocation of PKC ε & PKC β is occurs more rapidly & earlier in flight than in ground samples. However, there was no significant difference in the translocation of PKC β between ground & flight. The results confirm our previous findings that the behaviour of PKC is modified in microgravity. However, only certain PKC isoforms appear sensitive to microgravity which may have important implications for signal transduction. The functional consequences of these changes were investigated in the "Cytokine" experiment on STS-84 which is currently under analysed. (Supported by CNES: 96/241 & 97/071/6751)

[105] INTRACELLULAR TRANSLLOCATION OF PROTEIN KINASE C (PKC) IN HUMAN PERIPHERAL BLOOD T-CELLS DURING MICROGRAVITY CULTURE. B.B. Hashemi¹, J.P. Hatton², D. Schmidt³, J.E. McClure⁴, & C.F. Sams⁵. NASA-Johnson Space Center, Houston, ¹ETSS Strasbourg - France, ²ESA ESTEC - The Netherlands.

Experiments performed during space flight and utilizing ground-based hypogravity model systems indicate a dramatic reduction in the responsiveness of T-cells to activation signals. T-cells activated by soluble lectins or anti-TCR antibodies fail to express surface activation markers or to proliferate compared with 1-g culture. We and others have shown that addition of phorbol esters as co-stimulators can partially overcome this inhibition suggesting that perturbed ability to properly activate and translocate PKC within T-cells may play an important role in altered T-cell response to activation in microgravity.

In the current study we investigate the effects of microgravity on the distribution of PKC-β1 and PKC-δ isoforms in T-cells stimulated with bead-immobilized anti-TCR or with a combination of phorbol ester and calcium ionophore. T-cells were isolated from healthy donors and cultured in microgravity or in a 1-g reference centrifuge of the Biocor facility on Space Shuttle flight STS-84. Cells were activated in-flight for one hour with the appropriate stimuli, they were subsequently treated with fixatives, and finally they were stored at 4 °C for return to Earth. Post-flight immunofluorescence labeling was performed at JSC and fluorescence microscopy of samples demonstrate very good preservation of antigenicity and specific labeling of PKC isoforms. Scanning confocal microscopy of the cells reveals dramatic translocation of PKC-β1 and PKC-δ to the plasma membrane and to the cell-cell contact site in activated samples. Unstimulated cells exhibited a more uniform distribution of PKC in the cell cytoplasm and a small degree of localization with structures resembling the microtubule cytoskeleton. To our knowledge, these data are the first report of fluorescence microscopy evidence for PKC translocation in microgravity samples.

[106] MICROGRAVITY AND SIGNAL TRANSDUCTION PATHWAY IN SPERM – BIORACK ON SHUTTLE TO MIR. J.S. Tash, J.J. Fritch, M.E. Landis, G.E. Bracho. Dept. Molecular & Integrative Physiology, Univ. of Kansas Medical Center, Kansas City, KS.

Long term habitation in microgravity (μG) raises the question whether fertilization processes occur normally in the μG environment. Activation of sperm motility during fertilization is coupled to changes in phosphorylation of flagellar proteins. Sea urchin sperm then respond to the presence of the egg by chemotactic interactions with peptides released from the egg jelly coat that temporally activate both protein kinase and protein phosphatase signaling cascades. Previous European Space Agency (ESA) studies demonstrated that bull sperm swim with higher velocity and linearity in μG than at 1G. In experiments conducted on STS-81 and STS-84 using the ESA Biocor, flagellar protein phosphorylation during initiation of sea urchin sperm motility occurred significantly faster in μG than at 1G. The primary target for the response to μG is a 130 kDa phospholipase-D-containing protein (FP130) that is tightly bound to the flagellar axoneme. When sperm were activated in the presence of the egg peptide spermat, the normal biphasic increase in phosphorylation followed by a decrease in phosphorylation was observed in 1G. However, in μG the biphasic response was blocked and only a slow steady rise in FP130 phosphorylation was observed. This suggests that μG alters the balance between protein kinase and protein phosphatase activity in sperm. These results are consistent with a positive gravitropic response in sperm whereby gravitational pull on the sperm head interacts with the cytoskeleton much in the same way that has been proposed for statolith interactions with signaling pathways and the cytoskeleton in plants. These findings have important implications for fertilization in space and emphasize the need to examine early fertilization processes in μG. To date, no studies have been conducted to analyze efficiency of fertilization in microgravity. Rather, studies have focused on post-fertilization embryonic development where information on efficiency of fertilization has been ignored. In species where fertilization efficiency is low such as humans, changes in processes related to fertilization will probably have dramatic effects on fertilization outcomes. (Supported by NASA NAG-2-1016 and NIH HD- HD-33994)
SESSION H: ORAL SESSION - SPACEFLIGHT EXPERIMENT RESULTS III
CELL AND ORGANELLE POSITIONING OF GRAVITROPIC MOSS PROTONEMATA IN MICROGRAVITY. V. D. Kern, and F.D. Sack. Dept. of Plant Biology, Ohio State Univ., Columbus, OH.

Protonemata of the moss Ceratodon purpureus are unique in that a single apical cell both senses and responds to gravity and to directional light. Apical cells of protonemata grow by oriented tip growth which is negatively gravitropic in the dark and positively phototropic in unilateral red light. Gravitropic sensing in Ceratodon probably involves amyloplasts that sediment, and its phototropic response is phytochrome-mediated. The Collaborative US and Ukrainian Experiment (CUE) was a suite of plant experiments on STS-87 (Nov. - Dec. 1997). The BRIC SPM-A experiment examined the positioning of cell growth of amyloplasts in dark-grown Ceratodon protonemata that developed entirely in microgravity for 7 or 14 days. This study also determined the extent of phototropism in the absence of gravitropism. Engineers at KSC provided customized hardware (see accompanying abstract) that allowed petri dish cultures to be illuminated with unilateral light provided by red LEDs (660 nm ± 20 nm). Following initial cultivation in darkness for 7 d, a phototropic time-course (4 h to 7 d) was obtained during this 16-day flight. All cells were fixed chemically while still in microgravity. At the higher light intensity employed (1 μmol m⁻²s⁻¹), all protonemata grew either towards or away from the light i.e. both positive and negative phototropism took place. These results are consistent with ground-based experiments showing that red light illumination at intensities ≥140 nmol m⁻²s⁻¹ suppresses gravitropism. Ground-based work has also established that a modulated gravitropism is expressed at intensities ≤100 nmol m⁻²s⁻¹. The effects of exposure to 50 nmol m⁻²s⁻¹ unilateral red light will be discussed. At 1 g on earth, upright, dark-grown protonemata display a characteristic plastid zonation in which specific populations of non-green plastids sediment towards gravity in specific zones. Since dark-grown cells in microgravity would display neither gravitropism nor amyloplast sedimentation, it was predicted that both cell orientation and amyloplast position would be random. However, both cell position in older cultures and amyloplast distribution in all cells were non-random in microgravity. Microgravity-grown cells resembled, although not identical to, cells rotated on a clinostat (1 rpm) in ground-based experiments. The results point to the existence of endogenous forces that direct cell growth and amyloplast position in the absence of gravity. (Supported by NASA: NAG1-017)

AUTOTROPIC STRAIGHTENING AFTER GRAVITROPIC CURVATURE OF LEPIDIUM ROOTS IN MICROGRAVITY. B. Stankovic1, F. D. Sack1, A. Johnson1, F. Antonsen2, D. Volkmann3
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Autotropic or autonomic straightening is the loss of gravitropic curvature in plant organs following a withdrawal of a constant g-stimulus. Autotropic straightening of Lepidium (cress) roots occurs when gravitropically-curved roots are rotated on a clinostat (PI Physiol 117: 893-900). To determine whether autotropism also occurs in space, microgravity-grown cress roots were laterally centrifuged in-flight, and then returned to microgravity using Biorack hardware on Shuttle to Mir Mission 5 (STS-81). Roots were centrifuged at 4 different g-doses (0.1 and 1 g for 15 or 75 min). Roots in all 4 treatments underwent gravitropic curvature and subsequent straightening in microgravity, as a result of the loss of gravitropic curvature in older regions of the root and the coordinated alignment of new growth. Control roots grown entirely in microgravity exhibited minor changes in root angle. These results indicate that both microgravity and clinostat rotation can be equivalent in stimulus withdrawal with respect to the induction of autotrophic straightening. Cress roots are the only plant organ shown to undergo autotrophic straightening in both microgravity and clinostat. The observed root straightening in space rules out the hypothesis that autotropism represents a commitment to a pre-stimulus orientation with respect to gravity and instead suggest that there is a default tendency towards axiality which is expressed following a withdrawal of a constant g-stimulus. (Supported by NASA: NAG2-1023 to F.S., and by DARA: 50 9429 and MWF to D.V.)

MICROGRAVITY EXPERIMENTS WITH ARABIDOPSIS IN BIORACK SUPPORT A STATOLITH-BASED MODEL FOR GRAVIVERCEPTION. J.Z. Kiss, R.E. Edelmans, and P.C. Wood. Department of Botany, Miami University, Oxford OH.

In order to help resolve some of the controversy associated with ground-based research that has supported the starch-statolith theory of gravity perception in plants, we performed spaceflight experiments with Arabidopsis during the January 1997 and May 1997 missions of the Space Shuttle. This project was flown in the Biorack, which is a multiuser facility developed by the European Space Agency and serves as a small laboratory for studying cell and developmental biology in plants and small organisms. Seedlings of wild-type (WT) Arabidopsis, two reduced-starch strains, and a starchless mutant were grown in microgravity and then were either a 30, 60, or 90 minute gravity stimulus on a centrifuge. This experiment on gravitropic sensitivity/responsiveness was performed the "right way" in that brief gravitational stimuli were provided, and the seedlings were allowed to express the response without further unilateral gravity stimuli. Thus, the complications of ground experiments performed with clinostats were avoided. Our spaceflight results support previous ground-based studies of these and other mutants in that we found a positive correlation with increasing amounts of starch and increasing sensitivity to gravity. (This work was supported by grant NAG 2-1017 from NASA.)

MORPHOLOGICAL, CELLULAR AND MOLECULAR ANALYSIS OF ETIOLATED SOYBEAN TISSUE FROM THE GENE EXPRESSION (GENEX) STS-87 SPACEFLIGHT EXPERIMENT. W.C. Piastuch1, K.M. Johnson1, H.G. Levine1, E.C. Stryjewski2, L.H. Levine1, J.A. Sharek3, O. Martynenko2, and V. Prima1
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A total of 192 seeds of soybean (Glycine max cv. McCall) were imbibed and grown etiolated for six days in NASA's Biological Research In Canister (BRIC) hardware. Morphological characteristics including fresh weight, root and shoot lengths, and the degree of lateral root branching were significantly different between flight and ground tissues. Carbohydrate analysis has shown altered partitioning and concentrations in the flight tissues with substantial starch differences in elongated hypocotyl tissue. Preliminary molecular analyses show flight vs. ground expression differences for PAL, CHS, and CSR gene products involved in secondary metabolite production. Additionally, HPLC has indicated concentration changes in several secondary metabolites. Although previous Shuttle middeck experiments from our laboratory (STS-68, STS-63) have shown differences in etiolated soybean seedling growth, morphology, carbohydrate concentrations and enzyme activities in flight-grown tissues, an overriding factor in these experiments was the presence of significantly higher levels of ethylene in the flight growth containers measured post-flight. Our modified protocols resulted in: a) equivalent levels of ethylene for flight and ground controls (as measured by gas sampling after 5 days on-orbit growth and post-flight), b) elimination of previously encountered mechanical shearing and seedling contact with metal and wet surfaces, and c) complete recovery of intact tissues. (This work supported by NASA contract NAS10-12180, Dynamac Corp.)
[111] EFFECTS OF MICROGRAVITY ON PATHOGENESIS AND DEFENSE RESPONSES IN SOYBEAN TISSUES. E. Hilaire\(^1\), M. Ryba-White\(^1\), O. Nedukha\(^2\), E. Kordyum\(^2\), J.A. Guikema\(^3\), and J.E. Leach\(^1\). \(^1\)Dept. of Plant Pathology, Kansas State University, Manhattan; \(^2\)Institute of Botany, Kiev, Ukraine; \(^3\)Division of Biology, KSU. Microgravity profoundly impacts plant cell development and physiology; these alterations may have a significant impact on interactions between plants and microorganisms. Anecdotal information from previous spaceflight experiments indicates that plants grown in microgravity are more susceptible to microbial invasion. We established an experimental system to quantitatively evaluate the impact of growth in microgravity on both the disease susceptibility and active resistance of soybean seedlings to the pathogenic fungus, \textit{Phytophthora sojae}. The experiment (SOYPAT) was part of the Collaborative US/Ukrainian Experiment (CUE), Space Shuttle Mission STS-87. Relative to ground controls, space-grown soybean seedlings inoculated with a virulent strain of \textit{P. sojae}, R25, showed more severe disease symptoms, including tissue browning and maceration. Microscopic examination of root tissues sampled 7 days into flight revealed that colonization by R25 was more extensive in space-grown tissues than in ground controls. In the ground samples, R25 hyphae and haustoria were predominantly observed in the root hair zone, and did not penetrate the stele. In contrast, in flight samples R25 penetrated root zone roots and hyphae were observed in the stele. Thus, soybean seedlings are more susceptible to invasion and disease by \textit{P. sojae} R25. To determine if microgravity affects the ability of plants to build an active defense response, seedlings also were inoculated with \textit{P. sojae} strain R1. R1 induces a defensive response in this soybean cultivar (cv. Williams R2). Preliminary data suggests that the seedlings did resist fungal invasion. Although some symptoms were observed in R1-inoculated seedlings relative to uninoculated controls, the R1-inoculated seedlings exhibited significantly less symptoms than seedlings inoculated with R25 in both ground and flight conditions. The colonization of the soybean seedlings by R1 and the accumulation of enzymes typical of plant defensive compounds in ground vs. flight samples will be evaluated to determine if microgravity alters the resistance response. (Supported by NASA: NAG10-0142.)

[112] REPEATED SEED-TO-SEED EXPERIMENTS WITH \textit{BRASSICA RAPA} ON THE MIR SPACE STATION. M.E. Musgrave\(^1\), A. Kuang\(^2\), Y. Xiao\(^1\), G.E. Bingham\(^1\), L.G. Briarty\(^2\), M.A. Levinsikh\(^2\), V.N. Sychev\(^2\), and I.G. Podolsky\(^2\). \(^1\)Dept. Plant Pathol. & Crop Physiol., Louisiana State Univ., Baton Rouge, LA; \(^2\)Space Dynamics Lab., Utah State Univ., Logan, UT; \(^3\)Dept. of Life Sci., Univ. Nottingham, Nottingham, UK; \(^4\)Institut für Medizin/Biologische Probleme, Moscow, Russia. Recent short-duration experiments on reproductive development in \textit{Arabidopsis} and \textit{Brassica rapa} had shown that if the plant environment is adequately controlled on orbit to provide sufficient ventilation, seeds can develop normally. The opportunity to observe how these short-term successes in reproductive development would apply to a long-duration growth period in space came during the NASA-5 increment of the Shuttle-Mir program, May-October 1997, with the Greenhouse 3 experiment. Dry seeds of \textit{Brassica rapa} were planted on orbit in the modified Svet greenhouse by astronaut C. Michael Foale. These seeds germinated and developed into plants that bloomed and produced new seeds, which were subsequently planted on orbit to start a second generation. This process was repeated twice on orbit, and the dry seeds that were harvested are currently being compared with those that have been produced in a post-flight ground control. While on orbit, seeds that were produced in space were planted alongside seeds that had been brought to the Mir station from earth. Additional analyses are being conducted on fixed and frozen samples taken on orbit from these first and second generation space plants. This unique material will permit the comparison of plants in their first and second generations in microgravity and will begin to allow us to assess possible deleterious effects of multiple generations on orbit. Nevertheless, the results confirm that gravity is not required for any portion of the \textit{Brassica rapa} life cycle. Supported by NASA grant NAG2-1020 to MEM, NATO grant CRG960089 to LGB, and by GEB through NASA contract NAS2-13659.

[113] A COMPARISON OF SPACEFLIGHT AND GROUND CANOPY GAS EXCHANGE MEASUREMENTS. O. Monje\(^1\), G.E. Bingham\(^1\), B.K. Eannes\(^1\), W.F. Campbell\(^1\), V. Sychev\(^2\), M.A. Levinsikh\(^2\), and I. Podolsky\(^2\). \(^1\)Plants, Soils & Biometeorology Dept., Utah State University; \(^2\)Institute of Biomedical Problems, Moscow. Gas exchange data collected in the SVET Greenhouse aboard Mir was used to determine canopy photosynthesis, transpiration, and growth rates in microgravity. These measurements were repeated in a ground study where Mir Cabin air was simulated. The simulated cabin air included 1 \(\mu\)mol mol\(^{-1}\) ethylene in order to reproduce ethylene induced changes in canopy morphology. Carbon and water vapor fluxes from wheat (cv. Superdwarf) were measured using the Gas Exchange Measurement System (GEMS). The wheat canopy was grown in solid substrate (Balkanine) in a mockup of the SVET Greenhouse. An automatic watering system consisting of a datalogger and the heat pulse soil moisture probes from the GEMS was used to approximate the watering regime during the flight experiment. Canopy level growth rates, transpiration rates, and the mass balance of water between the flight and ground experiments were similar. (Supported: NASA Grant NCC 2-831 and the Space Dynamics Laboratory, USU.)

[114] WATER MANAGEMENT LESSONS FROM PLANT FULL LIFE CYCLE EXPERIMENTS ON MIR. G.E. Bingham\(^1\), S.B. Jones\(^1\), D. Or\(^1\), I. Podolsky\(^2\), V. Sychev\(^2\), \(^1\)Plants, Soils & Biometeorology Dept., Utah State University; \(^2\)Institute of Biomedical Problems, Moscow. Seven full life cycle plant experiments have been completed in the Svet-GEMS greenhouse on the Mir Orbital Station using both wheat and Brassica. An 8th experiment is planned for the last quarter, 1998. These experiments, where seed is planted in space and plants are allowed to grow for the full life cycle, closely simulate the experiences that space farmers will face operating a bioregenerative life support system. During these experiments, the root media (both Balkanine and Turface) were launched dry and wet up in space before planting. We discuss the substrate wetting and water management issues observed during these long-term growth experiments. Data demonstrating the importance of monitoring both water control system status and actual substrate water content is presented. Significant differences in hydraulic conductivity and water content distribution were observed between gravity and microgravity experiments. This difference could cause a reduction in root zone oxygen levels as the optimal water content shifts in space. Substrate media shape and chemical status are also shown to significantly impact water management strategy. Our work shows that it is impossible to simulate space substrate water content conditions in ground control experiments, using the same substrate. (Supported: NASA Grant NCC 2-831 and the Space Dynamics Laboratory, USU.)
SESSION I: CONCURRENT ORAL SESSION - CELL BIOLOGY AND BIOTECHNOLOGY/ INSTRUMENTATION II
[115] MICROGRAVITY INDUCED OSTEOPOROSIS STUDY ON STS-80 SPACE FLIGHT. K.E. Forkeyhim, 1,2 and E.B. Schenker, 1, 2 International Space University (ISU96), Vienna, Austria. 1Israel Aerospace Medicine Institute, Jerusalem, ISRAEL. 2University of Manitoba, Faculty of Medicine, Manitoba, CANADA.

Introduction: Space flight is a unique environment which causes a variety of physiological changes, including osteoporosis. The exact mechanism by which microgravity causes osteoporosis is unknown, but it has been shown that the severity of osteoporosis is dependent on mission length. This study compared in vitro osteoblast proliferation and function in microgravity and on Earth.

Methods: Osteoblast cells from the MC3T3 mouse cell line were launched on the STS-80 space shuttle flight in a quiescent state by culturing the cells in a medium containing 2% fetal calf serum (FCS). Once microgravity was reached, the cells were placed in a fresh medium with 11% FCS and allowed to proliferate for 72 hours and 45 minutes. The cells were then fixed with glutaraldehyde. Osteoblast cells on Earth were treated in a similar manner for comparison.

Results: The space flight samples contained 31% less cells than the ground controls and metabolized 35% less glucose than the ground samples. In addition the space flight cells were found to be smaller and have fewer processes than the ground cells. The f-actin cytoskeleton of the space flight cells was also found to be spindle-shaped and have less actin fibers radiating from the center of the cells in comparison to the ground controls.

Conclusion: These findings indicate that osteoporosis induced by space flight may be partially attributed to a change in the osteoblast's proliferation, function and morphology.

[116] THE ROLE OF GRAVITY IN REGULATING THE PRODUCTION OF EPIDERMAL GROWTH FACTOR. E.M. Durban and S. Das. Division of Oral Pathology, University of Texas-Houston, Dental Branch.

Epidermal growth factor (EGF) is a versatile molecule with numerous documented in vivo effects. EGF is synthesized by diverse tissues as a large membrane-bound precursor protein from which mature EGF and other EGF-like polypeptides can be produced by proteolytic cleavage. We observed in previous studies that EGF production in rodents is a gravity sensitive process that is affected in vivo by gravitational changes. Mice exposed to hypergravity (centrifugation corresponding to 2.9 or 3.5g) for 2 weeks experienced a 5-10-fold reduction in intracellular salivary EGF levels, a difference which was maintained after 4 weeks of centrifugation. In contrast, intracellular salivary EGF levels increased in flight animals (rats, STS-54, 6-day mission) by at least two-fold in comparison to 1g controls. The effects of microgravity on EGF production was specific as the levels of another growth factor, transforming growth factor alpha, were not affected. These results indicate that EGF synthesis, storage, or secretion can be affected by gravitational changes. We now address the question of whether alterations in EGF production by gravity are the result of a direct cellular response. The mouse submandibular salivary gland (SSG) was used as indicator organ as it produces vast amounts of EGF under the regulation of testosterone (DHT) and triiodothyronine (T3). Dissociated SSG cells were prepared for primary culture following our previously published protocols. Cells were cultured using a collagen matrix as support either at 1g or under simulated microgravity in a NASA-designed Rotating Wall Vessel (RWV). Cultures with DHT (1.7 x 10^-7 M; T3, 1.5 x 10^-8 M) and without hormones were sampled at time intervals for EGF levels. No significant difference in EGF levels were detected between simulated microgravity and 1g controls. These results suggest that, in the absence of systemic influences on potential mediator molecules, EGF production is not altered by gravity via a direct effect on the cells. (Supported by NASA: NAG5-4710)

[117] NEURAL STEM-LIKE CELLS GROWN IN A SIMULATED MICROGRAVITY ENVIRONMENT. H.P. Low, T.M. Savarese and W.J. Schwartz. 1Dept of Neurology, and 2Cancer Center, Univ of Massachusetts Medical School, Worcester.

Neural stem cells are self-renewing, multipotential cells that give rise to differentiated neuronal and glial populations. Such precursors have been identified in a number of neural structures including the striatal subventricular zone. These cells proliferate in a chemically-defined, serum-free medium containing epidermal growth factor (EGF) as clusters of cells in suspension ("neurospheres"). Our objective is to investigate the biology of embryonic murine neural precursor cells grown in a simulated microgravity environment using High Aspect Ratio Vessels (HARVs).

We found that simulated microgravity induces the formation of multiple, large three-dimensional tissue-like oid structures per vessel within 2 to 3 days of culture. Their size (1 to 2 mm in length) is 20 to 25 times larger than individual neurospheres. Such structures are always formed with horizontal (not vertical) rotation, but never in stationary cultures or in the absence of EGF. Histological analysis shows that each structure is a "shell" of cells that surrounds a necrotic cavity. Compared to neurospheres, cells in the tissue-like structures are more pleomorphic, including some with nucleomegaly. This microgravity-induced phenotype is reversible, i.e., cells from structures will form neurospheres when re cultured in flasks.

Immunocytochemistry for the proliferating cell nuclear antigen (PCNA) reveals a layer of proliferating cells localized on the outer surface of the structures. Staining for nestin, an intermediate filament protein that characterizes neural stem cells, shows a similar distribution. These observations, in addition to scanning electron microscopic images, suggest that structure formation is a primary event and not due to an aggregation or collision of neurospheres in the HARVs.

We believe that simulated microgravity may provide a unique in vitro model for future clonal growth and lineage analyses of neural stem cells. (Supported by NASA: NAG8-1358.)

[118] MECHANISMS OF LYMPHOCYTE FUNCTION INHIBITION IN MICROGRAVITY. D. Risin, D. Cooper, A. Sundaresan and N.R. Pellis. Biotechnology Program, NASA/JSC and Wyle Laboratories, Houston, TX and Division of Immunology, La Jolla Institute for Allergy and Immunology, San Diego, CA.

Understanding the mechanisms of suppression of immunological functions in space is important for developing effective strategies for preventing and correcting immune impairments in astronauts, especially in long-term space missions. Microgravity-induced immunosuppression at least partially could be due to the direct effects of changes in gravity on lymphocyte functions. We have shown earlier that simulated and true microgravity (MG) inhibits lymphocyte locomotion in type I collagen (Pellis et al., 1994, 1997). Approximation of microgravitational conditions at ground level has been attained by using rotating wall vessel bioreactor. In this study, we have demonstrated that simulated MG dramatically inhibits the polyclonal activation of human lymphocytes induced by PHA, CD2/CD28, CD3/CD28 or CD2/CD28 antibodies. The same defect in activation was observed in mixed lymphocyte cultures and in the specific recall to tetanus toxoid in human lymphocytes and to Borrelia burgdorferi in murine T-lymphocyte lines. The calcium ionophore ionomycin did not restore inhibited lymphocyte locomotion and activation, indicating that calcium flux is unaffected by simulated MG.

Direct activation of protein kinase C (PKC) by phospholipid ester (PMA) substantially restored inhibited lymphocyte locomotion and activation. The restoration of lymphocyte locomotion was nearly to normal values (84%). This suggests that the defect occurred in the signal transduction pathway upstream of the activation of protein kinase C. Our preliminary data show that the restoration of locomotion by PMA is independent of the DNA synthesis. Thus, these results indicate that simulated MG, and potentially true MG, causes a fundamental defect in signal transduction that results in blunted locomotion and loss of proliferative response to activation signals. (Supported by NRO OLMSA-02 and NSCORT #NAG5-4072 grants.)

Studies of lymphocyte activation during space flight and in ground-based models of hypogravity, including clinostatic rotation, show a dramatic reduction in the response to activating signals. Specifically, peripheral blood mononuclear cells activated by lectins or soluble monomonal antibodies are blocked very early in the activation sequence, whereas purified T-cells activated by phorbol ester plus ionomycin bypass this block and enter cell cycle progression. We have developed an activation system for purified T-cells using anti-CDR antibodies bound to polystyrene beads that exhibits the same inhibitory effects of clinostatic rotation on T-cell activation as occurs in the PBMC models. The T-cell activation response in this system has been characterized as a function of antibody concentration. This activation system provides a unique opportunity to investigate T-cell specific activation and signal transduction events sensitive to microgravity in the absence of accessory cells.

(C.L. Adams is supported by the National Research Council.)


Biorack is an ESA developed multi-stations facility entirely designed for Biological research under Microgravity conditions. Biorack flew three times onboard the ESA developed Spacelab module, first of all on the first German Spacelab mission D-1 (FLA or STS-22), in October 1985, then on the first International Microgravity Laboratory (IML-1/STS-42) in January 1992. Biorack made its third Spacelab trip onboard the second International Microgravity Laboratory (IML-2/STS-65) in July 1994. On all those missions, the variety of biological organisms flown in Biorack was ranging from bacteria, yeast, cell cultures, frog and sea-urchin embryos to fruit flies, nematode worms and plant seedlings.

After the IML-2 Mission, NASA and ESA agreed to have the BioRack Facility flown on three of the "Shuttle to MIR Missions" (S/MM) which was a series of missions called for the US Space Shuttle to dock with the Russian MIR Space Station. The BioRack facility was initially developed as a Spacelab payload and its configuration has to be slightly modified to be adapted and integrated into the SpaceHab module which was used on those three Mir docking Missions. A single SpaceHab module was flown on the first mission (S/MM-03 or STS-76, launched on 22 March 1996) and a double Spacehab module was used on the second (S/MM-05 or STS-81, launched 12 January 1997) and third (S/MM-06 or STS-84, flown in May 1997) missions. A total of thirty one experiments were flown during the three BioRack S/MM flights. The BioRack facility performed flawlessly during the three missions, including the new variable speed, centrifuges. An overview of the BioRack Facility Performance, derived from downlinked and recorded data as well as a summary of the BioRack experiment operations during these three Missions will be presented.

ESA is currently developing a new biological research facility called BioPack which is primarily intended to fly in the Orbiter Middeck or in the SpaceHab module. BioPack will have a static rack as well as three independent, variable speed, centrifuges, a cooler and a freezer; it will use standard ESA BioRack Type I and Type II containers.

[121] THE C.E.B.A.S. MINI MODULE ON STS-89: FIRST SUCCESSFUL SPACEFLIGHT OF A CLOSED AQUATIC ECOSYSTEM. V. Brieum, M. Andrikse, F. Pass and D. Voeste. Ruhr-University of Bochum, Faculty of Biology, C.E.B.A.S. Center of Excellence, Bochum, FRG.

The C.E.B.A.S. MINI MODULE, a closed aquatic ecosystem integrated into a middeck locker and consisting of a Zoological (animal tanks), a Botanical (plant bioreactor), Microb (bacteria filter) and an Electronic Component (data acquisition/control system) was flown on the STS-89 space shuttle mission in January 1998 for 9 days. Pre-flight the plant bioreactor was loaded with 53 g of Ceratophyllum demersum and the animal tanks with 4 adult pregnant females of the fish Ziphophorus helleri, 200 juveniles of the same species with less than 1 week of age, 38 large and 30 juvenile Bionautella glabrata water snails. The filter compartment was filled with 200 g of lava gran inculated with laboratory strains of ammonia oxidizing bacteria. After an adaptation period of 5 days the system was closed and integrated into the spacehab one day before launch. Video recordings of the animals were automatically taken for 10 minutes in 2 hour-periods, the tapes were changed daily by the astronauts. The housekeeping data and water parameters were within the expected range and were highly reproducible in comparison to the ground reference. After 9 days under space conditions the plant biomass increased to 117 g. The plants were all found in very good conditions. All 4 adult females were retrieved in different pregnancy stages and in a good physiological condition. The juvenile fishes had a survival rate of about 33%. This expected number was also found in the ground reference experiment. 97% of the snails survived and produced more than 250 neonates and 40 spawning packs. All samples were distributed according to a defined schedule and satisfied all scientific needs of the involved 12 principal investigators. This was the first successful spaceflight of an artificial aquatic ecosystem containing vertebrates, invertebrates, higher plants and microorganisms self-sustained by its inhabitants only. So, C.E.B.A.S. is a promising candidate for the early space station utilization. (Supported by DLR (grant #WS50WBD9319-3) and the Ministry of Science of Research of the State of Nordrhein-Westfalen (grant # IVA1216-00588).

[122] SIGNAL TRANSDUCTION IN T LYMPHOCYTES - A COMPARISON OF THE DATA FROM SPACE, THE FREE FALL MACHINE AND THE RANDOM POSITIONING MACHINE. M. Cogoli-Greuter,1, M. Schwarzenberg1, P. Pippia1, M.A. Melouf1, G. Cosso2, and A. Cogoli1. Space Biology, ETHZ, Technoparkstrasse 1, CH-8093 Zurich, Switzerland, and 2 Department of Physiological, Biochemical and Cellular Sciences, University of Sassari, via Muroni 25, I-07100 Sassari, Italy.

Several experiments in space show that mitogenic T cell activation is lost at 0 g. Immunocytochemistry indicates that such effect is associated with changes of the cytoskeleton. Biochemical studies suggest that the lack of expression of the interleukin-2 receptor is one of the major causes of the loss of activity. In fact, interleukin-2 is the third signal required for full activation. In order to deepen our investigations we are now working with the free-fall machine, FFM, invented by D. Medsal, and with the random positioning machine, RPM, or three-dimensional clinostat, developed by T. Hoson. The FFM produces periods of free-fall lasting approximately 800 ms followed by bounces of 15-30 g lasting 45-60 ms. The RPM eliminates the effect of gravity by rotating the biological specimen randomly around two orthogonal axes. Mononuclear cells, purified from the buffy-coat of human peripheral blood by gradient centrifugation on Ficol, were incubated for 72 hours at 37 °C either in simulated microgravity or at 1 g. Activation was determined by 2 hr pulse of methyl-3H-thymidine 72 hr after exposition to concanaval A. While the FFM failed to reproduce the results obtained with T lymphocytes in space, the data from the RPM are in good agreement with those in real microgravity. In fact, the inhibition of the mitotic index in the RPM is 89% compared to static controls. The RPM (as the FFM) can carry markedly larger specimens than the fast rotating clinostat and thus allows to conduct comprehensive studies to select suitable biological objects for further investigations in space.

The study with the FFM was conducted on behalf of ESA with its financial support (Purchase Order 161398). The RPM was purchased with a grant of the Italian Space Agency, ASI.
SESSION J: CONCURRENT ORAL SESSION - 
SPACE BIOMEDICINE AND 
STRUCTURAL SYSTEMS

Introduction: Artificial gravity may be useful for maintaining bone and muscle integrity and for providing a force background in which astronauts could ambulate normally during very long duration space missions. Previous studies, in which subjects were located at the center of a rotating room which was spinning at 10 rpm, have demonstrated rapid motor adaptation to effects of transient Coriolis forces on leg movements. Since astronauts aboard a rotating spacecraft would experience variations in the artificial gravity vector as well as Coriolis forces, we studied leg movements in subjects seated off-center in a rotating artificial gravity environment.

Methods: Subjects (n=8) made toe pointing movements to a visual target (approximately 35 cm straight ahead) in an otherwise dark room. Movements were made before, during and after counterclockwise rotation at 10 rpm while standing against a wall approximately 3 m from the center of the rotating room. The 3D spatial position of the toe was recorded with an OPTOTRAK.

Results: During initial rotation trials, the path of the moving toe deviated ~1 cm in the direction of the Coriolis forces generated but returned to baseline within 40 trials. Initial post-rotation movements were mirror symmetric to initial per-rotation movements but with path deviations of ~2.5 cm. Baseline performance was again restored within 40 trials.

Conclusions: Adaptation to rotation occurs off-center as well as on-center leg movement. These results suggest that adaptation of leg movement to rotating environments will likely be possible under conditions, such as those aboard rotating spacecraft, in which, in addition to transient Coriolis forces, variable artificial gravity vectors will also be present. Supported by NASA grants NAGW-4031, NAGW-4375, NAGW-4374, and NAGW-4735.

[124] MEASUREMENT OF ENERGY BALANCE ON FOUR LMS ASTRONAUTS DURING SPACE FLIGHT. T.P. Stein1, M.J. Leskiw1, M.D. Schluter1, R. Grebebeck2, H.W. Lane and R.W. Hoyt3.

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Energy expenditure was measured by the doubly labeled water method (H218O) on the four payload crew during two consecutive six day time periods (Flight days 3 to 9 and 9 to 15) on the recent Life and Microgravity Sciences (LMS) mission. Energy intake was determined by monitoring the astronauts diets during the study period. Energy balance was calculated from the difference between energy expenditure and intake. Results: The mean energy expenditure was 34.2 ± 1.4 kcal.kg1.d1, energy intake was 24.4 ± 1.4 kcal.kg1.d1 giving a net negative energy balance of -9.7 ± 4.3 kcal.kg1.d1. The negative energy balance is statistically significant from baseline (p < 0.05). Conclusions: (1) The astronauts were unable to maintain energy balance. (2) Dietary intake was very low on this mission.

[125] REDUCTION OF THIN FILAMENT DENSITY AND LENGTH IN HUMAN SOLEUS MUSCLE AFTER 17 DAY SPACEFLIGHT. D.A. Riley1, J.L.W. Bain1, J.L. Thompson1, R.H. Fitte1, J.J. Widrick1, S.W. Trappe1, T.A. Trappe1, and D.L. Costil2.

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Soleus muscle fibers were examined electron microscopically from pre- and post-flight biopsies of 4 astronauts orbited 17 days during the Life and Microgravity Sciences mission, June 1996. Spaceflight did not change thick filament packing density (~1062 filaments/μm2) or spacing (~32.5 nm) in atrophic muscles fibers. Pre-flight thin filament density (2978/μm2) decreased significantly to 2215/μm2 in the A band due to 17% fewer filaments and 9% more short filaments. Normal fibers had 13% short thin filaments. The 26% decrease in thin filaments correlated directly with Widrick et al. unpublished findings on the same muscles of a 32% average increase in the velocity of shortening of slow fibers with no detectable fast myosin. The lower thin filament density was calculated to increase thick to thin filament spacing from 17 to 23 nm. It is postulated that decreased myofilament packing density promotes earlier crossbridge detachment and faster velocity. Atrophic muscles may be more susceptible to sarcosore reloading damage because force per thin filament is estimated to increase by 23%. (Supported by NASA: NAS9-18768 and NAG2-956 and NIH: U01NS33472).


Lockheed Martin, Ames Research Center, Moffett Field, CA.

Rat dams and neonates were flown on STS-90 (Neurolab) for a 16.5 day mission. Neonates were loaded into the Research Animal Holding Facility at postnatal day 7 (PN7, P7 Group) or into the Animal Enclosure Module at PN13 (P13 Group). Preflight procedures were developed to select healthy and comparable animals for flight and control groups. Procedures for the P7 Group included daily food and water consumption data, observations to establish the precise time of birth, cross-fostering at PN2 to meet Principal Investigators' requirements of weight and gender, and Litter Selection based on food, water, and litter weight gain data. Procedures for the P13 Group included daily food and water data collection, Litter Selection and cagemold. Vivarium housed control groups and simulated (sim-) flight habitat control groups were treated identically to the flight group with a four and eight day delay, respectively. For the P7 Group, neonate weights were identical across the flight, vivarium and sim-caged groups at following PN2 operations and at cagemold. In flight, significant mortality occurred among the neonates and, at cage unload, the surviving flight animals were approximately half the weight of the vivarium and sim-caged neonates. A portion of the P7 animals was maintained for 30 days after cage-unload. At 30 days post-landing, the flight animals were not significantly different in weight from the ground control animals.

For the P13 Group, neonate weights at cagemold were not significantly different between the flight and sim-caged group; the vivarium-housed animals were slightly, but significantly, smaller than the other groups. At cage unload, the flight and sim-caged neonates were not significantly different from each other, while the vivarium-housed animals were slightly, but significantly, heavier than the other two groups. These data demonstrate the efficacy of the preflight data collection procedures to select healthy and comparable animals for flight and ground control groups. In the P7 Group, despite neonate mortality on orbit, surviving animals gained weight rapidly and matched their ground control cohorts within 30 days. For the P13 Group, all flight and ground control animals were healthy upon cage unload. (Supported by NASA ARC Life Sciences Division)

The objective of this study was to determine the efficiency of isometric exercise in attenuating the loss of muscle mass and function after 2 weeks of hindlimb unloading (HU). Rats were isometrically trained for 13 minutes, 3 times/day during HU. After 2 weeks of HU, the soleus (S) and gastrocnemius (G) muscles were removed, weighed, and bundles of fibers were prepared. Single fibers from the S and red gastrocnemius (RG) were isolated to the day of the experiment and placed between a motor arm and force transducer and fiber diameter, peak absolute force, peak force per cross-sectional area (Pc), and maximal unloaded shortening velocity (Vmax) were determined. After 2 weeks of HU, the wet mass to total muscle mass ratio of the S and G were 43% and 29% less than weight bearing (WB) rats, respectively. The diameter and absolute force of the S fibers were 33% and 67% less than WB, respectively. Vmax was elevated 30% relative to WB rats while Pmax was unaffected by 2 weeks of HU. The diameter and absolute force of the RG fibers were 36% and 45% less than WB rats, respectively. However, F0 was elevated 32%. F0 was unaffected by 2 weeks of HU.

Isometric exercise during 2 weeks of HU attenuated 59% of the decrease in muscle weight to body weight ratio of the S. 75% of the reduction in fiber diameter, 48% of the decrease in absolute force, and 64% of the increase in Vmax was unaffected by isometric training. Isometric training attenuated 74% of the decrease in muscle weight to body weight ratio of the G, and 84% and 96% of the decrease in the RG fiber diameter and absolute force, respectively. Pmax was elevated by 12% while Vmax in the RG fibers was unaffected by isometric training.

In conclusion, isometric training is effective in attenuating disuse atrophy of both the S and G muscles. However, attenuation of atrophy was greatest in the G muscle, which is affected to a lesser degree by disuse. (Supported by NASA: SBR9704 and NAGW-4376 and R. H. Fitts.)


The ability of IGF-1 to ameliorate spaceflight induced inhibition of bone formation was examined. Twelve maturing male Sprague-Dawley rats (40.0 d.o.) were flown for 10 days aboard STS-77. Sixteen vivarium housed rats served as ground controls. Four days prior to flight (L-4), 2mL osmotic pumps were surgically implanted in the rats with half delivering 1.4 mg/day rhIGF-1, while the other half saline. Tetracycline (50mg/kg i.m.) was injected at L-1. After sacrifice the right humerus (mid-diaphysis) and tibia (TFT) were prepared for histology. New bone formation relative to cortical bone area (BFr/AcT.Ar) was quantified.

The spaceflight rats gained 64% and 84% more weight during the 10 day experiment than the vivarium ground controls for IGF-1 and saline treated animals, respectively. This increased mass gain likely counteracted any spaceflight induced inhibition of bone formation.

When BFr/AC T.Ar for the IGF-1 treated rats is divided by BFr/AC T.Ar for the saline treated animals, the effect of IGF-1 is similar for spaceflight and ground control rats. IGF-1's relative effect for spaceflight and ground control animals, respectively is: tibia endosteme (38%, 44%); periosteum (28%, 14%), humerus endosteme (38%, 39%); periosteum (38%, 25%). These consistent data suggest that the efficacy of IGF-1 is not altered by spaceflight unloading. However, IGF-1 efficacy is altered by tail suspension unloading (Bikle et al. 1994).

The inhibition of bone formation at the endocortical surface of the humerus was an unexpected result. A similar inhibition of formation of trabecular bone at the proximal tibia has been previously reported (Tobias et al. 1992). Tobias suggests that the inhibition of formation may be a mechanical result of a greater bone diameter (greater moment of inertia, I, from increased periosteal formation). This spaceflight experiment refutes this hypothesis because the inhibition occurred for both spaceflight and ground control rats. (Supported by Chiron and NASA grants NAGW-1197, NAGW-2328 & NGT2-52239)

[129] PROGRAMMED ADMINISTRATION OF PARATHYROID HORMONE INCREASES BONE FORMATION IN HINDLIMB UNLOADED OVARIECTOMIZED RATS. R. T. Turner1, G. L. Evans1, J. M. Carolina1, B. Halloran2, E. Morey-Holton1. 1Dept. of Orthopedics and Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN; 2VA Medical Center, San Francisco, CA; and NASA Ames Research Center, Moffett Field, CA.

The beneficial effects of parathyroid hormone (PTH) treatment to reverse osteopenia are well established but technical difficulties associated with administering the hormone under weightless conditions have prevented evaluation of this endocrine therapy as an intervention to prevent bone loss during prolonged spaceflight. We developed a method for the programmed administration of parathyroid hormone to rats that can be applied to spaceflight experiments. We validated this method in hindlimb unloaded ovariectomized (OVX'd) rats.

PTH was administered to weight bearing and hindlimb unloaded OVX'd rats with osmotic pumps programmed to deliver 20 μg of human PTH (60 μg/kg/day) during a daily one hour infusion for 7 days. Hindlimb unloading of OVX'd rats decreased longitudinal growth, decreased osteoblast number, increased osteoclast number, and resulted in a further decrease in cancellous bone volume compared to weight bearing OVX'd rats. These changes were nearly identical to those observed in OVX'd rats following spaceflight (Endocrinology 138:1567, 1997). PTH treatment had dramatic effects on selected cancellous bone measurements; the hormone maintained cancellous bone volume in OVX'd weight bearing rats and greatly reduced cancellous bone loss in OVX'd hindlimb unloaded rats. Importantly, PTH treatment increased retention of a baseline fluorochrome label, osteoblast number and bone formation in the proximal tibial metaphysis regardless of the level of mechanical usage. These findings demonstrate that programmed administration of PTH, a method which could be applied to animal models during spaceflight, is effective in increasing osteoblast number and bone formation and has beneficial effects on bone volume in the absence of weight bearing and gonadal hormones.

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[130] MODULATION OF ACTIN REORGANIZATION AND GENE EXPRESSION IN OSTEOSTEALS BY FLUID FLOW IS REGULATED BY INTRACELLULAR CALCIUM. R. L. Duncan3, N. X. Chen1, C. H. Turner1, D. B. Burr1, and F. M. Pavalko2. 1Dept. of Orthopaedic Surgery, 2Anatomy, Physiology and Biophysics, Indiana University Medical Center, Indianapolis, IN.

It has been previously demonstrated that osteoblasts respond to fluid flow, but not physiological levels of strain, with an increase in expression of the early response gene, c-fos, and cyclooxygenase-2 (COX-2). Both of these proteins have been associated with the skeletal response to mechanical loading, in vivo. Two early cellular responses to flow which postulate mediate this fluid-induced expression of c-fos and COX-2 are actin cytoskeletal reorganization and an increase in intracellular calcium, ([Ca^2+]). When osteoblast-like MC3T3-E1 cells, grown on fibronectin coated glass slides, were subjected to 12 dynes/cm² flow, c-fos and COX-2 were maximally expressed at 0.5 and 3 hr, respectively. Accompanying this increase in expression was a dramatic increase in actin stress fiber formation. Disruption of stress fiber formation with either cytochalasin D or a fragment of α-actinin which prevents actin binding to focal adhesions completely abolished the increase in expression of c-fos and COX-2.

We next examined the role of [Ca^2+], on these responses to flow. MC3T3-E1 cells, pretreated with the intracellular Ca²⁺ chelator, BAPTA-AM, prior to application of fluid, failed to form actin stress fibers or express c-fos or COX-2. However, block of Ca²⁺ entry with the mechanosensitive channel blocker, Gd³⁺, or the L-type Ca²⁺ channel inhibitor, nifedipine, had no effect on these responses. When intracellular Ca²⁺ stores were depleted with thapsigargin, or IP₃, release inhibited with neomycin, stress fiber formation and gene expression were abrogated. These data indicate that [Ca^2+], is essential for mediating flow-induced responses in osteoblasts. However, it appears [Ca^2+], levels are mediated, not through Ca²⁺ entry, but via the phospholipase C pathway. (Supported by NASA: NAGS-4971.)
SESSION K: CONCURRENT POSTERS IV
Animal Development and Growth II

Two experiments were conducted in which pre-incubated Japanese quail embryos were exposed to ground based activities prior to launch as well as launch dynamics. In Experiment 1, shuttle launch centrifugation profile (g force), vibration, or a combination of both had no effect on the viability of embryos allowed to develop up to days 6 and 16 of incubation when compared to controls. Experiment 2 simulated preflight and ground control activities anticipated for a later flight mission (NASA 2/MIR21). These activities included exposing pre-incubated quail embryos to transport, shuttle launch profile g forces and vibrations followed by storage at 12 to 19°C in the CRIM (Commercial Refrigerator Incubator Module). Embryos allowed to incubate for 0, 3, 7, 10, 14, and 16 d were unaffected by the pre-flight protocol and launch dynamics with development similar to the controls. The embryo's ability to utilize calcium from the eggshell was not impaired by pre-flight conditions and launch dynamics in either experiment. It is concluded that quail embryo survival and shell calcium utilization were not affected by conditions that occurred on the ground in preparation for a space flight, including launch dynamics, transport, and storage.

(Supported by NASA (ARC): NAG 2-1001.)


As part of an ongoing study on early postnatal development of the mouse utriculus, we have been re-examining the question of early synaptic innervation. We chose to examine the developmental stages of PD0 (day of birth), PD4, PD7, PD10, and PD28, in order to correlate these data with our morphological studies on the transition from supporting cells to hair cells, and with subsequent type I and type II hair cell development (Ritsch et al. 1998). We are also interested in these early stages as a means to establish baseline data for future spaceflights involving developing mammals.

Multiple samples were taken from each of the 5 postnatal stages. Dissector counts of synaptic ribbons were made as described previously in a study of the chinchilla crista ampullaris (Lysakowski & Goldberg, 1997). Results revealed that synaptic innervation in both types of hair cells proceeds at an orderly rate. Our results differ from a previous study of synaptic innervation in cats in which the numbers of synaptic ribbons in type I hair cells decreased 93% from birth to adulthood (Favre & Sans, 1979).

The average number of ribbons per hair cell is initially high, 41.7 ribbons per hair cell at PD0, decreases during the perinatal period to 10.3 (PD4) and 7.8 (PD7), and then increases with each postnatal day to 13.2 (PD10) to 22.4 (PD28). Immature hair cells had many fewer synaptic ribbons than mature hair cells at each developmental stage. There was also some tendency for type II hair cells to have more synaptic ribbons than type I hair cells, especially later in development. Comparing our results to the previous study by Favre and Sans, the differences may perhaps be explained either by a species difference, or by our use of serial sections, or by our use of the dissector method compared to their use of a ratio method of counting.

(Supported by NASA NAG5-4593 and NIH RO1 DC2290.)


Embryonic neural crest cell migration enables the development of a diverse array of cell and tissue types, including craniofacial cartilage and bone, odontoblasts, ganglia, and endocrine and connective tissues. Although the effect of microgravity on motile behavior of some cell types has indicated that specific alterations occur, the effect on neural crest cells has not been investigated. The object of this investigation was to examine motility responses of neural crest cells to simulated microgravity using a High Aspect Ratio Vessel (HARV) bioreactor. Neural folds were dissected from stage 8 chick embryos and neural crest cells were allowed to attach to disks and emigrate. The disks with attached cells were rotated in a HARV bioreactor for 3 hours. Computer assisted video image analysis was used to examine parameters of neural crest cell motility before and after rotation using sequential images. From the analysis it was determined that migration rate of cells after exposure to microgravity was significantly reduced in comparison to the rate measured before rotation. Correlated with this was a significant decrease in dynamic cellular motile activity, indicated by diminished rates of dynamic change in area and perimeter. These findings support the notion that a simulated microgravity environment affects the migratory behavior of neural crest cells.

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SESSION K: CONCURRENT POSTERS IV
Animal Gravity Sensing II
[134] ELECTRICAL RESONANCE IN THE HORNET CUTICLE. W. Thornton and J. Ishay. 1 Dept. Physiol. and Pharmacol., Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv 69978, Israel, and 2 Dept. of Internal Medicine, Division of Cardiology, University of Texas, Medical Branch, Galveston, Texas, 77555-0553, USA.

We have found extensive evidence of various electrical phenomena in the cuticle of the Vespa orientalis including voltage, current, resistance and surprisingly large capacitance. As part of these and other investigations we also found electrical resonance in a preliminary study. The complex electrical impedance of 6 abdominal regions was then measured repetitively using a matrix of 4 colloidal silver electrodes in 6 young (one day old) and 7 adult (several days old) killed and cold preserved hornets. Electrode diameter was 2-3 mm and measured temperature 23-24°C. A known sinusoidal current ranging from 30-1500 Hz was applied to the electrodes from a high impedance source, and phase and magnitude of the resulting voltage compared to the applied current by oscilloscopic means. Characteristic features of resonance were found in all samples and electrode locations. Typical results from one abdominal area with a total of 4 each determinations in 13 preparations was: mean resonant frequency (Fo), 601 ±165 vs 562±84 Hz and mean band width (6dB) 31.1 ±10 vs. 16.1±3.9% Fo in young vs adults. Such behavior is consistent with electrochemical resonance in a piezoelectric system. Piezoelectricity has been reported in other insects. It is also consistent with the cuticular microstructure we find in this species. Such piezoelectric structure might also explain the large electrical capacitance found in these rather small insects. While this frequency is in the same region as synchronized group motor activity (vibration) common to this social insect, it has not yet been demonstrated whether such a resonant mechanism in the cuticle system plays sensory or other role or this is an intriguing epi-phenomenon. The role of the resonance phenomenon and gravity sensing by hornets is discussed.

[135] SHORT LATENCY VESTIBULAR EVOKED POTENTIALS (vEPs) IN TWO MAMMALIAN SPECIES. T.A. Jones, S.M. Jones, and M. Bothwell. Dept. of Surgery/Otolaryngology, School of Medicine, University of Missouri, Columbia.

The role of gravity in the ontogeny of mammalian gravity receptors remains a compelling issue to resolve. In birds there is physiological, morphological and behavioral evidence that embryonic exposure to microgravity can lead to abnormalities in peripheral vestibular end organs (Ferrin et al., 1996; Guryeva et al., 1993; Jones et al., 1993; Kostal et al., 1993). A number of anatomical studies have suggested that the microgravity environment may also alter the pattern of development in the mammalian vestibular periphery (Ross, 1993). However, no information regarding the physiological status of the vestibular periphery is available from these studies. One reason for the lack of information may be the difficulty associated with making direct, objective assessments of vestibular peripheral function in mammals. In the present study, we describe noninvasive recordings of vEPs in the mouse and rat. VEPs are compound action potentials of the vestibular nerve (and central relays) elicited by pulsed linear cranial translation. In both species, response onset occurred within 1.5 ms of the stimulus onset. Responses were unaffected by intense (122dBSPL) wide-band (20 to 50,000Hz) white noise forward masking, whereas auditory responses to intense clicks (112dBsPL) were eliminated under the same conditions. The masking paradigm precluded any significant participation of auditory neurons in the response to linear translation and masking was used during all vEP recordings. Responses were similar in both species. They included a series of positive and negative peaks that occurred within 8 ms and disappeared upon bilateral labyrinthectomy. As we have shown in the bird, the present results indicate that vEPs can be used in mammals to assess the functional status of vestibular neurons. We currently employ vEPs in studies of mammalian vestibular ontogeny and, in the future, mammalian vEPs will be used to evaluate the effects of altered gravitational environments. (Supported by NASA NAG5-4607).


Two behavioral paradigms are being used to identify gravitaxic mutants of the fruit fly Drosophila melanogaster. One assay, termed the climb test, involves gently hanging flies to the bottom of a vial. Wild type flies show a negative gravitaxis response and climb rapidly up the wall of the vial. The second assay involves use of a gravitaxic maze which flies can only traverse by making eight consecutive up/down movement choices. The behavior of wild type flies in these mazes has been studied and defined so that flies with aberrant responses can be identified and selected for further study.

Mutagenic screening of more than 30,000 flies has been performed to date, using several different screening modes. Currently a collection of 6000 viable mutant lines generated by chemical mutagenesis is being systematically analyzed. Several lines with altered gravitaxial responses have been identified so far and are under further study. Details of the screening protocols and aberrations identified will be presented.

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SESSION K: CONCURRENT POSTERS IV
Animal Structural Systems III
[137] EFFECTS OF LOCAL INSULIN-LIKE GROWTH FACTOR-I OVER-EXPRESSION AND PERIODIC WALKING ON SKELETAL MUSCLE MASS DURING HINDLIMB UNLOADING. S.E. Gordon1, R.J. Schwartz2, M.L. Fiorotto1, and F.W. Booth1. 1University of Texas Medical School at Houston, 2Baylor College of Medicine, and 3USDA/ARS Children's Nutrition Research Center, Houston, TX. Although treadmill walking and an exogenously enhanced IGF-I level are proposed as potential countermeasures to skeletal muscle atrophy in astronauts during long-term spaceflight, the combined effect of these two countermeasures on skeletal muscle homeostasis during unloading has yet to be investigated. The purpose of this investigation was to examine combined effects of skeletal muscle IGF-I over-expression and periodic treadmill walking on gastrocnemius muscle mass during hindlimb unloading in mice. Adult male and female 52-wk old mice from a transgenic line (TG; over-expressing IGF-I in muscle) or from the corresponding wild-type (WT) FVB parent strain were used. Transgenic mice harbored the human IGF-I gene driven by the regulatory regions from the skeletal ut-Aactus gene. IGF-I mRNA and protein are highly over-expressed in the gastrocnemius but not the soleus muscles of these animals. Experimental groups were: weight-bearing WT (n = 8); tail-suspended WT (n = 6); weight-bearing TG (n = 9); tail-suspended TG (n = 8); tail-suspended TG with periodic walking (n = 5). The tail-suspension protocol lasted 2 weeks and periodic walking consisted of exercising on a motor-driven treadmill twice daily for 30 min (60 min/day) at 5.7 m/min with a 0% grade. There was no effect of local IGF-I expression alone on the extent of gastrocnemius muscle atrophy with unloading. Furthermore, the combination of local IGF-I over-expression and the periodic walking protocol only attenuated unloading-induced gastrocnemius muscle atrophy by 12%. Conversely, walking attenuated unloading-induced atrophy by 38% in the soleus muscle, which did not express the IGF-I transgene. These results indicate that exogenously enhanced local IGF-I level alone may not attenuate unloading-induced gastrocnemius atrophy in mice. Moreover, fiber type-dependent characteristics such as the extent to which a muscle is activated during treadmill walking (soleus > gastrocnemius) may be more important for atrophy protection when the IGF-I and walking countermeasures are not optimized in the same muscle simultaneously. (Supported by the National Space Biomedical Research Institute.)


Clenbuterol is a specific β2 agonist used in Europe as a bronchodilator to treat asthma and other respiratory conditions. The normal therapeutic dose for clenbuterol in healthy adult male subjects is 20 μG twice daily. Additionally, clenbuterol has marked anabolic effects such as increasing muscle mass and protein content and decreasing fat deposition, leading to abuse by athletes to enhance performance. Furthermore, clenbuterol as well as other β-adrenergic agonists have been investigated as potential countermeasures to microgravity-induced skeletal muscle atrophy. A simple and sensitive procedure utilizing gas chromatography/mass spectrometry (GC/MS) for the identification and quantitation of clenbuterol in plasma and urine has been developed. This improved method utilizes trimethylboroxine for the derivatization of clenbuterol thereby yielding abundant diagnostic ions with high m/z values. The GC-MS method was compared to an EIA method. The applicability of both methods for the detection and quantitation of clenbuterol in biological tissues such as kidney, liver, and skeletal muscle samples, was demonstrated successfully. Method validation and standardization data will be discussed. Approximately 24 hrs post injection of (1 mg/kg), clenbuterol concentrations in rat kidney and liver samples were determined to be 65.1 and 60.4 ng/g, respectively. However, in plasma, significantly lower levels of clenbuterol were detected with concentrations ranging between 300 to 900 pg/ml. Additionally, clenbuterol concentrations in gastrocnemius skeletal muscle samples were approximately half the level observed in kidney and liver (33 ng/g), whereas brain concentration of clenbuterol was similar to that of kidney and liver (60.10 ng/g). Lower doses of clenbuterol showed its effect on muscle mass was significantly higher in predominately fast muscles (plantaris, ext. dig. longus), intermediate in the gastrocnemius, and less in predominately slow muscles (soleus, adductor longus). These methods should be especially useful in explaining the mechanisms underlying the tissue-specific effects of this drug. (Supported by NASA 1N4A4A38 & NAG9-971; NIH GM08248 & RR03034; and BIO-TEK Instruments, Winooski, VT.)


Bone formation is reduced in rat hindlimbs unloaded by tail suspension (Wronski and Morey-Holton, 1987). To determine the role of nutrition on bone formation, we studied the bone morphology of the tibia and humerus after tetracycline labeling in 3 groups of 200 g male rats housed in identical cages. Group 1 was ambulatory (C). Group 2 had unloaded hindlimbs (S). C and S were fed semipurified diets (AIN76A) with 0.5% calcium (Ca) and 0.6% phosphorous (P) (Dyets, Inc.). Group 3, a body weight (BW) control for S, was ambulatory and fed the same diet with fewer calories per g (D) than C or S. Dietary mineral in D was maintained by the addition of Ca and P. After 2 and 4 weeks, BW, bone formation (BFR) and mineral apposition rates (MAP) of the tibias and humeri were measured. BW, g in S were 246 ± 13 and in D 253 ± 7 after 2 wks and 287 ± 10 in S and 288 ± 9 in D after 4 wks, less than in C (232 ± 19 at 4 wks, p < .05). After 2 wks tibia BFR were reduced in S, but not in D (.029 ± .007 vs .043 ± .005, p < .05); after 4 wks, BFR were further reduced in S and also in D (.020 ± .003 vs .028 ± .005). Humerus BFR was the same in all groups except at 4 wks when D was less than S (.020 ± .005 vs .028 ± .006, p < .05). Changes in MAP were similar to those in BFR. We conclude that 1) bone is more sensitive to unloading than to nutrition and 2) bone effects from nutrition are generalized and not localized to weightbearing bones. (Funds NASA Space Act Agreement with ESA, Inc. RS01778 and NASA NCC2-389)
SESSION K: CONCURRENT POSTERS IV
Biotechnology/Instrumentation III
DIFFERENTIATION OF HUMAN SALIVARY EPITHELIAL CELLS IN SIMULATED MICROGRAVITY. S. Das¹, C.W. Patrick, Jr.², M. Miller³, T. Thompson¹, and E.M. Durban¹. ¹Oral Pathology, Univ. of Texas-Houston, Dental Branch, and Plastic Surgery, U. T. M. D. Anderson Cancer Center.

Normal salivaion is essential to oral and digestive health. Salivary tissue loss can have devastating consequences that reduce the quality of life of the individual. Restoration of salivary function is desirable, but consistent clinical modalities for this purpose are not available. As a first attempt to engineer salivary tissue suitable for homologous transplantation, we assessed whether simulated microgravity supports salivary cell differentiation. We used as a model a human salivary cell line (HSG) with stem cell properties inducible to acinar, myoepithelial, or keratinizing cells with 5-azacytidine, dibutyryl c-AMP, and retinoic acid respectively. HSG cells were cultured in a NASA-designed Rotating Wall Vessel (RWV) both with either collagen-coated Cytodex beads or biodegradable microspheres of poly(DL-lactic-co-glycolic acid), and in the absence of a matrix. Stationary 1g controls were likewise maintained. In some experiments, acinar cell differentiation was specifically induced with 5-azacytidine. HSG cells generated large 3-D organoids within 1 week of culture in the presence or absence of a matrix. Samples were analyzed for the production of amylase, an acinar cell product, and epidermal growth factor (EGF), a ductal cell product produced constitutively by HSG cells. Amylase was produced by all cells grown in the RWV only. Addition of 5-azacytidine to RWV cultures did not enhance amylase levels significantly. EGF was produced only by cells maintained at 1g. These results indicate that simulated microgravity stimulates the differentiation of human salivary cells towards the acinar cell phenotype, thus providing a model system for both engineering human salivary tissue and studying the regulation of salivary stem cell behavior. (Supported by the NIDR: RO3DE11185 to E.M.D.).

DISTANT OPERATIONAL CARE CENTER (DOCC) - A MODULAR MEDICAL FACILITY TO MAINTAIN HUMAN HEALTH AND PERFORMANCE IN SPACE. D. Hurwitz¹,² E.B. Schenker¹,² and K.E. Forkheim¹,³. ¹International Space University (ISU96), Vienna, Austria. ²Israel Aerospace Medicine Institute, Jerusalem ISRAEL. ³University of Manitoba, Faculty of Medicine, Manitoba, Canada.

Introduction: Commencing with the launch of Gagarin and the first small steps of Armstrong, to regular flights of the Space Shuttle and the marathon stays on Mir, human activity in space is increasing. The consequences for space medicine continue to multiply as new medical technologies and innovations are required to support a high quality of human performance for each unique mission and remote habitat.

Methods: The goal of this International Space University (ISU) project was to outline the design of the Distant Operational Care Center (DOCC), a modular medical facility to maintain human health and performance in space environments through prevention, diagnosis, and treatment of medical problems. The DOCC is adaptable to meet the requirements of different remote human habitats while maintaining a high quality of human performance.

Results: A survey of medical problems likely to occur in space was utilized to derive a list of medically oriented hardware, supplies, and expertise needed for the DOCC. Several technological advances in the medical field, including telemedicine, virtual reality, and artificial intelligence, make possible improved crew diagnosis and treatment in space. The DOCC also includes a ground segment which provides the necessary infrastructure to maintain, upgrade, and provide medical expertise to the DOCC and encompasses issues pertaining to policy, law, business, and management.

Conclusions: This paper assesses the cost and risks associated with the DOCC, identifies potential terrestrial applications, and identifies potential investors and customers. The ISU-DOCC Design Project offers an enormous incentive for future medical care enhancements.

To better understand how cells sense mechanical stress, we have undertaken a genetic analysis of the osmosensing MAP kinase pathway in S. cerevisiae called the HOG pathway. This pathway is composed of two putative membrane osmosensors that sense a loss in turgor pressure and cause the activation of a protein kinase cascade that in turn induces an osmoregulation response. We have identified several genes that when mutated suppress the osmosensitive growth phenotype of a mutant in the HOG pathway MAP kinase kinase Pbs2p. Loss of these genes has no effect on direct downstream responses of the HOG pathway such as osmoregulation. Curiously, these genes encode signaling proteins in another MAPK cascade, the pseudohyphal pathway. In addition, increases in external osmolality induce activation of the pseudohyphal pathway in a pbs2 mutant but not in wild type. Inappropriate activation of the pseudohyphal pathway under osmotic stress thus appears to explain part of the osmosensitivity of the pbs2 mutant. Unexpectedly, loss of the HOG pathway sensor Sho1p (but not the other osmosensor) suppresses the osmosensitivity of the pbs2 mutant, suggesting that activation of the pseudohyphal pathway by osmotic stress is mediated by the osmosensor Sho1p. Together, these results suggest that downstream signaling components in a pathway may function to maintain the specificity of signaling from upstream membrane mechanosensors. Maintenance of signaling specificity could be mediated either through down-regulation of other pathways or by formation of specific signaling protein complexes that block inappropriate molecular interactions, possibilities that are currently under investigation.

(Supported by NASA: NAGW-5007 and by the NIH and ACS.)


Skeletal muscle hypertrophy is promoted by in vivo administration of β-adrenergic receptor (BAR) agonists. These compounds presumably exert their physiological action through the BAR, and alterations in the population of BAR could potentially change the ability of the cell to respond to the BAR agonists. Since the intracellular chemical signal generated by the BAR is cyclic AMP (cAMP), experiments were initiated in primary chicken muscle cell cultures to determine if artificial elevation of intracellular cAMP by treatment with forskolin would alter the population of functional BAR expressed on the surface of muscle cells. Chicken skeletal muscle cells after 7 days in culture were employed for the experiments because muscle cells have attained a steady state with respect to muscle protein metabolism at this stage. Cells were treated with 0-10 μM forskolin for a total of three days. At the end of the 1, 2, and 3 day treatment intervals, the concentration of cAMP and the BAR population were measured. Receptor population was measured in intact muscle cell cultures as the difference between total binding of [3H]cAMP-12177 and non-specific binding of [3H]cAMP-12177 in the presence of 1 μM propranolol. Intracellular cAMP concentration was measured by radioimmunoassay. The concentration of cAMP in forskolin-treated cells increased up to 10-fold in a dose dependent manner. Increasing concentrations of forskolin also led to an increase in BAR population, with a maximum increase of approximately 50% at 10 μM. This increase in BAR population was apparent after only 1 day of treatment, and the pattern of increase was maintained for all 3 days of the treatment period. Thus, increasing the intracellular concentration of cAMP leads to up-regulation of BAR population. The effect of forskolin on the quantity and apparent synthesis rate of the heavy chain of myosin (mhc) were also investigated. A maximum increase of 50% in the quantity of mhc was observed at 0.2 μM forskolin, but higher concentrations of forskolin reduced the quantity of mhc back to control levels.


The blood-brain barrier (BBB) protects the brain from transient changes in blood composition and maintains homeostasis in the brain parenchyma. The permeability of the BBB is affected by developmental, physiological and pathological processes. Therefore, it is important to understand how it is induced, maintained and potentially modulated. Several two-dimensional in vitro models of the BBB exist, however, there are few models in which a three-dimensional environment is allowed. We used the normal human astrocytes (NHA) and bovine aortic endothelial cells (BAEC) were cultured, alone or together as co-cultures, for 7 days attached to Cytodex-3 microcarrier beads in the microgravity-based High Aspect Ratio Vessel (HARV) bioreactor. The HARV enhances 3-dimensional spatial freedom. After 48 hr, multi-bead aggregates are seen. Scanning electron micrographs showed NHA and BAEC organized into3-dimensional tissue-like aggregates, with bead-to-bead bridging of cells. Transmission electron micrographs revealed 2 distinct cell types with several cell-cell junction between them: these may be tight or adherence junctions. Immunocytochemical analysis confirmed the cellular heterogeneity, as demonstrated by expression of glial fibrillary acidic protein and von Willebrand’s factor. Glucose utilization decreased from 146 ± 33 to 91 ± 13 (mg/dl) over the initial 72 hr of simulated microgravity, and remained constant thereafter. These results suggest that the co-cultures were maintained in a metabolically active state. Thus, the microgravity-based rotating bioreactor appears to allow NHA and BAEC to be co-cultured resulting in a novel 3-dimensional model of a BBB. (Supported by grants from NASA: NCCW-0083 and NIH/RCMI 3G12 RR03034)

[145] EFFECT OF HYPERGRAVITY ON OSTEOBLAST C-FOS GENE EXPRESSION. Y. Fujita, and A. Sato. Space Utilization Research Programme, NASA, JAPAN.

Epidermal growth factor(EGF)-activated signal transduction system is influenced by gravity. EGF-induced c-fos gene expression is suppressed in microgravity, while hypergravity has a stimulatory effect on EGF-induced c-fos gene expression in human A431 epidermoid carcinoma cells. To investigate the effect on EGF-activated signal transduction system of hypergravity in bone cells, we examined the expression of c-fos gene in serum-deprived mouse MC3T3-E1 osteoblastic cells centrifuged at altered hypergravity, using RT-PCR with Sequence Detector. Results confirm that the EGF-induced c-fos gene expression is increased gravity-dependently at altered hypergravity, while little change of c-fos gene expression was observed in EGF-untreated serum-deprived cells. This results may indicate that hypergravity stimulates synergistically the EGF-activated signal transduction system of osteoblast.
SESSION K: CONCURRENT POSTERS IV
Plant Biology III
[146] EFFECT OF HYPERGRAVITY AND SIMULATED HYPOGRAVITY ON LEAF-PLANTLET DEVELOPMENT AND ASEXUAL REPRODUCTION OF *KALANCHOE DAIGREMONTIANA*. M.C. Pedrosa and D. Durzan. Dept Plant Biology, Univ of Lisbon, Portugal and Dept Environmental Horticulture, Univ California, Davis.

The effect of gravitational stimuli (hyper- and hypo-gravity) on plant development and asexual reproduction was studied using *Kalanchoe daigremontiana* as experimental model system. This species is a Crassulaceae that reproduces asexually by forming leaf-plantlets in leaf indentations, with a high sensitivity to environmental stimuli. *In vitro* plantlet cultures were used as source of explants. Leaf-plantlets and leaves were exposed to multiples (20 xg to ≤ 600 xg) and submultiples (≤ 2 x10^4 xg) of Earth’s gravity, for different periods of time (5 min to 15 days). Hypergravity for 15 days did significantly affect leaf-plantlet production from mature leaves. A tendency for asexual reproduction from leaf-derived-plantlets to increase with hypergravity was recorded. Plantlet production at 1 xg, from leaf-derived-plantlets exposed to hypergravity (2nd generation of leaf-plantlets), was lower for plantlets developed above 20 xg. Hypergravity treatments of 5 min to several hours were effective in increasing the number of plantlets formed per each leaf and the rate of asexual reproduction. For cultured leaf-plantlets, asexual reproduction also increased with the increase of hypergravity. Leaf culture under simulated hypogravity decreased leaf-plantlet production per leaf and asexual reproduction. Plantlet production from plantlets exposed to simulated hypogravity was lower than for controls. Negative effects of hypogravity were more pronounced under photoperiod than in darkness. Compared to unit gravity, developmental aberrations were found in hypogravity and related to programmed cell death by the TUNEL assay for individual cells.

(Supported by PRAXIS XXI 3/3.1/CTAE/1930/95 and INVOTAN 3/B/96/P0)


Super-Dwarf wheat, Triticum aestivum L., plants grown onboard the Russian Space Station, MIR, were 100 % sterile. The cause of sterility was not readily apparent, but atmospheric samples taken from the MIR Orbital Station indicated ethylene concentrations of 1-2 ppm. At NASA/ARC and Logan, Utah, Super-Dwarf wheat was exposed to 0, 1, 2, 3, 10 or 20 ppm of ethylene gas. Scanning electron microscopic examination showed that the pistils, stamens and lociculae ceased floral development at the same stage of ontogeny as those grown onboard the Russian Space Station, MIR. Laser scanning confocal microscopic examination of nuclei in the ethylene-treated pollen grains exhibited zero, one, two but rarely three nuclei, responses mimicking those observed from the MIR-grown wheat plants. These data suggest that the level of ethylene found onboard the MIR Orbital Station was sufficient to inhibit seed set.

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


Selection of viable, high quality seed is critical to the success of food production on Martian or Lunar bases. Selection of high quality seed for space flight experiments improves experiment uniformity and cost efficiency. In the present work on cabbage (*Brassica oleracea*) seed quality was determined based on the ratio of Anaerobic to Aerobic (ANA Ratio test) ethanol production. A high ANA ratio indicates high seed quality. To elucidate biochemical mechanisms at work, the influence of ambient oxygen was examined by performing seed moisture content studies in an oxygen-purged nitrogen gas glove-box. The changes in ethanol production after grinding seed were examined. A hypothesis was formulated that seed-integrity-related changes in ethanol production may be due to changes in the combined-functionality of at least two components of metabolism: alcohol dehydrogenase enzyme (ADH) activity and electron transport of oxidative phosphorylation. A shift in electron transport, mitochondrial membrane integrity, or ADH activity could explain seed-quality-related changes in total ethanol production in the ANA test conditions. Tetrazolium staining tests were performed to assess non-specific dehydrogenase activity in four seed conditions (Control Intact, Control Ground, Aged Intact, Aged Ground). Both oxygen and moisture were contributing factors in ethanol production. The optimal moisture content for ethanol production in the aerobic treatments was 0.3 g H2O/g seed dry weight. Water levels above 0.8 g H2O/g seed dry weight increased ethanol production, while oxygen inhibited ethanol production in treatments with less than 1.8 g H2O/g seed dry weight. Grding decreased ethanol production in aged seed but decreased ethanol production in control seed. Tetrazolium staining tests on ground seed at high moisture content were positively correlated with ethanol production. (Supported by NASA GSRP #NGT10-525607.)


Although many studies have been conducted on plant regeneration from seeds, seedlings and tissue culture in systems adaptable to microgravity, none have addressed the question of plant regeneration from stem cuttings for such an environment. Sweetpotato can be easily regenerated through stem cuttings. This research compares growth of such cuttings under clinorotation conditions of altered gravity with growth under 1-g. Sweetpotato stem cuttings (15 cm with approximately six nodes) of breeding line Tu-I2-155 were placed in a plant growth unit in both horizontal and vertical (control) orientation on clinostats. Cuttings were sampled at 7 and 14 days, photographed (stills and digital cameras) and fixed using a modified Karnovsky's fixative. Measurements were made on roots and leaf buds. Sections were cut using a DuPont Sorvall MT2B ultramicrotome, stained with uranyl acetate and lead citrate, and viewed at 60KV using a Phillips 201 transmission electron microscope. The study elucidated the rate and nature of changes in root and leaf bud initiation/emergence and amyloplast development and, in preparation for flight, provides a database of this information.

(Supported by NASA: NAG10 0209 and USDA/CSREES ALX-PS-1)
SESSION K: CONCURRENT POSTERS IV
Collaborative Ukrainian Experiment II

Plants grow in the spaceflight environment, however they sometimes exhibit alterations in growth, biomass partitioning and metabolism. One possible mechanism involves the overproduction of the gaseous plant hormone ethylene. Here we describe studies using soybean seedlings to determine the effect of spaceflight and clinorotation on ethylene production and subsequent effects on growth and biomass partitioning. Soybean (Glycine max cv. McCall) seedlings were germinated and grown in Biological Research In Canister (BRIC) hardware as part of the Collaborative Ukrainian Experiment (STS-87, 11/19-12/5, 1997). Half of the canisters had packets of KMnO4 (Purafill®) to remove ethylene. Gas samples were taken periodically on orbit for subsequent analysis on the ground. A similar configuration was used for 1 g ground control and ground-based clinorotation (1 rpm) studies. On the basis of shoot and root biomass, clinorotation resulted in no difference in CO2 concentration, yet there was a 50% increase in ethylene concentration in the headspace of the canisters relative to the stationary controls. Ethylene was scrubbed out effectively in both the clinorotated and stationary canisters by KMnO4. Root growth was greater in the clinorotated plants with and without the removal of ethylene, indicating that the enhanced root growth was due to clinorotation effects and not ethylene. On the basis of shoot plus root biomass, spaceflight resulted in a nearly five-fold increase in ethylene concentration and a doubling of CO2 concentration in the canisters relative to the ground controls. Overall seedling growth was much lower than in previous spaceflight experiments, however it appeared that biomass partitioning between hypocotyl and root was not influenced by spaceflight. (This work was supported by NASA Grants NAG10-0142 (CSB and JAG) and NAGW-4984 (NSCORT/NC State University) and NASA contract NAS10-11624 (Dynmac)).

[152] INFLUENCE OF MICROGRAVITY ON LOCALIZATION AND RELATIVE CONTENT OF Ca++ IN SOYBEAN SEEDLINGS. O. Nedash1, E. Kordyun1, C. Brown2, W. Piasuch1, I.Ovrunkaya1. 1 Institute of Botany, Kiev, Ukraine, 2 North Carolina State University and Dynmac Corporation, Raleigh, USA, 3 Dynmac Corp., Kennedy Space Center, USA.

The effect of microgravity on the redistribution and relative content of free and weakly bound calcium in different cells of the hypocotyl and cotyledons in soybean seedlings were established in Space Shuttle Mission STS-87. Localization of Ca++ was studied using the electron-cytochemical pyroantimone method in different cells of hypocotyl and cotyledon tissue in 6-day-old seedlings grown in space in the presence or absence of ethylene. Ethylene removal was achieved by using KMnO4 packets in the Biological Research Canisters (BRICs). Ethylene in the BRICs affected growth and development of hypocotyls and cotyledons and was reflected in the changes in cell ultrastructure. The relative content of Ca++ in the hyaloplasm of parenchyma cells of the hypocotyl hook was higher under the influence of ethylene in both ground control and microgravity than that in same cells without ethylene. The mesophyll of cotyledons developed more quickly in seedlings grown in microgravity than in 1 g ground control. Regardless of the presence of ethylene in the BRICs, storage proteins in the vacuoles of the mesophyll cells were completely lysed. Precipitated granules in the vacuoles and into intercellular space in seedlings grown in microgravity decreased in comparison to the ground control. The hypothesis that changes in calcium balance in plant cells can cause the acceleration of seedling growth and development in microgravity will be discussed.


Brassica rapa plants were grown for 17 days under spaceflight conditions. We examined the effect of microgravity on galacto-, sulfo-, and phospholipid composition, as well as fatty acid and pigment content in isolated thylakoid membranes. Chloroplasts were prepared from fresh leaves and fixed by boiling in isopropyl alcohol. Lipids were extracted from isopropanol-fixed material according to a procedure modified from Bligh and Dyer. The content of chlorophyll a and b was determined in lipid extracts by the method of Vernon. Lipids were extracted and separated from the chloroplasts by thin-layer chromatography on silica gel. Lipids were identified on developed chromatograms using of both unspecific methods such as UV fluorescence and procedures using of iodine vapors and specific reagents reactive on certain chemical. RF values of individual spots were compared with those of certain marker lipids and with those cited in literature. The samples for gas-liquid chromatography assay of lipid fatty acid content were prepared by methanalysis in methyl alcohol and sulfuric acid in soldered ampules at 80°C for 1 hour. Methyl esters were extracted with hexane. The content of following higher fatty acids was determined: palmitic acid, hexadecenoic acid, stearic acid, linoleic and linoleic acid. The index of the unsaturation was determined. It was found that chlorophyll a/b ratio, phospholipid composition and index of unsaturation of fatty acids in plants grown in space flight was changed compared with ground control plants.
[154] ULTRASTRUCTURE AND DNA CONTENT OF *BRASSICA RAPA* APICAL ROOT CELLS OF PLANTS GROWN IN MICROGRAVITY. G.I. Martyn, E.L. Kordyum, V.A. Zaslavsky, E. Hilare, and J.A. Guikema. Institute of Botany, Nat. Acad. Sci. of Ukraine, Kiev, Ukraine, and Kansas State University, Manhattan, Kansas, USA.

*Brassica rapa* seeds were germinated during the microgravity conditions of orbital spaceflight aboard STS-87, and plants were harvested at 6 and 13 days after planting. These plants were fixed on orbit, and were returned to earth for examination by transmission electron microscopy. In this report, we focus on the tips of both primary and lateral roots, examining meristematic cells, statocytes, and secretory cells of the root cap, as well as the cells of the meristematic, elongation, and differentiation zones above the root cap. Most of the characteristics of these cells were similar to the relevant ground control tissue, including the size, shape, and position of nuclei and the structure of the chromatin within the nuclei. Similarly, the extent of cell division within the meristematic regions was unchanged, and chromosomal structure observed by electron microscopy was the same. Some differences were observed, however. Space-grown statocytes were characterized by a distribution of amyloplasts throughout the cell, a decreased starch content of the amyloplasts, and a localization of smooth and branched endoplasmic reticulum membranes on the distal side. Space-grown secretory cells were highly vacuolated and showed a lowered golgi content, suggesting a reduction in mucilage production. Space-grown rhizodermal cells also showed an increase in vacuole content, and a decrease in the volume of endoplasmic reticulum membranes. It is possible that microgravity-induced changes in carbohydrate metabolism may impact protein biosynthesis and cause the alterations which we have observed.


A goal in our laboratory has been to perform a detailed comparison of embryo development in microgravity and 1-g. Because previous experiments with Arabidopsis had demonstrated the need for well-ventilated chambers to encourage normal reproductive development on orbit, we were fortunate to have our experiment on embryo development in *Brassica rapa* assigned to the Plant Growth Facility (PGF) hardware on shuttle flight STS-87 in November 1997. Air flow through the PGF's plenum resulted in a steady stream of filtered cabin air through the mesh-covered windows in the ends of the chambers. The PGF was used to grow *Brassica rapa* plants from seed (3 chambers) or to continue growing plants that had been started for 13 days on the ground (3 chambers). Both sets of plants flowered on orbit during the 16 day mission, and the older plants were pollinated on successive days to produce populations of silique of different ages. Beginning on Flight Day 2 of the STS-87 mission, the individual Plant Growth Chambers (PGCs) were removed daily from the PGF by a payload specialist. Flowers that were in anthesis were marked by encircling the pedicel of the flower with narrow tape, and then pollinated using a bee stick that had been loaded with pollen collected from other flowers in the same PGC. Color coding of the tape identified the date of pollination, thus allowing the identification of silique age post-flight. Images downloaded during the flight confirmed the development of silique on orbit, and during post-flight dissections, all of the expected embryo stages up to mature embryos were found in both the spacecraft and ground control material. Pollen viability as assessed by fluorescein diacetate staining immediately post-flight was 93% (ground control, 94%). The results demonstrate that no step in the reproductive process is dependent on gravity. Supported by NASA grant NAG-100139 to MEM.


The influence of spaceflight on the photosynthetic apparatus of 14- and 28-day-old *Brassica rapa* plants was examined by comparing plants grown aboard the Shuttle "Columbia" with those grown under similar conditions on Earth. The 14-day plants were watered and germinated on orbit, whereas the 28-day plants were 12 day old at the time of launch. This experiment was conducted in the Plant Growth Facility (PGF), and utilized three Plant Growth Chambers (PGCs) for each age group. Soon after landing, 0.5 g of the 1st tier of the true leaves were harvested from each PG, and were examined separately to allow comparison by PGC. The 14 day materials showed normal green color, whereas the 28 day materials from flight were yellow.

Fifteen leaves from each PGC were used for chlorophyll fluorescence induction assays. Leaves were used for thylakoid membrane isolation, and these were used to measure photochemical activity (photosystems 2 (PS2) and 1 (PS1), Chl a/b ratios, pigment absorption spectra, and both the excitation and emission spectra spectra of low temperature chlorophyll fluorescence. Measurements were done in duplicate (spectral measurements) or triplicate (photochemical activities). We observed a reduced size of the light harvesting antenna for PS2, no change in the PS2 photochemical activity, and a reduced total photochemical activity for PS1 in the spaceflight samples. The ratio of Qb-non-reducing to Qb-reducing reaction centers of PS2 was unchanged and equal to 20-25% as it is usually observed for non-stressed plant material. Changes in the kinetics of Chl fluorescence induction for flight samples suggest a decrease in the intersystem electron transport rate, and alterations analyses of low temperature Chl fluorescence spectra suggest differences in the organization of pigment-protein complexes.
SESSION K: CONCURRENT POSTERS IV
Spaceflight Experiment Results IV

Twenty-four male, Fischer rats were flown in the Research Animal Holding Facility (RAHF) to support Neurolab Adult Neuronal Plasticity Experiments. The flight animals were exposed to 16 days of microgravity.

To assure the health and well being of the animals, body weight gain, and food and water consumption was monitored. Animal selection for pre-flight surgical implants was based on similar body weight gain and food and water intake. Over the course of the 16 day mission food and water intake data was used to assess the health and well being of the animals. At landing the flight animals had similar body weight gain and food consumption as the ground controls.

Development of these experiments included ground testing to insure that the health and well being of the animals would not be compromised by the habitat. Data from the RAHF Housing Test (a mission length ground test) showed no significant difference of body weight gain, and food and water consumption between the RAHF housed animals and controls. No significant difference of body weight gain, and food and water consumption was observed between the animals with a biotelemetry implant and those without.

(Sponsored by NASA ARC Life Sciences Division)

[158] BACTERIA EXPOSED TO LONG-TERM SPACE FLIGHT CONDITIONS SHOW ALTERED RESPONSES TO ANTIBIOTICS.
M.A. Juergensmeyer1 and E.A. Juergensmeyer1, 2) Kansas State University, Manhattan, KS 2) Judson College, Elgin, IL

As humans explore the rest of our solar system, we will of course take our bacterial symbiotes and pathogens with us. It is therefore necessary to have an understanding of the way bacteria will respond to a non-Earth environment. The most obvious environmental change in a space habitat is the lack of gravity. Previously, bacteria cultured in microgravity have shown an enhanced growth rate, and an increased resistance to antibiotics. The mechanism of resistance has not been identified, as the resistance is quickly lost upon return of the bacteria to Earth. Short-term exposure of Escherichia coli to microgravity showed that while antibiotic resistance increased during flight, transformation rates and efficiency were not affected. To further characterize antibiotic resistance, we have exposed stationary phase cultures of E. coli, Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa to microgravity on the Space Station MIR (STS-79/MIR/STS-81 and STS-86/MIR/STS-89). Upon their return to Earth, each culture was challenged with 12 different antibiotics of varying modes of action, to determine minimal inhibitory concentration and minimal bactericidal concentration. In contrast to previous reports, we find that space-flown bacteria are frequently less resistant to antibiotics than ground controls. However, each bacterium responds differently to the suite of antibiotics, showing and increase in resistance to some, a decrease in resistance to others, and occasionally a change in resistance. No overall pattern is discernible at this time. Supported by NASA grants NAGW 1197 and NAGW 2328.

[159] CHROMOSOME MECHANICS IN FUNGI UNDER SPACE CONDITIONS. A. Hahn1, VD Kern2, and B. Hock1. 'Technical University Munich, Germany, and 2Ohio State University, Columbus.

Spore color mutants of the fungus Sordaria macrospora AUERSW were crossed under space flight conditions on the Shuttle-To-MIR-Mission S/MM 05 (STS-81). The arrangement of spores of different colors in the ascii allowed conclusions on the influence of space conditions on sexual recombination in fungi. Experiments on a 1gx centrifuge in space and in parallel on the ground were used for controls. The samples were analyzed microscopically upon their return to Earth. Each fruiting body was assessed separately. Statistical analysis of the data showed a significant increase in gene recombination frequencies caused by the heavy ion particle stream in space radiation. The lack of gravity did not influence crossing-over frequencies.

The single cell gel electrophoresis (SCGE) assay or comet assay was adapted for the analysis of DNA damage in hyphae of S. macrospora. The principle of this highly sensitive method is based on the conversion of single strand breaks and alkali-labile sites to double strand breaks by alkaline lysis. The cells are subjected to electrophoresis, stained, and microscopically evaluated. Increasing damage leads to increasing migration of DNA from the nuclei. Hyphae of the flown samples were assessed for DNA strand breaks. No increase in damage was found as compared to the ground samples. By treating cultures with x-rays, it was shown that S. macrospora is able to repair radiation-induced DNA strand breaks within hours.

(Supported by DLR, Germany: 50 WB 9630)
SESSION K: CONCURRENT POSTERS IV
Space Biomedical Results II
[160] EPSTEIN-BARR VIRAL REACTIVATION IN SPACECRAFT AND IN GROUND-BASED SPACE ANALOGS, S.K. Mela1, D.J. Lugg2, D.A. Payne2, S.K. Tying3, and D.L. Pierson4. 1Enterprise Advisory Services Inc.; 2Australian Antarctic Division, Hobart, Australia; 3Univ. TX Medical Branch, Galveston TX; and 4NASA Johnson Space Center, Houston TX.

Reactivation of latent viruses may pose an important health risk for people living and working in extreme environments such as space and its ground-based analogs. Compromise in immune function under such conditions may increase the incidence and duration of viral reactivation and shedding. We studied EBV shedding patterns in saliva samples from 37 subjects (30 M and 7 F): 11 from the Space Shuttle, 2 from the Mir space station, 16 from an 8-month winter-over study in Antarctica, and 8 from two closed-chamber studies. Samples collected on the Space Shuttle were stabilized and stored at ambient temperature until landing; samples from the Antarctic and chamber studies were stored at -70°C until analyzed. Each sample was concentrated with a 100K Microspin concentrator; EBV DNA was extracted with Qiagen's QIAamp 96 spin blood kit, amplified by PCR, and detected with the Digene SHARP Signal detection system. We analyzed 1,600 samples, 344 from the Space Shuttle, 65 from Mir, 642 from Antarctica, and 418 from chamber subjects. EBV DNA was detected in 13% of the samples from Space Shuttle crews, 18% from Mir, and 17% each from Antarctica and chamber studies. Individual shedding patterns varied widely, but EBV DNA was present more often in samples collected before and during each test period than afterward. In the Antarctic subjects, viral shedding was correlated with reduced cell-mediated immunity (CMI), as assessed with Multitest CMI skin test on the forearms. More than 80% of the subjects had reduced CMI at all 5 measurement times. We conclude that extreme environmental conditions and the stresses associated with total physical isolation caused a decline in CMI, resulting in an increase in EBV reactivation.

(Supported by NASA 106-20)

[161] VARIATION IN CENTRAL VENOUS, ESOPHAGEAL AND ABDOMINAL PRESSURE IN HUMANS DURING PARABOLIC FLIGHT: PRELIMINARY RESULTS. G. Pantalos1, S. Hart2, J. Mathias3, M.K. Sharp1, D. Watenpaugh1, J. Buckey2, S. Parnis4 and A. Hargens1. 1Univ. of Utah, Salt Lake City, UT; 2UTMB, Galveston, TX; 3Houston; 4Univ. of North Texas, Fort Worth; 5Dartmouth, Lebanon, NH; 6Texas Heart Inst., Houston; 7NASA/ARC, San Jose, CA.

Immediate entry into weightlessness of space, increases cardiac stroke volume despite reduction in central venous pressure (CVP). We hypothesized that the mechanism for this change is release of gravitational force on the chest wall and other massive tissues resulting in increased cardiac transmural pressure via reduction in intrapleural pressure. The increase in transmural pressure promotes increased cardiac filling at a lower CVP. The objective of this study was to test this hypothesis by simultaneously measuring intraesophageal pressure (IEP, closely approximates intrapleural pressure), intraabdominal pressure (IAP), and CVP in human subjects exposed to 1-G, 1.8-G and 0-G range of acceleration during parabolic flight onboard the NASA KC-135. Four male subjects were instrumented preflight with a multi-sensor, acceleration-insensitive nasogastric pressure catheter (Millar) with one sensor at the mid-thoracic level (T-3) and a second in the proximal duodenum. A second catheter was inserted peripherally with the sensor in the superior vena cava at the mid-thoracic level to measure CVP. For each set of parabolas, subjects assumed the launch, supine 6° head-down tilt, or seated postures with 1-G data recorded prior to a "push-over" and parabolic flight. Descriptive results from post-flight review are reported at present. Weightlessness reduced CVP and IEP below -1G values (3-4 mm Hg) for all recumbent postures, but, increased CVP and IEP (0-4 mm Hg) in the seated posture. Weightlessness decreased IAP (3.5 mm Hg) for all postures. During hyperacceleration, CVP and IEP usually increased above 1-G values, more so in recumbent than in the seated posture, and IAP increased (4-8 mm Hg) independent of posture. Reductions in CVP, IEP, and IAP during weightlessness for the recumbent postures support the hypothesis. Elevation of CVP and IEP during weightlessness for the seated posture may result from headward shifting of abdominal contents and blood. The change in pressures with entry into weightlessness in the head-down tilt posture questions the validity of this ground-based model for acute response to weightlessness. (Supported by NASA: NAGW 4338)

[162] EFFECT OF RESISTANCE TRAINING ON GLUT-4 CONTENT IN SKELETAL MUSCLE OF HUMANS SUBJECTED TO 20 DAYS OF BED REST. I Tabata1, Y. Suzuki1, T. Fukunaga1, and T. Yokozeki1. 1Nat'l Inst. of Health and Nutrition, 'Faculty of Med. and 2Graduate School of Arts and Sciences, Univ. of Tokyo, Japan.

This study was done to assess the effects of inactivity on GLUT-4 content in skeletal muscle of young men, and to evaluate resistance training as a counter-measure to inactivity-related changes in GLUT-4 content in skeletal muscle. For 20 days, the control subjects (n=4) remained in a -6° head-down tilt at all times throughout bed. The subjects in the training group (n=5) also remained at bed rest except during resistance training that consisted of thirty 3-sec isometric maximal voluntary contractions using leg press exercise to recruit the extensor muscles of the ankle, knee, and hip. Muscle biopsy samples were obtained from the lateral aspect of vastus lateralis (VL) muscle just before and after the bed rest. After bed rest, cross-sectional area of VL muscle of the control group decreased (-7.3±4.0 %) at borderline level (p<0.05), while bed rest did not affect cross-sectional area of the training group significantly. GLUT4 content in VL muscle of the control group was significantly decreased after the bed rest (pre-bed rest:473±48 cpm/µg membrane, post-bed rest:398±66 cpm/µg membrane, p<0.05), while it significantly increased in the training group (pre-bed rest:510±158 cpm/µg membrane, post-bed rest:663±189 cpm/µg membrane, p<0.01). In conclusion, the present study demonstrated that GLUT-4 in VL muscle decreased by ~16% after 20 days of bed rest, and isometric resistance training during bed rest induced a 30% increase above the pre-bed rest value of GLUT-4.

(Supported by NASA, Japan)


Cardiovascular alterations observed during exposure to microgravity result in impairment of baroreflex activity partially as a result of fluid and electrolyte shifts. Some of the physiological observations that have been made in astronauts are mimicked by the tail-suspended rat model (30° head-down tilt). The Dahl salt-sensitive rat has been frequently used as a model of salt-induced hypertension. We examined the effects of 7 day simulated microgravity (tail-suspended) and the subsequent post-suspension on metabolic parameters and baroreflex activity in Dahl salt-sensitive (SS) rats on a high salt (8% NaCl) diet. Baroreflex-heart rate response parameters were altered by the 7 day suspension and subsequent recovery. The heart rate (HR) range, BP2, and lower HR plateau values were elevated in suspended animals compared to their parallel controls. The curvature coefficient and gain values were reduced. There were no differences in food intake, water consumption and urine production between suspended and control rats. However, urinary calcium was reduced in suspended animals, both during and post-suspension. These data suggest that dietary sodium supplementation may serve as a countermeasure against the hypotension observed after simulated microgravity via a mechanism that involves calcium retention. (Supported in part by NIH grant RR03034 and NASA grant NCCW-0083.)
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