

SIXTEENTH ANNUAL MEETING

AMERICAN SOCIETY FOR GRAVITATIONAL AND SPACE BIOLOGY

ASGSB-CSA-ELGRA Combined Meeting
October 25-28, 2000
Montréal, QC, Canada

SHORT PROGRAM

Wednesday, October 25

15:00 Registration Opens
19:00 Student Mixer
19:30 ASGSB Governing Board Meeting

Thursday, October 26

07:30 Registration Opens
08:30–08:45 Opening Remarks and Welcome
08:45–10:15 Symposium I – Consequences of Contamination of the Spacecraft Environment
10:15–10:45 Break
10:45–12:15 Symposium I, *cont.*
12:15–14:00 Lunch and Committee Meetings
14:00–15:30 Concurrent Posters – Session I
 A. Space Life Sciences Training Program
 Undergraduate Student Poster Competition
 B. Graduate Student Poster Competition
15:30–17:00 Concurrent Posters – Session II
 A. Space Life Sciences Training Program
 Undergraduate Student Poster Competition
 B. Graduate Student Poster Competition
19:00–21:00 Reception

PROGRAM – 2000 ANNUAL MEETING

Friday, October 27

- 09:00–10:30 Symposium II – Psychosocial Issues in Long-Term Space Flight
- 10:30–11:00 Break
- 11:00–12:30 Symposium II, *cont.*
- 12:30–14:00 Lunch and Committee Meetings
- 14:00–16:00 Concurrent Oral Sessions
- I. Animal Development, Physiology and Gravity Sensing
 - II. Advanced Life Support and Biotechnology
- 16:00–17:30 Concurrent Posters – Session III
- C. Animal Development, Physiology and Gravity Sensing
 - D. Cell Biology
 - E. Plant Development, Physiology and Gravity Sensing
 - F. Advanced Life Support and Biotechnology
 - G. Spaceflight Experiment Results
 - H. Spaceflight Physiology and Medicine
- 18:30–21:00 Banquet and Keynote Speaker; Business Meetings
- 21:00 ASGSB Governing Board Meeting

Saturday, October 28

- 08:30–10:30 Concurrent Oral Sessions
- III. Space Flight and Space Medicine
 - IV. Plant Development and Physiology
- 10:30–11:00 Break
- 11:00–13:00 Concurrent Oral Sessions
- V. Spaceflight Results
 - VI. Cell Biology
- 13:00–14:00 Lunch
- 14:00–15:30 Concurrent Posters – Session IV
- C. Animal Development, Physiology and Gravity Sensing
 - D. Cell Biology
 - E. Plant Development, Physiology and Gravity Sensing
 - F. Advanced Life Support and Biotechnology
 - G. Spaceflight Experiment Results
 - H. Spaceflight Physiology and Medicine
- 15:30–17:00 Minisymposium – Current Ground-Based Models

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PROGRAM

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- 15:00 Registration Opens
19:00 Student Mixer
19:30 ASGSB Governing Board Meeting

Thursday, October 26

- 07:30 Registration Opens
08:30 Opening Remarks and Welcome

Symposium I Consequences of Contamination of the Spacecraft Environment

08:45 –12:15 Moderator: Richard Wassersug

| Time | | Page |
|-------------|--|-------------|
| 08:45 | Microbiological Contamination of Spacecraft. Duane L. Pierson, R.J. Bruce, T.O. Groves, N.D. Novikova and A.N. Viktorov. [1] | 4 |
| 09:30 | Contamination of Spacecraft Environment: Immunological Consequences. William T. Shearer. [2] | 4 |
| 10:15 | Break | |

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| 10:45 | Individual Variation in Human DNA Repair Genes: Consequences for the Space Traveler. Barry W. Glickman, M. Khaidakov and A. Mortimer. [3] | 4 |
| 11:30 | Plants, Plant Pathogens and Microgravity—A Deadly Trio. Jan Leach. [4] | 4 |
| 12:15 | Lunch and Committee Meetings | |

Concurrent Posters I

14:00 – 15:30

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

**A. Space Life Sciences Training Program
Undergraduate Student Poster
Competition**

| Poster # | | Page |
|-----------------|---|-------------|
| A01 | A Novel Red-Light-Based Photosensory System That Mediates Positive Phototropism in <i>Arabidopsis</i> Roots. N.J. Ruppel, R.P. Hangarter and J.Z. Kiss. [5] | 6 |
| A03 | Molecular Evolutionary Patterns in Microbial Natural Product Biosynthetic Gene and Enzyme Sequences: Search for Adaptive Significance. H.E. Page, C.L. Peterson and J.V. Lopez. [6] | 6 |
| A05 | The Effects of Modified Biological Research in a Canister (Bric) Spaceflight Hardware on the Survivability and Development of the Tobacco Hornworm (<i>Manduca sexta</i>). M.R. Inzunza, K. Anderson and O. van den Ende. [7] | 6 |
| A07 | Synaptic Innervation in Rat Utricular Macula. A. Chu and A. Lysakowski. [8] | 6 |
| A09 | Three-Dimensional Reconstruction and Analysis of Root Cap Statolith Distribution in <i>Arabidopsis thaliana</i> . R. Ehsanian, D.K. Bruck and J.D. Smith. [9] | 7 |

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|-----------------|---|-------------|
| A11 | Pesto Ground Control Experiments: A Gas Exchange System for Measuring Photosynthesis and Evapotranspiration. T. T. Tran, O. Monje and G.W. Stutte. [10] | 7 |
| A13 | Molecular Profiling of Planktonic and Biofilm Microbial Communities in Hydroponic Growth Systems. E. Nunez, J.L. Adams and M.S. Roberts. [11] | 7 |
| A15 | The 16 th Annual Spaceflight and Life Sciences Training Program at Kennedy Space Center, Florida. S. Potter, W. Hill, P. Currier, G. Koerner, J. Rebmann and A. Schlundt. [12] | 7 |

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| B. Graduate Student Poster Competition |
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| Poster # | | Page |
|-----------------|---|-------------|
| B01 | The Effect of Plastid Mutations on Gravitropism of Roots, Hypocotyls, and Inflorescence Stems of <i>Arabidopsis</i> . K. Yamamoto and J. Z. Kiss. [13] | 9 |
| B03 | Gauging the Internal Gas Content of <i>Brassica rapa</i> Siliques Grown in Space. K.L. Wilsen, J. Blasiak and M.E. Musgrave. [14] | 9 |
| B05 | Stress Response to Magnetic Levitation (Low-Gravity) and High Magnetic Fields in Transgenic <i>Arabidopsis</i> . A.N. Morgan, J. Yowtak, R.J. Ferl, J.S. Brooks, A.-L. Paul and M.W. Meisel. [15] | 9 |
| B07 | Changes in Osteoprogenitor Proliferation in the Rat Skeleton Due to Mechanical Unloading. N. Basso, Y. Jia, C.G. Bellows and J.N.M. Heersche. [16] | 9 |
| B09 | ???Tropism: When a Primary Root Encounters a Barrier to Downward Growth. G.D. Massa and S. Gilroy. [17] | 10 |
| B11 | Rapid Discrimination among Individual DNA Molecules in Microliter Volumes. W. Vercoutere, S. Winters-Hilt, H. Olsen, D. Deamer, D. Haussler and M. Akeson. [18] | 10 |
| B13 | Maternal Behaviour Under Hypergravity Conditions in CD-1 Mice. M. Simeoni, D. Santucci and E. Alleva. [19] | 10 |
| B15 | Contribution of Sympathetic Activity to Lypolysis during Exposure to Increasing Hypergravity Loads. M.M. Moran, T.P. Stein and C.E. Wade. [20] | 10 |

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| B17 | Starch Conversion to Sucrose at Night. S.E. Weise and T.D. Sharkey. [21] | 11 |

Concurrent Posters II

15:30 – 17:00

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

A. Space Life Sciences Training Program
Undergraduate Student Poster
Competition

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| A02 | Magnetic Levitation as a Low Gravity Environment. J. Yowtak, A.N. Morgan, R.J. Ferl, J.S. Brooks, A.-L. Paul and M.W. Meisel. [22] | 13 |
| A04 | Molecular Mapping of the Lazy-2 Gravitropic Response Gene of Tomato. J. Well, A. Madlung, K. Krutovskii, R. Meyer, T.J. White and T.L. Lomax. [23] | 13 |
| A06 | The Stability of Liquid Water in Porous Rocks in a Mars-like Environment. C. Paty, C. McKay, D. Catling and J. Heldmann. [24] | 13 |
| A08 | Changes in Statocyte Structure and Amyloplast Starch in <i>Arabidopsis thaliana</i> Columella Cells after Growth under Hypergravity Conditions. S.D. Hopkins and J.D. Smith. [25] | 13 |
| A10 | Carbohydrate Deposition in <i>Raphanus sativus</i> L. cv. Cherry Belle Shoots: Preliminary Ground Studies for the Rasta Spaceflight Experiment. H.N. Goldsmith, E.C. Stryjewski, G.W. Stutte, W. McLamb and D. Reed. [26] | 14 |
| A12 | Soil Water Potential Affects Crop Growth Rate of Wheat Trough Changes in Leaf Area, Not Photosynthesis. H.-T. Wang, O. Monje and G.W. Stutte. [27] | 14 |
| A14 | Development of Defined Media for Rapid Physiological Profiling of Microbial Communities. F.R. Perez, J.L. Garland and M.S. Roberts. [28] | 14 |

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| A16 | SLSTP 2000, Controlled Biological Systems Group, Student Research Projects Support NASA's Bioregenerative Life Support Program. D. Muhlestein and G. Koerner. [29] | 14 |

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| B. Graduate Student Poster Competition |
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| B02 | Chimeric Calcium/Calmodulin-Dependent Protein Kinase: Role of the Neural Visinin-like Domain in Regulating Autophosphorylation and Calmodulin Affinity. P.V.Sathyannarayanan, C.R.Cremo, W.F.Siems and B.W.Poovaiah. [30] | 16 |
| B04 | Development of the Nervous System and its Control of Gravity-Dependent Behaviours in Larvae of Bivalve Molluscs. J.T. Plummer, D.L. Jackson and R.P. Croll. [31] | 16 |
| B06 | Innervation of Rat Vestibular Maculae in Hypergravity: an <i>In Vivo</i> and <i>In Vitro</i> Study. S. Gaboyard, E.Scarfone, J. Lehouelleur and A. Sans. [32] | 16 |
| B08 | A Putative Role for the Cerebellum in Avian Vestibular Responses to Linear Translation. S. Irons-Brown, S.M. Jones and T.A. Jones. [33] | 16 |
| B10 | Effects of Short-Duration Microgravity on <i>Drosophila melanogaster</i> (Fruit Fly) Activity. M.S. Miller and T.S. Keller. [34] | 17 |
| B12 | Long-Term <i>In Vivo</i> Delivery of Recombinant Human Insulin-like Growth Factor-1 by Tissue-Engineered Skeletal Muscle Implants for Treating Disuse Muscle Atrophy in Mice. P.H.U. Lee, X.Y. Wang and H.H. Vandenberg. [35] | 17 |
| B14 | Mechanical Stress and Wounding Elicit Nitric Oxide Production in <i>A. thaliana</i> Wild-Type and Nitrate Reductase Mutants. H. Garcês, D.J. Durzan and M.C. Pedroso. [36] | 17 |
| B16 | Kinetics and Location of Phototropism in <i>Zea mays</i> L. Roots. C. Wolverton, J.L. Mullen, H. Ishikawa and M.L. Evans. [37] | 17 |
| B18 | Video Capture of Green Fluorescent Protein Reporting <i>In Vivo</i> , Real Time Gene Expression. M.S. Manak, A-L. Paul, P.C. Sehnke and R.J. Ferl. [38] | 18 |
| 19:00 | Reception | |

Friday, October 27

Symposium II
Psychological Issues in Long-Term
Space Flight

09:00 –12:30 Moderator: Gerry Sonnenfeld

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| 09:00 | Psychosocial Issues in Long-Term Space Flight: Overview. Larry Palinkas. [39] | 20 |
| 09:45 | Psychosocial Adaptation, Social Interaction Processes and Performance of Crews During SFINCSS Isolation Study: Cultural, Gender and Personal Factors. Judith Lapierre. [40] | 20 |
| 10:30 | Break | |
| 11:00 | Psychosocial Issues in Space: Results from Shuttle/Mir. Nick Kanas, V. Salnitskiy, E. Grund, D.S. Weiss, V. Gushin, O. Kozerenko, A. Sled and C.R. Marmar. [41] | 20 |
| 11:45 | Issues for the Future. Gro Sandal. [42] | 20 |
| 12:30 | Lunch and Committee Meetings | |

Concurrent Oral Sessions I and II

Oral Session I
 Animal Development, Physiology
 and Gravity Sensing

14:00 – 16:00 Moderator: Michael Wiederhold

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| 14:00 | (Micro-)Gravity Actions as Identified from Developmental Biology Experiments in Space and Their Interpretation. H.-J. Marthy. [43] | 22 |
| 14:15 | The Effects of Microgravity on the Swimming Behaviour of Starfish Larvae. B.J. Crawford and D.L. Jackson. [44] | 22 |
| 14:30 | A Critical Period for Vestibular Development in Zebrafish (<i>Danio rerio</i>). S.J. Moorman, R. Cordova and S.A. Davies. [45] | 22 |
| 14:45 | Functional Changes in Central Vestibular Relay Circuits Following 2g Centrifugation. S.M. Jones, L. Warren, R. Shukla, A. Browning, C.A. Fuller and T.A. Jones. [46] | 22 |
| 15:00 | Tests to Determine the Adequacy of NASA's Rodent Food Bars for Use in Long-Term Space Flight Experiments. J.E. Barrett, D.S. Yu and B.P. Dalton. [47] | 23 |
| 15:15 | POMC and Endorphine Are Induced by Hypergravity in Rat Brain. Y. Kumei, H. Shimokawa, R. Shimokawa, M. Terasawa, B. Linsuwanont and K. Ohya [48] | 23 |
| 15:30 | Early and Late Effects of Perinatal Hyper-Gravity Exposure on the Developing CNS. E.M. Sajdel-Sulkowska, L.A. Baer, G.-H. Li, G.M. Sulkowski, A.E Ronca and C.E. Wade. [49] | 23 |
| 15:45 | Gravitaxic Behavior in <i>Drosophila melanogaster</i> . J.D. Armstrong, M.J. Texada, E.L. Carter, E.S. Kuo, C.M. Nadorff and K.M. Beckingham. [50] | 23 |

Oral Session II
Advanced Life Support
and Biotechnology

14:00 – 16:00 Moderator: Charles Barnes

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| 14:00 | Programmable Plants: Development of an <i>In Planta</i> System for the Remote Monitoring and Control of Plants for Long-Term Life Support. C.S. Brown. [51] | 25 |
| 14:15 | Boundary Layers around Plant Leaf and Root Tissues Depend on Gravity. O. Monje, D.M. Porterfield and G.W. Stutte. [52] | 25 |
| 14:30 | Genetic and Environmental Influences on the Nutritive Quality of Spinach: A NASA ALS Candidate Crop. C.F. Johnson, R.W. Langhans, L.D. Albright, R.M. Welch, G.F. Combs, R.P. Glahn and R.M. Wheeler. [53] | 25 |
| 14:45 | Tissue Engineering in Zero Gravity. A. Cogoli. [54] | 25 |
| 15:00 | Use of the Rotating Bioreactor to Study Skeletal Mutations: Hereditary Multiple Exostosis. P.J. Duke, D. Montufar-Solis and J.T. Hecht. [55] | 26 |
| 15:15 | Fluid Handling and Management Experiment (FHAME). T.M. Crabb, R.C. Morrow and T.K. Klemp. [56] | 26 |
| 15:30 | Shear Forces and the Proper Control. J.J.W.A. van Loon, E. Folgering, J.P. Veldhuijzen and C.V.C. Bouten. [57] | 26 |
| 15:45 | Advanced Versatile Tools for Life Sciences Research in Space. P. Todd, J. Vellinger, A. Sharpe, R. Ormsby, H. Platt, K. Barton and M. Deuser. [58] | 26 |

Concurrent Posters III

16:00 – 17:30

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

**C. Animal Development, Physiology
and Gravity Sensing**

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| C01 | Microgravity Effects on Fertilized Eggs Have No Incidence, after Landing, on the Further Larval Development and Reproduction in the Amphibian <i>Pleurodeles</i> Walzl. H. Membre, A. Bautz, D. Durand, C. Aimar, A.M. Bautz and C. Dournon. [59] | 28 |
| C03 | Microgravity-Induced Malformations of the Body Correlate with the Depression of the Static Vestibuloocular Reflex. E. Horn. [60] | 2 8 |
| C05 | Gravity Stimulation Changes Withdrawal Reflex in Rats. K. Toda, Y. Kawauchi, Y. Kumei, F.H. Nasution and K. Makita. [61] | 2 8 |
| C07 | Hindlimb-Suspension, Water Deprivation and Salt-Loading Affect Angiotensin-Converting Enzyme (Ace) Expression in Rat Choroid Plexus. E. Vila-Porcile, C. Carcenac, C. Maseguin, J.-M. Gasc and J. Gabrion. [62] | 2 8 |
| C09 | Lack of Vestibular Otolith Participation in Human Orthostatic Blood Pressure Control. D.E. Watenpugh, A. Cothron, S.L. Wasmund, W.L. Wasmund, R. Carter III, N.K. Muentner and M.L. Smith. [63] | 2 9 |
| C11 | Regional and Muscle Specific Effects of a β -Adrenergic Agonist in Hindlimb Suspended Rats. D.A. von Deutsch, I.K. Abukhalaf, L.E. Wineski, S.A. Pitts, R. Roper, L.D. Kataria, D.C. Jackson, D.E. Potter and D.F. Paulsen. [64] | 2 9 |

D. Cell Biology

| Poster # | | Page |
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| D01 | Vitamin D Production in the Rotating Wall Vessel (RWV). F. Lewis, E.N. Benes, X.-C. Wang, P.L. Allen, T.G. Hammond and L.A. Cubano. [65] | 31 |
| D03 | L-Nmma Suppresses Nitric Oxide Production and Apoptosis in <i>Taxus brevifolia</i> Cells. M.C. Pedroso and D.J. Durzan. [66] | 31 |
| D05 | Altered Gravity Increases PGE ₂ Production through Activation of COX-2 mRNA Expression in Mouse Osteoblast Like MC3T3-E1 Cells. A. Sato, M. Fujita, M. Kanematsu, M. Narato, H. Kumagai, S. Kamigaichi and M. Takaoki. [67] | 31 |
| D07 | The Effect of Microgravity on Cytoskeleton Architecture and Proliferation of Human Breast Cancer Cell Line MCF-7. J. Vassy, S. Portet, D. Schoevaert, M. Beil, G. Millot, G. Gasset and F. Fauvel-Lafève. [68] | 31 |
| D09 | Clinostat Rotation Culture Modulates Gene Expression of Osteoclastogenesis-Regulating Factors via a Cyclic-Amp Dependent Mechanism. M. Kanematsu, H. Takai, M. Takaoki and A. Sato. [69] | 32 |
| D11 | Human Osteoblast Differentiation Is Expedited in Culture in a Magnetic Field. L. Yuge, T. Kumagai, I. Hide, S. Hiyama, M. Kanno, Y. Kumei, S. Takeda, Y. Ikuta, M. Sugiyama and K. Kataoka. [70] | 32 |
| D13 | Gravity Sensitivity of T-Cell Activation: The Actin Cytoskeleton. B.B. Hashemi, J.E. McClure and D.L. Pierson. [71] | 32 |
| D15 | Development of a Microgravity Cell Culture Platform for the Study of Bone Cell Metabolism onboard the NASA Shuttle. L. Misener, D. Sindrey, T. Smith, S. Pugh, D. Kusljic and P. Kwong. [71A] | 32 |

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E. Plant Development, Physiology
and Gravity Sensing

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| E01 | Oxygen Effects on Pollen Germination and Tube Orientation. J. Blasiak, D. Mulcahy and M. Musgrave. [72] | 34 |
| E03 | Changes in Cotyledon Cell Ultrastructure during <i>Brassica rapa</i> Seed Development in Microgravity. A. Kuang and M. E. Musgrave. [73] | 34 |
| E05 | Effects of Lithium Ions on Elongation and Gravitropic Responses of Primary Roots of Maize. T. J. Mulkey. [74] | 34 |
| E07 | The Actin Network in Lentil Root Statocytes. D. Driss-Ecole and G. Perbal. [75] | 34 |
| E09 | Microscopic Analysis of Sweetpotato Root Tips Propagated from Stem Cuttings Maintained in Either Vertical or Horizontal Clinorotation. C.S. Williams, D.G. Mortley, C.E. Morris, C.F. Davis, S.D. Gamble and J.W. Williams. [76] | 35 |
| E11 | Ultrastructure of <i>P. patens</i> Tip Caulonemata Cells: Untreated, Cold Grown, Severed, Oryzalin Treated, UV-A Treated. E. B. Tucker, V. Sookhdeo and L Yin. [77] | 35 |
| E13 | The <i>rib1</i> Mutant Is Resistant to Indole-3-Butyric Acid, an Endogenous Auxin in <i>Arabidopsis Thaliana</i> . C.S. Waddell and J. Poupart. [78] | 35 |

F. Advanced Life Support and Biotechnology

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| F01 | Regenerable Seed Plugs from Formed Plant Fiber. R.C. Morrow, C.J. Ehle and T.M. Crabb. [79] | 37 |
| F03 | Evapotranspiration by Salad Crops in Controlled Environments. D.E. Ciolkosz and G.D. Goins. [80] | 37 |
| F05 | Astrium – A New Name in Space Life Sciences. P. Kern and U.M. Kuebler. [81] | 37 |

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| F07 | IBIS: An Instrument Dedicated to Perform Biological Experiments in Microgravity. D. Thierion, G. Gasset, D. Chaput, A. Labarthe, B. Eche and M. Viso. [82] | 37 |

G. Spaceflight Experiment Results

| Poster # | | Page |
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| G01 | Expression of Fas/CD95 in Spaceflown Lymphocytes (Jurkat). L.A. Cubano and M.L. Lewis. [83] | 39 |
| G03 | Are There Two Mechanisms Underlying Space Motion Sickness? D.G.D. Watt. [84] | 39 |
| G05 | Payload Late Access Survey Preliminary Results. C. Martin-Brennan and R.C. Morrow. [85] | 39 |
| G07 | Effect of Microgravity on Root Regeneration, Ultrastructures, and Carbohydrate Content of Sweetpotato Stem Cuttings. D.G. Mortley, C.S. Williams, C.F. Davis and J.W. Williams. [86] | 39 |

H. Spaceflight Physiology and Medicine

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| H01 | Long-Term Effects of Microgravity on Human Sleep, Cytokine, and Endocrines. H. Moldofsky, F. Lue , J. MacFarlane, C.-G. Jiang, L. Poplonski, I. Ponomoreva, I. Larina and R. Gorczynski. [87] | 41 |
| H03 | Dose and Dose Rate Effects of Proton Radiation on Lymphocyte Populations in Blood and Spleen. D.S. Gridley, M.J. Pecaut and G.A. Nelson. [88] | 41 |
| H05 | Cytokine Synthesis by T Cells Collected from Apheresis Donors Receiving G-CSF. B.-N. Lee, M. Korbling, W.T. Shearer and J.M. Reuben. [89] | 41 |
| H07 | The Adrenal/Gonadal Response to a 5-Hour HDT Is Prompter in Men Than in Women. F. Strollo, G. Spera, E.V. Cosmi, M. Morè, A. Mambro and G. Rioldino. [90] | 41 |
| 18:30 | Banquet and Keynote Speaker; Business Meetings | |
| 21:00 | ASGSB Governing Board Meeting | |

Saturday, October 28

Concurrent Oral Sessions III and IV

Oral Session III
Space Flight and Space Medicine

08:30 – 10:30 Moderator: Stephen Keith Chapes

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| 08:30 | Effects of Spaceflight and Hindlimb Suspension Unloading on Rat Neuromuscular Development. D.A. Riley, B.L. Huckstorf, G.R. Slocum, J.L.W. Bain, P.M. Reiser, F.R. Sedlak, W. Liebl and M.T.T. Wong-Riley. [91] | 43 |
| 08:45 | Scientific Outcome of the Russian/French Cooperation on the Bion Flights. M. Viso. [92] | 43 |
| 09:00 | Muscle Collagen Gene Expression and Protein Adaptation Following 14 Days of Spaceflight in BION 11 Rhesus Monkeys (<i>Macaca mulatta</i>). D.A. Martinez, V.R. Edgerton, R.E. Grindeland, D.E. Gallagher, J.D. Tanksley, K.M. Shea and A.C. Vailas. [93] | 43 |
| 09:15 | Developing Protocols for Recombinant Adeno-Associated Virus-Mediated Gene Therapy in Space. S. Ohi, A. Aguilar and B.C. Kim. [94] | 43 |
| 09:30 | Evidence for a Th2 Shift Associated with Spaceflight: Immune Modulation by Stress Hormones. R.P. Stowe, D.L. Pierson, C.F. Sams and A.D.T. Barrett. [95] | 44 |
| 09:45 | Effects of Low Dose Proton Irradiation on Three Models of Behavior. M.J. Pecaut, A.L. Smith, E.D. Zendejas, C.N. Zuccarelli, P. Haerich and G.A. Nelson. [96] | 44 |
| 10:00 | Neurobehavioral Effects of Hypergravity Conditions in CD1 Mouse Strain. D. Santucci, G. Corazzi, N. Francia, A. Antonelli, L. Aloe and E. Alleva. [97] | 44 |
| 10:15 | Effects of Simulated Microgravity on Nitric Oxide Synthase Expression and Nitrate/Nitrite Content in Different Arteries of the Rat. J. Ma, C.I. Kahwaji, N.D. Vaziri, Z. Ni and R.E. Purdy. [98] | 44 |
| 10:30 | Break | |

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| <p>Oral Session IV Plant Development and Physiology</p> |
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08:30 – 10:30 Moderator: Mary Musgrave

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| 08:30 | Functional Characterization of the ARG1 Gene Family in <i>Arabidopsis thaliana</i> . K. Boonsirichai, E. Rosen, J. Sedbrook and P. H. Masson. [99] | 46 |
| 08:45 | Gravitropism: Cross-Talk between Calcium/Calmodulin and Hormone Mediated Signaling. B.W. Poovaiah and T. Yang. [100] | 46 |
| 09:00 | Gravisensitivity in Space Grown Lentil Seedling Roots. G. Perbal and D. Driss-Ecole. [101] | 46 |
| 09:15 | The Gypsi Mutants: A New Group of Gravity Mutants in <i>Arabidopsis</i> . S.E. Wyatt, A. Rashotte, G. Muday and D. Robertson. [102] | 46 |
| 09:30 | Reduced Gravity Response in the <i>Arabidopsis</i> Mutant <i>RCN1</i> Is Due to Increased Levels of Auxin Transport. A.M. Rashotte and G.K. Muday. [103] | 47 |
| 09:45 | InsP ₃ Signaling During Plant Gravitropism. I.Y. Perera, I. Heilmann, W.F. Boss and P.B. Kaufman. [104] | 47 |
| 10:00 | PH Signaling in the Gravitropic Response of <i>Arabidopsis</i> Roots. S. Gilroy, J.M. Fasano, R. Hirsch, P. Minnich and S.J. Swanson. [105] | 47 |
| 10:15 | Use of a Gravity Clamp to Reveal a Multiphasic Motor Response in Maize Root Gravitropism. M.L. Evans, J.L. Mullen, C. Wolverson and H. Ishikawa. [106] | 47 |
| 10:30 | Break | |

Concurrent Oral Sessions V and VI

**Oral Session V
Spaceflight Results**

11:00 – 13:00 Moderator: Alan Mortimer

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| 11:00 | Sleep and Circadian Rhythms in Space—Short-Term Versus Long-Term Missions. T.H. Monk. [107] | 49 |
| 11:15 | Testosterone Excretion during and after Space Flight on the Shuttle. T.P. Stein and M.D. Schluter. [108] | 49 |
| 11:30 | Residual Acceleration on Space Laboratories: Characterization, Effects on Fluid Flows and Implications for Microbiology. E.S. Nelson and K. Jules. [109] | 49 |
| 11:45 | Reporter Gene Expression during Spaceflight and in Controlled Inductive Environments. R.J. Ferl, M.S. Manak, C.J. Daugherty and A-L. Paul. [110] | 49 |
| 12:00 | Composition and Physical Properties of Starch in Microgravity-Grown Plants. K.H. Hasenstein, O.A. Kuznetsov, C.S. Brown, W.C. Piastuch and H.G. Levine. [111] | 50 |
| 12:15 | Locomotor Behaviour of Bivalve Larvae in the Absence of Gravity. D.L. Jackson and R.K. O'Dor. [112] | 50 |
| 12:30 | Rootzone Hypoxic Responses Result from Inhibition of Gravity Dependent Oxygen Transport in Microgravity. D.M. Porterfield, O. Monje, W. Stutte and M. E. Musgrave. [113] | 50 |
| 12:45 | Germination and Elongation of Flax in Microgravity. H.G. Levine, K. Anderson, A. Boody, D. Cox, O.A. Kuznetsov and K.H. Hasenstein. [114] | 50 |
| 13:00 | Lunch | |

Oral Session VI
Cell Biology

11:00 – 13:00 Moderator: Augusto Cogoli

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| 11:00 | The Tension-Driven Gating Transition in the Bacterial Mechanosensitive Channel, MscL. S. Sukharev, M. Betanzos, C.-S. Chiang and H.R. Guy. [115] | 52 |
| 11:15 | Spectrin-like Proteins Associate with the Actin-Organized Endoplasmic Reticulum Aggregate in the Spitzenkörper of Gravitropically Tip-Growing Cells. M. Braun and A. Sievers. [116] | 52 |
| 11:30 | Microgravity-Induced Inhibition of Apoptosis in Peripheral Blood Mononuclear Cells and Changes in PKC Isoforms. D. Risin, A. Sundaresan and N.R. Pellis. [117] | 52 |
| 11:45 | The Effect of Gravitational Perturbation on the Expression of Genes Regulating Growth and Metabolism in a Human Lymphoblastoid Cell Line (Jurkat Cells). K. Singh, L. Cubano and M. Lewis. [118] | 52 |
| 12:00 | Expression of Structural and Metabolic Stress Genes in Human Leukemic Lymphocytes Subjected to Glucose Deprivation and Vibrational Stress. N. Myers. [119] | 53 |
| 12:15 | Reciprocal Trophic Interactions between Human Retinal Precursors and Retinal Pigment Epithelium (RPE) and its Physiologic Relevance in Tissue Replacement. K. Dutt, R. Lawrence, R. Kumar and T. Lindsay. [120] | 53 |
| 12:30 | Preliminary Studies in Support of a Space Shuttle Flight Experiment Evaluating the Ability of rhIGF-1 to Attenuate Space Flight-Induced Skeletal Muscle Atrophy. B.C. Creswick, J. Shansky, P.H.U. Lee, X.Y. Wang and H.H. Vandenberg. [121] | 53 |
| 12:45 | Effect of Microgravity on the Cytoskeleton of Cultured Nervous Cells. B. Uva, M.A. Masini, M. Sturla, P. Prato and G. Tagliaferro. [122] | 53 |
| 13:00 | Lunch | |

Concurrent Posters IV

14:00 to 15:30

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

**C. Animal Development, Physiology
and Gravity Sensing**

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| C02 | Effects of Hypergravity on the Development of the Motor System in Crickets (<i>Acheta domesticus</i>). S. Böser and E. Horn. [123] | 55 |
| C04 | Factors Influencing the Susceptibility of Anurans to Motion Sickness. R.J. Wassersug, T. Naitoh and M. Yamashita. [124] | 55 |
| C06 | Hypergravity Induces Fos and CRH Expression in Rat Hypothalamus. R. Shimokawa, H. Shimokawa, M. Terasawa, B. Linsuwanont, Y. Kumei and K. Ohya. [125] | 55 |
| C08 | Effects of Gender and Hindlimb Unloading on Bone Histomorphometry in Adult Rats. G.L. Evans, S. Lotinun, T. Hefferan, E. Morey-Holtan and R.T. Turner. [126] | 55 |
| C10 | A Non-Invasive Analysis of Musculoskeletal Collagen Metabolism from Urine of Rhesus Monkeys during 14 Days of 2g Hypergravity. A.C. Vailas, T. Hoban-Higgins, C.A. Fuller, R.E. Grindeland, K.M. Shea and D.A. Martinez. [127] | 56 |
| C12 | Effects of Hypergravity and Adrenalectomy on Total Body Bone Mineral Content in Male Rats. B. Girtten, M. Moran, L. Baer, S. Pruitt, C. O'Brien, S. Arnaud and C. Wade. [128] | 56 |

D. Cell Biology

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| D02 | Nuclear Translocation of Nuclear Factor Kappa B (NF- κ B) and Vitamin D Receptor During Rotating Wall Vessel Culture of Human Renal Cells. X-C. Wang, P.L. Allen, E.N. Benes, L.A. Cubano and T.G. Hammond. [129] | 58 |
| D04 | Antibiotic Resistance in Bacteria Exposed to Simulated Spaceflight Environments. M.A. Juergensmeyer and E.A. Juergensmeyer. [130] | 58 |
| D06 | The Effects of Hypergravity on the Expression of Nitric Oxide Synthase by Endothelial Cells: Role of the F-Actin Cytoskeleton and c-jun N-Terminal Kinase. F.N. Bosah, G.L. Sanford and S. Harris-Hooker. [131] | 58 |
| D08 | Locomotory Function in Lymphocytes Is Affected by Microgravity-Induced Signal Transduction Lesions Involving Protein Kinase C. A. Sundaresan, D. Risin and N.R. Pellis. [132] | 58 |
| D10 | <i>Pleurochrysis carterae</i> Is an Excellent Model to Study Biomineralization and Gravitaxis in Space. D. Montufar-Solis and P.J. Duke. [133] | 59 |
| D12 | 3D-Clinostat Drives P38 MAPK Cascade in Cultured Human Osteoblasts. K. Kataoka, L.Yuge, T. Kumagai, I. Hide, S. Hiyama, M. Kanno, Y. Kumei, S. Takeda, Y. Ikuta and M. Sugiyama. [134] | 59 |
| D14 | Acoustic Wave Biosensor Technology for Probing the Effects of Gravity on Transcription. C. N. Jayarajah and M. Thompson. [135] | 59 |

E. Plant Development and Gravity Sensing

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| E04 | Differential Tissue-Specific Expression of a Western Red Cedar Dirigent Multigene Family in <i>Arabidopsis</i> : Phenolic Radical Coupling in Vascular Plants. M. Kim, J.-H. Jeon, L.B. Davin and N.G. Lewis. [137] | 61 |

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| E08 | Plant Circumnutations in Space: A Reappraisal of Time Sequences from the Spacelab-1 Experiment HEFLEX. T. K. Bardal, A. Johnsson and D.K. Chapman. [139] | 61 |
| E10 | Analysis of the Gravisensing System of <i>Chara</i> by Intracellular Magnetophoresis. O. A. Kuznetsov and K. H. Hasenstein. [140] | 62 |
| E12 | Effect of Slowly Rotating Clinostat on the Root System Development in Rapeseed (<i>Brassica napus</i>) Seedlings. J. Aarouf, P. Coulomb and G. Perbal. [141] | 62 |

F. Advanced Life Support and Biotechnology

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| F04 | MEXSY—A Modular Tool Kit for Life Science Experiments in Space. U.M. Kuebler and P. Kern. [143] | 64 |
| F06 | Evaluation of Nutrient Delivery System Design Concepts for Microgravity Using KC-135 Parabolic Flights. E.C. Stryjewski, I. Eraso, O. Monje, W.T. McLamb, D.W. Reed, R.N. Stuckey and G.W. Stutte. [144] | 64 |

G. Spaceflight Experiment Results

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| G08 | Human Parathyroid Hormone (1-84) Stimulates Bone Formation in Rat Bone Marrow Cultures During Spaceflight. D.R. Sindrey, D. Kusljic and P.C. Kwong. [148] | 66 |

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| H04 | Immunophenotype of Lymphocytes in Peripheral Blood of Apheresis Donors Mobilized by Granulocyte Colony-Stimulating-Factor (G-CSF). J.M. Reuben, B.-N. Lee, M. Korbling and W.T. Shearer. [150] | 68 |
| H06 | Effects of Lower Body Suction (LBNP) with Synchronous Graded Head- Down Tilting. H.G. Hinghofer-Szalkay, B. Haditsch and P. Pilz. [151] | 68 |

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| Minisymposium Current Ground-Based Models |
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15:30 – 17:00 Moderator: Marianne Cogoli-Greuter

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| 15:30 | Clinostats and Bioreactors. David M. Klaus. [152] | 70 |
| 16:00 | Human and Rodent Models. Didier Schmitt. [153] | 70 |
| 16:30 | Limitations of Models. Neal Pellis. [abstract unavailable] | |

ABSTRACTS

Symposium I
**Consequence of Contamination
of the Spacecraft Environment**

[1]

MICROBIOLOGICAL CONTAMINATION OF SPACECRAFT.

D.L. Pierson¹, R.J. Bruce², T.O. Groves², N.D. Novikova³, and A.N. Viktorov³. ¹NASA Johnson Space Center, ²Enterprise Advisory Services, Inc., Houston, TX, USA, and ³Institute of Biomedical Problems, Moscow, Russia.

The International Space Station (ISS) Phase 1 Program resulted in seven U.S. astronauts residing aboard the Russian Space Station Mir between March 1995 and May 1998. Collaboration between U.S. and Russian scientists consisted of collection and analyses of samples from the crewmembers and the Mir and Shuttle environments before, during, and after missions that lasted from 75 to 209 days in duration. The effects of long-duration space flight on the microbial characteristics of closed life support systems and the interactions of microbes with the spacecraft environment and crewmembers were investigated. Air samples were collected using a Russian or U.S.-supplied sampler (SAS, RCS, or Burkard,) while surface samples were collected using contact slides (Hycon) or swabs. Mir recycled condensate and stored potable water sources were analyzed using the U.S.-supplied Water Experiment Kit. In-flight analysis consisted of enumeration of levels of bacteria and fungi. Amounts of microorganisms seen in the air and on surfaces were mostly within acceptability limits; observed temporal fluctuations in levels of microbes probably reflect changes in environmental conditions (e.g., humidity). All Mir galley hot water samples were within the standards set for Mir and the ISS. Microbial isolates were returned to Earth for identification of bacterial and fungal isolates. Crew samples (nose, throat, skin, urine, and feces) were analyzed using methods approved for the medical evaluations of Shuttle flight crews. No significant changes in crew microbiota were found during space flight or upon return relative to preflight results. Dissemination of microbes between the crew and environment was demonstrated by DNA fingerprinting. Some biodegradation of spacecraft materials was observed. Accumulation of condensate allowed for the recovery of a wide range of bacteria and fungi as well as some protozoa and dust mites.

[2]

CONTAMINATION OF SPACECRAFT ENVIRONMENT:

IMMUNOLOGICAL CONSEQUENCES. William T. Shearer.

Departments of Pediatrics and Immunology, Baylor College of Medicine and Texas Children's Hospital, Houston TX.

Space flight immunology is the study of human and animal immune responses under the conditions of space travel (stress, isolation, microgravity, containment, microbial contamination, radiation) or in suitable ground models. A large body of circumstantial evidence is suggesting that immune responses are altered by space travel itself or in ground models, possibly to the point of creating immune deficiency. Contamination of the spacecraft environment by microbial organisms made more virulent by space travel could be predicted to possibly tip the balance between opportunistic or latent infections and the host defense system. The detection system must include assessment of the 4 components of the immune system: 1) antibodies, 2) T-cells, 3) phagocytes, and 4) complement. Simple and accurate in-flight screening tests must be devised to test these components of immunity in functional assays such as: 1) specific antibody production, 2) T-cell responses to neo- and recall antigens, 3) neutrophil superoxide production, and bactericidal capacity of complement. By devising this system of surrogate markers to detect an immune imbalance during space travel, it would be possible to create a countermeasures program with agents of immunoreconstitution that would prevent the collapse of immune responses and restore normal immune function. Such agents include drugs, immunoglobulins, and stem cell autotransplants, all of which are currently being used in immunosuppressed patients on Earth. Exploration of alterations of immune responses in space is an essential component of interplanetary space travel.

(Supported by a grant from the National Space Biomedical Research Institute.)

[3]

MOLECULAR STUDIES OF MUTATION SUGGEST THAT THE EARTH'S SPACE ENVIRONMENT IS NOT MUTAGENIC.

B.W. Glickman, M. Khaidakov, and A. Mortimer^b. Centre for Environmental Health, University of Victoria, Victoria BC; ^bSpace Science Program, Canadian Space Agency, Ottawa.

Somatic mutation levels were measured and the nature of mutations characterised in five Russian cosmonauts with recent long-term spaceflight experience and four age-matched trainees using the clonal *HPRT* assay. *Hprt* mutant frequencies in both cosmonaut and trainee groups were very similar, 17.2 ± 0.6 and $17.6 \pm 4.7 \times 10^{-6}$, respectively. However, these values are about twice that of the age-corrected values established for healthy, unexposed subjects in Western countries (Tates *et al.*, 1991; Branda *et al.*, 1993), low lower than the *HPRT* mutant frequencies observed in cosmonaut samples in our previous study (Khaidakov *et al.*, 1997). A total of 124 mutant clones were sequenced and the mutational spectra in the cosmonauts and trainees were essentially similar. However, they were significantly different from the Western mutational spectrum ($p=0.031$ and 0.038), and exhibited a higher incidence of splice errors and complex mutations. These data suggest that the space environment is not genotoxic at the *HPRT* locus. Interestingly, the uniformly high *hprt* mutant frequencies observed in the Moscovite samples (Khaidakov *et al.*, 1997; Curry *et al.*, 1997; Jones *et al.*, 1995) indicate a higher mutagenic burden in Russia, possibly reflecting a combination of environmental and dietary factors.

[4]

PLANTS, PLANT PATHOGENS, AND MICROGRAVITY—

A DEADLY TRIO. J.E. Leach. Department of Plant Pathology, Kansas State University, Manhattan.

Plants grown in spaceflight conditions are more susceptible to colonization by plant pathogens. The underlying causes for this enhanced susceptibility are not known. Possible causes are that the formation of structural barriers or the activation of plant defense response components are impaired in spaceflight conditions. Either mechanism would result from altered gene expression of the plant. Because of the tools available, past studies focused on limited physiological responses or biochemical pathways. With recent advances in genomics research, new tools, including microarray technologies, are available to examine the global impact of growth in the spacecraft on the plant's gene expression profile. In ground-based studies, we have developed cDNA subtraction libraries of rice that are enriched for genes induced during pathogen infection and the defense response. Arrays of these genes are being used to dissect plant defense response pathways in a model system involving wild-type rice plants and lesion mimic mutants. The lesion mimic mutants are ideal experimental tools because they erratically develop defense response-like lesions in the absence of pathogens. The gene expression profiles from these ground-based studies will provide the molecular basis for understanding the biochemical and physiological impacts of spaceflight on plant growth, development and disease defense responses. This, in turn, will allow development of strategies to manage plant disease for life in the space environment.

**Concurrent Posters
I–A
Space Life Sciences Training Program
Undergraduate Student Poster
Competition**

[5]

A NOVEL RED-LIGHT-BASED PHOTSENSORY SYSTEM THAT MEDIATES POSITIVE PHOTOTROPISM IN *ARABIDOPSIS* ROOTS. N.J. Ruppel¹, R.P. Hangarter², J.Z. Kiss¹. ¹Botany Dept., Miami Univ., Oxford, OH 45056; ²Biology Dept., Indiana Univ., Bloomington IN 47405.

The interaction between light and gravity is critical in determining the final form of a plant. However, while phototropism has been well-characterized in stems and stem-like organs, there have been relatively few studies of root phototropism. Our experiments suggest that there are two photosensory systems that elicit phototropic responses in roots of *Arabidopsis thaliana*: a previously identified blue-light photoreceptor system mediated by phototropin (= NPH1 protein) and a novel red-light-based mechanism. Results from three independent types of experiments (i.e., time course, unilateral illumination, orientation studies with light from above and below) confirmed the novel red-light response. The phototropic responses in roots are much weaker than the graviresponse, which competes with and often masks the phototropic response. It was through the use of mutant plants with a weakened graviresponse that we were able to identify the activity of the red-light-dependent phototropic system. In addition, the red-light-based photoresponse in roots is even weaker compared to the blue-light response. While it appears that the positive phototropism is controlled by phytochrome, studies are in progress to determine which member(s) of the phytochrome family is involved in mediating this response. (Financial support was provided by NASA grant NAG 2-1017 and the Howard Hughes Summer Internship program at Miami University.)

[7]

THE EFFECTS OF MODIFIED BIOLOGICAL RESEARCH IN A CANISTER (BRIC) SPACEFLIGHT HARDWARE ON THE SURVIVABILITY AND DEVELOPMENT OF THE TOBACCO HORNWORM (*MANDUCA SEXTA*) M.R. Inzunza¹, K. Anderson², O. van den Ende². ¹Spaceflight and Life Sciences Training Program, ²The Bionetics Corporation, NASA Kennedy Space Center, FL.

Tobacco Hornworms (*Manduca sexta*) are used extensively in life sciences research. The *M. sexta* larva and pupa have been used in many endocrine and physiology studies. Determining the effects of gravity or microgravity on these *M. sexta* pupa systems is the focus of some biochemical research. In order to study the *M. sexta* in microgravity, a containment system was developed that would provide the *M. sexta* pupa with a safe and non-restrictive environment. The Biological Research in a Canister (BRIC) is flight hardware designed to accommodate various biological experiments for flight on the shuttle. It is small, passive and inexpensive. *M. sexta* pupa have already flown twice in the BRIC hardware. The initial spaceflight proved that modifications to the BRIC were necessary to allow an acceptable amount of gas exchange for the *M. sexta* due to their sensitivity to environments with low oxygen (O₂) and high carbon dioxide (CO₂). The unmodified BRIC does not allow for ample gas exchange because it traps ambient CO₂ in the BRIC. The BRIC hardware was modified from both lids being solid to both lids being perforated. The new modified lids are covered with gas permeable, hydrophobic membranes. These experiments will determine the effects of increased CO₂ and decreased O₂ on the *M. sexta* survivability and development in the modified and non-modified BRIC's. Conclusive analysis indicates that *M. sexta* pupae exposed to decreased O₂ and increased CO₂ levels in the non-modified BRIC slows or stops the pupa's development. *M. sexta* pupa in the modified BRIC developed normally.

[6]

MOLECULAR EVOLUTIONARY PATTERNS IN MICROBIAL NATURAL PRODUCT BIOSYNTHETIC GENE AND ENZYME SEQUENCES: SEARCH FOR ADAPTIVE SIGNIFICANCE. H.E. Page¹, C.L. Peterson², and J.V. Lopez². ¹Florida Atlantic University, Boca Raton, and ²Division of Biomedical Marine Research, Harbor Branch Oceanographic Institute, Ft. Pierce.

Understanding gene and protein sequence evolution and their effects on enzymatic structure and function is important for the development of new pharmaceuticals, such as the polyketide class of antibiotics. Results from experiments performed aboard space shuttle flights have clearly indicated that microgravity can provide a beneficial environment for protein growth and presumably drug development. This project employed reverse genetics to study the phenomenon of prokaryotic evolution as an indirect vehicle of drug discovery. Because of their importance, the genes and gene products coding for the biosynthesis of natural products must adapt quickly to environmental pressures. Besides genetic mutation, it is hypothesized that horizontal transfer and positive selection of natural product biosynthetic genes play important roles in microbial evolution, and that significant sequence and structural differences between shallow and deep-water homologues will exist if enzymes of deep-water microbes have had to adjust to increased hydrostatic pressures. These phenomena can be detected by modern molecular methods and inferred based on newly derived and known sequence data. This same logic would be applicable to microbes in a microgravity environment, since they are expected to adapt to a reduced pressure system. To test this hypothesis, new bacterial strains from diverse marine sponges were isolated and inherent polyketide synthase gene fragments were PCR-amplified and cloned. Biosynthetic and housekeeping loci were compared to determine unusual patterns of evolution and infer protein structural differences from shallow versus deep sources.

[8]

SYNAPTIC INNERVATION IN RAT UTRICULAR MACULA. A. Chu and A. Lysakowski*. Dept. of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago IL 60612.

The afferent innervation pattern of the utricular macula has been examined in a previous study (Fernández et al., 1990). Afferents were found to be of three types. Calyx afferents constitute ≈6% of the total afferents, are restricted to the striola and are probably comparable to "M" fibers (Ross et al., 1986). Dimorphic afferents constitute ≈92% of the total and are found throughout the sensory epithelium. Bouton afferents comprise a relatively small ≈2% of the total population and are found in the extrastriolar region. Given this diversity of afferent innervation, we are interested in the regional pattern of normal synaptic innervation in the adult rat, to provide background data to interpret our hypergravity experiments. Hypergravity experiments are being done to study synaptic plasticity, and we are using a variable linear force to prevent adaptation in the irregular afferents.

Multiple samples were taken from each rat utricular macula. Each sample spanned the entire sensory epithelium and included material from all three regions: striola, juxtastricola, and medial and lateral extrastriola. Dissector counts of synaptic ribbons and calyceal invaginations were made as described previously in a study of the chinchilla crista ampullaris (Lysakowski and Goldberg, 1997). Preliminary results from utricular macula indicate that there are slightly lower numbers of synaptic ribbons per hair cell in the rat and that these numbers do not vary by region, but that calyceal invaginations are more numerous in the striola, compared to the extrastriola. Synaptic ribbons in type II hair cells are larger and occur in clusters more often in the striola compared to the extrastriola, similar to our results in the crista.

We will also present preliminary results of our hypergravity experiments.

(Supported by NASA NAG5-4593 and NIH R01 DC2521.)

[9]

THREE-DIMENSIONAL RECONSTRUCTION AND ANALYSIS OF ROOT CAP STATOLITH DISTRIBUTION IN *ARABIDOPSIS THALIANA*. R. Ehsanian^{1,2}, D.K. Bruck¹, J.D. Smith³. ¹Dept. of Biology, San Jose State University, San Jose CA; ²Lockheed Martin Space Operations, Moffett Field CA; and ³NASA Ames Research Center, Moffett Field CA.

Living organisms have evolved mechanisms for sensing and reacting accordingly to the Earth's gravity. Specialized gravity-sensing cells, statocytes, serve the important function of providing higher plants with the directional cue needed for proper growth and development. Amyloplasts in the statocytes of the root cap are redistributed due to changes in the gravitational environment leading to the hypothesis that they function as the statoliths for higher plants. The overall focus of this research in our lab was to determine the mechanisms by which plants adapt to changes in the gravitational environment. We hypothesized that the position and size of the amyloplasts would change due to the change in the gravitational environment. The hypergravity research facilities at NASA Ames Research Center were used to expose seedlings of *Arabidopsis thaliana* to chronic hypergravity stimulation. By means of the ROSS program at the Ames Center for Bioinformatics, three-dimensional reconstructions made from electron micrographs were used to elucidate relative intracellular and intercellular positions of the amyloplasts. This method is less subjective and more accurate than traditional morphological techniques used to reconstruct and analyze ultrastructure. The ability to visualize and measure the entire cell in a three dimensional environment helps to determine the effects of gravity on amyloplast position with much greater certainty than through traditional morphometric methods. The results of these analyses help show how plastid position is related to size, and thus lead us to a mechanism by which higher plants adapt to changes in the gravitational environment.

This research was supported by NASA Ames Research Center.

[11]

MOLECULAR PROFILING OF PLANKTONIC AND BIOFILM MICROBIAL COMMUNITIES IN HYDROPONIC GROWTH SYSTEMS. E. Nunez, J. L. Adams and M. S. Roberts. Dynamac Corporation, ALS Controlled Biological Systems Group, Kennedy Space Center FL 32899

Microbial biofilms have been shown to occur in association with the surfaces of many plant species. Included among these are candidate crops under evaluation by the Advanced Life Support (ALS) program for use in long-term space missions. Long-term space missions will require the capability to grow food in space and to recover nutrients, water, and air from recycled waste. A viable alternative for food production and resource recovery in space utilizes hydroponic nutrient-delivery systems. The high density of plants within an enclosed hydroponic system may, however, promote the development of biofilms which may compete with plants for nutrient uptake or harbor potentially pathogenic organisms. The presence of potential pathogens within a bioregenerative system could indirectly affect the efficiency of the life support system by impairing plant growth or directly impact astronaut health by allowing the growth of human pathogens. We describe here our initial efforts to quantify the population size, species richness and community composition of the biofilm component in the planktonic and biofilm communities of wheat crops in ALS hydroponic growth systems. Microbial diversity within these systems was evaluated using molecular-based techniques to profile microbial diversity in both sessile and planktonic populations. Biofilm-traps incorporating porous, scintered glass beads (Siran Carriers; Jaeger Biotech Engineering, Inc.) were used to sample nutrient solution biofilms. Planktonic and biofilm populations from each of three nutrient solution treatments (Hoaglands, filtered compost leachate, and unfiltered compost leachate) were evaluated using Terminal Restriction Fragment Length Polymorphism (T-RFLP). This DNA-based approach for community analysis allows the detection of both dominant and non-dominant 'culturable' populations in addition to the characterization of 'non-culturable' bacteria. Our results indicate that there are distinct microbial communities inhabiting the planktonic and biofilm environments within the hydroponic growth systems.

[10]

PESTO GROUND CONTROL EXPERIMENTS: A GAS EXCHANGE SYSTEM FOR MEASURING PHOTOSYNTHESIS AND EVAPOTRANSPIRATION. T. T. Tran¹, O. Monje², and G.W. Stutte². ¹University of Illinois, Urbana, IL ²Dynamac Corporation, Kennedy Space Center, FL.

The PESTO (Photosynthesis Experiment System and Testing and Operation) spaceflight experiment aims to measure photosynthesis and transpiration in microgravity. A four-chamber gas exchange system was designed, programmed, and assembled for conducting PESTO ground control experiments. The gas exchange system will measure ground-based data to be used for comparison with microgravity-based data obtained in the BPS (Biomass Production System, Orbitec, Madison, WI) aboard the International Space Station. The gas exchange system consists of a mixing tank, a plant growth chamber, mass flow meters, and two infrared analyzers to measure the CO₂/H₂O concentrations. The mixing tank allows the concentration of CO₂ to be varied between 300 to 2000 µmol/mol with +/- 10 µmol/mol accuracy. The mass flow of air into the chambers ranged between 2-8 Liters per min. CO₂ recovery tests and measurements of chamber leak rates were performed. In addition, relative measures of net photosynthesis and transpiration of wheat canopies grown at 3 different water potentials were made using the gas exchange system. These measurements agreed with measures of net assimilation rate and leaf area index obtained from growth analysis data.

(This work was supported in part by NASA's Fundamental Biology Program (NCC-0034) and Spaceflight and Life Sciences Training Program.)

[12]

THE 16TH ANNUAL SPACEFLIGHT AND LIFE SCIENCES TRAINING PROGRAM AT KENNEDY SPACE CENTER, FLORIDA. S. Potter¹, Dr. Walter Hill⁴, P. Currier², G. Koerner³, J. Rebmann³, Dr. Al Schlundt⁴, ¹NASA, Kennedy Space Center (KSC), ²The Bionetics Corporation, ³Dynamac Corporation, ⁴Tuskegee University, College of Agricultural, Environmental, and Natural Sciences.

The six-week Spaceflight and Life Sciences Training Program (SLSTP) teaches students how to successfully design and conduct biological research and operations in space, and how to assess the environmental impacts of a launch site. Students gain hands-on research and team experience both in the laboratory and in the field.

Thirty students (including two from the Canadian Space Agency) explored current research methods and opportunities in space life sciences with NASA researchers and engineers that are developing flight and ground-based experiments. The students learned biological, hardware, and management operations for life science payloads on the shuttle and International Space Station. Other hands-on studies included various experiments in Advanced Life Support bioregenerative life support, and ecological studies that assist in preserving the 147,000 acres of wetlands habitat that surround the KSC facility.

The curriculum also provided an overview of the field of space life sciences. The students were instructed on NASA's future long-term space exploration objectives, how life science plays an integral role in all of these endeavors, and how this mission can only be accomplished through collaborations and teamwork.

Students received six semester hours of college credit from Tuskegee University, NASA's primary academic partner in this program. The SLSTP is sponsored by NASA Headquarters' Life Sciences Division, Office of Spaceflight, and Minority University Research and Education Division; and NASA's Kennedy Space Center.

**Concurrent Posters
I–B
Graduate Student Poster Competition**

[13]

THE EFFECT OF PLASTID MUTATIONS ON GRAVITROPISM OF ROOTS, HYPOCOTYLS, AND INFLORESCENCE STEMS OF *ARABIDOPSIS*. K. Yamamoto, J. Z. Kiss. Department of Botany, Miami University, Oxford OH 45056.

Gravity is one of the stimuli that is important in plant growth and development. The sites of gravity perception are the columella cells in roots and endodermal cells in hypocotyls and inflorescence stems. Since plastids likely play a role in these cells for graviperception, we investigated gravitropism in plastid mutants of *Arabidopsis*. The *arc* (accumulation and replication of chloroplasts) mutants (*arc6* and *arc12*) are known to have variations in plastid number, size, and morphology in leaf mesophyll cells. However, in this study, we observed plastid mutations not only in mesophyll cells but also throughout the entire plant body. Seedlings and inflorescence stems of both wild-type and *arc6* were fixed and sectioned for anatomical studies. Plastid numbers per cell were examined in these sections with light microscopy. In the wild-type, an average of 5 plastids was observed per cell in columella and endodermal cells. However, in *arc6*, an average of only 1 - 2 plastids was found, and these were larger in size compared to those of the wild-type. Time course of curvature studies were conducted with seedlings and inflorescence stems of *arc12*, *arc6*, and their wild-types. Results demonstrated that gravisensitivity differed depending on the plant organ even though mutations were expressed through the entire plant body. The organs that demonstrated inhibition of gravisensitivity were as follows: hypocotyls of light-grown seedlings and inflorescence stems of *arc6*, roots of light-grown seedlings and inflorescence stems of *arc12*. Studies are currently in progress to clarify the effects of variation in number and size of plastids on gravitropism in *Arabidopsis*.

(Special thanks to Dr. Kevin Pyke for providing the *arc* mutants and to NASA grant NAG 2 - 1017 for financial support.)

[14]

GAUGING THE INTERNAL GAS CONTENT OF *BRASSICA RAPA* SILIQUES GROWN IN SPACE. K.L. Wilsen, J. Blasiak and M.E. Musgrave. Biology Department, University of Massachusetts, Amherst, MA 01003.

Experiments conducted previously on the Mir space station revealed that although *Brassica* siliques formed normally in the microgravity environment, both the ripening process and seed quality were affected. Because the internal atmosphere of gases in the silique is of great importance for normal seed development, we are developing a protocol to determine the internal gas content of *Brassica* siliques grown on board the space station. The goal is to establish the combined effects of microgravity, the man-made cabin atmosphere and lack of convection on the relative concentrations of gases inside a maturing silique. Compared to ambient atmosphere (21% O₂, 325 ppm CO₂, <5 ppb ethylene), the atmosphere around developing seeds inside the silique is reduced in O₂ (7-12%) and highly elevated in CO₂ (6000-8000 ppm) and ethylene (3-12 ppm).

Further, we will devise a method for sampling and preserving siliques in such a manner that gas profiles of the preserved material, determined by means of gas chromatography, are representative of gases present at the time of sampling. Eventually, this technology will be used to gauge the internal gas concentrations of siliques grown and harvested on ISS. Post-flight analyses of gases must reflect real time gas compositions of plants growing on board. Ultimately, this information will be used to understand the factors inhibiting healthy plant reproduction in space.

(Supported by NASA: NAG2-1375.)

[15]

STRESS RESPONSE TO MAGNETIC LEVITATION (LOW-GRAVITY) AND HIGH MAGNETIC FIELDS IN TRANSGENIC *ARABIDOPSIS*. A.N. Morgan¹, J. Yowtak¹, R.J. Ferl³, J.S. Brooks², A.-L. Paul³, and M.W. Meisel¹. ¹Dept. of Physics and NHMFL, Univ. of Florida, Gainesville; ²Dept. of Physics and NHMFL, Florida State Univ., Tallahassee; and ³Dept. of Hort. Sci. and Biotech. Program, Univ. of Florida, Gainesville.

Genetically engineered *Arabidopsis thaliana* provides a means to examine the effects of low-gravity and strong magnetic fields at the cellular level. The plants are engineered with a transgene gene containing the *Adh* promoter and GUS reporter gene. The *Adh* gene is sensitive to a variety of environmental stresses and induces GUS expression in stressed tissues. GUS activity is evaluated qualitatively for cellular distribution by staining the plant with an appropriate substrate to produce a blue color in tissue regions where there are concentrations of the GUS enzyme. A quantitative measure of GUS activity in the plant tissue is obtained using spectrofluorometric assays. *Arabidopsis* plants were magnetically levitated in magnetic field gradients to simulate a milli-gravity ($\approx 10^{-3}g$; $g=9.8 \text{ m/s}^2$) environment. Control specimens were exposed to earth's gravity (*i.e.* 1 g) and 2.5 hours of a homogeneous magnetic field from 0 to 25 Tesla. High levels of GUS activity were found in both levitated plants and plants that experienced strong homogeneous magnetic fields. For the homogeneous field specimens, increased GUS activity is found above fields of 17 Tesla in both leaf and root tissue, with the GUS activity in the leaf tissue approaching the levels seen during hypoxic stress induction. Qualitative and quantitative data will be presented and the potential use of magnetic levitation as an earth-based low-gravity environment for staging preliminary experiments will be discussed.

(Research supported, in part, by the National Science Foundation through the In-House Research and REU Programs of the National High Magnetic Field Laboratory (NHMFL) and by NASA: NAG10-0145.)

[16]

CHANGES IN OSTEOPROGENITOR PROLIFERATION IN THE RAT SKELETON DUE TO MECHANICAL UNLOADING. N. Basso, Y. Jia, C.G. Bellows and J.N.M. Heersche. Faculty of Dentistry, University of Toronto, Toronto.

A lack of mechanical stimulation associated with space flight or tail suspension in rats results in a reduction in bone matrix production and bone formation. This may be due to a disruption/delay of the development of cells along the osteoblast lineage pathway. Our experiments address specifically the effects of *in vivo* unloading conditions on the proliferative capacity of osteoprogenitors. Using the NASA model of tail suspension, hind limbs of three-month-old female Sprague-Dawley rats were unloaded for 14 days. Normally loaded control rats were pair-fed. Explants were prepared from the proximal femur, calvarium, and proximal humerus and outgrowth cells were collected after 13 days and used to initiate experiments. Cells were plated at a density 3000 cells per dish and grown in medium either unsupplemented or supplemented with 10 nM dexamethasone (dex) or 3 μ M progesterone (prog). Preliminary data revealed no significant difference in the number of fibroblastic colony forming units (CFU-F, a measure of total number of progenitors) between cell populations derived from suspended and control rats in any of the cell populations examined. We observed a decreased number of alkaline phosphatase positive progenitors (CFU-AP) to CFU-F observed in cells isolated from femur (dex treated) and calvarium (all treatment groups), but not in humerus, of suspended animals. The data suggests that hind limb unloading results in a decreased proportion of CFU-AP and that dex-dependent progenitors are affected by unloading to a greater extent than prog-dependent progenitors in femur and calvarium. Evaluation of bone nodule numbers, indicative of CFU-osteoblast (O), and of bone histomorphometry to evaluate bone formation parameters is in progress.

[17]

???TROPISM: WHEN A PRIMARY ROOT ENCOUNTERS A BARRIER TO DOWNWARD GROWTH. G.D. Massa and S. Gilroy. Dept of Biology, Penn State University.

In nature, roots may encounter various obstacles as they grow through soil. To examine this interaction of roots with rigid barriers, experimental protocols have been designed and tested using *Arabidopsis thaliana*. A growing root appears to sense an obstacle, possibly by integrating gravity and touch stimuli, and a growth response is initiated. The response requires reorientation of the growing root tip until unobstructed vertical growth can resume. As the root interacts with the barrier, different regions of the root coordinate, letting the tip maintain a set angle while bend formation allows elongation to occur parallel to the obstacle surface. We have studied the response kinetics of *Arabidopsis* roots growing under these conditions, and have found that root growth rate is unaltered by interaction with a barrier. The angle of the root tip, however, changes from 90° (vertical) to a set angle of approximately 140° as the root tracks across the surface. In addition, other species were examined and their responses to barriers support conclusions drawn from *Arabidopsis*. Cellular localization of sensing was examined using laser ablation, which allowed the selective removal of peripheral cap and columella cells, and demonstrated the importance of both cell types for a normal ???tropic response. Using *Arabidopsis* plants transformed with ion-sensitiveGFP's we are examining the fluorescence patterns during ???tropic and are comparing these with the effects of isolated gravity and touch stimuli. Suggestions for naming the ???tropic response will be solicited.

This research was supported by NSF.

[19]

MATERNAL BEHAVIOUR UNDER HYPERGRAVITY CONDITIONS IN CD 1 MICE. M Simeoni, D Santucci and E Alleva. Behavioural Pathophysiology Section, Laboratorio di Fisiopatologia di Organo e di Sistema, Istituto Superiore di Sanita, Viale Regina Elena 299, I-00161 Rome Italy.

Investigations in the micro-gravitational environment of space will potentially provide us with new insights into biological processes, as well as a capacity to safeguard the health of animals and humans in future space ventures. Hyper- or micro-gravity induced changes in maternal behaviour will in part determine the mother's success in rearing young under these conditions. To identify maternal behavioural items vulnerable to altered gravity we examined the effects of hypergravity exposure during lactation in mice, a species whose small body size makes them particularly suitable for use in space biology. Primiparous and multiparous CD-1 females were exposed with their pups to a centrifugal force of 2g for 1 hour daily from postnatal day (PND) 3 to 10, and detailed behavioural observations recorded on PND 3, 5, 7 and 10 over the hour before, during, and after rotation. Preliminary analyses (PND 3 and 5) indicate that certain maternal behaviours such as licking the pups and nest-building are repressed under hypergravity conditions. Results also suggest that maternal experience is a factor in the behavioural response to hypergravity, with terziparous dams spending less time in contact with pups than primiparous mothers while rotating on PND 5. A detailed understanding of the behavioural response to hypergravity in nursing mothers will aid in our attempts to optimise the nest environment in future altered-gravity experiments on space missions.

[18]

RAPID DISCRIMINATION AMONG INDIVIDUAL DNA MOLECULES IN MICROLITER VOLUMES. W. Vercoutare¹, S. Winters-Hilt², H. Olsen¹, D. Deamer¹, D. Haussler² and M. Akeson^{1,2*}. ¹Department of Chemistry and Biochemistry, and ²Center for Biomolecular Science and Engineering, University of California, Santa Cruz 95064.

[We are developing a lightweight instrument that can discriminate between individual DNA molecules. The apparatus consists of two 70- μ l chambers separated by a lipid bilayer barrier in which a single protein channel α -hemolysin is inserted; a potential is applied and the resulting ionic current through the channel is recorded by standard patch-clamp instrumentation. A signature current blockade is produced each time a molecule of DNA enters the pore. Hundreds of DNA molecules can be detected in a minute-long recording. Recognition of current signatures and identification of DNA is done in part by using a machine learning algorithm called a 'Support Vector Machine'. This system can be used to distinguish differences in length, composition and concentration of DNA. No modification of DNA is necessary, unlike other high-resolution DNA techniques. Most recently we used DNA hairpins to model duplex DNA, and have shown that single base-pair and single nucleotide differences can be resolved. This instrument would be useful for detecting damage to DNA caused by radiation. The size of this device and the sensitivity to small differences in DNA make this promising for use in laboratories with close confines such as a space station. (Supported by NASA: NAG5-9403; and NIH: HG01826-01.)

[20]

CONTRIBUTION OF SYMPATHETIC ACTIVITY TO LYPOLYSIS DURING EXPOSURE TO INCREASING HYPERGRAVITY LOADS. ^{1,2}M.M. Moran, ³T.P. Stein and ¹C.E. Wade. ¹NASA Ames Research Center, Moffett Field, CA 94035, ²San Jose State University, San Jose, CA 95192, and ³University of Medicine and Dentistry of New Jersey, Stratford, New Jersey 08084.

An increase in catecholamine release from the sympathetic nerves results in lypolysis. The focus of this study was to determine the role of sympathetic catecholamines in the fat loss that occurs during exposure to hypergravity. The study was conducted using 150 g male Sprague-Dawley derived albino rats. Rats were centrifuged at either 1.25 G (n=8), 1.5 G (n=16), 2 G (n=8), or remained at 1 G (n=16). Epididymal fat pads were weighed on day 14 on the study, and urinary epinephrine (E) and norepinephrine (NE) were collected daily and individual samples were pooled. Mean urinary E (picoMoles/day) on days 11-14 in the 2.0 G group (3807 \pm 322) was significantly (p \leq 0.05) higher than the 1.5 (2179 \pm 243), 1.25 (1676 \pm 86), and 1.0 (1749 \pm 77) G groups. There were no differences in urinary E between the 1.5, 1.25, and 1.0 G groups. Mean urinary NE (picoMoles/day) on days 11-14, was significantly higher in the 2.0 G group (12888 \pm 774) than the 1.5 (8149 \pm 604), 1.25 (5743 \pm 301), and 1.0 (6258 \pm 426) G groups. NE from the 1.5 G group was higher than the 1.25 and 1.0 G groups and no differences were found in NE between the 1.25 and 1.0 G groups. Mean epididymal fat mass (g/100 g body mass) in the 2.0 (0.694 \pm 0.05), 1.5 (0.780 \pm 0.2), and 1.25 (0.802 \pm 0.04) G groups were significantly less than the 1.0 G (0.905 \pm 0.02) group. The inverse relationship between fat mass and catecholamines suggest that an increase in sympathetic activity contributes to the loss of fat mass that occurs during exposure to hypergravity.

[21]

STARCH CONVERSION TO SUCROSE AT NIGHT. S.E. Weise, and T.D. Sharkey. Dept of Botany and Wisconsin Center for Space Automation and Robotics, Univ of Wisconsin-Madison.

During photosynthesis fixed carbon is partitioned either as starch in the chloroplast or as sucrose in the cytosol. Transitory starch is synthesized in chloroplasts during the day and is broken down at night and exported to the cytosol to make sucrose. The pathway for transitory starch breakdown, export from the chloroplast, and sucrose synthesis at night is currently unknown. We have isolated chloroplasts from bean leaves one hour after onset of the dark portion of the photoperiod and analyzed the sugars and sugar phosphates that were exported into the surrounding medium after a 2.5 hour incubation in the dark. We found that maltose and/or another glucose containing disaccharide was exported at a rate of $58 \pm 11 \text{ nmol mg}^{-1} \text{ chl hr}^{-1}$, and glucose was exported at a rate of $8 \pm 4 \text{ nmol mg}^{-1} \text{ chl hr}^{-1}$. We found no G6P, F6P, or Fructose present. We observed a small amount of G1P present continually ($10 \pm 3 \text{ M}$). Exogenous glucose seems to have an inhibitory effect on maltose export while exogenous ATP seems to have the opposite effect, stimulating maltose export. When chloroplasts are incubated with radio labeled glucose a small amount of the labeled glucose is found to be incorporated into the exported maltose. From these results and earlier work by other groups we present a hypothesized pathway for transitory starch breakdown at night. This work is the first step in a larger project to better understand plant carbohydrate metabolism regulation in day vs. night. This will provide insight into engineering continuous photoperiod tolerance, which will be of great importance in future Advance Life Support Systems.

**Concurrent Posters
II-A
Space Life Sciences Training Program
Undergraduate Student Poster
Competition**

[22]

MAGNETIC LEVITATION AS A LOW GRAVITY ENVIRONMENT. J. Yowtak¹, A.N. Morgan¹, R.J. Ferl³, J.S. Brooks², A.-L. Paul³, and M.W. Meisel¹. ¹Dept. of Physics and NHMFL, Univ. of Florida, Gainesville; ²Dept. of Physics and NHMFL, Florida State Univ., Tallahassee; and ³Dept. of Hort. Sci. and Biotech. Program, Univ. of Florida, Gainesville.

Several means, such as parabolic flights, drop towers, and clinostats, have been used to produce earth-based hypogravity environments. Another method to achieve a milli-gravity ($\approx 10^{-3}g$; $g = 9.8 \text{ m/s}^2$) environment for extended periods of time is magnetic levitation of diamagnetic materials. Under these conditions, the net magnetic force on the levitated object balances the force of gravity the object experiences, resulting in a net force of zero. It is noteworthy that water and many organic materials are diamagnetic and, therefore, may be magnetically levitated. The simple physical explanation of this process will be described in order to communicate the potential of using this technique for a variety of pre-flight staging experiments. Specifically, transgenic *Arabidopsis thaliana* plants have been magnetically levitated using resistive magnets at the NHMFL to investigate the effects of this type of low-gravity environment on the transgene (*Adh/GUS*) expression (see abstract of Morgan, *et al.* ASGSB-CSA-ELGRA). The *Adh/GUS* transgene is sensitive to microgravity and other environmental stresses (see abstract of Ferl *et al.* ASGSB-CSA-ELGRA). In support of those investigations the magnetic susceptibilities of the various parts of the plant tissue have been measured and have been used to model the forces experienced by a levitated plant. A computer simulation provides a resultant force value of each element of the specimen, and, when summed together, these elements balance the gravitational pull to produce levitation. An estimate of the actual forces may be obtained and, depending on the physical size of the specimen, yield values on the order of milli-g or less.

[Research supported, in part, by the NSF through the In-House Research and REU Programs of the National High Magnetic Field Laboratory (NHMFL).]

[23]

MOLECULAR MAPPING OF THE LAZY-2 GRAVITROPIC RESPONSE GENE OF TOMATO. J. Well, A. Madlung, K. Krutovskii R. Meyer, T.J. White, and T.L. Lomax. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis OR 97331-2902

Shoots of the single gene *lazy-2* (*lz-2*) mutant of tomato exhibit a reversed gravitropic response in the presence of red light. Our laboratory has previously shown this phenotype to be regulated by the red light photoreceptor, phytochrome (Gaiser, J.C. and Lomax, T.L. 1993. *Plant Physiol.* 102: 339-344). Isolation of the corresponding gene would provide a unique opportunity to elucidate the mechanism by which light and gravity interact to regulate plant architecture. We are attempting to isolate the *Lz-2* gene using a map-based cloning strategy. Using a mapping population generated by crossing the *lz-2* mutant in *Lycopersicon esculentum* (domestic tomato) with *L. pennellii* (a wild tomato relative), we have localized the *Lz-2* gene to the centromeric region of chromosome 5 in tomato (Behringer, F.J. and T.L. Lomax 1999. *J. Heredity* 90: 489-493). Proximity to the centromere complicates mapping due to reduced recombination, therefore we have generated a new backcross mapping population using *L. pimpinellifolium*, a more closely-related wild tomato species. Here, we report the results of screening that population with molecular markers that are closely linked with the *Lz-2* gene. In a parallel approach to identifying the *Lz-2* gene, we are also using transposon tagging with an *Ac/Ds* transposon insertion that is closely linked to the *Lz-2* locus. Analysis of the tagged progeny for the *lazy-2* mutant phenotype will be described.

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

[24]

THE STABILITY OF LIQUID WATER IN POROUS ROCKS IN A MARS-LIKE ENVIRONMENT. C. Paty¹, C. McKay², D. Catling³, J. Heldmann⁴. ¹Physics and Astronomy, Bryn Mawr College, PA, ²PhD AstroGeoPhysics, University of Colorado, ³PhD Atmospheric, Oceanic and Planetary, Physics, University of Oxford, ⁴MS Space Studies, University of North Dakota.

On Mars today, low temperature and pressure limit the stability of liquid water. The effects of pore sizes and atmospheric pressure on liquid water in rocks were studied to examine the possibility of liquid water existing on Mars inside rocks and in pore spaces in the soil. Porous rocks from the Antarctic and from the Negev Desert (Israel) were used along with the simulated Mars dirt JSC Mars-1 and a control material of known pore size distribution. The relationship between relative humidity and the water-containing pore size in equilibrium (i.e. not evaporating) was determined by placing the samples in salt solution controlled relative humidity chambers and measuring their water mass at equilibrium. The relationship between atmospheric pressure and water stability in pores was then determined by placing the same samples in a pressure chamber with only water vapor present on a scale and measuring their water mass at equilibrium. Due to the effects of small pores on the surface tension, and hence the vapor pressure, of water, it is possible that the temperature and pressure requirements for liquid water stability in pores are significantly different than those for flat surfaces.

(Supported by the NASA Ames Astrobiology Academy.)

[25]

CHANGES IN STATOCYTE STRUCTURE AND AMYLOPLAST STARCH IN *ARABIDOPSIS THALIANA* COLUMELLA CELLS AFTER GROWTH UNDER HYPERGRAVITY CONDITIONS. S.D. Hopkins¹ and J.D. Smith². ¹Lockheed Martin Space Operations, Moffett Field CA, ²NASA Ames Research Center, Moffett Field CA.

The purpose of this morphological study was to determine if amyloplast starch content and size were reduced in *Arabidopsis* root caps after growth in a hypergravity environment. During a past space flight experiment it was shown that, in space and on a clinostat, statolith mass in white clover (*Trifolium repens*) seedlings was dependent on the gravity environment; in an under-stimulated environment (i.e. space flight) it was shown that the volume of statocyte amyloplasts was increased [Smith et al., (1997) *Plant J.* 12:1361-1373]. With that in mind we propose the hypothesis that in an over-stimulated environment (i.e. hypergravity on a centrifuge) the statocyte amyloplast volume would decrease. *Arabidopsis* seedlings were grown on vertical agar plates in an incubator at 25°C for two days under constant illumination from fluorescent lights. After 48 hours the fluorescent lights in the incubator were turned to the off position and the experimental groups were taken to the NASA Ames 1 ft. diameter centrifuge where they were subjected to either 2 g, 4 g or 8 g chronic centrifugation at 25°C in total darkness. After 48 hours of exposure to hypergravity the seedlings were chemically fixed, and the root caps were excised and processed further for transmission electron microscopy. Negatives of longitudinal root tip images were scanned and quantitative morphometrics were completed using NIH Image. Initial observations have shown that the overall root cap and the gross plant morphology remained unchanged by chronic exposure to hypergravity. Preliminary morphometric analysis of columella cell stories 2 and 3 for the 1 g and 8 g samples showed no significant difference in amyloplast starch content; however, the area fraction of amyloplast per cell was changed. Further analysis is underway to substantiate these findings. Continuing investigations will focus on vacuole, mitochondria and nucleus area fractions as well as comparisons between cell story and statocyte development.

(This research was supported by NASA Ames Research Center.)

[26]

CARBOHYDRATE DEPOSITION IN *RAPHIANUS SATIVUS* L. CV. CHERRY BELLE SHOOTS: PRELIMINARY GROUND STUDIES FOR THE RASTA SPACEFLIGHT EXPERIMENT. H. N. Goldsmith¹, E. C. Stryjewski², G. W. Stutte², W. McLamb³, D. Reed³. ¹Brown University, Providence, RI, ²Dynamac Corporation and ³Bionetics Corporation, Kennedy Space Center, FL.

As part of ground-based testing for the RASTA (Radish Assimilation in Spaceflight Testbed Atmosphere) experiment, amyloplast sedimentation and carbon partitioning in *Raphianus Sativus* L. cv. Cherry belle were characterized for future microgravity studies. Radish plants were grown on a clinostat to simulate microgravity and reduced gravity (.005g), and at 1g. Plants were harvested at 3, 4, 5, 6, 7, and 8 days after planting (DAP) and the stems were fixed, dehydrated, and embedded in paraffin. The samples were then sectioned (12 µm) and stained with a Schiff/PAS reaction to reveal starch location. Three and 4 DAP plants were found to contain too much starch to determine sedimentation whereas these resources were depleted in 7 and 8 DAP plants. Five and 6 DAP plants contain adequate levels of starch for sedimentation studies. Analysis to determine a minimum gravity level for amyloplast sensing revealed that starch sedimented in 1g as well as reduced gravity, but were dispersed in microgravity. Therefore the gravi-sensing system in radish is sensitive to a signal 1/200 the strength of unit gravity. Although starch in the endoderm did respond to gravity, the starch in the mesophyll did not. The starch of the mesophyll tissue was found to be dispersed in all 3 treatments, indicating that these are storage tissues and not gravity sensitive. The amount of starch in the mesophyll, however, was greater in 1g than in microgravity, suggesting that less starch reserves for radish growth may be available to the plants in space.

(This work was supported in part by NASA's Fundamental Biology Program (NCC-0034) and Spaceflight and Life Sciences Training Program.)

[28]

DEVELOPMENT OF DEFINED MEDIA FOR RAPID PHYSIOLOGICAL PROFILING OF MICROBIAL COMMUNITIES. F.R. Perez,¹ J.L. Garland² and M.S. Roberts². ¹Dept. of Biochemistry, Univ. of Arizona; ²Dynamac Corp., KSC, FL.

Recycling of reusable resources will be vital in planning long-term space missions. Microbial communities, used for recycling within successful bioreactors, will have to be accurately characterized. One easy and rapid method for characterization is using physiological profiling. BiOLOG Inc. produces the MT2 plate designed to conduct such profiling of pure microbial cultures. The goal of this study was to develop an experimental plate more suitable for community-level profiling. Microbial communities were selected for their preference to four amino acids as carbon sources and then plated in the presence of different recipes of growth supporting nutrients (GSN), two redox dyes as carbon metabolism monitors, and different carbon sources. High concentrations of GSN lead to higher ($P < 0.05$) false response absorbencies (0.70 ± 0.06 nm; SD) compared to a low concentration of GSN (0.55 ± 0.05 nm) in control wells. There were no significant differences in the level of response between the different recipes used for the GSN comparing Buffered Mineral Salts (BMS) in the absence (1.0 ± 0.01 nm) or presence of yeast extract (1.0 ± 0.01 nm), casamino acids (0.93 ± 0.01) or both additions (0.90 ± 0.01 nm). In comparing redox dyes, tetrazolium violet (1.0 ± 0.01 nm) was more sensitive in experimental wells compared to methylthiazolylidiphenyl (0.45 ± 0.005 nm). MT2 plates (1.1 ± 0.015 nm) responded relatively nonspecifically to carbon sources compared to our experimental plates (0.43 ± 0.005 nm). Therefore the MT2 plates might not be functionally relevant for the profiling of microbial communities. These findings show that our experimental plates using BMS alone at a low concentration with tetrazolium violet dye allowed for physiological profiling whereas the MT2 commercial plates were less suitable.

(Supported by NASA's SLSTP at KSC and by the NIH through a MARC grant [GM08718-02] at Univ. of Arizona).

[27]

SOIL WATER POTENTIAL AFFECTS CROP GROWTH RATE OF WHEAT THROUGH CHANGES IN LEAF AREA, NOT PHOTOSYNTHESIS. H.-T. Wang¹, O. Monje² and G. W. Stutte². ¹BioServe Space Technologies, Boulder, CO ²Dynamac Corporation, Kennedy Space Center, FL.

Plant growth conditions must be optimized for use in advanced life support systems during space flight. Wheat cv. Apogee was grown at three soil water potentials (ψ_s): -0.5 kPa, -0.3 kPa, and -0.1 kPa for 24 days. Measurements of leaf area and dry mass were made at 5, 9, 14 and 18 DAP and used to determine the leaf area index (LAI), net assimilation rate (NAR), and crop growth rate (CGR). ψ_s did not have a significant effect on plant development until after 9 DAP, at which time growth was affected by ψ_s . Plants grown at -0.1 kPa exhibited the largest CGR and LAI and had the greatest biomass, suggesting that -0.1 kPa was the optimal ψ_s . These results are in contrast to those observed with Superdwarf wheat. Growth analysis indicated that changes in CGR were due to changes in leaf area, rather than changes in photosynthesis. There was no significant difference between chlorophyll fluorescence measurements: Fv/Fm, qN and qP of plants grown at different ψ_s , which further indicated that ψ_s had little effect on photosynthesis. Slight differences in starch content of plants grown at different ψ_s were observed. This study provided baseline data characterizing the effect of ψ_s on wheat growth for the PESTO experiment.

(This work was supported in part by NASA's Fundamental Biology Program (NCC-0034) and Spaceflight and Life Sciences Training Program.)

[29]

SLSTP 2000, CONTROLLED BIOLOGICAL SYSTEMS GROUP, STUDENT RESEARCH PROJECTS SUPPORT NASA'S BIOREGENERATIVE LIFE SUPPORT PROGRAM. D. Muhlestein¹, G. Koerner², ¹Crop Physiology Laboratory, Utah State University, ²Dynamac Corporation, Kennedy Space Center, FL. KSC Investigators: J. Adams, K. Corey, I. Eraso, P. Fowler, J. Garland, G. Goins, M. Hummerick, V. Kremins, H. Levine, O. Monje, M. Roberts, V. Rygalov, R. Strayer, L. Strejewski, G. Stutte, S. Young. Students: J. Bamford, A. Brown, R. Marino, L. Martinez, J. Metz, C. Nash, E. Nunez, F. Perez, T. Tran, H. T. Wang.

Thirty undergraduate students participated in the sixteenth annual Spaceflight and Life Sciences Training Program (SLSTP) held at Kennedy Space Center. Ten students worked with NASA and contract scientists on experiments related to controlled biological systems (CBS). The CBS group encompasses plant gravitational and space biology, and aspects of advanced life support research, using bioregenerative systems. Experimentation by CBS students covered wide areas of the continuum necessary for successful plant based life support, including areas of plant physiological response, microbial ecology, resource recovery, and engineering systems for growing plants on extended missions in space. In addition to lab activities students were exposed to seminars and tours encompassing all of the ongoing life science research at KSC and in other NASA and university programs. SLSTP is supported by NASA.

**Concurrent Posters
II-B
Graduate Student Poster Competition**

[30]

CHIMERIC CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE: ROLE OF THE NEURAL VISININ-LIKE DOMAIN IN REGULATING AUTOPHOSPHORYLATION AND CALMODULIN AFFINITY. P.V.Sathyarayanan, C.R.Cremo, W.F.Siems and B.W.Pooavaiah. Washington State University, Pullman, WA-99164.

Calcium/calmodulin-modulated proteins have been implicated in playing a role in the transduction of the gravity signal. Previously, we reported the expression of chimeric Ca^{2+} /calmodulin dependent protein kinase (CCaMK) in roots (J. Biol. Chem, 274:12001-8,1999). CCaMK is characterized by a serine-threonine kinase domain, an autoinhibitory domain, a calmodulin (CaM) binding domain and a neural visinin-like domain with three EF-hands. The neural visinin-like Ca^{2+} -binding domain at the C-terminal end of the CaM-binding domain makes CCaMK unique. Using EF-hand deletions in the visinin-like domain, we found that the visinin-like domain regulated Ca^{2+} -stimulated autophosphorylation of CCaMK. To investigate the effects of Ca^{2+} -stimulated autophosphorylation on the interaction with CaM, the equilibrium binding constants of CCaMK were measured by fluorescence emission anisotropy using dansylated CaM. Binding was eight-fold tighter after Ca^{2+} -stimulated autophosphorylation. This shift in affinity did not occur in CCaMK deletion mutants lacking Ca^{2+} -stimulated autophosphorylation. A variable CaM affinity regulated by Ca^{2+} -stimulated autophosphorylation mediated through the visinin-like domain is a new regulatory mechanism for CCaMK activation and CaM-dependent protein kinases (J. Biol. Chem, in press). Using MALDI-TOF mass spectrometry, threonine 267 was identified as the Ca^{2+} -stimulated autophosphorylation site of CCaMK. Our experiments demonstrate the existence of two functional molecular switches in a protein kinase regulating the plant kinase activity, namely a visinin-like domain acting as a Ca^{2+} -triggered switch and a CaM-binding domain acting as an autophosphorylation triggered molecular switch. These molecular switches may help this kinase in transducing calcium transients into phosphorylation signals in plant roots.

(Supported by NASA and NSF.)

[31]

DEVELOPMENT OF THE NERVOUS SYSTEM AND ITS CONTROL OF GRAVITY-DEPENDENT BEHAVIOURS IN LARVAE OF BIVALVE MOLLUSCS. J.T. Plummer, D.L. Jackson, and R.P. Croll. Dept. of Physiology and Biophysics, Dalhousie University, Halifax N.S. Canada.

The effects of microgravity on the behaviour of larval mussels (*Mytilus edulis*) were studied in the Aquatic Research Facility (ARF) during the ten-day STS-77 mission. Locomotion patterns observed in microgravity differed significantly from those seen in normal gravity. Larvae in microgravity frequently altered their swimming directions and the dimensions of their helical swimming paths. Gravity was determined to be the primary orientation cue for these animals, as no directional orientation was exhibited by larvae in space. These findings suggest that microgravity either unmasks behaviours not expressed in normal gravity or causes certain behaviours to developed abnormally. In an effort to understand these effects on behaviour, we are currently examining the development of the larval nervous system and its innervation of peripheral target tissues such as the locomotory cilia of the velum. To date we have used immunocytochemistry and other histochemical techniques to provide a comparative study of the normal positions and morphologies of neurones which contain a range of transmitters including serotonin, catecholamines and various peptides such as FMRFamide, APGWamide and small cardiac peptide (SCP) in bivalve larvae. We are also developing techniques to permit detailed studies of the statocysts, the gravireceptive organs in these organisms. This work on normal larval development in *M. edulis* and other bivalves will form the foundation for studies of the effects of microgravity on neural development in a future ARF flight.

(Funding provided by the Canadian Space Agency.)

[32]

INNERVATION OF RAT VESTIBULAR MACULAE IN HYPERGRAVITY; AN *IN VIVO* AND *IN VITRO* STUDY. S. Gaboyard, E.Scarfone, J. Lehouelleur and A. Sans. INSERM 432 Univ. Montpellier.

The primary captors of the peripheral vestibular system, the sensory hair cells are innervated by neurones of Scarpa's ganglion during the perinatal period. We have studied hair cell activity as an epigenetic factor influencing development and neuronal plasticity while modifying the gravitational field, the primary stimulus. In a new 3-D co-culture preparation, explants of vestibular maculae and ganglion from 3-day old rats are embedded in an extracellular matrix gel, permitting orientation and process growth in three dimensions. The preparation was flown on the Photon-12 mission. The time course of neurite regeneration has been presented previously (ARO 2000, 571). We submitted the co-cultures to hypergravity (2g). Neurofilament staining showed no change in the time course of neurite growth when compared to 1g. We also studied the *in vivo* effects of hypergravity on afferentation. Neurofilaments were stained in preparations taken from developing rats subjected to 2g. As *in vitro*, *in vivo* studies revealed no change in the time course of innervation. The unchanged time course of development agrees with morphological studies of synapse formation following weightlessness (Ross, M.D., J. Vest. Res., 1993), and electrophysiological studies of the major potassium conductances in hair cells *ex vivo* (Lennan, G.W.T., 749, ARO Abstracts, 1999). However, in the microgravity study the number of synaptic bodies on hair cells was elevated, and in hypergravity the current density of the potassium current of type I cells was greatly increased. We conclude that an altered gravitational field may not influence the time course of vestibular development, but could modify the functional interactions of the signalling pathway between mechano-electrical transduction and Scarpa's neurones.

(Supported by CNES & EPR Languedoc-Roussillon.)

[33]

A PUTATIVE ROLE FOR THE CEREBELLUM IN AVIAN VESTIBULAR RESPONSES TO LINEAR TRANSLATION. S. Irons-Brown¹, S.M. Jones², and T.A. Jones^{1,2}. ¹Dept of Physiology, and ²Surgery/ENT, University of Missouri-Columbia.

There have been a number of studies that have identified the higher order central relays involved in generating auditory brain stem responses (ABR). Similarly, vestibular responses elicited by acceleration pulses, also known as vestibular evoked potentials (VsEPs), are generated by peripheral and central relays. Specifically, VsEP response components P1 and N1 are generated by the vestibular portion of the peripheral eighth nerve, whereas response components beyond P2 are generated by central relays (Nazareth and Jones, 1998, J. Vestib. Res. 8, 233-252). However, little is known about which central relays are involved. The purpose of the present study was to determine the involvement of the cerebellum in generating avian vestibular evoked potentials (VsEPs). Linear acceleration pulses were used to elicit VsEPs before and after aspiration of the cerebellum in eleven- to fourteen-day-old chicks (*Gallus domesticus*). Response peak latencies (e.g., P1, P2, P3, P4) and amplitudes (e.g., P1/N1, P2/N2, P3/N3, P4/N4) were then measured and compared between pre and postoperative conditions. Animals were grouped into two categories: 1) those where more than 50% of the cerebellum was removed, and 2) those where less than 50% had been removed. The results revealed, on average, that both groups of animals demonstrated major reductions in the amplitude of P2/N2. In animals where more than 50% of the cerebellum had been removed, reductions were also apparent for P4/N4. No changes were seen for response peak latencies. The results support the hypothesis that the cerebellum is involved in the generation of VsEPs to linear acceleration stimuli.

(Supported by NASA NAG5 4607 and NIH R01 DC04477.)

[34]

EFFECTS OF SHORT DURATION MICROGRAVITY ON DROSOPHILA MELANOGASTER (FRUIT FLY) ACTIVITY. M.S. Miller and T.S. Keller. Dept of Mechanical Engineering, Univ. of Vermont, Burlington.

The effects of microgravity on *Drosophila melanogaster* (fruit fly) activity was examined during six KC-135 flights and one Nike-Orion sounding rocket launch. Previous investigator's experiments on Cosmos satellites and Space Shuttle missions have shown a significant decrease in the life span of male fruit flies after microgravity exposure. Understanding the mechanism(s) behind this reduced life span could lead to important advances in the understanding of the aging process. The increased aging is hypothesized to be induced by an increased locomotor activity, driven by the *Drosophila*'s negative geotactic response. This response is the tendency of the flies, when stimulated, to walk in the opposite direction of Earth's gravitational vector. During microgravity exposure, *Drosophila* may become more active since they are confused by the lack of gravity and begin searching for the gravity vector. Two separate systems were used to determine fruit fly activity. A video system monitored 32 flies housed separately in either linear or circular tracks. An infrared system monitored an additional 240 flies, all housed in linear tracks, using 480 pairs of emitters and detectors. Locomotor activity is determined by counting the number of times than an infrared beam is broken per time period. Most *Drosophila* examined have had a normal geotactic response, but a small number of flies with a large negative geotactic response or positive geotactic response have been flown to determine the effect of this trait. During the KC-135 flights, the infrared data on the normal geotactic flies showed a significant increase in activity ($p < 0.01$) during portions of the microgravity exposure when compared to activity at 1 g. The non-normal geotactic flies showed no increased activity during microgravity and their activity level was significantly different from the normal geotactic flies ($p < 0.01$) throughout microgravity. Based on this data, *Drosophila* activity appears to increase during microgravity and may be related to the flies' geotactic response.

(Supported by NASA: NGT -5135.)

[36]

MECHANICAL STRESS AND WOUNDING ELICIT NITRIC OXIDE PRODUCTION IN A. THALIANA WILD-TYPE AND NITRATE REDUCTASE MUTANTS. H. Garcês^{1,2}, D.J. Durzan² and M.C. Pedroso^{1,2}. ¹Dept. Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Portugal, and ²Dept. Environmental Horticulture, University of California, Davis, U.S.A.

Nitric oxide (NO) is a signaling molecule that plays several important roles in plants. We investigated if mechanical stress (centrifugation) and wounding altered NO production in *Arabidopsis thaliana* leaves. We postulated that NO originated either from a putative nitric oxide-synthase (NOS), or by nitrate reductase (NR) activity. Leaves from wild-type and G⁺4-3 double NR mutant (*nia1, nia2* mutant defective in the assimilation of nitrate from N. Crawford, UC San Diego) were centrifuged, and/ or wounded and then labeled with DAF-2 DA (4,5-diaminofluorescein diacetate) to visualize *in vivo* NO production. Results with the wild-type (control) and mutant indicated that the increase of NO production in leaves caused by centrifugation and wounding was not a product of NR activity. Moreover, endogenous NO production in both clones was suppressed by the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA). This was consistent with previous results in *Taxus* and *Kalanchoë* (Pedroso et al., 2000, J. Exp. Bot. 51: 1027-1036), reaffirming that a putative NOS activity is present in plants.

(Supported by Fundação para a Ciência e a Tecnologia, contract PRAXIS XXI 3/3.1/CTAE/1930/95 and GGPXXI/BD/3377/96.)

[35]

LONG-TERM IN VIVO DELIVERY OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR-1 BY TISSUE-ENGINEERED SKELETAL MUSCLE IMPLANTS FOR TREATING DISUSE MUSCLE ATROPHY IN MICE. P.H.U. Lee, X.Y. Wang, H.H. Vandenburg. Dept. of Pathobiology, Brown Univ. School of Medicine, Providence RI.

It is well known that space flight causes significant muscle atrophy in both humans and animals. Long-term systemic delivery of therapeutic growth factors, such as insulin-like growth factor-1 (IGF-1), at pharmacological levels may be an effective countermeasure for attenuating or preventing space flight-induced skeletal muscle atrophy. The goal of this study was to demonstrate that recombinant human IGF-1 (rhIGF-1) can be delivered long-term *in vivo* in a murine model using tissue-engineered skeletal muscles made from genetically modified primary mouse myoblasts. A retroviral vector was used to stably transduce primary mouse myoblasts (PMM) with the rhIGF-1 gene under the control of a constitutively expressed promoter. These cells (PMM-IGF1) were tissue-engineered into bioartificial muscles that secreted 673.8±146 ng/implant/day *in vitro*. These rhIGF-1 secreting bioartificial muscles were implanted subcutaneously into mice while sham operated mice served as controls. Serum rhIGF-1 levels were assayed every two weeks for 65 days. rhIGF-1 was detected in the serum as soon as 23 days after implantation. By 51 days, serum levels had reached as high as 565 ng/ml, whereas sham control animals had no detectable levels for the duration of the study. Sustained or increased rhIGF-1 levels were detected in implanted mice until the end of the study at 65 days post-implantations. This study demonstrates the feasibility of long-term sustained delivery of rhIGF-1 in mice using tissue-engineered skeletal muscle. rhIGF-1 delivered in this manner can be used in a hindlimb suspension model for demonstrating its potential application for treating space flight-induced skeletal muscle atrophy.

(Supported by AG15415, HL60502, and NASA NAG2-1205.)

[37]

KINETICS AND LOCATION OF PHOTOTROPISM IN ZEA MAYS L. ROOTS. C. Wolverton¹, J.L. Mullen², H. Ishikawa¹, M.L. Evans¹. ¹Dept. of Plant Biology, Ohio State University, Columbus, and ²Dept. of Biology, Indiana University, Bloomington.

It is well established that roots of many species are capable of generating differential growth in response to unilateral light treatment. The importance of this root phototropic response has recently been highlighted as it relates to the characterization of the gravitropic sensitivity of *Arabidopsis* mutants with reduced levels of starch (Plant Physiol 122: 453). The demonstration of interactions between root gravitropism and phototropism raises fundamental questions regarding the nature of root phototropism: Where is light perceived in the root? Where is the location of differential growth? What are the kinetics of the response?

To address these questions, we applied image analysis software to the study of phototropism in roots of *Zea mays* L. cv. "Merit." Surprisingly, we found that application of unilateral white light results in differential growth primarily in the central elongation zone (CEZ) within the first hour after light application. This contrasts with the localization of differential growth primarily to the distal elongation zone (DEZ) in roots undergoing gravitropism. As a first step toward elucidating the site of photoperception in roots, we removed the root cap and illuminated with unilateral light. This decapping procedure eliminated phototropic curvature under the fluence rate utilized for these experiments.

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[38]

VIDEO CAPTURE OF GREEN FLUORESCENT PROTEIN REPORTING *IN VIVO*, REAL TIME GENE EXPRESSION.

M. S. Manak, A-L. Paul, P. C. Sehnke, and R. J. Ferl. Department of Horticultural Sciences and the Biotechnology Program, University of Florida, Gainesville FL 32611-0690.

Green fluorescent protein (GFP) is a reporter gene that provides the unique ability to collect *in vivo* data on gene expression patterns in a non-destructive manner. When coupled to a stress inducible promoter, such as that of *Arabidopsis* Alcohol dehydrogenase (Adh) gene, GFP can be used to monitor a plants response to stress in real time. The *in vivo* stress response can be captured with digital video imaging equipment to detect the wavelength of light being admitted by GFP. Current data captured with this method include detailed digital footage of whole *Arabidopsis* plants showing stress induced gene expression patterns over a 40day period. The positive control was transgenic *Arabidopsis* with a CaMV 35S promoter/ GFP fusion which causes expression through out *Arabidopsis* from five day old seedlings to mature seven week old plants. The negative control was transgenic *Arabidopsis* with a promoterless GFP construct, which exhibits no GFP expression. *Arabidopsis* transformed with an Adh promoter/ GFP fusion construct show specific expression patterns unique to each environmental stress applied, hypoxia, cold, drought, ABA. This stress response gene/ GFP reporter gene expression system coupled with a digital camera data collection system provides the opportunity to observe *Arabidopsis* gene responses, in real time and via telemetry, to stresses encountered as a result of space flight missions. In a larger sense, this technology would allow ground-based scientists to observe gene expression patterns in orbital, lunar, Martian, or other extraterrestrial environments, without the need for crew time or sample return.

(Supported by NASA: NAG10-0145.)

Symposium II
Psychological Issues in Long-Term
Space Flight

[39]

PSYCHOSOCIAL ISSUES IN LONG-TERM SPACE FLIGHT: OVERVIEW. L.A. Palinkas. Dept of Family and Preventive Medicine, Univ. of California, San Diego.

Anecdotal evidence of the individual and interpersonal problems that occurred during the Shuttle-Mir Space Program (SMSP) and other long duration Russian/Soviet missions, and studies of personnel in other isolated and confined extreme (ICE) environments suggest that psychosocial elements of behavior and performance are likely to have a significant impact on the outcome of long duration missions in space. This impact may range from individual decrements in performance, health and well-being, to catastrophic mission failure. This paper reviews our current understanding of the psychosocial issues related to long duration space missions from three different perspectives: individual, interpersonal and organizational. Individual issues include personality characteristics that predict for successful performance, the stress of isolation and confinement and its effect on emotions and cognitive performance, adaptive and maladaptive coping styles and strategies, and requirements for the psychological support of astronauts and their families during the mission. Interpersonal issues include the impact of crew diversity and leadership styles on small group dynamics; adaptive and maladaptive features of ground-crew interactions; and processes of crew cohesion, tension and conflict. Organizational issues include the influence of organizational culture and mission duration on individual and group performance, and managerial requirements for long duration missions. Improved screening and selection, leadership, coping and interpersonal skills training, and organizational change are key elements to the prevention of performance decrements on long duration missions.

(Supported by NASA: NAG5-4571.)

[41]

PSYCHOSOCIAL ISSUES IN SPACE: RESULTS FROM SHUTTLE/MIR. N. Kanas¹, V. Salnitskiy², E. Grund¹, D.S. Weiss¹, V. Gushin², O. Kozerenko², A. Sled², and C.R. Marmar¹. ¹Univ of California and Dept of Veterans Affairs Medical Center, San Francisco; and ²Institute for Biomedical Problems, Moscow.

Important psychosocial issues involving tension, cohesion, leader support, and in-group/out-group interactions were evaluated in a 4½ year study involving 5 U.S. and 4 Russian Shuttle/Mir space missions. During the in-flight phase of the missions, weekly mood and group climate questionnaires were completed by 5 astronauts, 8 cosmonauts, and 42 U.S. and 16 Russian mission control subjects. Where appropriate, the results from this study were corrected for Type I errors due to multiple significance testing. All 6 tests of the hypothesized displacement of tension and negative emotions from crewmembers on-orbit to mission control personnel on Earth were significant, and 5 of the 6 tests of displacement from mission control personnel to management were significant. In contrast, there were few findings that supported our hypothesized changes in tension, cohesion, and leader support in crew and ground subjects as a function of various time models (e.g., 1st vs 2nd half, triphasic). There were several significant differences in response between Americans and Russians and between crewmembers and mission control personnel. These findings suggest that countermeasures need to be developed to deal with displacement, cultural differences, and crew-ground interactions in order to improve the interpersonal climate of future international space missions. These countermeasures would have special relevance for pre-launch training and in-flight support, and they should include both crewmembers and ground personnel who are involved with the missions. The impact of factors other than time on interpersonal functioning during the course of the missions needs to be explored. In the future, we plan to evaluate the influence of stressful events and other incidents on crew tension, cohesion, and leader support.

(Supported by NASA contract #NAS9-19411.)

[40]

PSYCHOSOCIAL ADAPTATION, SOCIAL INTERACTION PROCESSES AND PERFORMANCE OF CREWS DURING SFINCSS ISOLATION STUDY: CULTURAL, GENDER AND PERSONAL FACTORS. J. Lapierre, Health Science Module, Université du Québec à Hull.

Objectives of this study were to understand and identify key elements related to positive psychosocial adaptation to the environment, to explore and compare the social interactions of different crews and to develop an explanation of the concept of performance, as described and experienced by crewmembers. Grounded theory and the Fourth Generation Evaluation Research methodology were used. Questionnaires reflecting processes over time, interviews, SFINCSS information material, focus groups and participant observation during 110 days comprised the data-gathering strategies. QSR NUD*IST (Qualitative Solutions and Research-Non Numerical Unstructured Data Indexing Searching and Theorizing) was the software used to analyze the data. External stresses related to operational, ethical and managerial issues, and internal stresses related to human interactions and behaviors have been critical in adaptation. Psychosocial support to crews must be redefined in a proactive-participative way with crewmembers. Passive support and unilateral management of crisis during SFINCSS were insufficient to reduce the negative impact of the incidents on the collective mission and to prevent one crewmember from leaving at day 60. The three main crews differed in social interaction processes in relation to both internal and external life. Cultural and personality elements seem to explain such differences. Specific attitudes, behaviors and perception of gender-oriented roles in mixed crews during SFINCSS show that women's roles in space missions are culturally defined. Styles of leadership and decision-making inside the three crews varied. Results indicate a need for integrated knowledge development in the area of human interactions, especially for long-term missions. If support to astronauts is to effectively promote adaptation, it cannot be developed without the input of those receiving it. Finally, new sets of criteria should be given weight to guide astronaut selection.

[42]

ISSUES FOR THE FUTURE. G.M. Sandal. Dept. of Psychosocial Sciences, University of Bergen, Norway.

Psychosocial factors are likely to play an increasingly important role in determining mission success as the duration of space flight increases and crews become more heterogeneous. On the International Space Station (ISS) astronauts and cosmonauts will form one international crew although living in different national modules. The scenario of rotating, multinational crews presents a challenge for mission planners in the field of optimizing performance and interaction within and between crews. Cultural variability both within the astronaut corps and in the more complex environment of multinational operations has been a neglected concept. In recent years progresses have been made in validating the ability of psychometric testing to predict performance in demanding, socio-technical environments and training principles have been outlined. Documentation of the cross-cultural applicability and fairness of such methods remains an important issue. There have been few opportunities to validate the effectiveness of countermeasures in relation to astronaut performance or psychosocial parameters in space. Empirical evidence have been based on simulations and research on performed on personnel operating in environments that involve many similar stressors to those experienced by astronauts in space, such as aviation, deep diving and polar research stations. It is now apparent that attempts to transfer experiences gained from such analogue environments to space require a thorough evaluation of the threats, risks, and human behaviors specific to the fields. Collection of comparable data in space is needed to establish the validity of data gained from these settings.

**Concurrent Oral Sessions
I
Animal Development, Physiology and
Gravity Sensing**

[43]

(MICRO-)GRAVITY ACTIONS AS IDENTIFIED FROM DEVELOPMENTAL BIOLOGY EXPERIMENTS IN SPACE AND THEIR INTERPRETATION. H.-J. Marthy, CNRS, Observatoire Océanologique, 66650 Banyuls sur mer, France.

Looking backwards in time, the hypothesis appears still well justified that crucial events in animal development, such as fertilization, body pattern lay out, germ layer formation or particular organogenetic processes could hardly occur in a correct manner in the absence of gravity. It was (and still is) astonishing, therefore, to learn from various experiments, performed on various aquatic and terrestrial organisms in development, that the morphogenetic role of gravity is minor or even neglectable. Thus, in all cases studied so far (amphibians, fishes, insects, echinoderms), *viable juveniles* result from eggs or embryos maintained in real micro-gravity conditions for a portion or for the whole period of development. Inversely, in all these cases, some deviations from the “normal” way of development are reported, giving evidence that eggs and embryos “sense” apparently a particular gravity situation as well known for “single-(animal and human)-cell systems *in vitro*”. In contrast yet, the proliferating and differentiating “multi-cellular organisms” seem capable to overcome the perturbations and achieve, supposedly due to coordinating intercellular signals (regulation signals), the state of viable and finally reproducible individuals. From the reported morphological and physiological deviations, no far reaching generalizations as to the morphological role of gravity in animal development can be made. However, a few, apparently (micro-)gravity related phenomena can be identified in the different models, which allow similar statements. Among others, these are in particular: - early developmental stages are more sensitive against gravity alteration than more advanced ones - cell adhesiveness appears gravi-sensitive and - “multi-cellular systems” regulate morphogenetic deviations throughout development, which leads to viable juveniles. The phenomena, supporting such conclusions, are overviewed in the different models.

(Support to the author is provided by CNES and CNRS of France.)

[45]

A CRITICAL PERIOD FOR VESTIBULAR DEVELOPMENT IN ZEBRAFISH (*DANIO RERIO*). S.J. Moorman, R. Cordova, and S.A. Davies. Dept. of Anatomy, Case Western Res Univ, Cleveland, OH.

A critical period is defined as the briefest period of time during development when stimulus deprivation results in long lasting or permanent sensory deficits. Analogies to the visual and auditory systems suggested the existence of a critical period during vestibular development. We have used a bioreactor designed by NASA to simulate microgravity for cells in culture to determine a critical period of vestibular development in zebrafish. Zebrafish eggs were collected within 3 hours of having been laid and fertilized. Experiment #1; eggs were placed in the bioreactor at 3, 24, 30, 36, 48, or 72 hours post-fertilization (hpf) and maintained in the bioreactor until 96 hpf. Experiment #2; eggs were placed in the bioreactor immediately after they were collected and maintained in the bioreactor until 24, 36, 48, 60, 66, 72, or 96 hpf. Beginning at 96 hpf, all larvae had their vestibulo-ocular reflexes (VOR) evaluated once each day for 5 days. Only larvae that hatched from eggs that were placed in the bioreactor prior to 30 hpf in the experiment #1 or removed from the bioreactor later than 66 hpf in experiment #2 had VOR deficits that persisted for at least 5 days. These data suggest a critical period for vestibular development in the zebrafish that begins prior to 30 hpf and ends after 66 hpf. To confirm this, zebrafish eggs were placed in the bioreactor at 24 hpf and removed after 66 hpf. VORs were evaluated in these larvae once each day for 5 days beginning at 96 hpf. These larvae had VOR deficits that persisted for at least 5 days. It should be noted that none of the larvae in the first experiment of in this last experiment had any obvious or significant changes in morphology of the inner ear. Overall, these data support the idea that there is a critical period for functional maturation of the zebrafish vestibular system. The developmental period identified includes the time frame during which the primary afferent neurons are born, innervate their central and peripheral targets and remodel their central projections.

(Supported by NASA: NAG2-1356.)

[44]

THE EFFECTS OF MICROGRAVITY ON THE SWIMMING BEHAVIOUR OF STARFISH LARVAE. B.J. Crawford and D.L. Jackson. Dept. of Anatomy, U.B.C. Vancouver, B.C. Dept. of Biology, Dalhousie University, Halifax, N.S. Canada.

During the course of early development, embryos/larvae of the starfish *Pisaster ochraceus* develop distinct swimming patterns. During and shortly after hatching the embryos tumble about on the bottom of the culture dishes and make short excursions into the water column. By the early-to-mid gastrula stages healthy embryos swim in straight lines through the water column and rotate on an animal vegetal axis which passes through the long axis of the embryo. Once the mouth has formed, the larvae, which are heavier than water, spend a lot of time oriented vertically in the water column with the expanded anterior end upwards. When this behaviour is occurring, the larvae change the pattern of rotation. The axis of rotation still passes through the posterior part of the embryo but the anterior end describes a wide circle around the rotational axis, thereby allowing the animal to present a larger surface area to resist gravity (parachute behaviour). Thus, the later swimming behaviour may enable orientation to gravity despite the fact that the larvae have no obvious organ for detecting it. In the vertical orientation the preferred direction of rotation is clockwise (CW). When swimming horizontally the larval rotation is primarily CW but they will change from CW to antiCW and back. The swimming behaviour of larvae raised in the Aquatic Research Facility aboard STS-77 was recorded and analysed using a PC-based image analysis system (Optimas® 6.0). Larvae raised in a 1G centrifuge appeared to orient themselves along the gravity gradient and to exhibit parachute behaviour. Larvae raised in μ G swam in randomly oriented tracks, the majority of which were straight. Many of these tracks exhibited oscillations with a period of 2-5 seconds which corresponds to the period of rotation of the larvae exhibiting parachute behaviour in controls. The results confirm that these organisms have a method of detecting gravity which may be due to differences in density in different regions of the embryo. It also suggests that the parachute behaviour is innate and can develop without gravitational clues.

(Supported by Canadian Space Agency.)

[46]

FUNCTIONAL CHANGES IN CENTRAL VESTIBULAR RELAY CIRCUITS FOLLOWING 2G CENTRIFUGATION. S.M. Jones¹, L. Warren², R. Shukla¹, A. Browning¹, C.A. Fuller² and T.A. Jones¹. ¹School of Medicine, University of Missouri, Columbia; ²SNPB, University of California, Davis, CA 95616.

Linear vestibular evoked potentials (VsEPs) were used to characterize peripheral and central vestibular function in birds following embryogenesis at 2G centrifugation or at elevated levels of vibration (+20dBre: background levels). Additionally, peripheral and central vestibular responses were characterized in normal hatchlings exposed to 2G centrifugation for 7 days. Linear VsEP response peak latencies, amplitudes, thresholds and input/output functions were quantified. Birds vibrated throughout embryogenesis and up to one-week post-hatch revealed no changes in linear VsEP compared to control siblings. Birds centrifuged at 2G throughout embryogenesis also evidenced no changes in the linear VsEP measured at hatch (P0). Significant changes were seen, however, for linear VsEPs of post-hatch birds placed at 2G for 7 days beginning on post-hatch day 5. Linear VsEPs for these animals displayed significant reductions in response amplitudes for central neural relays of gravity receptors (P2, N2 and P3). In contrast, peripheral nerve responses (i.e., P1, N1) were not significantly altered with the 7-day exposure to 2G. Thus, there was no evidence of generalized changes in peripheral gravity receptor excitability or in the rate of maturation in developing animals under increased levels of gravity or vibration. If gravity level plays a critical role in shaping peripheral vestibular ontogeny at magnitudes between 1 and 2G, then it must operate on more subtle physiological features than can be resolved by the VsEP. In contrast, exposure to elevated gravity during post-hatch periods does alter central vestibular function thus providing direct evidence for central vestibular adaptation to the gravitational environment. The fact that central functional change was observed in hatchlings and not embryos raises the possibility that the first 2-weeks post-hatch may be a critical period of “heightened developmental sensitivity” to hypergravity.

(Supported by NASA: NAGW 1275, 3910 and NAG 2-1032.)

[47]

TESTS TO DETERMINE THE ADEQUACY OF NASA'S RODENT FOOD BARS FOR USE IN LONG-TERM SPACE FLIGHT EXPERIMENTS. J.E. Barrett, D.S. Yu, B.P. Dalton. NASA Ames Research Center, Moffett Field CA.

Long-term space flight studies with rodents will require a diet that is nutritionally adequate, nutritionally stable during storage, and does not crumble. Rodent feeding studies and numerous other experiments are being conducted in order to determine the effectiveness of NASA's current shuttle rodent food bar in preparation for use in long term (45-90 day tests) aboard the International Space Station. Beginning at about 7 weeks of age, male and female Sprague Dawley and Fischer 344 rats were housed in vivarium cages and fed either food bars or lab chow control diet for at least 100 days. Food bar acceptance by the animals was closely monitored, and no apparent decrease in food intake occurred as rats were switched from the weanling diet to food bar or chow. Growth, food and water intake, and necropsy values were obtained. Preliminary results indicate that there were physiological differences in some rat groups fed the food bar when compared to rats fed the control diet. Differences were noted in food and water intake in some groups, and in male Fischer body weights. Blood chemistry variables, some organ weights and organ to body weight ratios were significantly different for some groups. There were indications that lipid metabolism and storage were affected in rats fed the food bar diet. Cholesterol, abdominal and epididymal fat, blood urea nitrogen, and liver weights were significantly different for three of the four groups when compared with controls. However, the differences observed in the various parameters were inconsistent from group to group. Carcasses of the rats are being analyzed for moisture, fat and protein content. The food bars are tested periodically in order to monitor nutrient stability during storage. Additional rodent-feeding studies with mice are planned in order to obtain more information about food bar effectiveness as a diet for use in long-term studies.

(Supported by NASA: UPN393-25.)

[49]

EARLY AND LATE EFFECTS OF PERINATAL HYPER-GRAVITY EXPOSURE ON THE DEVELOPING CNS. E. M. Sajdel-Sulkowska^{1,2}, L. A. Baer³, G-H Li², G. M. Sulkowski⁴, A. E Ronca⁵, Charles E. Wade⁵. ¹Dept. of Psychiatry, Harvard Medical School and ²Brigham and Women's Hospital, Boston MA; ³Lockheed Martin Engineering and Sciences, Moffett Field CA; ⁴Harvard Medical School, Cambridge MA; ⁵Life Sciences Division, NASA/Ames Research Center, Moffett Field CA.

The results of previous experiments demonstrated that rats could successfully survive developmental exposure to 1.5G from gestational day 11 (G11) through postnatal day 21 (P21). However, their growth was compromised, with forebrain and cerebellum size decreased as compared with stationary control animals. The present study was designed to define the time-course of the hypergravity effect. Timed-pregnant Sprague-Dawley rats were exposed to continuous centrifugation at 1.5 G (HG; n=35) from G11 until one of six time points: P6, P9, P12, P18, P21, and P30. During the 41-day-long centrifugation, stationary controls (SC; n=34) were housed in the same room with HG rats. Neonatal body, forebrain, and cerebellum size were measured at each time point. All parameters were significantly affected (p>0.0001) in HG neonates at all times, but the degree and the time of maximal inhibition differed. HG neonates exhibited two time points at which maximal change was observed: maximal decrease in body mass was on P9 (26.77%) and P21 (23.79%); in brain size on P9 (13.47%) and P21 (10.42%); in cerebellar size on P6 (19.15%) and P21 (10.78%). These data support earlier speculations of Oyama and Platt (1967) that the general developmental effects of hypergravity are greatest just after birth and again at weaning. Furthermore, in the case of the cerebellum, the early effects of perinatal hypergravity exposure correspond to the critical period of granule cell proliferation, while the late effects correspond to cell differentiation.

(Supported by NASA grant NCC2-1042.)

[48]

POMC AND ENDORPHINE ARE INDUCED BY HYPERGRAVITY IN RAT BRAIN. Y. Kume¹, H. Shimokawa², R. Shimokawa³, M. Terasawa², B Linsuwanont², K. Ohya². ¹Dept. of Biochem., ²Dept. of Pharmacol., ³Dept. of Neuropathol., Tokyo Medical and Dental University, Tokyo, Japan.

The purpose of this study was to observe the hypergravity effects on the brain activity in rats. Wistar male rats of 5-week-age were exposed to 2G by centrifugal rotation for 10 min. Rats were sacrificed with perfusion. Sections were prepared for immuno-histochemistry and in situ hybridization. Hypergravity induced expression of POMC (proopiomelanocortin) and endorphine in the brain. The POMC gene encodes endorphine. POMC and endorphine was expressed in the arcuate nucleus of the hypothalamus. These histological changes were consistent with behavioral change that was observed as hypergravity-induced analgesia. Gravity change might induce opioid-related reaction in rat.

(Supported by NASDA and Japan Space Forum.)

[50]

GRAVITAXIC BEHAVIOR IN *DROSOPHILA MELANOGASTER*. J.D. Armstrong, M.J. Texada, E.L. Carter, E.S. Kuo, C.M. Nadorff and K.M. Beckingham. Dept. of Biochem. and Cell Biol., Rice Univ., Houston TX.

The fruitfly *Drosophila melanogaster* is an excellent model for genetic analysis of behavior. We are applying both traditional genetic and transgenic approaches to the study of gravitational responses as a route to identifying i) the molecular signaling pathways, ii) sense organs, and iii) the neuronal circuitry involved in gravity responses. Gravitaxic behavior in *Drosophila* is assayed using a vertical maze system with eight up/down choice points and nine final exit routes. Flies progress through the maze and distribution of a given mutant population across the exits is compared to controls. Supplementary tests eliminate mutants with problems in locomotion, anatomy, or general responsiveness. Two types of mutagen have been employed: EMS (chemically induced point mutations) and P{GAL4} (transposon insertion mutations). 750 mutant strains have been tested. Several mutant strains with maze exit positions i) much higher or ii) much lower than controls have been isolated. For some, the genes affected have been identified. These include an evolutionarily conserved PDZ-domain containing protein with a known cell signaling role downstream of the initial visual response. The known role of this protein in mediating fast cell signaling in vision is pleasingly suggestive of a similar fast sensory mechanism for gravity. Also identified are genes for several transcription factors, notably one involved in the development of the nervous system. A complementary targeted approach is also being pursued. A brain region termed the central complex (CC), interconnected to most, if not all, sensory modalities, has been directly linked to a range of behaviors in insects. We have studied 30 mutations that affect the neuronal circuitry of the CC and demonstrated a clear link between this neuronal structure and gravitaxic behavior. In summary, our studies in *Drosophila* have identified genes with potential roles in both the signaling pathways and neuronal circuitry required for gravitaxic responses and have identified at least one brain region involved in coordinating gravitaxic behavior. Further, they suggest that the mechanisms underlying gravitaxic responses are conserved across the animal kingdom. (Supported by a NASA Specialized Center for Research and Training [NSCORT] Grant at Rice University NAG5-4072.)

**Concurrent Oral Sessions
II
Advanced Life Support and Biotechnology**

[51]

PROGRAMMABLE PLANTS: DEVELOPMENT OF AN *IN PLANTA* SYSTEM FOR THE REMOTE MONITORING AND CONTROL OF PLANTS FOR LONG TERM LIFE SUPPORT. C.S. Brown, Dynamac Corporation and NSCORT in Gravitational Biology, NC State University. In order to align NASA's goal of solar system exploration with the emerging fields of genomics and nanoscience, we performed a feasibility study to define and prioritize the major issues associated with the generation and use of programmable plants. We explored the concept of bringing these two disciplines together for the development of plants with remote monitoring and control devices *in planta*. We developed an architecture for the design and use of robotic plants that could be programmed to express or repress inducible genes or modules of genes from remote signals. The plants, designed with receptors/receivers that would be activated by remotely generated signals, would thus be programmed from earth or from a satellite to initiate a bioregenerative life support system for an incoming or resident crew, to initiate production of pharmaceuticals or nutraceuticals, to generate biomass, or to produce fiber or plastics for construction. Development and integration of a fully biologically based life support system that is genetically engineered and remotely controlled to act in response to the changing needs of the crew/team would be of enormous significance in the quest to establish a human presence beyond Earth. The means to bring this about will include the application of functional genomics and nanotechnology.

(Supported by the NASA Institute for Advanced Concepts and the Kenan Institute for Engineering, Technology and Science.)

[53]

GENETIC AND ENVIRONMENTAL INFLUENCES ON THE NUTRITIVE QUALITY OF SPINACH: A NASA ALS CANDIDATE CROP. C.F. Johnson¹, R.W. Langhans², L.D. Albright¹, R.M. Welch³, G.F. Combs⁴, R.P. Glahn³, and R.M. Wheeler⁵. ¹Dept. Ag. and Bio. Engineering CEA Program, ²Dept. of Flor. and Orn. Horticulture, and ⁴Dept. Nutritional Sciences, Cornell University, Ithaca; ³USDA-ARS, Ithaca; and ⁵NASA, Kennedy Space Center.

Spinach, *Spinacia oleracea*, is among the candidate crops selected for NASA's Advanced Life Support (ALS) system. NASA has defined three areas of concern regarding spinach: 1) reputed high iron content, 2) high oxalic acid content, and 3) high nitrate content. Studies were performed to assess whether genetic (cultivar selection) and environmental (Controlled Environment Agriculture (CEA)) influences might be used to produce a spinach crop with improved nutritional value for humans.

In vitro digestion methods and human intestinal cell culture (Caco-2 cells) were utilized to assess iron bioavailability. Spinach digested with ascorbic acid showed increased iron bioavailability, demonstrating the importance of evaluating whole-meals rather than single food items for space flight diets.

Examination of the National Seed Storage Laboratory's 290 spinach cultivars showed a range in oxalic acid content from 750 to 1750 moles/g (dry weight basis) and range in nitrate content from 280 to 1200 moles/g (dry weight basis).

Nitrate levels were decreased to undetectable amounts and oxalate levels were decreased by one-half to two-thirds through the development of a new pre-harvest culture technique. The CEA techniques developed to improve the food-value of spinach were oriented toward large-scale production so the methods are commercially feasible, demonstrating the potential for CEA technology transfer to improve food quality for humans.

(Supported by NASA GSRP #NGT10-52607.)

[52]

BOUNDARY LAYERS AROUND PLANT LEAF AND ROOT TISSUES DEPEND ON GRAVITY. O. Monje¹, D.M. Porterfield², and G.W. Stutte¹. ¹Dynamac Corporation, Kennedy Space Center, FL; ²Department of Biological Sciences, University of Missouri-Rolla.

Changes in the behavior of fluids and gases in space can induce plant stress responses that confound plant spaceflight experiments. Microgravity alters the movement of heat, water vapor, CO₂ and O₂ between plant surfaces and their environment due to the absence of thermally driven, buoyancy dependent convective transport around leaf and root tissues. Based on this reduction of mass transport, we hypothesize that the thickness of boundary layers forming around leaf and root tissues should increase in microgravity. Furthermore, since these are direct physical changes in the gravity dependent behavior of fluids, measurement is amenable to the short periods of microgravity produced on KC-135 parabolic flights. Infrared transducers and a root oxygen bioavailability sensor were used to monitor changes in leaf temperature and rootzone oxygen transport as a function of gravity. Both thermal transport around the aerial leaf tissue and oxygen transport within the rooting matrix decreased in phase with changes in the force of gravity. These direct physical measurements demonstrate that changes in boundary layer conditions can arise in microgravity and suggest that stress-inducing reductions in thermal transfer, transpiration, and metabolic transport of gases (CO₂ and O₂) may occur in space. These effects might be alleviated in microgravity by the use of mechanical forced convection to drive thermal and mass transfer between plant tissues and the surrounding environment.

(Supported by NASA: NCC10-0027 to GWS, and a Missouri Research Board Grant to DMP.)

[54]

TISSUE ENGINEERING IN ZERO GRAVITY. A. Cogoli. Space Biology, ETH Zurich, Switzerland.

The purpose of this communication is to present a project recently selected by ESA within the application and commercialisation programme of the International Space Station. Team members from academic institutions are A. Bader, Hannover; S. Ambesi, Udine; P. Bruckner, Münster; R. Pörtner, Hamburg; A. Cogoli and I. Walther, Zurich; W. Müller is the industrial partner from Sulzer Medica, Winterthur. The objectives of the project are: to develop procedures of *in vitro* organogenesis of pancreatic islets, thyroid tissue, liver, vessels and cartilage; to study the mechanism of organogenesis in low-g; to define the requirements of a modular space bioreactor for medically relevant organ-like structures; to set up procedures for the production of implants for medical applications. It is believed that low-g may contribute in two aspects to progress in this field. First as a useful and non invasive tool to study important and still obscure biological events like signal transduction, gene expression, and cell proliferation. Second, low-g may favor the mass production of cells by obtaining higher cell densities per unit culture volume as well as a smooth cell-cell aggregation and three-dimension organogenesis in the absence of sedimentation and shear forces. The strategy adopted consists of a step by step approach: 1st All mammalian biological systems (single cells and tissues) will be optimized according to the team members' specific expertise. 2nd The biological studies will be accompanied by ground-based simulations at 1 g, at simulated microgravity in clinostats (mainly in the random positioning machine) and at hypergravity in centrifuges. 3rd Design of a modular bioreactor consisting of a central „servicing unit“ and of modules specific for each biological system. 4th Optimization of the bioreactor to space laboratory requirements according to one selected system. 5th Semi-automation of the bioreactor.

6th Further expansion of the bioreactor to other systems. 7th Flight opportunities for hardware and biological tests: Biopack, Modular Cultivation System, Biolab, BMTC Spacehab, International Space Station, sounding rockets. Preliminary tests in the random positioning machine will be followed by flights in space in 2001-2003.

[55]

USE OF THE ROTATING BIOREACTOR TO STUDY SKELETAL MUTATIONS: HEREDITARY MULTIPLE EXOSTOSIS. P.J. Duke¹, D. Montufar-Solis¹, J.T. Hecht². Dept. of Orthodontics¹, Dental Branch, Dept. of Pediatrics² Medical School, University of Texas Health Science Center, Houston TX.

Osteochondromas are the most common bone tumors. These cartilage capped, bony projections are benign, and in individuals with Hereditary Multiple Exostoses (HME), are related to mutations in the putative tumor suppressor genes EXT1 and EXT2. Ultrastructural and confocal microscopy studies of cultured chondrocytes from HME excised exostoses identified abnormal amounts of α -actinin microfilaments throughout the cell. These fibrils were also found in freshly excised exostoses. HME chondrocytes are difficult to study because they will not differentiate in the usual systems developed for chondrocyte culture, i.e., agar, agarose, and alginate beads.

The rotating bioreactor provides an environment for 3-D development of tissues. In the present study, human costochondral chondrocytes and chondrocytes from excised HME exostoses were expanded in monolayer culture, trypsinized, aggregated on a rotating table, and then cultured for 3 weeks in a bioreactor. The aggregation of the two cell types differed: HME cells formed smaller, tighter aggregates, and continued to aggregate when placed in the bioreactor. Control chondrocytes formed cartilage as shown by positive staining with type II collagen antibody, and Toluidine blue metachromasia. HME chondrocytes did not stain metachromatically, nor did cells have the histological appearance of cartilage, but the fibrous components previously observed were seen intracellularly. These studies suggest that the formation of exostosis projections from the growth plates of HME individuals is related to the cell's inability to form normal cell-cell or cell-matrix contacts, and to receive and respond correctly to cartilage differentiation signals.

(Supported by Shriners Hospital for Children grant #15955 to JTH and by UT Dental Branch Pilot Research Grant to JD.)

[57]

SHEAR FORCES AND THE PROPER CONTROL. J.J.W.A. van Loon¹, E. Folgering², J. P. Veldhuijzen³, C.V.C. Bouten². ¹DESC, OCB-ACTA-VU, Amsterdam, Netherlands. Web site: <http://www.desc.med.vu.nl>. ²Eindhoven University Technology, MATE, Eindhoven, Netherlands; ³ACTA-VU, Oral Biology, Gr. Oral Cell Biology, Amsterdam.

To draw any conclusions from microgravity, experiments it is important to have a proper control. To define this we have to understand the artifacts involved in such studies. Based on Einstein's equivalent principal, a 1xg control can either be a sample that remains on Earth or that is put into an on-board centrifuge. It was with facilities like Biorack that one could use an on-board 1xg control. One of the never addressed differences between ground 1g and in-flight 1g is the shear force generated in on-board centrifuges while this shear force might be one of the main artifacts in spaceflight and on-ground studies involving centrifuges. Shear forces are generated in two ways. Static shear and fluid shear. Fluid shear is best known from the circulatory system where the endothelium is exposed blood flows. Static shear forces are generated in materials exposed to e.g. accelerations. The latter being the main issue of this numerical study. Although differently shear forces have, *in vitro*, an impact on both attached and free-floating cells. They may even have a significant effect in animal centrifuge studies.

We calculated that for some experiment set-ups in the past as well as for some future ISS facilities the level of shear force in on-board centrifuges could be as much as 95% of the total force. Some of the differences reported between ground 1xg and in-flight 1xg centrifuge could have been caused by this phenomenon.

The artifact should be dealt with for future missions and hardware designs as well as for the interpretation of previous data.

(Supported by SRON and the NIVR combined grant # MG-051.)

[56]

FLUID HANDLING AND MANAGEMENT EXPERIMENT (FHAME). T.M. Crabb, R.C. Morrow, and T.K. Klemp. Orbital Technologies Corporation, 1212 Fourier Dr, Madison WI.

FHAME was developed to test and demonstrate liquid/gas phase control in fluid management subsystems such as the humidity control (HCS) and nutrient delivery (NDS) subsystems of the Biomass Production System (BPS). Both these subsystems use close-looped pressure control that maintains negative pressures to oppose the natural capillary forces across a porous interface. The FHAME hardware was developed as a simplified apparatus to observe fluid flow in BPS subsystems, test water loading and priming characteristics of the HCS and NDS, and evaluate the effects of rooting media particle size and composition on NDS operation. The ability to test fluid subsystems in microgravity independently of other components will allow these subsystems to be optimized for function and reliability. The FHAME hardware is 123 cm x 41 cm x 55 cm high. Its weight is estimated to be less than 11 kg. The unit is configured to fly in one standard crew transfer bag (CTB). The estimated power requirement is less than 30 watts. FHAME components include a computer with hard drive and floppy drive, a reservoir, pumps, a degasser module, LCD cameras, a data display, pressure sensors and soil moisture sensors. Most of these components and subsystems are identical to those used in the BPS flight unit. The gas/liquid separation components (including bubble detectors) and soil moisture sensors represent new components. Test modules (i.e. humidity control and nutrient delivery modules) are sent as separate stowage items and plugged into the test platform on the FHAME unit. The FHAME unit will be useful for on-orbit testing of different NDS technologies and optimization of NDS configurations. Initial test protocols have been defined to demonstrate key subsystem and component operations for future NASA ISS payloads, such as the plant research unit (PRU). These protocols cover evaluation of liquid/gas separation functionality; evaluation of autoprime for the NDS and HCS; demonstration of automatic prime maintenance; evaluation of rooting matrix particulate size on loading and distribution of water; and evaluation of seed wetting and germination on-orbit.

[58]

ADVANCED VERSATILE TOOLS FOR LIFE SCIENCES RESEARCH IN SPACE. P. Todd, J. Vellinger, A. Sharpe, R. Ormsby, H. Platt, K. Barton and M. Deuser. Space Hardware Optimization Technology, Inc., Greenville IN.

A family of versatile research tools is available for life sciences research in space. Individual instruments range from laboratory-tested concepts to flight proven technology. The *Avian Development Facility* is designed to incubate avian eggs and process them robotically, rotating eggs and preserving embryos at specified stages of development. Its numerous robotic functions also make it capable of incubating and processing cell cultures, insect specimens, developing invertebrates and plant seedlings. In addition to specimen rotation a rotating carousel provides 0 to 1 g. The envelope provides full environmental control including humidity, temperature, CO₂ and oxygen in a closed environment. In the *Avian Hatchling Habitat* embryos hatch out of eggs in special holders transferred from the Avian Development Facility. This habitat includes an automated avian waste management system, downlink video, lighting and atmosphere control. Studies of vestibular function, reproduction, feeding, etc. can be performed with minimal crew assistance. The *Advanced Animal Habitat* is designed to maintain rats or mice in single or grouped housing. Functions include video observation, light cycle and atmosphere control, automated delivery of food and water, waste collection, glovebox access, and biotelemetry compatibility. This habitat is designed to operate on the ISS Centrifuge. The *Cell Culturing Cassette* is a robotic cell culture reactor system capable of supporting culture volumes up to 50 mL with options for rotation, oxygenation, and robotic sampling of cells and/or medium at programmable specified times. The *Advanced Separator* is a circular array of multiple controllable fluids-mixing contactors applicable to a wide variety of experiments that can be initiated in a 1 mL volume. Multiple cassettes can be flown in a single mid-deck locker. All of these tools are mid-deck locker based and uplink and/or computer controlled, and their applications are not necessarily limited to those mentioned in this report.

(Supported in part by NASA SBIR program awards.)

**Concurrent Posters
III–C
Animal Development, Physiology and
Gravity Sensing**

[59]

MICROGRAVITY EFFECTS ON FERTILIZED EGGS HAVE NO INCIDENCE, AFTER LANDING, ON THE FURTHER LARVAL DEVELOPMENT AND REPRODUCTION IN THE AMPHIBIAN *PLEURODELES* WALT. H. Membre, A. Bautz, D. Durand, C. Aimar, A.M. Bautz and C. Dournon. EA 2401 Génétique et Interactions Cellulaires en Reproduction, Univ Henri Poincaré, Nancy, France.

Our goal was to show if natural fertilization and normal development could occur in space and after landing. Female *Pleurodeles* were inseminated on the ground, then laying was induced by hormonal treatment in space. Eggs were obtained at a 18°C constant temperature in the three FERTILE experiments during the 1996 Cassiopée, 1998 Pégase, and 1999 Perseus French missions aboard the Mir station. The distribution of peptidase-1 genes, a polymorphic sex linked enzyme, in progenies stated the actual fertilization in microgravity and ruled out parthenogenesis or gynogenesis. During the first cleavage of segmentation, microvilli of µg-, space-1g- and ground-1g-eggs, observed under SEM in the vicinity of the animal pole, were different in length, diameter and distribution, attesting an influence of microgravity on these cells. Recovered at the hatching stages after landing and reared in the laboratory at room temperature, the young larvae underwent metamorphosis and became adult without obvious abnormalities. The rate of development and the morphology were similar in these animals and in controls. As expected, the sex ratio was equal to 1. No sex reversal was observed. Born-in-space males were firstly mated with ground-control females, and then, depending on their fertility, mated with born-in-space females. All the mating gave progenies that normally developed. Analysis of the F2 generation is in process.

(Supported by CNES: 96/0265, 793/98/7208, 97/071 and 793/99/7443)

[61]

GRAVITY STIMULATION CHANGES WITHDRAWAL REFLEX IN RATS. K. Toda¹, Y. Kawauchi^{1,2}, Y. Kumei³, F.H. Nasution⁴ and K. Makita⁵. Sect. Cognitive Neurobiol¹, Dept. Anesthesiol², Sect. Bio-Matrix³, Orthodontic Science, Division of Oral Health Sciences⁴ and Systemic Organ Regulation⁵, Tokyo Med. Dent. Uni., Tokyo 113-8549, Japan.

The purpose of the present study is to reveal the effect of gravity stimulation on the nociceptive responses in the rat. 1.5G or 2.0G gravity stimulation was applied during 10 minutes in Wistar albino rats. We selected eight sites for noxious stimulation using von Frey type filament. The threshold intensities of withdrawal reflex were measured before and after the application of gravity stimulation. Naloxone-HCl (0.1mg/gip) was used to test whether endogenous-opioids are concerned with those gravity-induced effects. The threshold value was significantly increased in all the stimulating sites tested after 2.0G stimulation, but not after 1.5 G stimulation. Naloxone blocked analgesic effects in case of 2.0G-gravity stimulation. The present study indicates that high gravity can provoke naloxone-reversible analgesic effects in rats. This analgesic effects is considered to be a stress-induced analgesia.

[60]

MICROGRAVITY-INDUCED MALFORMATIONS OF THE BODY CORRELATE WITH THE DEPRESSION OF THE STATIC VESTIBULOOCULAR REFLEX. E. Horn. Gravitational Physiology, Department of Neurobiology, University of Ulm, Ulm, Germany.

Studies in an amphibian (*Xenopus laevis*) have demonstrated that the development of the roll-induced static vestibuloocular reflex (rVOR) was significantly depressed by a 9- to 10-day microgravity exposure but that it recovered completely during 1g-readaptation within 5 weeks. This decrease was explained by a developmental retardation of the network underlying the vestibuloocular reflex. It was also found that microgravity induced malformations of the body; affected tadpoles developed tails which curved upwards. These lordotic malformations might be an unspecific effect of microgravity on the developing animals. Alternatively, it might be caused by a depressed sensitivity of the vestibular system because the vestibular system influences the base activity level of trunk muscles via the vestibulospinal pathway. If this hypothesis is right a correlation between the extent of malformation and the extent of rVOR depression is likely. A respective correlation analysis was performed with data recorded in tadpoles which flew on the space missions STS-55 and STS-84. The rVOR amplitudes (= angle between the maximal up and down movement of the eye during a complete 360° lateral roll) of lordotic tadpoles with 0g-experience were compared with those recorded from both normally developed tadpoles with microgravity experience and normal tadpoles from the 1g-ground and 1g-flight controls. The comparison showed a significant lower rVOR amplitude in lordotic tadpoles than in normal ones. No difference was found in normally developed tadpoles with µg-experience and the respective 1g-controls. Based on the correlations between anatomical, physiological and behavioral development in the time domain and the organization of vestibuloocular and vestibulospinal projections, microgravity induced malformations of the body are probably an expression (1) of the tonic influence of the vestibular nuclei on the motor system and (2) of a non-synchronous development of trunk and body muscles.

(Supported by DLR, grants 50QV8925-5 and 50WB9553-7.)

[62]

HINDLIMB-SUSPENSION, WATER DEPRIVATION AND SALT-LOADING AFFECT ANGIOTENSIN-CONVERTING ENZYME (ACE) EXPRESSION IN RAT CHOROID PLEXUS. E. Vila-Porcile¹, C. Carcenac¹, C. Masseguin¹, J.-M. Gasc² and J. Gabrion¹. ¹Inst. des Neurosci., UMR CNRS 7624, Univ. P.-&-M. Curie, Paris, France (e.mail: jgabrion@snv.jussieu.fr), ²INSWEM U36, Collège de France, Paris.

Angiotensin I-converting enzyme (ACE, EC 3.4.15.1) is a transmembrane ectopeptidase that generates angiotensin II (AII), a vasopressor and aldosterone stimulating peptide, from angiotensin I, and is mainly expressed in vascular walls, lungs and adrenals but also in choroidal epithelial cells, in brain. When immunodetected in choroid plexus by immunofluorescence and immunogold methods with the specific polyclonal Y4 antibody¹, the enzyme is associated to the plasma membrane and asymmetrically distributed at the apical pole of the cells in control rats. It colocalizes, at the level of microvilli membranes, with angiotensin II, detected with the specific BAII-01 antibody (gift from J. Sealey, Cornell U., NY). Adaptation to head-down suspension (HDS) induces cellular and molecular alterations in choroidal cells^{2,3} in response to central hypovolemia, which results from body fluid shifts. In this study, the choroidal ACE expression was observed also impaired by HDS. ACE was strongly reduced in choroid plexus of rats suspended for 14 days and more again, in rats suspended for 28 days, suggesting that conversion of AI into AII was reduced in these conditions. The altered ACE distribution was restored after a 2-day return to orthostatic position, the enzyme appearing even overexpressed. When detected in choroid plexus of rats after 5 or 7 days of water deprivation or salt-loading (2% NaCl in drinking water), ACE was reduced and heterogeneously distributed under both conditions. The most altered pattern was noted in salt-loaded rats. In all these experimental conditions, known to affect the water balance, most of the choroidal apical proteins were down-regulated. As AII is normally found in CSF, it might be suggested that reduced levels of choroidal ACE noted during such conditions could result in AII decrease in CSF and might be involved in the cerebral fluid balance during hypovolemia. (Supported by CNES and CNRS funding.)¹Mounier *et al.*, *Kidney Int.*, 1987, 32:684-690; ²Gabrion *et al.*, *Brain Res.*, 1996, 734:301-315; ³Davet *et al.*, *J. Appl. Physiol.*, 1998, 84:19-29.

[63]

LACK OF VESTIBULAR OTOLITH PARTICIPATION IN HUMAN ORTHOSTATIC BLOOD PRESSURE CONTROL. D.E. Watenpugh, A. Cothron, S.L. Wasmund, W.L. Wasmund, R. Carter III, N.K. Muenter, and M.L. Smith. Department of Integrative Physiology, University of North Texas Health Science Center, Fort Worth.

Evidence exists that the vestibular system influences the cardiovascular system. We hypothesized that supine-to-upright vestibular otolith organ stimulation contributes significantly to human cardiovascular responses to orthostasis. Twelve blindfolded subjects underwent the following three 60° upright tilting procedures for three min each, in random order: 1) with the head kept in line with the body, such that the head and body moved from horizontal to 60° above horizontal (0 to 0.87 Gz otolith organ stimulation); 2) with the neck flexed passively from 0° to 30° relative to the body during 60° tilt, such that the head moved from horizontal to 90° above horizontal (0 to 1 Gz otolith organ stimulation); and 3) with the neck flexed 30° during supine baseline conditions, and the neck then passively extended to -30° during 60° body tilting, such that the head remained at 30° above horizontal throughout body tilting (constant 0.5 Gz otolith organ stimulation). This tilt condition provided the cardiovascular challenge of whole body tilting without any concurrent increase in otolith Gz stimulation. All 3 types of tilt increased thoracic impedance, muscle sympathetic nerve activity (160-180%, N = 8 of 12), arterial pressure (~13%), and heart rate (~19%) relative to supine conditions (all P < 0.04). None of these responses differed significantly between the 3 tilt conditions. These data suggest the otolith organ does not play an important role in human.

[64]

REGIONAL AND MUSCLE SPECIFIC EFFECTS OF A β -ADRENERGIC AGONIST IN HINDLIMB SUSPENDED RATS. D.A. von Deutsch^{1,2,3}, I. K. Abukhalaf^{1,2,4}, L.E. Wineski^{1,3}, S.A. Pitts^{1,3}, R. Roper^{1,2}, L.D. Kataria^{1,2}, D.C. Jackson^{1,2}, D.E. Potter^{1,2} and D.F. Paulsen^{1,3}. ¹NASA Space Medicine and Life Science Research Center, ²Department of Pharmacology and Toxicology, ³Department of Anatomy, ⁴Clinical Research Center, Morehouse School of Medicine, Atlanta GA.

Exposure to microgravity and prolonged bed-rest results in head-ward fluid shifts. The impact of these changes on skeletal muscle are of particular interest. Clenbuterol (Cb) was used because it is the most potent anabolic agent amongst the β_2 -adrenergic family of drugs. The regional, dose-dependent, and muscle specific effect(s) of clenbuterol in hindlimb-suspended and non-suspended mature male rats were investigated. In non-suspended rats, 0.4 mg/kg clenbuterol has been observed to have a greater anabolic effect on muscle mass in the leg [(EDL), plantaris, soleus, and gastrocnemius] as opposed to the thigh [(ADL) and pectineus]. This regional effect was not apparent with the 1.0 mg/kg treatment in non-suspended rats. Specifically, the thigh muscle (ADL) was the least responsive to clenbuterol's (1.0 mg/kg) anabolic effect and showed the greatest reduction in mass to unloading. The leg muscles (plantaris and EDL) were the most responsive to clenbuterol (1.0 mg/kg) in suspended rats. Unloading and clenbuterol treatment resulted in distinctly different responses by the soleus, EDL, and plantaris muscles. Soleus showed a moderate anabolic effect of slower onset in response to clenbuterol in loaded muscle. Plantaris showed modest anabolic effect in loaded muscle, but the onset of clenbuterol's action was more immediate. Significant changes in polyamine levels were observed in loaded and unloaded muscles as well as between clenbuterol treated and untreated muscles. Clenbuterol (1.0 mg/kg) reduced the loss of body weight in both suspended and non-suspended rats and resulted in a significant reduction in the amount of mesenteric fat (more than 50% reduction).

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**Concurrent Posters
III-D
Cell Biology**

[65]

VITAMIN D PRODUCTION IN THE ROTATING WALL VESSEL (RWV). F. Lewis, E.N. Benes, X-C. Wang, P.L. Allen, T.G. Hammond and L.A. Cubano. Nephrology Section, Environmental Astrobiology Center and Center for Bioenvironmental Research, Tulane University Medical Center and VA Medical Center, New Orleans LA.

Vitamin D has a billion dollar a year pharmaceutical market. 1-alpha-hydroxylase, which catalyzes production of the active 1-25-diOH form of vitamin D in the kidney, is expensive to manufacture, and there are currently no biological sources for its active form. Several lines of evidence demonstrate that 1-alpha-hydroxylase is expressed and regulated during rotating wall vessel culture of human renal cortical epithelial cells in passage 4. First, assay of 1-25-diOH production from 25-OH vitamin D demonstrates 1-alpha hydroxylase functional activity in cells during culture in the vessel. Second, RT-PCR assay of 1-alpha hydroxylase gene product shows significant up-regulation in the vessel at 4 hour, which is maintained at 48 hours. This gene expression is further increase by addition of parathyroid hormone, which activates vitamin D production in vivo, demonstrating regulation of the response. In contrast to conventional flask culture, each component of the vitamin D pathway in the renal proximal tubule is maintained in during Rotating Wall Vessel culture including megalin, which delivers vitamin D binding protein into the cell, 1-alpha-hydroxylase, vitamin D and PTH receptors. In particular our cell line will be very useful industrially and in the treatment of vitamin D deficiencies as result of renal failure.

(Supported by NASA NRAs 9-811 and 8-1362.)

[67]

ALTERED GRAVITY INCREASES PGE₂ PRODUCTION THROUGH ACTIVATION OF COX-2 mRNA EXPRESSION IN MOUSE OSTEOBLAST LIKE MC3T3-E1 CELLS. A. Sato¹, M. Fujita¹, M. Kanematsu¹, M. Narato², H. Kumagai², S. Kamigaichi¹ and M. Takaoki¹. ¹Space Utilization Research Programme, National Space Development Agency of Japan, Tsukuba-shi, Ibaraki 305-8505, Japan. ²Advanced Engineering Services Co., Tsukuba-shi, Ibaraki 305-0032, Japan.

Prostaglandin E₂ (PGE₂) is a bone-resorbing and bone-forming substance produced by osteoblasts. Altered gravity regulates bone remodeling. The purpose of this study was to clarify effects of altered gravity on the PGE₂ production and cyclooxygenase (COX) mRNA expression in osteoblast-like MC3T3-E1 cells. The cells were exposed to simulated microgravity or hypergravity for 1.5, 3, 6, 12 and 24 h. Their PGE₂ production and COX-1 and COX-2 mRNA expression were assessed. Results indicate that; 1) simulated microgravity obtained in a clinostat demonstrated 6- and 14- fold increase in PGE₂ level in culture media of cells after 3 and 6 h culture compared with those of cells in control (stationary culture); 2) COX-1 mRNA expression did not significantly change but COX-2 mRNA expression increased 3.5- and 1.5- fold after 1.5 and 3 h, then declined to 1/2-fold 24 h later in cells exposed to simulated microgravity; 3) Hypergravity (5, 10 and 20g) induced the increase in PGE₂ level in culture media and in expression of COX-2 mRNA in cells; 4) Nabumetone (35-100iM), a COX-2 inhibitor completely blocked altered gravity-induced PGE₂ production of cells. These results indicate that both clinorotation and centrifugation could modify bone remodeling by COX-2 mRNA expression, suggesting altered gravity might be functioning as a mechanical stressor in cells.

[66]

L-NMMA SUPPRESSES NITRIC OXIDE PRODUCTION AND APOPTOSIS IN *TAXUS BREVIFOLIA* CELLS. M.C. Pedroso^{1,2} and DJ. Durzan². ¹Dept. Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Portugal, and ²Dept. Environmental Horticulture, University of California, Davis, U.S.A.

In plants, nitric oxide (NO) plays a signaling and controlling role in cell proliferation, stress responses, disease resistance, ethylene emission, senescence and cell death (Pedroso et al., 2000, J. Exp. Bot. 51: 1027-1036). Both protective and deleterious effects have been reported (Beligni and Lamattina, 1999, Trends Plant Sci. 4: 299). The effect of centrifugation on NO production was investigated in oocyte-derived haploid callus cultures of *Taxus brevifolia*. Samples were incubated at 1 g (controls), 20 and 150 g, for 3h in culture medium, with or without: a nitrate or nitrite supplementation; NO donors (sodium nitroprusside, SNP, and S-nitroso-N-penicillamine, SNAP); NO-synthase inhibitors (N^G-monomethyl-L-arginine, L-NMMA, and N^G-nitro-L-arginine, L-NNA), their enantiomers; and a NO scavenger (carboxy-PTIO). Cells were then processed for detection of NO, DNA fragmentation and apoptosis (Pedroso and Durzan, 2000, Ann. Bot. in press). NO was visualized in the cytosol and plastids. L-NMMA and L-NNA reduced (0.5-1 mM) or suppressed (at 5 mM) NO production, DNA fragmentation and apoptosis, in both centrifuged and non-centrifuged cells. Results show that plant NO production can be regulated by NOS inhibitors and that arginine, substrate together with oxygen for putative NOS activity, represent a new focal point for the control of a wide range of plant responses including the response to mechanical forces.

(Supported by PRAXIS XXI 3/3.1/CTAE/1930/95 and Center for Plant Biotechnology)

[68]

THE EFFECT OF MICROGRAVITY ON CYTOSKELETON ARCHITECTURE AND PROLIFERATION OF HUMAN BREAST CANCER CELL LINE MCF-7. J Vassy¹, S Portet¹, D Schoevaert¹, M Bei², G Millot¹, G Gasset³ and F Fauvel-Lafève¹. ¹IUH Saint Louis, Paris, France; ²Universitätsklinikum, Ulm, Germany; and ³GSBM, Toulouse, France.

As cells are sensitive to mechanical forces, microgravity might act on stress-dependent cell changes. We proposed that the integration of environmental factors might induce specific cytoskeletal architecture patterns. The latter were characterized by quantitative image analysis.

The human breast cancer cells MCF-7 were flown on a Photon capsule. Cells were fixed in a paraformaldehyde glutaraldehyde mixture after 1.30, 22 and 48 h in orbit. 1g in flight controls were compared to microgravity experiments.

Postflight, immunofluorescent localizations were performed to visualize cell proliferation (Ki67), signal transduction (phosphotyrosine), microtubules and intermediate filaments (cytokeratins). Microfilaments were visualized using fluorescent phalloidin and DNA using chromomycin A₃. Confocal microscopy and image analysis were used to quantify the number of proliferating cells and mitosis, the modifications of cytoskeleton networks and chromatin texture.

In microgravity, phosphotyrosine signal transduction were reduced and number of proliferating cells were increased in correlation with mitosis. Numerous cells showed diffuse microtubules. Perinuclear cytoskeleton networks were relaxed and chromatin texture modified.

In conclusion, the increase of the mitosis period can be explained by an alteration of the microtubule self-assembly in microgravity, involving reaction-diffusion processes. Relaxation of perinuclear cytoskeleton network and modification of chromatin distribution are in agreement with basic predictions of cellular tensegrity.

[69]

CLINOSTAT ROTATION CULTURE MODULATES GENE EXPRESSION OF OSTEOCLASTOGENESIS-REGULATING FACTORS VIA A CYCLIC-AMP DEPENDENT MECHANISM. M.Kanematsu¹, H.Takai², M.Takaoki¹ and A.Sato¹. ¹National Space Development Agency of Japan, Ibaraki, Japan. ²National Institute for Longevity Science, Aichi, Japan.

Bone loss observed in astronauts may be induced by an acceleration of osteoclastic bone resorption as well as a decline of osteoblastic bone formation. Recently, an osteoclastogenesis inhibitory factor (OPG) and an osteoclast differentiation factor (RANKL) were cloned and identified as the products for osteoblasts/bone marrow stromal cells. In the last year, we reported that gene expression level of RANKL was increased while that of OPG was decreased revealed by northern analysis in mouse bone marrow-derived stromal cell line (ST2) cultured on a single axis clinostat. The modulation was not due to alteration in mRNA stability. The present study was undertaken to clarify the mechanism how the modulation of RANKL and OPG gene expressions observed in clinostat culture was induced.

Osteotropic factors such as PGE₂ and PTH has been shown to up-regulate RANKL gene expression, and the effect was suggested to be mediated by cAMP. The clinostat culture caused an increase in the intracellular cAMP level in ST2 cells. Both of forskolin, an intracellular cAMP elevating agent, and db-cAMP mimicked the modulation of RANKL and OPG gene expression in clinostat culture. The modulation of these gene expressions in clinostat culture was blocked by a PKA inhibitor (H89). The modulation was not blocked by a cyclooxygenase inhibitor.

Our results showed that clinostat culture increase RANKL while decrease OPG gene expression via a cAMP dependent mechanism. This modulation was not mediated by an autocrine loop for PGE₂.

[71]

GRAVITY SENSITIVITY OF T-CELL ACTIVATION: THE ACTIN CYTO-SKELETON. B.B. Hashemi^{1,2}, J.E. McClure^{1,2}, and D.L. Pierson¹. ¹Life Science Research Laboratories, NASA - Johnson Space Center, Houston, TX 77058, and ²National Space Biomedical Research Institute, Baylor College of Medicine, Houston TX 77030.

Experiments performed during space flight indicate an inhibition of human peripheral T-cell activation in microgravity culture [Hashemi et. al. FASEB J. 1999 13, (4) 2071]. This inhibition correlates with a lack of activation-induced polarization of the Microtubule Organizing Center (MTOC) towards the activation site. The results indicate that changes in the gravity environment of T-cells from 1g can have dramatic effects on their functional responses.

The actin cytoskeleton plays a crucial role in signal transduction and activation response of T-cells [Destin et. al. Nature Immunology 2000 1, (1) 23]. In the current study, we evaluate the polymerization state of actin in the Jurkat Leukemia T-cell line cultured under hypogravity and hypergravity conditions. When Jurkat cells are exposed to hypogravity culture by clinorotation, they exhibit an impairment in the activation-induced polymerization of F-actin; a response that occurs readily in T-cells that are cultured in 1g. Furthermore, exposure of T cells to hypergravity culture has a dramatic effect on the actin cytoskeleton. A 30-minute exposure of Jurkat cells to as little as a 2g hypergravity culture results in a significant decrease in cellular F-actin. Exposure to higher centrifugal forces in the range of 100-300g for as little as 10 minutes results in substantially lower levels of cellular F-actin. These results are consistent with our earlier findings of inhibition of T-cell activation responses during space flight, and they have significant implications for gravitational biology as they suggest an important role for the actin cytoskeleton in gravity sensitivity of T-cell activation.

(This work was supported by NASA grant No. NAG 2-1357.)

[70]

HUMAN OSTEOBLAST DIFFERENTIATION IS EXPEDITED IN CULTURE IN A MAGNETIC FIELD. L. Yuge¹, T. Kumagai¹, I. Hide¹, S. Hiyama², M. Kanno¹, Y. Kumei³, S. Takeda⁴, Y. Ikuta¹, M. Sugiyama¹, and K. Kataoka¹ ¹Faculty of Medicine, Hiroshima University, Hiroshima, Japan. ²Faculty of Dentistry, Hiroshima University, Hiroshima, Japan. ³Faculty of Dentistry, Tokyo Medical and Dental University, Tokyo, Japan. ⁴Dept. of Molecular Genetics, National Institute of Neuroscience, Tokyo, Japan

We developed a new cell stimulation method in which magnetic microparticles (MPs) were introduced into the cytoplasm of cultured human osteoblasts and the cells were cultured in a magnetic field. We examined the differentiation of osteoblasts with respect to MAPK-cascade, osteogenesis and morphology including histological detection of minerals. After exposure to the magnetic field, the cells containing MPs became larger and were elongated along the axis of the magnetic pole. The expression of bone specific regulatory factors such as osteopontin, bone sialoprotein (BSP) and osteocalcin, and formation of hydroxyapatite crystals were seen earlier and more frequently in this group of osteoblasts than in the other groups (cells alone without magnetic field, cells containing MPs but without magnetic field, and cells alone with magnetic field). The differences were statistically significant at p<0.001 by analysis of variance on a day-by-day basis. P38 activity was increased (increasing phosphorylation activity) in this environment, whereas MAPK/ERK and SAPK/JNK phosphate activity were not changed in any group. The precisely quantitative and stable stimulus induced by a magnetic field developed in the present study offers a new approach to elucidate the entire process of osteoblast differentiation to form hydroxyapatite crystals.

(Supported by Japan Space Forum form NASDA, Space Utilization Research from ISAS, the Ministry of Education, JPTA, and the Magnetic Health Science Foundation.)

[71A]

DEVELOPMENT OF A MICROGRAVITY CELL CULTURE PLATFORM FOR THE STUDY OF BONE CELL METABOLISM ONBOARD THE NASA SHUTTLE. L. Misener, D. Sindrey, T. Smith, S. Pugh, D. Kusljic, P. Kwong. Millenium Biologix Inc., Kingston, Ontario, Canada; Allelix Biopharmaceuticals Inc., Mississauga, Ontario, Canada.

The study of bone cell metabolism in space has been compromised by the lack of microgravity (μ g) hardware that successfully addresses the biological requirements of bone cell cultures in this challenging environment. We have designed a unique cell culture platform (OSTEOTM), utilizing Millenium's synthetic bone substrate (OsteologicTM), which supports mineralization by osteoblasts (OB) and resorption by osteoclasts (OC) in μ g. To address diverse experimental requirements, including the need for independent control of OB and OC environments, the system was designed to generate 192 data points from 12 independent bioreactors. In evaluating conditions influential in cell culture performance, the most important parameters affecting cell growth were: fluid dynamics, bio-compatible materials, sterility, gas exchange, temperature stability, and physical launch forces. Medical grade plastics were tested for biocompatibility with bone cell cultures. Hematoxylin/Eosin staining of cell monolayers and tetracycline labeling of mineralized matrix showed polystyrene as the most compatible material, followed by polysulfone and acrylic. Sterilization via hydrogen peroxide vapour was adopted to avoid the toxic residues left by ethylene oxide. BMS cells cultured, with and without 5% CO₂, in open slides and within the closed pathway system, revealed that normal growth and mineralization could be achieved with CO₂ dependent media without the need for CO₂ gassing. The culmination of this work has lead to the development of a proven system, operated by astronaut John Glenn on the NASA Shuttle STS-95 in October 1998, for the successful in vitro study of bone cell metabolism in μ g conditions.

**Concurrent Posters
III–E
Plant Development, Physiology and
Gravity Sensing**

[72]

OXYGEN EFFECTS ON POLLEN GERMINATION AND TUBE ORIENTATION. J. Blasiak, D. Mulcahy and M. Musgrave. Biology Department, University of Massachusetts, Amherst MA 01002.

Chemical gradients and structural features within the pistil have been previously proposed as factors determining the directionality of pollen tube growth. In this study, we examine the behavior of pollen of eight species germinated in a dynamic oxygen gradient. While the germination rates of some species decreased directly with decreasing oxygen tension, other species showed no decrease in germination at oxygen tensions as low as 2 kPa. In one species, germination was consistently greater at decreased oxygen tensions than at ambient atmospheric levels. In three of the eight species tested, the developing pollen tube showed clear directional growth away from the more oxygenated regions of the growth medium, while in one species growth was towards the more oxygenated region. The remaining four species showed random tube growth. The pattern of oxytropic responses among the taxa suggests that this tropic behavior is both widespread and phylogenetically unpredictable.

(Supported by NASA: NAG2-1020 and NAG2-1375.)

[74]

EFFECTS OF LITHIUM IONS ON ELONGATION AND GRAVITROPIC RESPONSES OF PRIMARY ROOTS OF MAIZE. Timothy J. Mulkey. Life Science Dept., Indiana State Univ., Terre Haute IN 47809

Rubbing-induced ethylene and conversion of ACC (1-aminocyclopropane-1-carboxylic acid) to ethylene in relation to thigmotropism is reduced by treatment with lithium (*Pl. Physiol.* 72:522). In animal systems, Li inhibits resynthesis of PIP₂ (*Biochem. J.* 180:655) and inhibits inositol-1-phosphate phosphatase (*J. Biol. Chem.* 255:10896); but plant phosphatases appear less sensitive to Li ion exposure (*Pl. Physiol.* 76:40). Previous work in our laboratory has demonstrated a role for auxin-induced ethylene production in root growth and gravitropic responses and has suggested involvement of the second messenger system in auxin-associated responses of root. This study examines the effects of Li ions on elongation and gravitropic responses of maize roots. Li ions at concentrations greater than 0.1 mM promotes the elongation rate of primary roots of maize. Additionally, asymmetric application of Li to the lower surface of the root delays positive gravicurvature or results in negative gravicurvature. Li ions alter *in vitro* protein phosphorylation patterns; Li treatment results in phosphorylation patterns similar to those observed in auxin-stimulated/ethylene-inhibited tissue. These results will be discussed in relation to auxin-ethylene interaction and protein phosphorylation in the putative second messenger system of hormone action during elongation and gravitropic responses.

[73]

CHANGES IN COTYLEDON CELL ULTRASTRUCTURE DURING *Brassica rapa* SEED DEVELOPMENT IN MICROGRAVITY.

A. Kuang¹ and M. E. Musgrave². ¹Department of Biology, University of Texas-Pan American, Edinburg TX 78539; ²Biology Department, University of Massachusetts, Amherst MA 01003.

Prior analysis of mature *Brassica rapa* seeds produced on the Mir station showed that seed production in microgravity resulted in reduced protein in the storage reserves, and a retention of starch as a storage product. Using immature seeds (8-15 days post pollination) of *Brassica rapa* L. cv. 'Astroplants' produced on the shuttle during the Collaborative Ukrainian Experiment on STS-87, we compared the progress of storage reserve deposition in cotyledon cells during early stages of seed development. Immature seeds were dissected from siliques immediately post-flight or following the ground control. These embryos were fixed in 2.5% glutaraldehyde and 1% formaldehyde, postfixed in 1% osmium, embedded in Spurr's resin, sectioned and stained for light and electron microscopy. Results showed that starch accumulation began in plastids at an early developmental stage and grain size and number increased with seed development. At the stage of cotyledon elongation, starch reached a maximum in both flight and ground control seeds. In the spaceflight seeds, starch was retained after this stage, while starch grains decreased in size in the ground control. Large and well-developed protein bodies were observed in cotyledon cells of ground control seeds at 13 days post-pollination, but their development was delayed in the spaceflight material. Numerous mitochondria were observed in cotyledon cells of ground control seeds at 8 days post pollination, suggesting the need for large amounts of energy for cell division and protein synthesis and deposition at later developmental stages. Degradation of starch grains may provide the main source of energy for these processes. Restricted energy supplies would retard protein deposition and cell division, resulting in lower cotyledon cell number, smaller sized protein bodies, and persistent starch grains observed in the mature seeds from Mir.

(Supported by NASA: NAG-100139 and NAG2-1375.)

[75]

THE ACTIN NETWORK IN LENTIL ROOT STATOCYTES. D. Driss-Ecole and G. Perbal. Laboratoire de Cytologie Expérimentale et Morphogénèse Végétale, Université Pierre et Marie Curie, 4 place Jussieu, F-75252 Paris Cedex 05, France.

Root statocytes are characterized by a strict structural polarity. A treatment by the actin-disrupting drug cytochalasin (B or D) demonstrates that the actin microfilaments (MFs) are involved in the distal positioning of ER and in the proximal location of the nucleus. We carried out a pre-embedding immunogold silver technique with a monoclonal anti-actin antibody and demonstrated that the amyloplasts (statoliths) are enmeshed in an actin web of short filaments arranged in different ways. Moreover myosin-related proteins were localized on the surface of statoliths by immunofluorescence.

Comparison of mean velocities of amyloplast movement in root transferred from 1 g centrifuge (root-tip-directed acceleration) to microgravity (S/MM-03 mission of Spacehab) and in inverted roots on earth allowed to determine the very low value of the force responsible for the movement of one amyloplast (0.016 pN). Taking into account the force produced by one molecule of myosin it can be hypothesized that there is a loss of a large part of energy during the basipetal movement in microgravity due to the fact that the MFs are not oriented parallel to the axis of the statocyte confirming our cytological observations. The analysis of the movement of the amyloplasts in microgravity showed that the amyloplasts can move individually. These observations raise the possibility that in the root statocytes the actin cytoskeleton is made of an interconnected network of fibrous units organized in a random fashion close to the percolation model proposed by Forgacs. Myosin-coated statoliths can move unidirectionally along several and short actin filaments during the basipetal movement. In 1 g the link between MFs and molecules of myosin can be disrupted implying that amyloplasts will move by their own weight.

(Supported by CNES 793/00/8103.)

[76]

MICROSCOPIC ANALYSIS OF SWEETPOTATO ROOT TIPS PROPAGATED FROM STEM CUTTINGS MAINTAINED IN EITHER VERTICAL OR HORIZONTAL CLINOROTATION. C.S. Williams, D.G. Mortley, C.E. Morris, C.F. Davis, S.D. Gamble and J.W. Williams. Center for Food and Environmental Systems for Human Exploration of Space and the George Washington Carver Agricultural Experiment Station. Tuskegee University, Tuskegee AL.

Although there has been some criticism of the system, clinostat rotation continues to be used as one of the major ground based models for simulation of microgravity. Sweetpotato has been identified as one of the potential food crops for human planetary space exploration. To that end, sweetpotato growth characteristics under simulated microgravity have important application to its growth in space. Stem cuttings were placed in growth chambers rotating on clinostats with either vertical or horizontal orientation for either 4, 7, or 14 days. Root tips were collected and immediately placed in fixative (3% glutaraldehyde, 1.5% paraformaldehyde in 0.1M sodium cacodylate buffer). Following overnight fixation, specimens were postfixed in 2% osmium tetroxide, en bloc stained with 0.5% uranyl acetate, dehydrated in a graded series of ethanol, and embedded in Spurr's Medium (under vacuum). Ultrastructural observations of the root tips, with emphasis on the statocytes, showed morphological and organizational changes, particularly in the starch-containing statoliths, within the cells consistent with the altered geotropism and direction of growth of the roots (vertical orientation = "downward"; horizontal orientation = "upward" or "outward").

(Supported by NASA:NAG10 0209 and USDA/CSREES ALX-PS-1. Ultrastructural Facility support was provided by NIH/RCMI 5-G12RR03059.)

[78]

THE *rib1* MUTANT IS RESISTANT TO INDOLE-3-BUTYRIC ACID, AN ENDOGENOUS AUXIN IN *ARABIDOPSIS THALIANA*. C. S. Waddell and J. Poupart. Dept. of Biology, McGill University, Montreal, Canada.

The presence of indole-3-butyric acid (IBA) as an endogenous auxin in *Arabidopsis* has been recently demonstrated. However, the *in vivo* role of IBA remains to be elucidated. We have characterized a semi-dominant mutant that is affected in its response to IBA, but shows a wild-type response to indole-3-acetic acid (IAA), the predominant and most studied form of auxin. This specificity of auxin response is unique amongst published auxin resistant mutants; it demonstrates that *rib1* can discriminate between the two known endogenous auxins of *Arabidopsis*. We have named this mutant *rib1* for *resistant to IBA*. Root elongation assays show that *rib1* is specifically resistant to IBA, to the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), and to auxin transport inhibitors. *rib1* does not display increased resistance to IAA or to the synthetic auxin naphthalene acetic acid (NAA), nor to other classes of plant hormones. *rib1* individuals also have other root specific phenotypes, including a shorter primary root, an increased number of lateral roots, and a more variable response than wild type to a change in gravitational vector. Adult *rib1* plants are morphologically indistinguishable from wild-type plants. These phenotypes suggest that IBA is a physiologically active auxin in roots with specific roles in root development and response to environmental stimuli. We propose that RIB1 could function in either IBA transport or response. This work was supported by a grant from the Natural Sciences and Engineering Council of Canada.

[77]

ULTRASTRUCTURE OF *P. PATENS* TIP CAULONEMATA CELLS: UNTREATED, COLD GROWN, SEVERED, ORYZALIN TREATED, UV-A TREATED. Edward B. Tucker¹, Vinoud Sookhdeo¹, and Lucy Yin². ¹Natural Sciences, Baruch College, CUNY, New York, NY and ²Biology Department, University of Massachusetts, Amherst MA.

The apical cells of *P. Patens* caulonemata are tip growing and in the dark they are negatively gravitropic. Our data indicated that sedimentation of chloro-amyloplasts preceded gravi-curvature, similar to that previously reported for *Ceratodon* (Walker and Sack, 1990; Planta 181: 71-77). Gravi-curvature occurred if cells were grown at 5°C, and was temporarily inhibited if cells were either treated with oryzalin or exposed to a flash of UV-A light. Gravi-curvature did not occur if cells were severed from the sub-apical cells.

Cells were grown on agar plates, wrapped in foil and placed vertically. They were not gravi-stimulated. Treatments were: untreated, grown at 5°C, severed, treated for 5 min with 0.1% oryzalin, or exposed to UV-A for 10s. Cells were fixed in the dark with 4% paraformaldehyde, 2% glutaraldehyde in 100 mM PIPES pH 6.8 and post-fixed with 2% OSO₄ plus 0.8% potassium ferricyanide. Tissue was *en bloc* stained with 2% uranyl acetate and embedded in Spurr resin. Sections were post stained with lead citrate. The ultrastructure of the apical cell demonstrated organelle domains: an apical ER zone, a chloro-amyloplast free zone, a chloro-amyloplast zone, a nuclear zone, and a vacuolar zone. Changes in organelle ultrastructure and distribution were observed on electron micrographs of the treated cells. For example, in untreated cells chloro-amyloplasts aligned in rows seemingly outlined by endoplasmic reticulum. In cold grown cells chloro-amyloplasts were larger while in severed cells they were smaller. Chloro-amyloplasts of oryzalin treated cells accumulated in the tip region. With all of these treatments the normal alignment of chloro-amyloplasts was altered.

(Supported by NASA grant NAG5-3743.)

**Concurrent Posters
III–F
Advanced Life Support and
Biotechnology**

[79]

REGENERABLE SEED PLUGS FROM FORMED PLANT FIBER. R.C. Morrow, C.J. Ehle and T.M. Crabb. Orbital Technologies Corporation, 1212 Fourier Dr, Madison WI.

A project was undertaken to develop techniques for producing seed plugs from formed plant fibers for Advanced Life Support (ALS) plant growing units. Plant plugs can place a significant impact on an ALS if they cannot be completely recycled. For example, an annual supply of seed-starting structures such as seed plugs would be on the order of 150 to 5000 kg for a six-person base, depending on the seeding technique used. The primary challenge was to develop regenerable plant supports that retain structural integrity in a wet, humid environment for one crop cycle, can be easily fabricated on-site, are non-toxic to plants, and do not require separation from the inedible plant waste stream. To produce plant plugs, wheat fiber was processed using a combination of dry and wet refining and then formed into mats using pulp-molding techniques. After testing fiber size combinations, fiber mats were formed into plant plugs for use in structural and biocompatibility tests. A variety of molds, both for "plug" and "linear wedge" configurations were also fabricated and tested in the pulp molding system. Tests conducted on the plant plugs included tensile (tear) strength testing, a four week plant growth study using lettuce and comparing growth to that obtained with commercially available inorganic plant plugs, and a germination study using a variety of crop plants. Plant tests showed that some of the wheat-based plugs showed a reduction in plant growth. However, wedge shaped linear plugs from wheat showed plant productivity equivalent to commercial plugs. Plugs fabricated from wheat fiber did release organic materials that could support microbial growth into the nutrient solution. However there was no indication of toxic materials being released. Leaching of the wheat fiber with hot water during the wet refining process removed significant amounts of organic carbon and inorganic nutrients. However, it appears that an additional leaching step is probably required. Germination tests showed that wheat plugs supported germination at about the same rate as commercial plugs. Plugs fabricated from coarse cut wheat fiber showed distinctly better plant development than did the plugs fabricated from fine cut wheat fiber.

[81]

ASTRIUM – A NEW NAME IN SPACE LIFE SCIENCES.

P. Kern¹, U.M. Kuebler¹. ¹Astrium GmbH, Friedrichshafen, Germany.

Astrium is the merger of former DaimlerChrysler Aerospace (DASA) of Germany with MatraMarconiSpace (MMS) of France and Great Britain. Both companies have a long history of self-standing space life science programs, as well as a joint LS project, for example the BIORACK which flew on 6 shuttle missions and was built by Matra/Toulouse and Dornier/Friedrichshafen, which now belong to ASTRIUM.

The presentation will focus on the actual status of the ASTRIUM Life Science Program and will give details on some of its present and future payloads:

- BIOLAB for ESA, ISS payload
- EMCS for ESA, ISS contribution to NASA
- LBNP for DLR, ISS contribution to NASA
- CARDIOLAB for DLR/CNES, ISS payload
- BIOBOX for ESA, shuttle/spacehab or foton payload
- SEXSY for DLR, shuttle/spacehab payload

MEXSY Astrium R&D, modular experiment system

[80]

EVAPOTRANSPIRATION BY SALAD CROPS IN CONTROLLED ENVIRONMENTS. D. E. Ciolkosz¹ and G. D. Goins². ¹Department of Agricultural and Biological Engineering, Cornell University. ²Advanced Life Support and Space Biology Laboratory, Kennedy Space Center

Water use by crops in Bioregenerative Life Support Systems (BLSS) is an important aspect of the performance of effective life support systems. Experiments were conducted to measure the daily evapotranspiration (ET) by three salad crops (Lettuce, Radish, and Spinach) which are candidates for use in BLSS. The plants were grown in a Nutrient Flow Technique (NFT) system in a controlled, closed environment under one of three different electrical light sources (fluorescent, red LED, red LED + blue). The plants were grown for 21 days, with shoot and root fresh mass and dry mass measured at day 21. The plants were grown under the same temperature regime, humidity level, nutrient concentration, and photosynthetic light level. Results indicate that, if system evaporation is discounted, water use in hydroponic lettuce is a function of the incident photosynthetic light level, and is not strongly affected by the level of thermal radiation. Results from the spinach and radish plants suggest a combined dependence on photosynthetic and thermal radiation levels. These results underline the importance of the interaction between light source and water use, which may be of crucial importance for Bioregenerative Life Support (BLSS) systems.

[82]

IBIS: AN INSTRUMENT DEDICATED TO PERFORM BIOLOGICAL EXPERIMENTS IN MICROGRAVITY. D. Thierion¹, G. Gasset², D. Chaput¹, A. Labarthe¹, B. Eche¹ and M. Viso³. ¹Centre National d'Etudes Spatiales, Toulouse, ²Groupement Scientifique de Biologie et de Médecine spatiale, Paris; ³Centre National d'Etudes Spatioales, Toulouse.

IBIS (Instrument de Biologie Spatiale) was design by the technical center of the French Space Agency (CNES) in Toulouse to answer some fundamental questions in biology and developmental biology addressed by the French scientific community. The instrument was specifically designed to fly on the Foton capsules. Despite the shaky history of the three performed flights the contribution of IBIS to study the effects of micro-gravity on living systems is very significant.

The Instrument is composed of three main compartments. The refrigerator can hold 32 pairs of experimental "cassettes". The temperature can be set from 0 to 22°C. The incubator can house simultaneously 16 pairs of "cassettes". One cassette lays on the continuous centrifuge and one on the micro-gravity tray. The temperature of this compartment can be set between 20 and 37°C. The intermediate compartment is used to enter and retrieve the cassettes. This area can be used also to house 5 cassettes at a temperature between 20 and 37°C. The "cassettes" are processed in pairs; one for the micro-gravity tray and the other for the centrifuge. Three types of cassettes are already designed and manufactured: two dedicated to animal cells and larvae, one to seed development. The hardware and its performance will be described and the past and future mission profiles will be presented.

Since the preparation of the biological samples in Plessetsk is possible the range and the quality of experiments which can be performed with IBIS, are significantly enhance.

**Concurrent Posters
III-G
Spaceflight Experiment Results**

[83]

EXPRESSION OF FAS/CD95 IN SPACEFLOWN LYMPHOCYTES (JURKAT). L.A. Cubano and M.L. Lewis. Microgravity Biotechnology Laboratory, University of Alabama in Huntsville.

Human lymphocytes flown on the Space Shuttle respond poorly to mitogen stimulation, and populations of the lymphoblastoid T cell line, Jurkat, manifest growth arrest, increase in apoptosis and time- and microgravity-dependent increases in the soluble form of the cell death factor, Fas/CD95 (sFas). Aging shares similarities with changes seen during spaceflight including loss of bone and reduced immune response. The potential role of apoptosis in population dynamics of space-flown lymphocytes has not been investigated. We flew Jurkat cells on STS-80 and STS-95 to determine whether apoptosis and the apparent microgravity-related release of sFas are characteristic of lymphocytes in microgravity. The effects of spaceflight and ground-based tests simulating spaceflight experimental conditions, including high cell density and low serum concentration, were assessed. Immunofluorescence microscopy showed increased cell associated Fas in flown cells. Results of STS-80 and STS-95 confirmed increase in apoptosis during spaceflight and the release of sFas as a repeatable, time-dependent and microgravity-related response. Ground-based tests showed that holding cells at 1.5 million/ml in medium containing 2% serum before launch did not increase sFas. Reports of increased Fas in cells of the elderly and the increases in spaceflown cells suggest possible similarities between aging and spaceflight effects on lymphocytes.

(Supported by NASA Grants NAG2-985, NCC8-132, and NASA Graduate Student Research Program Grant 97-GSRP-076.)

[85]

PAYLOAD LATE ACCESS SURVEY PRELIMINARY RESULTS.

C. Martin-Brennan¹ and R.C. Morrow², ¹The Bionetics Corporation, Kennedy Space Center, FL 32899 and ²Orbital Technologies Corporation, 1212 Fourier Dr, Madison WI.

A survey was conducted by the American Institute of Aeronautics and Astronautics (AIAA) Life Sciences and Systems Technical Committee as a result of the need to gather information regarding late installation requirements of current and future microgravity experiments on the Space Shuttle or International Space Station (ISS). Late installation is defined as the time when experiments are physically placed on the Space Shuttle at the launch pad. The Shuttle and ISS experiments that require late installation currently are installed and launched in the Space Shuttle middeck. There are currently four time windows that are offered to researchers to define their late installation requirement: 3 days to 24 hours before launch; 24 hours before launch; 24 to 18.5 hours before launch, and 18.5 to 15.5 hours before launch. These are the times when the experiments are loaded into the space shuttle at the launch pad; however, a researcher must be ready to go from the laboratory (e.g., Hangar L) to the launch pad 1.5 to 2.0 hours prior to the agreed upon time window.

Questions asked in the survey concerned the type research that the recipients foresaw conducting on the Space Shuttle or International Space Station, whether or not they saw a need for late installation of their experiments, and if so, what late installation timeframe was required, what is the scientific or engineering rationale for this late installation requirement, and what would be compromised if late installation requirements were not met. About 475 surveys were sent out to members of the ASGSB. A total of 57 responses were received, of which 36 required late access, 12 did not require late access and 9 were not sure. Of the 36 that required late access, 23 required access within 18.5 hours or less prior to launch.

[84]

ARE THERE TWO MECHANISMS UNDERLYING SPACE MOTION SICKNESS? D.G.D. Watt. Aerospace Medical Research Unit, McGill University, Montreal, Canada

Motion sickness likely occurs whenever there is a conflict between actual vestibular (and other) inputs and those predicted by the CNS based on past experience. Conflicts can be caused by (1) damage or disease, (2) acceleration environments that go beyond normal physiological range, and (3) altered processing of labyrinthine signals resulting from prolonged vestibular suppression. Previous studies have assumed that the main cause of space motion sickness is abnormal vestibular signals generated during movement in microgravity (mechanism 2). This study's objective was to determine if altered central processing (mechanism 3) also contributes. Each in-flight experiment lasted 43 min. and consisted of monitoring eye, head and upper torso rotation while 4 astronauts went about their normal activities. Post-flight, the data were filtered to reduce noise, artifacts were identified and removed, and eye position was converted to eye velocity. Finally, eye velocity was plotted relative to head velocity during periods of pure, yaw-axis head movement. During spontaneous, self-generated head rotations, the vestibulo-ocular reflex should keep the eyes stabilized relative to inertial space. Except during quick gaze refixations, eye velocity should be equal and opposite to head velocity. Analysis of Flight Day 1 data demonstrated a statistically significant decrease in eye stabilization, however. Furthermore, head velocities were mostly below 100°/sec, where visual tracking alone should have been able to maintain gaze on target, suggesting deliberate suppression of both visual and vestibular mechanisms. A more normal pattern was seen on Flight Days 7, 12, 13 and 15, and after landing. Results suggest that the astronaut has much in common with a passenger reading in the back seat of a car. In both cases, vestibular systems operate in altered acceleration environments beyond the normal range defined by evolution. This produces motion sickness. In addition, both seem to suppress vestibular function. The passenger does this to keep his or her gaze stable relative to the book. Why the astronaut does it is unclear. Whatever the reason, excessive and prolonged suppression leads to temporary changes in vestibular function and this second mechanism also produces motion sickness. (Supported by the Canadian Space Agency.)

[86]

EFFECT OF MICROGRAVITY ON ROOT REGENERATION, ULTRASTRUCTURES, AND CARBOHYDRATE CONTENT OF SWEETPOTATO STEM CUTTINGS. D.G. Mortley, C.S. Williams, C.F. Davis and J.W. Williams. Center for Food and Environmental Systems for Human Exploration of Space and G.W. Carver Agricultural Experiment Station, Tuskegee University, Tuskegee AL 36088, USA.

Sweetpotato stem cuttings grown in microgravity aboard STS-93 shuttle mission, were evaluated for root growth, alteration of amyloplasts, and carbohydrate content. Twelve cuttings (6 cm long) of 'TU-82-155' sweetpotato were grown in a Commercial Generic Bioprocessing Apparatus (CGBA) which provided a thermally controlled environment (23C). Cuttings were grown in Phytigel impregnated with a modified half-Hoagland nutrient solution. After five days in microgravity, cuttings were received within two hours upon return to earth and quickly processed. All cuttings produced fibrous roots and growth was quite vigorous and except a slight browning of some root tips, appeared normal. Roots tended to grow in a disoriented fashion as it sensed for gravity. There was a more even distribution of amyloplasts in ground controls compared to a more random distribution in flight samples. The concentration of sugars, glucose, fructose, and sucrose, and total starch content were all substantially higher in flight samples compared to ground based samples.

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

**Concurrent Posters
III–H
Spaceflight Physiology and Medicine**

[87]

LONG TERM EFFECTS OF MICROGRAVITY ON HUMAN SLEEP, CYTOKINE, AND ENDOCRINES. H. Moldofsky¹, F Lue¹, J MacFarlane¹, C-G Jiang¹, L Poplonski¹, I Ponomoreva², I Larina², R Gorczynski¹ Univ. of Toronto Centre for Sleep and Chronobiology, ² State Research Center of Russian Federation, IBMP.

Because of the possible adverse effects of disturbed sleep on health during long term stay in space, we studied sleep/wake-related changes in diurnal cytokine and neuroendocrine functions in prolonged microgravity. **Method:** 8 male cosmonauts/astronauts (mean age 45.5 y.) who participated in NASA4, MIR23 and 24 were assessed for sleep/wake-related aspects of cytokine and endocrine functions during baseline data conditions (BDC) preflight. 5 subjects who served on MIR for 4 to 6 mon. were studied during mid-flight and late-flight at about days 81, 82, 143, 144, then 1 wk. post flight. 3 to 6 mon. later, 3 subjects participated in post-flight 2 follow-up. In BDC serial venous blood samples, taken during wake and sleep, were assayed for plasma interleukin-1(IL-1), IL-1ra, and IL-6; plasma prolactin, growth hormone, and cortisol. Sleep, cytokines, and endocrine functions were compared among preflight, inflight, postflight 1 and postflight 2. **Results:** The EEG sleep showed increased awake time, increased movement arousals to stage 1 sleep, and decreased % stage 1 sleep during late flight vs. preflight. Within flight, time in slow wave (deep) sleep declined. During the first wk. postflight vs. preflight, there was increased sleep period time, increased awake time, reduced sleep efficiency, and reduced % stage 2 sleep. In comparison to preflight, plasma IL-1, IL-1ra increased, and IL6 tended to increase. IL-1 declined during post flight. IL-1 and IL-1ra returned to baseline in postflight 2. IL6 remained elevated during post flight 1 and on post flight 2 compared to preflight. Plasma prolactin increased in postflight 1 in comparison to pre and inflight. No changes occurred in growth hormone pre and postflight 1 and 2, and was undetected inflight. Plasma cortisol was unchanged inflight and post-flight 1, but decreased in postflight 2. **Conclusions:** Prolonged spaceflight results in sleep physiological disturbances and augmentation of cytokines IL-1, IL-1ra, IL-6 and prolactin. These sleep and proinflammatory changes during extended stays in micro-gravity conditions may contribute to skeletal and fatigue problems.

(Support: Canadian Space Agency No.9D007-5-8505/01-ST.)

[88]

DOSE AND DOSE RATE EFFECTS OF PROTON RADIATION ON LYMPHOCYTE POPULATIONS IN BLOOD AND SPLEEN. D.S. Gridley^{1,2}, M.J. Pecaut¹ and G.A. Nelson¹. Departments of ¹Radiation Medicine (Radiobiology Program) and ²Microbiology and Molecular Genetics, Loma Linda University and Medical Center, Loma Linda CA 92354.

The major goal of this study was evaluate the effects of proton radiation, the major form of radiation in deep space, at varying total doses and dose rates on lymphocyte populations in the blood and spleen. High energy protons (250 MeV, entry region of the Bragg curve) were delivered in a single fraction at total doses of 0.5, 1.5, and 3.0 Gy and at dose rates of 1 cGy (low-dose rate, LDR) and 80 cGy (high-dose rate, HDR) to the entire body of young adult, female C57BL/6 mice (n = 42). The animals were euthanized at 4 days post-exposure for quantification of specific lymphocyte populations by 4-color flow cytometry at 4 days post-exposure. A highly significant dose-dependent reduction was observed in the total leukocyte count and in the percentages and numbers of T (CD3+), B (CD19+), T helper (CD4+), and T cytotoxic (CD8+) cells in both the blood and spleen (p<0.001). A similar, but less pronounced, dose effect was found with natural killer (NK1.1+ NK) cells (p<0.01). In general, the numbers for each cell population were slightly lower with HDR than with LDR radiation. A significant dose rate effect was noted only in T and B cell percentages in the spleen (p<0.05), but not in the blood. These data indicate that, under the conditions of the present study, lymphocyte response to proton radiation is highly dependent upon the total dose and that dose rate effects are more likely to be evident in the spleen lymphocytes compared to those circulating in peripheral blood. These data are similar to our previous observations following whole-body photon (γ -ray, ⁶⁰Co) irradiation, thereby suggesting that data obtained with photons may be predictive of proton effects with respect to relative proportions and numbers of the measured lymphocyte populations.

(Supported by NASA: cooperative research agreement NCC-9-79.)

[89]

CYTOKINE SYNTHESIS BY T CELLS COLLECTED FROM APHERESIS DONORS RECEIVING G-CSF. B.-N. Lee¹, M. Korbli², W.T. Shearer³ and J.M. Reuben¹. ¹Depts of Laboratory Medicine and ²Blood and Marrow Transplantation, Univ. of Texas M. D. Anderson Cancer Center, and ³Dept of Pediatrics, Baylor College of Medicine, Houston TX.

Bone marrow aplasia can occur from damage to the hematopoietic stem cells following exposure to radiation. Mobilization of peripheral blood progenitor cells (PBPC) by granulocyte colony-stimulating factor (G-CSF) and collected by apheresis is safe, well tolerated for autologous and allogeneic transplantation, and a proven countermeasure for marrow failure. However, little is known of the ability of T cells in PBPC preparations to synthesize Th1 and Th2 cytokines. PBPC preparations were stimulated with PMA and superantigen (SEB) and the syntheses of Th1 and Th2 cytokines by T cells of donors were assessed before receiving G-CSF (Pre-GCSF), after receiving 6 μ g/kg body weight G-CSF subcutaneously q 12 h x 5 d (Post-GCSF), and during apheresis. Compared with pre-GCSF, Post-GCSF samples had significantly lower percentages of T cells synthesizing IL-2, IFN- γ , and TNF- α following stimulation with SEB, and not with PMA. Moreover, compared with pre-GCSF, the apheresis bag had significantly fewer T cells synthesizing IL-2 and TNF- α upon PMA stimulation and fewer T cells synthesizing IL-2, IFN- γ , and TNF- α with SEB stimulation. T cells from apheresis bag also had significantly fewer T cells synthesizing IL-2, IFN- γ , and TNF- α upon SEB stimulation when compared to the T cells from post-GCSF. The percentages of T cells synthesizing IL-10 were similar for all preparations. Therefore, while G-CSF can mobilize the progenitor cells from bone marrow, it reduces Th1 responses by T cells.

(Supported by NASA/NSBRI)

[90]

THE ADRENAL/GONADAL RESPONSE TO A 5-HOUR HDT IS PROMPTER IN MEN THAN IN WOMEN. F. Strollo¹, G. Spera², E.V. Cosmi³, M. More¹, A. Mambro¹ and G. Riondino¹. ¹INRCA Endocrine Unit, ²1st University Andrology Unit and ³1st University Gynecology Department, Rome, Italy.

Introduction: Based on our previous results, reversible hypoandrogenism occurs in male astronauts during space flight; data concerning women-astronauts are missing at the moment. This might be an additive factor wrt bone demineralization, of course.

The aim of the study was to try and confirm space results in men during a short on-ground experiment and to investigate upon the ovarian response to the same test.

Materials and methods: In the morning, after an overnight fast, 9 healthy male volunteers aged 33 to 55 years underwent an HDT test for 5 hours: HR, SBP and DBP measurements and circulating cortisol, LH, testosterone (T) and androstenedione (A) assays were performed immediately before and after the test. 9 healthy age-matched female volunteers followed the same protocol, excepted for T and A, substituted for by estradiol and estrone, a strong and weak estrogen hormone, respectively.

Results: Males showed a significant decrease in HR, SBP and DBP (p<.05), in cortisol (p<.01) and in A (p<.05). Females failed to show any significant changes in the parameters under study.

Discussion: (1) once again males showed a more homogenous and fast neuroendocrine response to tilting maneuvers; (2) only the weaker androgen A decreased significantly in men, possibly due to blood-shift dependent changes in liver blood flow; (3) T did not decrease significantly during the test, probably due to its longer half-life; and (4) overall female reaction seems to be slower, if any.

Conclusions: Five hours are probably too short a period for the testis to react to blood shift in terms of T production. Next short- and long-term bed rest European campaigns might enable us to confirm flight results during ground simulation studies and eventually to evaluate the time course of gonadal steroid changes.

**Concurrent Oral Sessions
III
Space Flight and Space Medicine**

[91]

EFFECTS OF SPACEFLIGHT AND HINDLIMB SUSPENSION UNLOADING ON RAT NEUROMUSCULAR DEVELOPMENT. D.A. Riley, B.L. Huckstorf, G.R. Slocum, J.L.W. Bain, P.M. Reiser, F.R. Sedlak, W. Liebl and M.T.T. Wong-Riley. Dept. Cell Biol., Neurobiol. and Anatomy, Medical College of WI, Milwaukee.

During the first month of life, muscle fiber types differentiate by transitioning from embryonic to adult myosin heavy chain expression, the motor innervation shifts from multiple to single innervation, and the motor nerve endings change from simple to complex morphology. Our studies examined neuromuscular development in 8 d old rats subjected to 9 d of unloading by spaceflight (STS-72 NIH.R3) or intermittent hindlimb suspension (4.5 h suspended, 1.5 h return to the dam for nursing 24 h/day). We predicted altered development of the antigravity weightbearing soleus and normal development of the non-weightbearing EDL muscle. For spaceflight, there were flight (FLT), asynchronous ground control (AGC), and vivarium ground control (VC) litters (10 rats/litter). For suspension, from one litter 5 rats were hindlimb unloaded (HU), and the other 5 were simultaneously removed but not suspended to control for isolation (IC). Another unperturbed litter provided vivarium controls. Fibers were typed by myosin ATPase histochemistry and myosin isoform specific immunostaining. Silver-cholinesterase staining showed the motor innervation. Body weights at 9 d for FLT, HU and IC were ~50% of VC. Unique to soleus, unloading accelerated fast IIA myosin production, delayed slow myosin expression and retarded standardized muscle weight gain and fiber growth. Muscle loading appears the major regulatory factor of fiber type differentiation. Elimination of multiple innervation was not delayed, but fewer motor nerve endings achieved complexity. Nerve impulse activity appears sufficient for resolving multiple innervation. Smaller endplates (muscle fibers) inhibited nerve ending growth.

(Supported by NASA NAG2-956 and NIH UO1NS33472.)

[93]

MUSCLE COLLAGEN GENE EXPRESSION AND PROTEIN ADAPTATION FOLLOWING 14 DAYS OF SPACEFLIGHT IN BION 11 RHESUS MONKEYS (*MACACA MULATTA*). D.A. Martinez¹, V.R. Edgerton², R.E. Grindeland³, D.E. Gallagher¹, J.D. Tanksley¹, K.M. Shea¹, and A.C. Vailas¹. ¹Connective Tissue Physiology Laboratory, University of Houston, TX, ²Dept. of Physiological Sciences, UCLA, CA, ³NASA-Ames Research Center, Moffett Field, CA.

Short-duration space flight and hindlimb unloading cause pronounced skeletal muscle atrophy and weakening of the contractile elements, thereby subjecting the muscle and supporting connective tissue to increased ground reaction forces during reloading. Collagen, the major component of the muscle ECM, aids in the transmission of contractile forces to tendons and bones and prevents tissue deformation. Very little is known regarding the plasticity of skeletal muscle collagen following acute spaceflight. Therefore, the objectives of this study are twofold: 1) To measure changes in muscle collagen gene expression from muscle biopsies following spaceflight [soleus (SOL) medial gastrocnemius (MG) and tibialis anterior (TA)] and 2) To assess the adaptive changes in muscle collagen concentration and maturation following micro-gravity. As a part of a larger study, lower limb pre-flight and post-flight muscle biopsy specimens were obtained from two rhesus monkeys flown on the Russian/US joint BION 11 Space Project for 14 days. The expression of the major muscle collagen genes, type I (COL1A2) and type III (COL3A1) were measured by quantitative-competitive RT-PCR. Collagen concentration (hydroxyproline) and maturation (HP and LP cross-link content) were assayed by reverse phase-HPLC. Muscle biopsy RT-PCR results indicate a significant decrease in COL1A2 (-25%) and COL3A1 (-16%) in the TA, but no significant changes in the SOL or MG muscles. Likewise, post-flight hydroxyproline concentration decreased significantly from pre-flight levels in the TA, but not in the MG or SOL. Post-flight HP and LP cross-link contents in the TA were significantly elevated compared to pre-flight contents with no changes in MG and SOL. These results indicate that muscle collagen adaptation may be dependent on muscle function and/or muscle duty cycle during spaceflight.

(Supported by NASA: NAG2-1089 and NAG2-1284.)

[92]

SCIENTIFIC OUTCOME OF THE RUSSIAN/FRENCH COOPERATION ON THE BION FLIGHTS. M. Viso. Centre National D'Etudes Spatiales, Paris.

Since 1975, the international cooperation on biosatellite which were launched by the Soviet Union and then Russia, was the main opportunity for the French scientists to obtain samples and data from flown animals (rats, monkeys, amphibians). This cooperation culminates with the flights of Bion 10 and of Bion 11. The early flights focused, for the French scientists, on bone physiology (Vico L., Alexandre C., Zerath E., Nagues C.) And muscle physiology (Desplanches, D., Mounier, Y., Falempin, M.). The opportunity was given to analyze recorded data from the monkey in flight to study the sleep and wake cycles (Lagarde D., Milhaud C.) And the alterations of attention (Rougeul-Buser A.). Several studies of the regulation of blood volume and body water regulation were performed (Gharib C., Gauquelin G.). All these studies were performed either on *Rattus norvegicus* or *Macaca mulatta* in cooperation and coordination with the Russian scientists of IMBP.

The last two flights focused on physiology with a special emphasis on neuro-physiology using *Macaca mulatta* as model. In various muscles, morphological and biochemical change of the fast fibers muscles (Desplanches D.) and functional changes at the muscle fiber level (Mounier Y.) were identified. A change in the nervous command of the antagonist muscles of the arm (Biceps/triceps) was observed (Falempin M.). The deep alteration of the myotendinous junction was also observed (Marini J.F.). The modification of the bone growth and the calcification was shown (Zerath E.) as for the first time the alteration of the cell metabolism (Marie P.).

The prominent results will be presented and discussed.

[94]

DEVELOPING PROTOCOLS FOR RECOMBINANT ADENO-ASSOCIATED VIRUS-MEDIATED GENE THERAPY IN SPACE. S. Ohi, A. Aguilar and B.C. Kim. Depts. of Biochemistry and Molecular Biology, Pediatrics and Child Health, Center for Sickle Cell Disease, Col. of Medicine and The Graduate School, Howard University, Washington DC.

With the advent of the era of International Space Station (ISS) and Mars exploration, our ultimate goal is to develop protocols for gene therapy which are suitable to humans on the earth as well as in space. Specifically, we are trying to cure the hemoglobinopathies β -thalassemia (Cooley's anemia), sickle cell disease, and space anemia by gene therapy. Toward the goal, exploiting non-pathogenicity of the adeno-associated virus serotype 2 (AAV2), the human parvovirus, we have so far accomplished the following: (1) recombinant AAVs (rAAVs) that harbored human α - and β -globin cDNAs, as well as an entire β -globin gene, were constructed; (2) the vectors were characterized in terms of infectivity, integration, and gene expression (e.g. Ohi, S., Kim, B.C., *J. Pham. Sci.* 85: 274, 1996); (3) we have established methods for purification of hematopoietic stem cells (HSCs) using immunomagnetic selection, and further, developed an easily harvestable, novel liquid suspension culture system (static culture, 1 G environment) for growing/ expanding HSCs without stromal cells; (4) the human globin cDNAs/ gene were efficiently expressed from the rAAVs in the mouse HSCs in culture; (5) procedures for bone marrow transplantation (BMT) with HSCs have been established. With the rAAV constructs and hematopoietic stem cell culture systems in hand, attempts are being made to cure the mouse model of β -thalassemia (C57BL/6-*Hbb*th/*Hbb*th, *Hb*^{th/mix}) by HSC transplantation (HST) as well as by gene therapy. The cystamine-cellulose acetate electrophoretogram of blood samples taken from the β -thalassemic mice following HST showed chimerism of hemoglobin species in the recipients, indicating a successful HST.

(Supported by grants from NIH, American Heart Association, and Armstead-Barnhill Foundation for Sickle Cell Anemia.)

[95]

EVIDENCE FOR A TH2 SHIFT ASSOCIATED WITH SPACEFLIGHT: IMMUNE MODULATION BY STRESS HORMONES. R.P. Stowe¹, D.L. Pierson², C.F. Sams², and A.D.T. Barrett¹. ¹Dept of Pathology, University of Texas Medical Branch, Galveston, and ²Life Sciences Research Laboratories, National Aeronautics and Space Administration, Lyndon B. Johnson Space Center, Houston TX.

Stress hormones, interleukin (IL)-10, Epstein-Barr virus (EBV)-genome load, and EBV-antibody titers were measured to determine if decreased cellular immunity, known to occur during spaceflight, would result in loss of control over EBV replication. To test this hypothesis, blood and urine samples were collected before and after flight from 29 astronauts who flew on 9- or 16-day missions. Compared to annual medical exams, antibodies to anti-EBV nuclear antigen were decreased while total immunoglobulin (Ig)-G was increased ten days prior to flight. At landing, plasma cortisol was decreased after 9-day flights but increased after 16-day missions. Significant increases were also found in urinary cortisol, total IgE, and EBV genome load after 16-day spaceflights. Moreover, EBV reactivation was associated with elevated levels of IL-10. These results suggest immune impairment in astronauts due to a stress-induced enhancement of Th2 cytokine synthesis, which may allow opportunistic infections to arise during spaceflight.

(Supported by NASA: NGT -51666, NAS9 -97005, UTMB-Sealy Center on Aging, and the Space Industrial Fellowship-Houston Advanced Research Center.)

[97]

NEUROBEHAVIORAL EFFECTS OF HYPERGRAVITY CONDITIONS IN CD1 MOUSE STRAIN. D. Santucci¹, G. Corazzi¹, N. Francia¹, A. Antonelli², L. Aloe², and E. Alleva¹. ¹Behavioural Pathophysiology Section, Laboratorio di Fisiopatologia di Organo e di Sistema, Istituto Superiore di Sanita', Viale Regina Elena 299, I-00161 Rome, Italy, and ²Istituto di Neurobiologia, Consiglio Nazionale delle Ricerche, V.le Carlo Marx 15, I-00137 Rome, Italy.

In mammals, alterations in vestibular functions, due to modifications of the gravitational environment, cause motion sickness (MS). In rat, a species incapable of emetic response, pica behaviour (i.e. the consumption of non nutritive substances, such as kaolin) has been considered an appropriate index of MS. Mice are a much smaller sized mammalian species than rats, thus more suitable for space missions.

In the present study, the effects of 1 hr of rotation-induced 2g-hypergravity on the behavior and picaism of adult male and female mice were evaluated. In addition, male NGF and BDNF levels in several brain areas were assessed. Results showed a clear decrease in spontaneous activity both during and immediately after the rotation, confirming that mice experienced motion sickness as a consequence of being subjected to these rotational stimuli. Sex differences emerged in pica behaviour, with females eating more kaolin than males regardless of the rotational exposure. Moreover, NGF levels, but not BDNF, were affected by hypergravity conditions with a significant increase in several brain areas such as frontal cortex, hypothalamus and hippocampus. Our preliminary study suggests that mouse may be a suitable species to investigate MS syndrome as well as general adaptive responses to altered gravitational stimuli.

[96]

EFFECTS OF LOW DOSE PROTON IRRADIATION ON THREE MODELS OF BEHAVIOR. M.J. Pecaut¹, A.L. Smith¹, E.D. Zendejas¹, C.N. Zuccarelli^{1,2}, P. Haerich², G.A. Nelson¹. ¹Department of Radiation Medicine (Radiobiology Program) or ²Department of Psychology, Loma Linda University Medical Center, Loma Linda, CA

In future long-term missions, (i.e. Mission to Mars or Lunar Missions) astronauts may be exposed to ionizing radiation. This radiation may cause decrements in astronaut behavior. Furthermore, radiation induced changes in behavior may be synergistically compounded by other psychological factors inherent to such missions (i.e. general mission related stress, living conditions in a closed environment, etc). Therefore, an accurate understanding of behavioral responses to ionizing radiation, and the effectiveness of Aluminum shielding, is required to ensure a safe working environment in space.

Female C57BL/6 mice were irradiated with 0, 3 or 4 Gy protons, with or without 15 g/cm² Aluminum shielding. Behavior testing occurred twice per week, on weeks 0, 1, 2, 4, 8 and 12 post-irradiation. Three separate tests were used to measure different aspects of behavior: Open Field (spontaneous locomotor and exploratory activity), Rotor Rod (muscle control and coordination), and Acoustic Startle (reflex response). All three tests have aspects of short and long term habituation.

Irradiation with 4 Gy significantly altered rotor rod and acoustic startle behaviors in weeks 0-2 post-irradiation. However, though several measures of open field behavior were analyzed, there was little or no effect of radiation. This suggests that there are possible proton radiation-induced short-term changes in motor control, startle reflex, and habituation. In contrast, there is little effect on long term learning, exploratory behavior.

(Supported by NASA cooperative agreement NCC-9-79.)

[98]

EFFECTS OF SIMULATED MICROGRAVITY ON NITRIC OXIDE SYNTHASE EXPRESSION AND NITRATE/NITRITE CONTENT IN DIFFERENT ARTERIES OF THE RAT. J. Ma, C.I. Kawwaji, N. D. Vaziri, Z. Ni and R.E. Purdy. Department of Pharmacology, College of Medicine, University of California, Irvine CA 92697.

Our previous work indicated that arterial nitric oxide synthase (NOS) expression might be altered by simulated microgravity. The aim of this work was to investigate the alterations in NOS expression and nitrate/nitrite content of different arteries from simulated microgravity rats. Male Wistar rats were randomly assigned to either control group or simulated microgravity group. For simulating microgravity, animals were subjected to hindlimb unweighting for twenty days. Rats were then euthanized and arterial tissues removed for determination of NOS expression and nitrate/nitrite content. Western blotting was used to measure endothelial constitutive NOS (ecNOS) and inducible NOS (iNOS) protein content. Total concentrations of nitrate/nitrite, stable metabolites of nitric oxide, were determined by the chemiluminescence method using the purge system of a Sievers Instruments Model 270B Nitric Oxide Analyzer. Compared with controls, isolated vessels from simulated microgravity rats showed a significant increase in both ecNOS and iNOS expression in carotid arteries and thoracic aorta, and a significant decrease in ecNOS expression of mesenteric arteries. The ecNOS and iNOS content of cerebral arteries, as well as ecNOS expression of femoral arteries, showed no differences between the two groups. The iNOS expression of mesenteric and femoral arteries could not be detected in this study. Concerning the nitrate/nitrite content, HU rats showed an increase in cerebral arteries, a decrease in mesenteric arteries, and no significant differences found in carotid artery, femoral artery and thoracic aorta. These data indicated that there were differential alterations in NOS expression and nitrate/nitrite content of different arteries after hindlimb unweighting. We suggest that these changes might represent the adaptations to body fluid redistribution during simulated microgravity.

(Supported by NASA: NAG9-1149.)

**Concurrent Oral Sessions
IV
Plant Development and Physiology**

[99]

FUNCTIONAL CHARACTERIZATION OF THE ARG1 GENE FAMILY IN *Arabidopsis thaliana*. K. Boonsirichai, E. Rosen, J. Sedbrook and P. H. Masson. Department of Genetics, University of Wisconsin, Madison WI, USA.

Mutations in the *Arabidopsis thaliana* ARG1 gene result in specific defects in root and hypocotyl gravitropism, without altering the plant's ability to grow or to respond to phytohormones or to lateral light stimulation. Hence, ARG1 appears to be involved in gravity sensing and/or early phases of gravity signal transduction (Sedbrook et al., 1999, Proc. Natl. Acad. Sci. USA 96: 1140-1145). This gene encodes a dnaJ-like protein that carries a coiled coil domain whose sequence shares homologies with a number of proteins known to interact with the microfilaments or microtubules in animal and plant cells.

Arabidopsis thaliana contains 2 other genes, named ARL1 and ARL2, which encode proteins that are very similar to ARG1. Interestingly, mutations in ARL2 result in a gravitropic phenotype very similar to the Arg1 phenotype. arg1,arl2 double mutants develop an intermediate gravitropic phenotype similar to that of single mutants, suggesting that both proteins interact in the gravity signal transduction pathway.

In an attempt to better understand the role of these dnaJ-like proteins in plant growth and development, we have generated antibodies against ARG1, and are using them, in combination with ARG1-GFP reporter fusions, in an attempt to localize the proteins within plant cells. We have also identified and are characterizing several genetic enhancers of the Arg1 phenotype. Interestingly, two of these enhancers result in a tendency for mutant roots to grow upward rather than downward, at least on hard agar surfaces. The results of these experiments will be discussed in view of the potential role(s) played by ARG1, ARL1 and ARL2 in gravitropism.

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[101]

GRAVISENSITIVITY IN SPACE GROWN LENTIL SEEDLING ROOTS. G. Perbal and D. Driss-Ecole. Laboratoire de Cytologie Expérimentale et Morphogénèse Végétale, Université Pierre et Marie Curie, 4 place Jussieu, F-75252 Paris Cedex 05, France.

Lentil seedlings (*Lens culinaris* L., var. Verte du Puy) were grown in microgravity or on a 1 g centrifuge for 26h in the frame of the S/MM05 mission of Spacehab. Then they were subjected to various centrifuge accelerations (from 0.39 to 0.93 g) for 9 or 22 min. The rate of curvature was measured on photographs taken by time-lapse photography (at interval of 10 min) for 3h in microgravity. The results obtained demonstrated that for any dose of stimulation, the gravitropic response was stronger for roots grown in microgravity than for roots grown on the 1 g centrifuge (before stimulation). The analysis of the dose response curve of the gravitropic response of these roots showed that growth in microgravity increased gravisensitivity by 67%. This difference of sensitivity could not be explained by the growth rate of the roots in both culture conditions (microgravity and 1 g on the centrifuge). Nor could the volume or the content of starch in the statoliths be accounted for.

The increased sensitivity of microgravity grown lentil roots appeared to be due to the distribution of the amyloplasts within the statocytes before stimulation.

(Supported by CNES 793/00/8103.)

[100]

GRAVITROPISM: CROSS-TALK BETWEEN CALCIUM/CALMODULIN AND HORMONE MEDIATED SIGNALING. B.W. Poovaiah and T. Yang. Washington State University, Pullman WA.

Calmodulin (CaM), a ubiquitous Ca²⁺-binding protein in eukaryote is a primary intracellular Ca²⁺ receptor, which transduces the second messenger Ca²⁺ signal by binding to and altering the activity of the other proteins. To better understand the downstream elements involved in Ca²⁺/CaM-mediated signal transduction, several approaches were used to clone and characterize genes that encode for CaM-binding proteins (Patil et al., PNAS 92: 4897-4901, 1995; Wang et al., Plant Mol. Biol. 31: 87-100, 1996; Plant Mol. Biol. 31: 683-687, 1996). Recently, two genes that are rapidly induced by the plant hormone auxin (*ZmSAUR1*; Yang and Poovaiah, J. Biol. Chem. 275: 3137-3143, 2000) and ethylene (NtER1; J. Biol. Chem. in press.) have been isolated. The CaM-binding assay revealed that both of the recombinant proteins (*ZmSAUR1* and NtER1) bind to CaM in a Ca²⁺-dependent manner suggesting a role for Ca²⁺/CaM in auxin and ethylene signal transduction. Synthetic peptides of amino acids corresponding to the potential CaM-binding region of *ZmSAUR1* and NtER1 were used for Ca²⁺-dependent mobility shift assays. In both cases synthetic peptides formed a stable complex with CaM only in the presence of Ca²⁺. CaM-binding experiments and comparisons of amino acid sequences of other SAURs and NtER1 homologs suggest that this is a general phenomenon. Auxin, ethylene, and calcium/calmodulin are known to be involved in controlling differential growth patterns in response to signals such as gravity and light. It is well established that SAURs are differentially expressed during gravitropic bending. Further characterization of SAURs and NtER1 and their interacting proteins should help in the overall understanding of gravity signal transduction in plants.

(Supported by NASA and NSF.)

[102]

THE GyPSi MUTANTS: A NEW GROUP OF GRAVITY MUTANTS IN *ARABIDOPSIS*. S. E. Wyatt¹, A. Rashotte², G. Muday² and D. Robertson³. NASA Specialized Center of Research and Training, ¹Department of Environmental and Plant Biology, Ohio University, Athens OH; ²Wake Forest University, Winston-Salem NC; ³North Carolina State University, Raleigh NC.

Horizontal gravistimulation for 3 h at 4°C does not cause curvature in *Arabidopsis* inflorescence stems. However, when the cold-treated stems are returned to a vertical position at 21°C, they bend in response to the cold, horizontal gravistimulation. These results indicate that gravity perception can occur at 4°C, that part of the response is sensitive to cold, but that the signal persists. Cold treatments abolished polar auxin transport, but the ability to transport auxin returned after plants were returned to room temperature. This suggests that cold acts upstream of auxin transport. Utilizing this cold effect on gravity signal transduction, we initiated a screen to select for mutants affected in the signal transduction and/or storage mechanism. We have identified three classes of mutants, designated Gravity Persistence Signal (GyPSi) mutants, with altered response after gravistimulation at 4°C. The first, GyPSi 1 (*gps1*) shows no response to gravistimulation at 4°C when returned to 21°C. *gps2* shows a positive response to the 4°C gravity stimulus (bends the wrong way). The *gps3* mutant is negatively gravitropic after return to room temperature, but continues to bend, apparently not recognizing the new vertical position. These mutants represent a new group of mutants specifically affected in an aspect of the signal perception/transduction pathway that occurs prior to auxin transport.

(Research supported by NASA grant # NAGW-4984.)

[103]

REDUCED GRAVITY RESPONSE IN THE *ARABIDOPSIS* MUTANT *rcn1* IS DUE TO INCREASED LEVELS OF AUXIN TRANSPORT. A.M. Rashotte and G.K. Muday. Dept. of Biology, Wake Forest University, Winston-Salem NC 27109.

The *Arabidopsis* mutant *roots curl in NPA 1 (rcn1)* was identified in a screen for plants forming root curls in the presence of the auxin transport inhibitor naphthylphthalamic acid (NPA) and the *RCN1* gene has been shown to encode a protein phosphatase 2A regulatory subunit. We found that roots of *rcn1* have slower gravity response than wildtype (Ws) roots in both the light and dark. Roots of *rcn1* show similar dose dependent inhibition of elongation by NPA as Ws roots. Since basipetal auxin transport in the root of *Arabidopsis* has been suggested to control gravity response, levels of basipetal IAA movement in *rcn1* were measured. The level of basipetal auxin transport in *rcn1* roots was nearly double that of Ws, with no apparent change in NPA sensitivity. Treatment of Ws plants with the protein phosphatase inhibitor cantharidin mimics these changes in gravity response and auxin transport observed in *rcn1*. These results suggest reduced gravity response and increased auxin transport in *rcn1* are due to the reduced protein phosphatase activity in this mutant. We postulate that elevated basipetal auxin transport in *rcn1* or Ws, treated with cantharidin, overwhelms the roots ability to either perceive or induce lateral auxin gradients that are essential for gravity response. To test this hypothesis, *rcn1* plants were grown on low levels of NPA, and the kinetics of gravity response were analyzed using a computer digitizer system. Low levels of NPA increased the rate of gravitropic bending and decreased basipetal auxin transport of *rcn1* roots to near wild-type levels. These experiments indicate that reduced protein phosphatase activity, obtained either genetically or with inhibitors, elevates basipetal auxin transport. This elevated basipetal auxin transport is tied to reduced gravity response, perhaps through overwhelming lateral auxin gradients in roots.

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[105]

PH SIGNALING IN THE GRAVITROPIC RESPONSE OF *ARABIDOPSIS* ROOTS. S. Gilroy, J.M. Fasano, R. Hirsch, P. Minnich and S.J. Swanson. Biology Department, Penn State University, State College PA.

Gravity perception, signal transduction, signal translocation and the growth response are integral components of root gravitropism yet how they are linked remains unknown. Alterations in wall and cytoplasmic pH could potentially mediate signal transduction and response elements during root gravitropism. We therefore developed the technology to visualize cell wall pH, using a pH-sensitive probe conjugated to a cellulose binding peptide from *Clostridium celluloverans*. In addition we monitored cytoplasmic pH using the pH probe BCECF-dextran and by observation of roots expressing a pH-sensitive GFP. We also simultaneously mapped the regional growth characteristics of the root by following individual cell elongation rates during the graviresponse. Following gravistimulation the wall pH of the root cap acidified rapidly, within 3 min of gravistimulation, and transiently (over 10-15 minutes) suggesting that changes in cap apoplastic pH correspond to the perception/signal transduction phase of the gravity response. There was also a slower, more sustained drop in the wall pH of first the distal and then central elongation zone. The cytoplasm of the gravity perceptive cells in the cap showed a rapid pH increase from 7.2 to 7.8, within 30 sec of gravistimulation. Blocking the cytoplasmic pH increase by releasing caged protons into the cap cells delayed the gravitropic response. The rapid timing and location of pH changes implies that activation of proton fluxes in the columella cells is close to the initial events of gravity perception in the root.

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[104]

InsP₃ SIGNALING DURING PLANT GRAVITROPISM.

I. Y. Perera,¹ I. Heilmann,¹ W. F. Boss¹ and P. B. Kaufman². ¹North Carolina State University and ²University of Michigan

Plant gravitropism is mediated by a cascade of biophysical and biochemical events which must be coordinated to orchestrate a growth response. As the site of both graviperception and response, the pulvinus of cereal grasses is an attractive model system to study the biochemical changes occurring during gravitropism. The involvement of phosphoinositides (PtdIns) in gravitropism was investigated in the internodal pulvini of maize and the leaf-sheath pulvini of oat. Rapid, transient increases in inositol 1,4,5-trisphosphate (InsP₃) were detected within seconds of gravistimulation fluctuating between the upper and lower sides of the gravistimulated pulvinus. This was followed by a long term increase in InsP₃ only on the lower side which correlated with the initiation of differential growth. Consistent with the increase in InsP₃, we also measured increased formation of PtdInsP₂ *in vitro* and an increase in PtdInsP 5-kinase specific activity on the lower side of the pulvinus, indicating an upregulation of the PtdIns pathway. To dissect the roles of the transient and sustained changes in InsP₃ in the gravitropic response we used cold temperature and the phospholipase C (PLC) inhibitor U73122. Cold temperature blocked the gravitropic response of oats. However, plants perceived the gravistimulus in the cold and could respond by bending when returned to room temperature. Both the short and long term InsP₃ changes were unaffected by the cold treatment. Pharmacological inhibition of PLC abolished gravitropic curvature by ~ 60 % and also eliminated the sustained increase in InsP₃ in the lower half of the pulvinus. Based on these data we propose a requirement for InsP₃ during both the perception and commitment to growth in the gravity signal transduction cascade. A mechanism combining both short and long term changes in InsP₃ could enable a plant to distinguish between transient movements, as caused by wind, and permanent lodging. The initial spike would serve as an initiation signal and the gradual increase could drive the requisite biochemical processes necessary for differential growth.

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[106]

USE OF A GRAVITY CLAMP TO REVEAL A MULTIPHASIC MOTOR RESPONSE IN MAIZE ROOT GRAVITROPISM. M.L. Evans, J.L. Mullen, C. Wolverton and H. Ishikawa. Dept. of Plant Biology, The Ohio State University, Columbus.

We recently developed a gravity clamp device ("ROTATO") for studying root gravitropism. The device uses video digitizer analysis of root growth and shape in conjunction with a rotating specimen stage to maintain a selected subsection of a root at any specified angle with respect to gravity (*Plant Physiol* 123: 665). When a maize root is stimulated using ROTATO to maintain the tip at a constant angle, the gravitropic response appears to occur in three phases. Phase I is a period of rapid curvature that continues for approximately 90 min. This is followed by Phase II, during which the rate of curvature is severely reduced, and then by Phase III, a prolonged period during which the rate of curvature is about half that of Phase I. Phase II is more prominent when the root tip is maintained at lower (e.g. 60 degrees) angles and is reduced or sometimes absent when the root tip is maintained at 90 degrees.

We used ROTATO to compare the kinetics of curvature when the root tip is maintained at 45 degrees or at 90 degrees. The lower angle of stimulation induces a slower rate of curvature. Phase III occurs at about the same time following the beginning of gravistimulation whether the root tip is maintained at 45 degrees or at 90 degrees. This indicates that Phase III is triggered at a certain time after stimulus initiation rather than by accomplishment of a certain amount of curvature.

Because the early phase of gravitropic curvature appears to be expressed in the distal elongation zone (DEZ) (*Plant Physiol* 102: 1203) we are testing the hypothesis that Phase I gravitropism is expressed in the DEZ while Phase III gravitropism is expressed in the central elongation zone (CEZ). We believe that our gravity clamp device will be a useful tool for quantitative analysis of root gravitropism.

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Concurrent Oral Sessions
V
Space Flight Results

[107]

SLEEP AND CIRCADIAN RHYTHMS IN SPACE—SHORT -TERM VERSUS LONG-TERM MISSIONS. T.H. Monk. Department of Psychiatry, University of Pittsburgh Medical Center.

This paper will discuss some of the issues relating to human sleep and circadian rhythms in space, and how these issues change as one moves from short duration to long duration missions. Regarding short duration missions, data are presented from a 17-day Space Shuttle mission (STS-78) in which four astronauts recorded sleep (polysomnography, sleep diary, wrist actigraphy), circadian rhythms (continuous rectal temperature, urinary cortisol, urinary melatonin), mood, alertness and performance (five/day) in two 72h measurement blocks, one starting 2d after launch, the other 12d after launch. Comparisons are made with pre-flight (L-7d) and post-flight (R+18d) baseline 72h measurement blocks. The mission was a single shift mission with no changes in sleep timing of more than 25 mins. The data demonstrated stability and good entrainment in the subjects' circadian rhythms, with baseline amplitude levels and no phase drift. However, sleep was shorter and less deep (less slow wave sleep) than it was on the ground. No major mood, alertness or performance decrements were observed. Regarding long duration missions, we shall discuss some suggestive results published by Frost and colleagues in their study of human sleep aboard the 1970s Skylab missions, and will also present data obtained from our own long-term (>120d) studies aboard space station Mir. In contrast to the shuttle mission, our Mir data came from an eventful mission (fire, near collision, collision, coolant leak, temperature fluctuations) in which deviations from Appendix K guidelines occurred and sleep was often profoundly disrupted. Using sleep diaries, and end of shift questionnaires, we show that disturbed nights of sleep with durations less than 5h can be associated with decrements in the following day's performance. Long duration missions appear to have consequences in both sleep and circadian rhythms which may not be observed when data from shorter duration missions are considered.

(Supported by NASA: NAG9-1036, NAS9-19407, NAS9-1036; and National Institute on Aging: AG13396, AG15136.)

[109]

RESIDUAL ACCELERATION ON SPACE LABORATORIES: CHARACTERIZATION, EFFECTS ON FLUID FLOWS AND IMPLICATIONS FOR MICROBIOLOGY. E.S. Nelson¹ and K. Jules². ¹Computational Microgravity Laboratory and ²Principal Investigator Microgravity Services, NASA Glenn Research Center, Cleveland OH.

The residual acceleration in space laboratories may be much smaller than the gravitational acceleration on earth, but it is not zero. The net acceleration that acts on an experiment arises from, e.g., orbital mechanics, atmospheric drag, and thruster firings, and it acts on fluids (and presumably microbes) in gravity-like ways.

In the past decade, a substantial knowledge base has developed to characterize the residual acceleration on various carriers and their effects on heat, mass and momentum transport. This presentation will show the measured accelerations on the Shuttle and the predicted microgravity environment on the International Space Station (ISS). The quasisteady acceleration level on the Shuttle has been measured by the Orbital Acceleration Research Experiment (OARE) to be on the order of a μg . Similar levels are anticipated on ISS, depending on the experiment location, based on finite-element models. Thruster firings may generate transient accelerations up to 1000 μg , but they only last for 1-3 seconds (although their effects on fluid systems may be more long-lived). The exceedingly complex vibratory component of the acceleration environment that is generated by, e.g., structural oscillation, the ergometer and equipment used by the life sciences community, spans a rich spectrum from 0.01 to 300 Hz, as measured by the Space Acceleration Measurement System (SAMS).

We will give special attention to problems in the fluids arena that are of relevance to gravitational biologists, particularly mixing, shaking, and convective transport. For example, the maximum velocity generated by a single-frequency uniaxial acceleration decreases with increasing frequency on fluids with density gradients in confined chambers. Other examples include jets and suspensions.

(Supported by NASA.)

[108]

TESTOSTERONE EXCRETION DURING AND AFTER SPACE FLIGHT ON THE SHUTTLE. T.P. Stein and M.D. Schluter. Dept. of Surgery, University of Medicine and Dentistry of New Jersey -SOM, Stratford NJ, 08084.

A report from the German space shuttle Deutsche-2 (D2) mission reported that testosterone activity was decreased by about 55% on four astronauts measured on plasma on mission days 4/5 of space flight. This study sought to extend these observations by measuring urinary testosterone secretion in a larger group of astronauts (n=9). The mean testosterone excretion was lower for the group as a whole (1.71 ± 0.29 vs 1.40 ± 0.20 mg/24 hr, p=ns). There were three outliers who showed a mean increase of $139 \pm 11\%$ with flight. The remainder showed a decrease $80 \pm 7.5\%$. Examination of the data showed that the baseline testosterone levels for the 3 with the increase were significantly lower than the other 7 subjects. Regression of baseline (preflight) testosterone against change with flight was statistically significant ($r^2=0.64$, $p<0.01$). The observations suggest that a decrease in urinary testosterone excretion with space flight is not found in subjects who have low baseline testosterone levels preflight.

[110]

REPORTER GENE EXPRESSION DURING SPACEFLIGHT AND IN CONTROLLED INDUCTIVE ENVIRONMENTS. R.J. Ferl¹, M.S. Manak¹, C.J. Daugherty and A-L. Paul¹. ¹Department of Horticultural Sciences and the Biotechnology Program, University of Florida, Gainesville FL 32611-0690

Transgenic *Arabidopsis* containing an Adh/GUS promoter reporter fusion gene were used to assay the influence of spaceflight on stress expression on shuttle flight STS-93. The *Arabidopsis* Adh gene is sensitive to a variety of metabolic and environmental stresses. Cold, ABA and hypoxia are among the common stress factors that induce Adh expression, with hypoxia being the most well characterized. Parallel experiments were conducted in the Orbiter Environment Simulator (OES) as negative controls for spaceflight effects. In addition, positive controls were conducted to evaluate the patterns of transgene expression under conditions known to activate the Adh gene promoter. The pattern of Adh/GUS expression in the negative controls is a simple absence of Adh/GUS gene product in the roots and minimal expression in the shoots. The plants exposed to spaceflight expressed Adh/GUS in roots in a pattern that is very similar to plants that have seen hypoxic stresses. However, there is a major difference between microgravity stress patterns of induction and those of hypoxic stress. Although both mild hypoxia and the spaceflight environment of STS-93 induce transgene expression in distal portions of roots, only hypoxia appears to facilitate the signal transduction to the shoot tissues. This divergence in expression patterns suggests that the transgene induction during spaceflight may be through a signal transduction pathway independent of hypoxia.

The next generation of transgene constructions will replace the GUS reporter with a gene for Green Fluorescent Protein (GFP). Constructions incorporating GFP facilitate real-time evaluations of transgene expression as GFP can be viewed non-destructively. The ability to view transgene expression in vivo, and at a distance, is a central feature in the design of future long-term gene expression missions and projects.

(Supported by NASA: NAG10-0145.)

[111]

COMPOSITION AND PHYSICAL PROPERTIES OF STARCH IN MICROGRAVITY-GROWN PLANTS. K.H. Hasenstein¹, O.A. Kuznetsov¹, C.S. Brown², W.C. Piastuch³ and H.G. Levine³, ¹Biology Dept., University of Louisiana at Lafayette LA 70504-2451; ²North Carolina State University; and ³Dynamac Corp., Kennedy Space Center, FL 32899.

The effect of spaceflight on starch development in soybean (*Glycine max L.*, BRIC-03) and potato (*Solanum tuberosum*, Astroculture-05) was compared with ground controls by biophysical and biochemical measurements. Space grown starch grains were on average 20-50% smaller in diameter than ground controls. The ratio $\Delta\chi/\Delta n$ (- difference of magnetic susceptibilities, Δn -difference of densities between starch and water) of starch grains was ca. 15% and 4% higher for space-grown soybean cotyledons and potato tubers, respectively, than in corresponding ground controls. Since the densities of particles were similar for all samples (1.36 to 1.38 g/cm³), the observed difference in $\Delta\chi/\Delta n$ was due to different magnetic susceptibilities and indicates modified structure of starch grains. When starch grains were enzymatically degraded with α -amylase, only 23% of the starch from the flight cotyledons was degraded compared to 48% in ground controls. The amylose content was 4 to 12% higher in space-grown tissues. The good correlation between the amylose content and $\Delta\chi/\Delta n$ suggests, that the magnetic susceptibility of starch grains is affected by their amylose content. Since some of the examined seedlings experienced increased ethylene concentrations during microgravity, soybeans from another flight (GENEX) experiment with normal levels of ethylene were analyzed and showed no difference to ground controls both in size distribution, $\Delta\chi/\Delta n$ and amylose content. Therefore the role of ethylene appears to be more important for changes in starch metabolism than microgravity.

(NASA grant NAG10-0190 and NASA contract NAS10-12180.)

[113]

ROOTZONE HYPOXIC RESPONSES RESULT FROM INHIBITION OF GRAVITY DEPENDENT OXYGEN TRANSPORT IN MICROGRAVITY. D.M. Porterfield¹, O. Monje², G.W. Stutte² and M. E. Musgrave³. ¹Biological Sciences, University of Missouri-Rolla, ²Dynamac Corporation, Kennedy Space Center, and ³Biology Department, University of Massachusetts, Amherst.

Whereas gravity driven convective movements of fluids and gases physically mediate physiological mass transport and exchange here on earth, in microgravity biological systems can become limited to slow diffusional transport processes. In roots limited transport of oxygen would decrease the bioavailability of metabolic oxygen (hypoxia), and consequently induce fermentative metabolism. Hypoxic metabolism has been seen in spaceflight exposed roots of *Arabidopsis* (Porterfield et al., *Plant Physiol* 113:685-693) dwarf wheat, and *Brassica* (Porterfield et al., *Adv Space Res* 26:315-318). In a recent experiment the metabolic status of root systems from two groups of *Brassica* plants grown on the Shuttle (STS-87) for 16 days was analyzed using alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) as enzymatic markers of hypoxia. ADH activity increased 47% and 475%, in the 15 day old and 30 day old plants, respectively, whereas PDC increased by 146% in the 30 day old plants. Support for the hypothesis that these metabolic responses are the result of microgravity physically inducing hypoxia in plant roots came from measurements made on a recent KC-135 flight experiment. Here a root oxygen bioavailability (ROB) sensor was used to directly measure changes in oxygen bioavailability as a function of gravity. The sensor was designed and built to be sensitive to convective changes and to simulate the metabolic oxygen depletion profiles in the rhizosphere around a growing root. The sensor reported that oxygen bioavailability decreased in phase with the changes in gravity as measured with an accelerometer. These results provide direct and compelling data supporting the microgravity convection inhibition model for explaining changes in plant physiological transport during spaceflight.

(Supported by NASA: NCC10-0027 to GWS, NAG-100139, NAG2-1020, NAG2-1375 to MEM, and a Missouri Research Board Grant to DMP.)

[112]

LOCOMOTOR BEHAVIOUR OF BIVALVE LARVAE IN THE ABSENCE OF GRAVITY. D.L. Jackson and R.K. O'Dor. Department of Biology, Dalhousie University, Halifax N.S. Canada.

As a force that draws zooplankton away from their food source near the ocean surface, gravity has influenced the evolution of behaviours that counteract sinking. Paradoxically, it has also been proposed that gravity can be exploited by heavy zooplankton such as bivalve larvae as a means of increasing feeding efficiency, notwithstanding the higher cost of locomotion. Gravity may act as a cue for orientation as bivalve larvae migrate throughout the water column, and is one of the forces that govern their vertically directed helical swimming pattern. In this initial study of the effects of microgravity on zooplankton, veliger stage larvae of the blue mussel *Mytilus edulis* were reared in the Canadian Space Agency's Aquatic Research Facility (ARF) during the ten day STS-77 mission. Video recordings of larval behaviour in both microgravity and in a normal gravity control centrifuge were made twice daily, and samples of larvae were preserved on Flight Days 3, 5, and 7. One group of larvae from each gravity treatment was returned alive from orbit.

Most larvae in microgravity continued to swim in a helical pattern, but changed direction and helix dimensions frequently. Larval swimming directions in microgravity were random, indicating that gravity is their primary orientational cue. Mean forward swimming speeds, pitch angles, and helix heights of larvae in microgravity were greater than their normal gravity counterparts. Larvae swimming downwards in normal gravity had greater translational and rotational swimming speeds than either upward swimmers or larvae in microgravity. The helix diameters of downward swimmers were also greater than those of larvae swimming in the absence of gravity. Larvae in microgravity required less energy to swim than larvae in normal gravity, and were able to move in higher helices and steeper pitch angles due to the lack of interactions between gravity and drag. These studies suggest that bivalve larvae have a much greater degree of control over their behavioural repertoire than has been previously considered.

[114]

GERMINATION AND ELONGATION OF FLAX IN MICROGRAVITY. H.G. Levine¹, K. Anderson², A. Boody², D. Cox³, O.A. Kuznetsov⁴ and K.H. Hasenstein⁴. ¹Dynamac Corp., KSC, FL 32899; ²Bionetics Corp., KSC, FL 32899; ³NASA, KSC, FL 32899; ⁴Biology Dept., Univ. of Louisiana, Lafayette LA 70504-2451.

Flax (*Linum usitatissimum L.*) seeds were flown on STS-101 in a risk mitigation "precursor" flight designed to assess critical technologies and protocols which will be used in the experiment "Magnetophoretic induction of curvature in roots" (MICRO) on STS-107. The intent of MICRO is to: (1) monitor the effect of high-gradient magnetic fields (HGMF) on inducing curvature in flax roots, and (2) study the effect of microgravity/HGMF on the development and structure of the plant cytoskeleton. Specific objectives for the STS-101 Precursor experiment were to: (1) evaluate the Micro-Effusion Delivery Unit for Space Applications (MEDUSA), and (2) determine the optimal combination of dispensed water volume and germination paper in the experimental chambers. Water was dispensed using MEDUSA units consisting of eight pistons that were rotated manually to deliver 320, 400, and 480 μ L water per set of eight seeds. The seeds were fixed 3 h after landing, corresponding to 33-34 h after the initiation of imbibition. Seed germination rates varied according to the amount of dispensed water and the germination paper (one or two layers of different thickness) employed. Seedling development was found to be a function of seed position relative to the site of water introduction, and there were differences in the observed patterns and root length between the flight and ground control experiments.

(Supported by NASA: NAG10-0190, NAS6-100.)

**Concurrent Oral Sessions
VI
Cell Biology**

[115]

THE TENSION-DRIVEN GATING TRANSITION IN THE BACTERIAL MECHANOSENSITIVE CHANNEL, MscL. S. Sukharev, M. Betanzos, C.-S. Chiang and H. R. Guy¹. Dept. of Biology, University of Maryland, College Park, and ¹NCI, NIH, Bldg. 12B, Bethesda MD.

The mechanosensitive channel of large conductance, MscL, is a ubiquitous molecular valve involved in turgor regulation in bacteria and a uniquely suitable model system for studies of force perception by receptor proteins¹. The solved crystal structure of MscL from *Mycobacterium tuberculosis*² provides a framework for the evaluation of conformations that permit opening of a large pore by membrane tension. Previous kinetic analysis suggested that expansion of the channel protein precedes the opening³. Following this lead, we built molecular models for the *E. coli* MscL in the closed, pre-expanded and open conformations and found that the channel gate can not be placed within the transmembrane region as was assumed before. Instead, we propose that the gate is formed by unresolved N-terminal segments (S1), each of which is connected to the transmembrane barrel via a flexible linker. A bundle of five S1 helices is postulated to disrupt when the channel opens. Disulfide trapping of engineered cysteines supports the bundle-like arrangement of cytoplasmic S1 segments in closed states and a tilted orientation of transmembrane M1 helices as the channel barrel expands. Patch-clamping in the presence of oxidizing or reducing agents shows that the S1 segments can be trapped in two predicted conformations, locking the channel in either closed or partially open state. Extension of the S1-M1 linker for one residue favors sub-conducting states, whereas further extension renders the channel insensitive to stretch. The mechanism reveals critical spatial relationships between the protein segments that transmit the force from the lipid bilayer to the channel gate.

¹ Sukharev, S. et al. *Annu. Rev. Physiol.* 59, 633-657 (1997)

² Chang, G. et al. *Science* 282, 2220-2226 (1998).

³ Sukharev, S. et al. *J. Gen. Physiol.* 113, 525-540 (1999).

[117]

MICROGRAVITY-INDUCED INHIBITION OF APOPTOSIS IN PERIPHERAL BLOOD MONONUCLEAR CELLS AND CHANGES IN PKC ISOFORMS. D.Risin¹, A. Sundaresan¹ and N.R. Pellis². Biotechnology Program, ²NASA/JSC and ¹Wyle Life Sciences, Systems and Services, Houston TX.

Our previous studies showed that modeled microgravity (MMG) inhibits programmed cell death (PCD) in lymphocytes. The inhibitory effect was demonstrated in three different experimental models (PCD induced by gamma-radiation in PBMC and activation-induced PCD in activated T cells after restimulation with PHA-M or PMA+ionomycin). Further analysis revealed no changes in expression of Fas, Fas ligand (FasL), bcl-2 and bax in activated T lymphocytes cultured in MMG versus stationary conditions. Recently it was demonstrated that PKC isoforms are actively involved in regulation of activation-induced cell death and in upregulation of FasL. Based on these findings we analyzed the possible role of changes in PKC isoform expression in MG-induced inhibition of PCD in PBMC. We have found that MMG selectively inhibits expression of PKC isoforms. Most prominent decrease was observed in PKC ϵ , it was less obvious in PKC δ and almost marginal and insignificant in PKC α . These results, initially obtained by Flow cytometry, were confirmed at the messenger RNA level by RT-PCR and at protein level by Western blot. Recent confocal microscopy studies demonstrated more uniform distribution of PKC ϵ in lymphocytes cultured in static conditions and less uniform and patchy distribution in MG. Changes in PKC isoform expression and distribution may be etiologic to the observed changes in PCD or merely a consequence of the physical environment.

(Supported by NRA grant OLMSA-02.)

[116]

SPECTRIN-LIKE PROTEINS ASSOCIATE WITH THE ACTIN-ORGANIZED ENDOPLASMIC RETICULUM AGGREGATE IN THE SPITZENKÖRPER OF GRAVITROPICALLY TIP-GROWING CELLS. M. Braun and A. Sievers. Botanisches Institut, Universität Bonn, Bonn, Germany.

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

(Supported by Deutsches Zentrum für Luft- und Raumfahrt, DLR.)

[118]

THE EFFECT OF GRAVITATIONAL PERTURBATION ON THE EXPRESSION OF GENES REGULATING GROWTH AND METABOLISM IN A HUMAN LYMPHOBLASTOID CELL LINE (JURKAT CELLS). K. Singh, L. Cubano and M. Lewis. Dept of Biology, University of Alabama, Huntsville.

Gravitational perturbation altered gene expression, increased glucose consumption and induced apoptosis in space flown Jurkats according to results from STS-95. This study investigated the effect of two components of space flight, centrifugal acceleration and simulated microgravity, on metabolic gene expression in Jurkat cells. Cells were subjected to a typical launch centrifugal acceleration, 3g of force for eight minutes, and a typical laboratory centrifugal force of 90g for five minutes. A rotating cell culture system was used to randomize the unidirectional vector of gravity over 360° to mimic the free fall and low shear force associated with microgravity. The metabolic genes analyzed were thioredoxin, transketolase, apolipoprotein C-IV, xanthine dehydrogenase, prostaglandin endoperoxide synthase 1, glyceraldehyde-3-phosphate dehydrogenase, and lactate dehydrogenase A. Gene expression was analyzed using RNA extraction and RT-PCR techniques. Comparison with a static control indicated no significant change in metabolic activity or metabolic gene expression of Jurkat cells in the RCS or when subjected to 3g or 90g of force. Apolipoprotein C-IV was up regulated significantly only at 0 hour in 90g and 3g, but not in the RCS. The conclusion is that the differential gene expression observed in space flown cells (STS-95) is the result of some other factor(s) of spaceflight or microgravity, not analyzed in this experiment.

Funded by NASA grant NAG2-985.

[119]

EXPRESSION OF STRUCTURAL AND METABOLIC STRESS GENES IN HUMAN LEUKEMIC LYMPHOCYTES SUBJECTED TO GLUCOSE DEPRIVATION AND VIBRATIONAL STRESS. N. Myers. University of Alabama in Huntsville

Human leukemic T-lymphocytes flown in microgravity showed disorganization of the cytoskeleton and metabolic changes. Post-flight analysis revealed that glucose utilization in flight cells was almost two times more than ground controls, yet growth of flown cells was significantly arrested (Lewis et al., 1998). One theory proposes that glucose use is increased in the process of recovery from spaceflight-induced stress. The structural and metabolic stress genes chosen for analysis included the Glucose Regulated Proteins (GRPs) 75, 78, and 94, ATF 6, actin, keratin, and two genes involved in cell growth, cFos and cMyc. Vibration tests were conducted at the Marshall Space Flight Center using the launch profile from STS-95 to allow comparison with results of spaceflown cells. Results showed no significant changes between gene expression in controls versus vibrated cells. However, the GRP genes were significantly upregulated in glucose-starved cells in ground-based tests demonstrating GRPs as useful markers for metabolic stress for better cell experiments in space.

[121]

PRELIMINARY STUDIES IN SUPPORT OF A SPACE SHUTTLE FLIGHT EXPERIMENT EVALUATING THE ABILITY OF rhIGF-1 TO ATTENUATE SPACE FLIGHT-INDUCED SKELETAL MUSCLE ATROPHY. B.C. Creswick, J. Shansky, P.H.U. Lee, X.Y. Wang and H.H. Vandenburg. Dept. of Pathobiology, Brown University School of Medicine, Providence RI.

Tissue-engineered avian bioartificial (A-BAM) muscles flown in the middeck Space Tissue Loss Module on two previous space shuttle missions demonstrated a reduction in muscle protein synthesis leading to myofiber atrophy (*EASEB J.* 13, 1031; 1999). Insulin-like growth factor-1 (IGF-1) is an anabolic growth factor that has the potential of being an effective pharmacological countermeasure for space flight-induced skeletal muscle atrophy in astronauts. Ground definition studies have been conducted in preparation for a space flight experiment designed to demonstrate the use of recombinant human IGF-1 (rhIGF-1) secreted by genetically modified C₂C₁₂ BAMs for attenuating space flight-induced skeletal muscle atrophy in this established *in vitro* model. The study aims to 1) investigate the effects of rhIGF-1 on A-BAMs, 2) demonstrate sustained high level secretion of biologically active rhIGF-1 by C₂C₁₂ BAMs, and 3) test the BAMs in the specially designed middeck Cell Culture Module (CCM) bioreactor. A-BAMs were tissue-engineered with embryonic chick myoblasts and treated with varying doses of rhIGF-1 for 16 days. A-BAMs treated with 100 ng/ml rhIGF-1 had a 151% increase in myofibers number and a 71% increase in mean myofiber diameter when compared to untreated control A-BAMs. BAMs made from C₂C₁₂ myoblasts that have been stably transduced with a retroviral vector containing the rhIGF-1 gene under the control of a constitutively expressed promoter were maintained in the CCM for 25 days. rhIGF-1 accumulated steadily to a final concentration of 55.3±11.6 ng/ml. The results of the preliminary experiments show that 1) avian BAM myofibers are responsive to rhIGF-1, 2) genetically modified C₂C₁₂ BAMs can secrete the desired level of rhIGF-1, and 3) the CCM is adequate for the planned space flight experiment.

(Supported by NASA NCC2-1062.)

[120]

RECIPROCAL TROPHIC INTERACTIONS BETWEEN HUMAN RETINAL PRECURSORS AND RETINAL PIGMENT EPITHELIUM (RPE) AND ITS PHYSIOLOGIC RELEVANCE IN TISSUE REPLACEMENT. K. Dutt, R. Lawrence, R. Kumar and T. Lindsay. Department of Pathology, Morehouse School of Medicine, Atlanta GA.

Age related macular degeneration and retinitis pigmentosa remain the leading cause of blindness. Tissue replacement as a therapy is a promising approach, however scarcity of tissues for replacement is a major obstacle. Since retinal, RPE interactions are critical to retinal development and functioning, we have explored co-culturing non-transformed human retinal precursors and RPE in monolayer and three-dimensional co-cultures in the NASA bioreactor. To elucidate the role of cell-cell interaction and trophic factors in retinal generation from multipotential precursors, immunophenotyping, Western blot, and RT-PCR were performed to identify cell types and trophic factors induced in response to a variety of conditions.

When multipotential retinal precursors were exposed to conditioned medium from RPE under serum free conditions, dose dependent and density dependent cell commitments towards multipolar neurons were noted. bFGF, BDNF, CNTF (1-100 ng/ml) act instructively to switch multipotential precursor cells towards photoreceptor (bFGF) and a multipolar and ganglionic pathway (BDNF, CNTF). BDNF, CNTF commit cells towards multipolar neuronal pathway with upregulation of dopamine receptors D₂, D₂D₃. Co-culture of retinal precursors and RPE in the bioreactor generated multiple cell types, including Muller cells, which are absent in monolayer co-culture. RT-PCR was performed to identify trophic factors secreted by RPE in monolayer and the bioreactor. Retinal and RPE co-cultures grown in the bioreactor might prove better for transplants. Identification of factors critical to retinal development will be important not only to generate 3D retina for transplants, but to provide cues to survival and functioning of transplants.

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[122]

EFFECT OF MICROGRAVITY ON THE CYTOSKELETON OF CULTURED NERVOUS CELLS. B. Uva, M.A. Masini, M. Sturla, P. Prato and G. Tagliaferro. Dipartimento di Biologia Sperimentale, Ambientale ed Applicata, Università di Genova, Viale Benedetto xv, 5, 16132 Genova, Italy.

Living beings have evolved their morphological and physiological systems in presence of the earth gravitational field. The cytoskeleton provides the cell with order and shape, but the cytoskeleton network is not a static structure, it is instead a highly dynamic complex of microtubules, microfilaments and intermediate filaments. They are responsible for organelles and vesicles transport within the cell, and are capable of rearranging themselves during the different physiological cell activities and during the different stages of the cell cycle. Provided that neuro-cellular systems use gravity to transfer signals and metabolites, the problem addressed by this preliminary research concerns possible alterations of the cytoskeleton that may occur in microgravity. The research was carried out with the aid of ground facilities (clinostat) on C6 glial cells in culture. The aspect of the cell cultures was observed by scanning electron microscope. The organisation of the cytoskeleton was studied by fluorescent immunohistochemistry observed at the confocal microscope. For the immunohistochemistry, antibodies to α -tubulin and actin were used. After 20 and 24 hours of microgravity the cells showed an altered aspect: the cytoplasmatic processes and the cell contacts were more numerous than in cultured cells maintained at 1g. The immunocytochemical analysis showed an altered organisation in the cells subjected to microgravity; the tubular system and the microfilaments were highly disorganised. By these preliminary data we may conclude that microgravity influences the cell shape and organisation.

(Research supported by ASI.)

**Concurrent Posters
IV–C
Animal Development, Physiology and
Gravity Sensing**

[123]

EFFECTS OF HYPERGRAVITY ON THE DEVELOPMENT OF THE MOTOR SYSTEM IN CRICKETS (*ACHETA DOMESTICUS*). S. Böser, and E. Horn. Gravitational Physiology, Department of Neurobiology, University of Ulm, Ulm, Germany.

Studies on NeuroLab (STS-90) with crickets (*Acheta domestica*) have shown that the development of the gravity related compensatory head movement was not influenced by microgravity while a central neuron which is probably involved in the transmission of information flow between the gravity sense organ and the neck muscles was affected. In general, proprioceptive reafferences caused by leg movements or central arousal are necessary for the performance of gravity related behavior in insects. They are responsible for a “physiological gating” of the nervous pathway connecting the gravity receptors with neck muscles. Thus, modifications of the gravity processing system induced by altered gravity may be, in part, influenced by changes of neurons linked to the motor system of the legs. We studied the effect of a 16-day 3g-exposure on two groups of GABA-immunoreactive neurons within the thoracic ganglia of crickets, (1) groups of interneurons located in clusters dorsally, and (2) 3 pairs of motoneurons, the common inhibitors (CI), located ventrally. CIs innervate wing and leg muscles. The topography of GABAergic thoracic neurons and the size of CI-somata were determined. In addition, the morphology of GABAergic terminals and the number of varicosities (boutons) was studied on thoracic muscles which are responsible for movements of the abdomen against the gravitational force. For comparison, muscles were chosen which are mainly involved in breathing or internal stabilization of the thorax. Hypergravity affected the development of thoracic CI-motoneurons in a stage-related manner. For all 3 CI-types from stage 4-larvae, the absolute sizes of the soma were significantly (u -test; $p < 0.02$) smaller in 3g-larvae than in the 1g-controls. Morphological studies on the development of neuromuscular connection sites revealed no difference between 1g- and 3g-reared larvae irrespective of whether they have anti-gravity properties (stabilization of abdomen posture) or not (intrinsic thoracic muscles).

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[125]

HYPERGRAVITY INDUCES FOS AND CRH EXPRESSION IN RAT HYPOTHALAMUS. R. Shimokawa¹, H. Shimokawa², M. Terasawa³, B. Linsuwanont², Y. Kumei³ and K. Ohya². ¹Dept. Of Neuropathol, ²Dept. of Pharmacol., ³Dept. of Biochem, Tokyo Medical and Dental University, Tokyo, Japan.

The purpose of this study was to clarify the histological changes induced by hypergravity exposure. Wistar male rats of 5-week-age were exposed to 2G by centrifugal rotation for either 10 min. Rats were sacrificed with perfusion. Sections were prepared for immunohistochemistry and in situ hybridization. Hypergravity induced Fos expression in the arcuate, paraventricular nuclei and dorsal hypothalamic area. These Fos expression were altered time-dependently and contrasted by expression of CRH (corticotropine releasing hormone), opioid and non-opioid-related protein.

(Supported by NASDA and Japan Space Forum.)

[124]

FACTORS INFLUENCING THE SUSCEPTIBILITY OF ANURANS TO MOTION SICKNESS. R.J. Wassersug¹, T. Naitoh² and M. Yamashita³.

¹Dept of Anatomy and Neurobiology, Dalhousie Univ, Halifax, Nova Scotia, B3H 4H7, Canada, ²Dept of Biological Science, Shimane Univ, Matsue 690-8504, Japan, ³Space Utilization Research Center, Institute of Space and Astronautical Science, 3-1-1, Yoshino-dai, Sagami-hara, Kanagawa, 229, Japan.

We examined the propensity for motion sickness in five anuran species (*Rhacophorus schlegelii*, *Bufo marinus*, *Xenopus laevis*, *Rana nigromaculata*, and *Hyla japonica*), concentrating our efforts on the treefrog *R. schlegelii*, because it had shown the greatest susceptibility to motion sickness in a previous study. We used parabolic flight as our provocative stimulus and fed all specimens a known volume of food 1.5 to 3 hr before flight. The presence of vomitus in a frog's cage was our indicator of motion sickness.

Only the treefrogs exhibited emesis. Significantly more emesis was observed in flight-exposed than control *R. schlegelii* ($p < 0.05$). There was no sex difference in susceptibility to motion sickness ($p > 0.5$), however, the *R. schlegelii* that vomited were slightly larger ($p < 0.02$) than those that did not. Among microgravity (μ g)-exposed *R. schlegelii*, those that vomited spent on average 85% more time airborne and tumbling in μ g than those that did not vomit ($p = 0.031$).

Our data support the view that postural instability and sensory conflict are elements of motion sickness in anurans. Specifically, conflicts between tactile, vestibular and visual input seem essential for producing motion-induced emesis in anurans.

Since the factors that induce motion sickness in *R. schlegelii* are the same ones that produce motion sickness in humans, arboreal frogs may be useful alternative models to mammals in motion sickness research.

(Research supported by Japan Space Forum and the Natural Science and Engineering Research Council of Canada.)

[126]

EFFECTS OF GENDER AND HINDLIMB UNLOADING ON BONE HISTOMORPHOMETRY IN ADULT RATS. G.L. Evans¹, S. Lotinun¹, T. Hefferan¹, E. Morey-Holtan² and R.T. Turner¹. ¹Mayo Clinic, Rochester, MN and ²NASA Ames Research, Moffett Field, CA.

Osteopenia induced by partial or complete mechanical unloading of the rat skeleton has been attributed to decreased bone formation. However, ovariectomized unloaded rats lost cancellous bone during hindlimb unloading (HLU) and spaceflight because of increased bone resorption. Using 6 month old Fisher intact male and female rats we evaluated HLU effects on skeletal changes over a 2 week period of time. Two baseline groups male and female were sacrificed at the beginning of the experiment to establish initial bone volume. The control rats were pair fed to their HLU group to correct for weight changes. Female and male HLU ($n = 12$) and weight bearing ($n = 10$) rats were housed individually in identical cages. Final body weights did not differ significantly between the control and HLU for either gender. Uterine and seminal vesicle weights decreased in the hindlimb unloaded rats compared to the baselines, but these changes were due to dietary restriction rather than unloading. There were pronounced gender differences in static and dynamic cortical and cancellous bone measurements with males having the greater cortical bone mass and females having the greater cancellous bone density (BV/TV). Two weeks of HLU resulted in similar decreases in cortical bone formation and in cancellous bone density and bone formation in male and female rats. Unexpectedly, unloading in males as well as females dramatically increased resorption of a pretreatment fluorochrome that had been incorporated into cancellous bone. Thus, increased bone resorption contributes to the bone loss which occurs in rats as a result of mechanical unloading.

(Supported by NASA Grant NAG9-1150.)

[127]

A NON-INVASIVE ANALYSIS OF MUSCULOSKELETAL COLLAGEN METABOLISM FROM URINE OF RHESUS MONKEYS DURING 14 DAYS OF 2G HYPERGRAVITY. A.C. Vailas¹, T. Hoban-Higgins², C.A. Fuller², R.E. Grindeland³, K.M. Shea¹ and D.A. Martinez¹.
¹Connective Tissue Physiology Laboratory, University of Houston, TX, ²University of California at Davis, CA, ³NASA-Ames Research Center, Moffett Field, CA.

Metabolic by-products of tissue metabolism measured in urine of astronauts, cosmonauts, and rhesus monkeys, suggests that an increased degradation of connective tissue occurs during short duration exposure to microgravity. Increases in the urinary excretion of hydroxyproline, collagen cross-links and mineral salts in astronauts is evidence that degradation of connective tissue can be enhanced by altered load environments, such as weightlessness. Little consensus data has been collected on the non-invasive measurement of collagen degradation products associated with an enhanced weight-bearing stress (*hyper-gravity*) on the skeleton. The purpose of this study is to assess the urinary collagen metabolic profiles of 6 rhesus monkeys during: 2 weeks of rest (pre-2G), 2 weeks of a 2G insult and 2 weeks of post-2G recovery. 24 hour collections were obtained from 6 individual rhesus monkeys. Urine volumes were recorded and stored at -85 °C. Urine hydroxyproline (Hyp) and collagen cross-links (HP and LP) were measured by rp-HPLC. Creatinine was measured using a kit assay. During the 2G exposure period, our results showed a significant elevation of HP and LP cross-links and Hyp per volume of urine during the first 7 days followed by a decline in urinary content of cross-links and Hyp from days 7-14. However, the 2nd week was still significantly elevated above resting pre-2G levels. Recovery to pre-2G collagen biomarker levels was not achieved until days 7-14 during the post-2G period. We conclude that non-invasive measurement of collagen biomarkers have merit and are excellent physiological indicators of collagen metabolism during 2G hypergravity in rhesus monkeys. Future daily, in-flight, non-invasive collections of urine would be a useful tool to assess musculoskeletal plasticity in astronauts and cosmonauts during extended flights. (Supported by NASA: NAG2-1089 and NAG2-1284.)

[128]

EFFECTS OF HYPERGRAVITY AND ADRENALECTOMY ON TOTAL BODY BONE MINERAL CONTENT IN MALE RATS. B. Girten, M. Moran, L. Baer, S. Pruitt, C. O'Brien, S. Arnaud¹ and C. Wade¹. Lockheed Martin and ¹NASA, NASA Ames Research Center.

The effects of 14 days of increased gravitational load, and the absence of adrenal stress hormones on total body bone mineral content (BMC) were examined in male Sprague-Dawley rats. Centrifugation at 2 Gs (2G) was used to increase the gravitational load, and bilateral adrenalectomy (ADX) was used to eliminate the production of adrenal stress hormones. Stationary groups at 1 G (1G) and sham operated (SHAM) animals served as controls. Thirty rats (n=6 or 8) made up the four experimental groups (1G SHAM, 1G ADX, 2G SHAM and 2G ADX). BMC was assessed by dual energy x-ray absorptiometry (DXA) and activity was determined through biotelemetry. Body mass and food intake were also measured. Multi-factorial analysis of variance (MANCOVA) and Newman Keuls post hoc tests were used to analyze significant effects (p<0.05) for the primary variables. Results indicated that BMC decreased significantly with increased G for both the SHAM and ADX groups. The BMC for the 1G ADX group was also significantly lower than the 1G SHAM group, however the 2G SHAM and ADX groups were not significantly different. There was a significant decrease in body mass with increased G and there was no ADX effect on body mass. When BMC was normalized for body mass changes, there were no significant group differences. Activity level decreased with body mass, and food intake data showed there was significant hypophagia during the first few days of centrifugation. These results suggest that the decrease in total body BMC seen with hypergravity may be based to a large extent on the differences in body mass induced by the 2 G load.

**Concurrent Posters
IV-D
Cell Biology**

[129]

NUCLEAR TRANSLOCATION OF NUCLEAR FACTOR KAPPA B (NF-KB) AND VITAMIN D RECEPTOR DURING ROTATING WALL VESSEL CULTURE OF HUMAN RENAL CELLS. X-C. Wang, P.L. Allen, E.N. Benes, L.A. Cubano and T.G. Hammond. Nephrology Section, Environmental Astrobiology Center and Center for Bioenvironmental Research, Tulane University Medical Center and VA Medical Center, New Orleans LA.

Rotating wall vessel culture induces re-expression of many tissue specific molecules normally lost when differentiated mammalian cells are placed into conventional 2-D tissue culture. The transcriptional pathways underlying these events are unknown. The vitamin D receptor is a nuclear transcription factor, which is up-regulated during RWV culture of human renal cells suggesting that it may be also act by nuclear translocation. Further, NF-kB has been implicated in shear stress responses, in a dynamic shear range identical to the residual shear in the RWV. This study examines directly whether the vitamin D receptor, and/or NF-kB are translocated to the nucleus during RWV culture. Human renal cortical cells in conventional 2-D culture were trypsinized and passaged into RWVs on carrier beads. Serial timed aliquots of cells had nuclear protein isolated by osmotic lysis techniques, and were probed for vitamin D receptor and NF-kB content by Western blot. Both transcription factors increased for 2 hours following initiation of RWV culture. These changes were independent of cell cycle and apoptotic changes. Hence, there is time dependent nuclear translocation of the vitamin D receptor and NF-kB following initiation of RWV culture of human renal cells.

(Supported by NASA NRAs 9-811 and 8-1362.)

[131]

THE EFFECTS OF HYPERGRAVITY ON THE EXPRESSION OF NITRIC OXIDE SYNTHASE BY ENDOTHELIAL CELLS: ROLE OF THE F-ACTIN CYTOSKELETON AND c-JUN N-TERMINAL KINASE. F.N. Bosah¹, G.L. Sanford¹ and S. Harris-Hooker². Depts of Biochemistry¹ and Medicine², Morehouse School of Medicine, Atlanta GA 30310.

Shear stress and mechanical force are known to induce a number of changes in vascular cell behavior including cell shape, cytoskeletal reorganization and gene expression. Hypergravity (Hgrav) is another physical force that been found to alter the behavior of several different cell lines. However, the underlying mechanisms of these effects have not been clearly defined. The objectives of this study were to determine the effects of Hgrav on inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) expression and to determine the intracellular signaling pathway that mediates these effects. Vascular endothelial cells (EC) were seeded in 25 mm² flasks at 37°C for 6hr. Next, cells were subjected to Hgrav by centrifuged at 6G, in the presence or absence of cytochalasin D for 24 and 48 hr. We also assessed whether the stress mediated protein kinase pathway mediates the cellular effects of Hgrav. This was done by examining the expression of activated (phosphorylated) c-jun N-terminal kinase (JNK) by cultures treated similarly. Hgrav increased the expression of iNOS and eNOS by 80% and 50%, respectively. Both NOS isotypes were dramatically decreased by treatment of EC with cytochalasin D. Expression of activated JNK was minimum in control (non-centrifuged) EC but was elevated in cells subjected to Hgrav. This elevation in activated JNK expression was blocked by treatment with cytochalasin D. Once JNK is activated, it phosphorylates other intracellular proteins including transcriptional factors. Additional studies are underway to further define how the JNK signaling pathway mediates the way changes in gravity may affect vascular EC behavior and function.

(Supported by NASA NGT2-52239, NCC9-53, and NIH/RCMI 3G12RR03034.)

[130]

ANTIBIOTIC RESISTANCE IN BACTERIA EXPOSED TO SIMULATED SPACEFLIGHT ENVIRONMENTS.

M.A. Juergensmeyer¹ and E.A. Juergensmeyer². ¹ Division of Microbiology, Montana State University, Bozeman MT; ² Biology Department, Judson College, Elgin IL.

Clinorotation of bacterial cultures does not induce the change in response to antibiotics seen after exposure to spaceflight conditions. This inability of clinorotation to simulate microgravity is seen in both motile and nonmotile cultures. To further refine these observations, we have attempted to isolate critical factors of the spaceflight environment, such as lack of a defined gravity vector, temperature fluctuations, and short periods of hypergravity. Motile and nonmotile bacterial cultures subjected to these factors, singly and in combination, still do not exhibit the same response to antibiotics seen after exposure to spaceflight.

[132]

LOCOMOTORY FUNCTION IN LYMPHOCYTES IS AFFECTED BY MICROGRAVITY-INDUCED SIGNAL TRANSDUCTION LESIONS INVOLVING PROTEIN KINASE C. A. Sundaresan¹, D. Risin¹ and N.R. Pellis². Biotechnology Program, ²NASA/JSC and ¹Wyle Life Sciences, Systems and Services, Houston TX.

Severely diminished migratory functions in lymphocytes in true microgravity (TG) and modeled microgravity (MMG) were previously demonstrated in our laboratory. However, activated lymphocytes proved resistant to the deleterious effects of microgravity. We hypothesized that microgravity caused a lesion in transmembrane signaling either at, or up-stream of, Protein Kinase C (PKC). When lymphocytes were stimulated with PMA, a phorbol ester which directly activates PKC, there was a substantial (87%) recovery in lymphocyte migratory function. However using the calcium ionophore ionomycin had no effect. Hence, calcium-independent PKC isoforms were implicated and investigated in this study. Investigation showed that PKC δ and ϵ were down-regulated substantially by greater than 60% both at the transcriptional and translational levels. This was demonstrated by RT-PCR analysis and Western blot analysis. Phospholipase c gamma (PLC- γ) is the the predominant enzyme in lymphocytes which facilitates hydrolysis of PIP₂ to IP₃ and DAG. Changes in Phospholipase C gamma activity were also observed in microgravity cultured lymphocytes where active PLC- γ was reduced to less than 50% of active enzyme seen in 1g (ground) cultured lymphocytes. Thus, there may be multiple effects from change in the physical environment that result in interrupted signal transduction of which changes occur in microgravity at the membrane level and in signal transduction that lead to immune suppression and need to be further elucidated.

(Supported by NRA grant OLMSA-02 and NSCORT grant #NAG5-4072).

[133]

PLEUROCHRYSIS CARTERAE IS AN EXCELLENT MODEL TO STUDY BIOMINERALIZATION AND GRAVITAXIS IN SPACE. D. Montufar-Solis and P.J. Duke. Dept. of Orthodontics, Dental Branch, UT Houston Health Science Center, Houston TX.

The negatively gravitaxic, marine coccolithophore *Pleurochrysis carterae* is an ovoid, biflagellate single celled alga, easily maintained in culture for long periods of time without requiring medium changes. Each cell is covered with calcifying scales, or coccoliths, which are produced and mineralized in the Golgi cisternae in a process that has been extensively studied and described by Marsh (*Protoplasma* 207:54-66). Previously, we reported on the development of an ISS experiment to determine any spaceflight-induced changes on *P. carterae*'s biomineralization, and the relationship of biomineralization and morphology to motility and gravitaxis during spaceflight. We established the ability of the cells to grow after a storage period in the dark at 4°C for 70 days. Due to delays in ISS construction, requirements for culture of *P. carterae* in space are being redefined to explore the option of using various shuttle facilities. In the current studies, cells were grown in various volumes; in sealed containers with different amounts of enclosed air; and under different light intensities. Cell number increased under almost all conditions, the single exception being no light. *P. carterae*'s tolerance of lengthy periods in the cold and dark prior to cultivation; its adaptability to a wide range of culture conditions with minimal requirements for light and gas exchange; and the added advantage of being a highly polarized single celled organism, make it an attractive cell type for spaceflight studies, whether on shuttle or ISS.

(Supported by NASA: NAG2-1261.)

[135]

ACOUSTIC WAVE BIOSENSOR TECHNOLOGY FOR PROBING THE EFFECTS OF GRAVITY ON TRANSCRIPTION. C. N. Jayarajah and M. Thompson, University of Toronto, Department of Chemistry, Toronto, ON, Canada

The development of an on-line biosensor method based on the thickness-shear mode (TSM) acoustic wave device for monitoring the crucial events in the transcription process, which leads to gene expression, is discussed. In transcription, the enzyme RNA polymerase must not only bind specifically to its promoter DNA sequence, but must also initiate the catalytic process of template dependent RNA synthesis. The network analysis of impedance measurements allows for characterization of TSM sensors upon binding of RNA polymerase and synthesis of mRNA on template DNA immobilized onto a gold electrode surface. With the TSM device, it is possible to measure kinetics of protein-DNA binding and RNA synthesis while controlling the dynamics of initiation events at various stages in real time on the basis of in vitro assays. Furthermore, the TSM device offers several advantages in being able to detect a series of binding events including transcription factors, mediators and inhibitors, such as drug molecules. As well, variations in RNA synthesis can be monitored based on the TSM response for changes in mass, density and viscoelasticity. Signaling is direct for the TSM device, and no tagging agents are required.

The TSM acoustic wave device is presented here as an optimal technique to investigate the effects of microgravity on transcription and to examine how gravity affects shear stress responses. Consequently, this biosensor method can be efficiently employed to identify gravity dependent genes, and transcription factors that undergo changes in microgravity. A low-gravity environment is useful to study fundamental physical and chemical processes associated with transcription. These studies will further our understanding of how an important environmental cue such as gravity triggers changes at a molecular level in gene expression and cell differentiation. Moreover, these results will contribute significantly to the human genome project and drug discovery.

[134]

3D-CLINOSTAT DRIVES p38 MAPK CASCADE IN CULTURED HUMAN OSTEOBLASTS. K. Kataoka¹, L. Yuge¹, T. Kumagai¹, I. Hide¹, S. Hiyama², M. Kanno¹, Y. Kumei⁴, S. Takeda³, Y. Ikuta¹ and M. Sugiyama¹. ¹Faculty of Medicine, Hiroshima University, Hiroshima, Japan. ²Faculty of Dentistry, Hiroshima University, Hiroshima, Japan. ³Faculty of Dentistry, Tokyo Medical and Dental University, Tokyo, Japan. ⁴Dept. of Molecular Genetics, National Institute of Neuroscience, Tokyo, Japan.

We developed a cell stimulation method using a 3D-clinostat, which is a multi-directional gravity device for simulating microgravity. We cultured osteoblasts in the 3D-clinostat, examining differentiation of osteoblasts in terms of MAPK-cascade, osteogenesis and morphology, including histological detection of minerals. After exposure to the multi-directional environment, the cells became larger and oval shaped. The expression of bone specific regulatory factors, such as osteopontin, bone sialoprotein (BSP) and osteocalcin, were delayed, as well as the formation of hydroxyapatite crystals in this group of osteoblasts. P38 activity was repressed due to decreasing phosphorylation activity in this environment, whereas MAPK/ERK and SAPK/JNK phosphate activity were not changed in any group. The precise quantitative and stable stimulus induced by a multi-directional gravity device in the present study offers a new approach to elucidate the entire process of osteoblast differentiation to form hydroxyapatite crystals.

(Supported by Japan Space Forum from NASDA, Space Utilization Research from ISAS, the Ministry of Education, JPTA, and the Magnetic Health Science Foundation.)

**Concurrent Posters
IV–E
Plant Development, Physiology and
Gravity Sensing**

[136]

ALTERATION OF ORCHARDGRASS EMBRYOGENESIS FROM LEAF SEGMENTS UNDER HYPERGRAVITY AND CLINOROTATION. J.K. McDaniel, Z. Tomaszewski and B.V. Conger. Dept of Plant and Soil Sciences, Univ. of Tennessee, Knoxville.

The effects of hypergravity and clinorotation on somatic embryogenesis in orchardgrass was studied using a system in which embryos initiate from single mesophyll cells in *in vitro* cultured leaf segments. The two innermost leaves were split along the midvein, cut transversely into 3 mm x 3 mm segments and plated serially onto Schenk and Hildebrandt medium containing 30 μ M dicamba. Segments from one leaf half were used for treatments and the corresponding "sister" or "mirror" segments from the other half served as controls. Embryogenesis was estimated by counting the number of plantlets (germinated embryos) from separate leaf segments. The response of each treated segment was compared to that of its corresponding control. Plant material was grown in either an environmental chamber or a greenhouse. Previous experiments showed a significant reduction in embryogenesis of centrifuged (3 or 5 g for 5 d) or fast clinorotated (50 rpm for 5 or 10 d) leaf segments in comparison to nontreated controls. Those treatments reduced embryogenesis by at least 50-70% regardless of leaf segment orientation to the g force. Hypergravity at 2 g for 5 d and slow clinorotation (1 rpm) had no adverse effect. Preincubation after plating for 5 d prior to fast clinorotation (50 rpm for 5 d) reduced embryogenesis about 30%. Preincubation for 6 d prior to centrifugation at 3 g for 6 d reduced embryogenesis by 35%, while preincubation for 3 d resulted in about 60% reduction. This was the same as that of nonpreincubated segments (reduction ~ 65%). Preincubation for 5 d prior to 2 g centrifugation for 5 d did not have a significant effect. The response of leaves taken from the growth chamber and greenhouse was similar.

(Supported by NASA grant NAG10-0221.)

[138]

GRAVITROPIC SENSITIVITY CAN BE RESTORED IN STARCH-DEFICIENT MUTANTS OF *ARABIDOPSIS* BY HYPERGRAVITY. K.J. Fitzelle, R.E. Edelman and J.Z. Kiss. Botany Dept., Miami Univ., Oxford OH 45056.

Despite extensive study of plant gravitropism, there have been few experiments which have utilized hypergravity as a tool to investigate gravisensitivity in flowering plants. Previous studies have shown that starch-deficient mutants of *Arabidopsis* are less sensitive to gravity compared to the wild-type (WT). In this report, the question addressed was whether hypergravity could restore the sensitivity of starch-deficient mutants of *Arabidopsis*. The strains examined include a WT, a starchless mutant, and a reduced starch mutant. Vertical orientation studies with dark-grown seedlings indicate that increased centrifugal acceleration improves orientation relative to the gravity vector for all strains, even the WT. For starchless roots, growth of seedlings under constant 5g acceleration was required to restore orientation to the level of the WT at 1g. In contrast, approximately 10g was required to restore the orientation of the starchless mutant hypocotyls to a WT level at 1g. Examination of plastid position in root cap columella cells of the starchless mutant revealed that the restoration of gravitropic sensitivity was correlated with the sedimentation of plastids toward the lower cell wall. Even in WT plants, hypergravity caused greater sedimentation of plastids and improved gravitropic capability. Collectively, these experiments support the hypothesis of a statolith-based system of gravity perception in plants. To our knowledge, this is the first report to use hypergravity to study the mechanisms of gravitropism in *Arabidopsis*.

(Financial support was provided by NASA grant NAG 2-1017 to JZK.)

[137]

DIFFERENTIAL TISSUE-SPECIFIC EXPRESSION OF A WESTERN RED CEDAR DIRIGENT MULTIGENE FAMILY IN *ARABIDOPSIS*: PHENOLIC RADICAL COUPLING IN VASCULAR PLANTS M. Kim, J.-H. Jeon, L.B. Davin and N.G. Lewis. Institute of Biological Chemistry, Washington State University, Pullman WA 99164-6340.

Dirigent genes encode the first known plant proteins that control the outcome of phenoxy radical coupling (monolignols): these are involved in the ubiquitous lignan biosynthetic pathway, and additional evidence suggests that related proteins house (arrays of) dirigent (monomer binding) sites are required for lignification. A multi-gene dirigent family (of at least 8 members) was obtained from western red cedar (*T. plicata*) together with the corresponding promoter regions. *Arabidopsis* plants were transformed individually with each dirigent promoter fused to a GUS-reporter gene. It was found that each promoter was differentially expressed in different tissues and organs, both spatially and temporally, but all were associated with various components of the vascular apparatus, as well as with specialized compartments, such as trichomes and flower sepals. The importance of these results, related to gravitational effects on vascular plants, is described.

[139]

PLANT CIRCUMNUTATIONS IN SPACE: A REAPPRAISAL OF TIME SEQUENCES FROM THE SPACELAB -1 EXPERIMENT HEFLEX. T. K. Bardal¹, A. Johnsson¹ and D.K. Chapman². ¹Department of Physics, Norwegian University of Science and Technology, Trondheim, Norway, ²Dynamac Corp, Kennedy Space Center, FL

Circumnutations are rhythmic movements of plant organ, often around the plumb line. In order to test the hypothesis of gravity-induced circumnutations an experiment of sunflower (*Helianthus*) plants was performed in Spacelab-1 in 1983. The experiment (acronym HEFLEX) comprised video recording of growth performed by the plants to compare with studies under 1-g conditions and on a clinostat. Circumnutations could be recorded in the absence of gravity but amplitude and period of the movements of the plants as well as the quantitative occurrence of circumnutation were changed. It was concluded that circumnutations were present in the absence of gravitropic stimuli.

The evolution of computers and analysis methods have inspired further work to characterise circumnutations in hypocotyls. Circumnutation of *Helianthus* have been further studied and *Arabidopsis* has been mapped, all in experiments on the ground. The period distribution in a single plant, as well as direction of rotation etc., have been found to be complicated. A re-evaluation of circumnutation data from space was therefore of interest in order to study details of the plant movements.

We have applied different analysis methods to study the frequency contents in the time series. Time-frequency transformation methods provide new insight in the details of the data from the experiment. We find that the sunflower hypocotyls in some cases show not only one dominating period but also short-period nutations. These nutations have a period of approximately 20-30 minutes in contrast to the long period nutations at about 100 min. An overall average of the periods might, therefore, give a too simplified picture of complicated movements. Furthermore, the analysis shows that the frequency of the nutations is time-dependant. These features were also found in the ground-based studies.

(Supported by Department of Physics, Norwegian University of Science and Technology, and NASA Contract NAS10-12180.)

[140]

ANALYSIS OF THE GRAVISENSING SYSTEM OF *CHARA* BY INTRACELLULAR MAGNETOPHORESIS. O.A. Kuznetsov and K.H. Hasenstein. Biology Dept., UL Lafayette, LA 70504-2451.

The movement of statoliths and rhizoid growth was measured by video microscopy in the absence and presence of the microtubular depolymerizer oryzalin, F-actin depolymerizer latrunculin, or myosin inhibitors BDM and ML-9 with or without a high gradient magnetic field (HGMF). Lateral displacement of statoliths in vertically oriented rhizoids caused curvature in the direction of the displacement. Application of oryzalin did not change the organization of the cell apex or behavior of statoliths but dramatically changed the cytoplasmic streaming in the basal portion of the cell from typically longitudinal to independent domains that circulated independently from one another around the cell axis. The growth rate gradually declined. Latrunculin immediately stopped growth and the statoliths sedimented to the apex. After magnetophoretic upward movement, sedimentation in latrunculin-treated rhizoids decreased insignificantly but saltatory movements were dramatically reduced. Although the combined activity of oryzalin and latrunculin B enabled the greatest upward displacement of statoliths by HGMF, they never moved past the nucleus. The viscoelastic properties of cell interior were deduced from the extent and rate of statoliths displacement under different force values. The elasticity of the cytoskeletal network interacting with a statolith was estimated to be 4×10^{-7} N/m. X-ray analysis by SEM confirmed the presence of Ba and S in statoliths, even in the plants grown on a Ba-deficient medium.

(NASA grant NAG10-0190.)

[141]

EFFECT OF SLOWLY ROTATING CLINOSTAT ON THE ROOT SYSTEM DEVELOPMENT IN RAPSEED (*BRASSICA NAPUS*) SEEDLINGS. J. Aarouf¹, P. Coulomb¹ and G. Perbal². ¹Laboratoire de Cytologie et Pathologie Végétales, Faculté des Sciences, 33 rue Louis Pasteur, F-84000 Avignon, France. ²Laboratoire de Cytologie Expérimentale et Morphogenèse Végétale, Université Pierre et Marie Curie, 4 place Jussieu, F-75252 Paris Cedex 05, France.

Seedlings of *Brassica napus* were grown in the light for 5 days on a slowly rotating clinostat (1 rpm) or in the vertical position (rotated at 1rpm). The results obtained demonstrated that clinorotation stimulated root growth and degradation of lipid reserves as well as synthesis of sucrose in the cotyledons. An increase of the biomass of the excised root system (23 %) was also observed on a clinostat after 15 days of culture in a medium containing 1 % sucrose. Clinorotation seemed therefore to act directly on the growth of the root system and provoked a higher sucrose translocation from source to sink, i. e., from the cotyledons to the root system. In fact, a faster transport of ¹⁴C-labeled sucrose from the cotyledons to the root system was observed. When the seedlings of *Brassica napus* were cultivated in darkness during 5 days, the results showed also a greater development of the primary root. However, breakdown of the lipid reserves and the sucrose level were not altered by clinostat.

Analysis of the meristematic activity and determination of the levels in IAA, ABA and zeatin in the primary root tips demonstrated that after 5 days on the clinostat, the increased length of the primary root in the light could be the consequence of an increase in both IAA and ABA contents followed by a higher meristematic activity.

Clinorotation was directly responsible for the increase of the root development independent of mobilization of lipid reserves from the cotyledons.

(Supported by CNES 793/00/8103).

**Concurrent Posters
IV–F
Advanced Life Support and
Biotechnology**

[142]

STARCH PARTITIONING IN *RAPHANUS SATIVUS*: PRELIMINARY GROUND STUDIES FOR THE RASTA SPACEFLIGHT EXPERIMENT. G.K. Tynes¹, I. Eraso¹, E.C. Stryjewski¹ and G. W. Stutte¹. ¹Dynamac, Kennedy Space Center.

Experiments were performed to characterize starch partitioning in *Raphanus sativus* L. (cvs. Cherry Belle and Reggae) for the RASTA (Radish Assimilation In Spaceflight Testbed Atmospheres) experiment. Plants were grown at elevated CO₂ levels and through a range of temperatures to simulate growing conditions that could be experienced on earth or on the space shuttle. Both cultivars were grown at 400, 1500, and 10,000 ppm of CO₂ and at temperatures ranging from 18°C to 30°C. Tissue samples were taken weekly and starch concentrations were enzymatically determined. Starch concentration in both roots and shoots decreased between 14 and 21 days after planting (DAP). Starch content of roots was typically higher than shoots at elevated CO₂ levels at 14 DAP. Temperature had no effect on starch concentration or partitioning at 7 DAP. In contrast starch concentrations were approximately 3X higher in shoots than roots at 14 and 21 DAP. Root starch appears to be inversely related to temperature at harvest. The results of CO₂ concentration and temperature of partitioning of starch were similar for both cultivars. By simulating conditions of the space flight environment we will be better able to differentiate microgravity effects from environmental stresses.

(This research was supported by a grant (NCC-0034) from NASA's Fundamental Biology Program.)

[144]

EVALUATION OF NUTRIENT DELIVERY SYSTEM DESIGN CONCEPTS FOR MICROGRAVITY USING KC-135 PARABOLIC FLIGHTS. E.C. Stryjewski¹, I. Eraso¹, O. Monje¹, W.T. McLamb², D. W. Reed², R. N. Stuckey³ and G. W. Stutte¹. ¹Dynamac and ²Bionetics, Kennedy Space Center, FL, and ³Space Shuttle Customer Integration Office, Johnson Space Center, Houston TX.

Prototype nutrient delivery systems and planting substrates were flown aboard NASA's KC135 to aid in the re-design of the Plant Growth Facility (PGF) nutrient delivery system to accommodate the RASTA (Radish Assimilation in Spaceflight Testbed Atmospheres) experiment. Two candidate nutrient delivery systems were tested: 1) a *volume wetting* system which relies on pressure to deliver nutrients and 2) a *surface wetting* system which relies on capillary action to draw nutrients into the substrate. PGF Plant Growth Chambers (PGC) using 0.5 µm porous tubes or perforated 1/8" polyethylene tubing embedded in either arcillite (Turface[™]) or porous foam (Oasis[™]) were constructed to test volume wetting. For surface wetting, vessels containing several wicking materials were constructed. In these systems, polyethylene tubing brought liquid to the surface of the wicking material. At the onset of each 0g segment, liquid was forced into each system and the uniformity of its distribution was determined. Liquid distribution from both the porous tubes and perforated tubing in arcillite and Oasis foam was uniform in 0g whereas pooling at the PGC base was evident in the ground controls. Uniformity of distribution was sensitive to the compactness of the arcillite sizes (0.5-1, 1-2 and 1-2.8mm) tested. The more compact the media, the better the distribution while this had little effect in the ground controls. Surface wetting nutrient delivery was ineffective in 0g. Mechanical stresses caused some of the tubes to slip and lose contact with the wicking material. Without this contact, the liquid simply bounced off the wicking material and was not absorbed. Since mechanical disturbances are expected during a space flight mission, a surface delivery system is not acceptable for the RASTA experiment. These results suggest that the PGF nutrient delivery system should consist of an embedded delivery system of perforated tubing running through an absorbent substrate.

(Supported by NASA's Fundamental Biology Program, NCC-0034.)

[143]

MEXSY – A MODULAR TOOL KIT FOR LIFE SCIENCE EXPERIMENTS IN SPACE. U.M. Kuebler¹ and P. Kern¹. ¹Astrium GmbH, Friedrichshafen, Germany.

The goal of Mexsy is to create a standard processing system for biotechnological experiments in a space environment. Terrestrial Life Science Experiments are normally performed with standard laboratory equipment like Petri dishes, culture flasks or complete bioreactors. This enhances the validation of the results, because of the repeatability and comparability for others, using the identical equipment and limits the overall cost due to mass production and the preparation times due to availability of the hardware.

The Modular Experiment System MEXSY offers a set of standardized modules, proven to be compatible with the special environment of microgravity as well as the additional safety, operational and logistics requirements related to manned space flight.

MEXSY covers all standard processes like: Closed containment of the culture, injection of organisms and reagents, gas and nutrient supply, control of temp, pH, pO₂, pCO₂, nutrient composition, sampling of media and biological material, optical access.

MEXSY is adapted to safety and operational constraints by: Automation for crewtime limitation, modularity and exchangeability of submodules, compatibility with existing infrastructure (e.g. incubators, refrigerators, freezers and centrifuges).

Potential Applications of MEXSY are cell cultivation, tissue engineering, multi-generation experiments, marine and freshwater habitats, pharmacology studies, toxicity monitoring and bio-compatibility testing.

Two MEXSY demonstrators were developed and built. The prototypes for a yeast bio-reactor and a modular mini-aquarium shall be presented at the meeting.

**Concurrent Posters
IV-G
Spaceflight Experiment Results**

[145]

RESERVE UTILISATION IN SEEDS OF *ARABIDOPSIS THALIANA* GERMINATED IN MICROGRAVITY. L.G. Briarty¹ and E.P. Maher².
¹Plant Science Div., School of Biological Sciences, Univ. of Nottingham, Nottingham GB, ²The Open Univ. in Scotland, 10 Drumsheugh Gardens, Edinburgh GB.

Investigating the effects of spaceflight conditions on basic plant metabolism is difficult or impossible to carry out, given the complexity and constraints of in-orbit experiments. Accurate, quantitative ultrastructural analysis of in-orbit fixed material is possible however using stereology, and such data allow some indirect conclusions to be made concerning physiological processes. In a study on the IML-1 mission we germinated seeds in closed containers, fixing the seedlings at a number of time-points by filling the containers with 1% buffered glutaraldehyde. Material was grown in the ESA Biorack, which allowed the use of in-flight 1g controls. Following return we used stereology to determine structural parameters of the main tissue and sub-cellular components in thin sections. Growth rates were similar in roots and shoots of 0g and 1g control seedlings, though by 86h 0g roots were slightly shorter than the controls. Cotyledon organisation was in general similar to light-grown material. The major effect of growth in microgravity appeared to be a significant slowing in the rate of storage lipid consumption, so that after 86h of germination the volume fraction of symplast occupied by lipid was 15.5% as opposed to 7% in 1g controls. Intercellular space volume was reduced to 0.95% in the 0g material compared to 1.4% in the controls (95% confidence). Since the seedlings were growing in closed containers it is concluded that the differences in lipid consumption rate may be the result of local anoxia in the absence of convection. This research was supported by the University of Nottingham National Westminster Bank Research Fund (to LGB) and by the UK Science and Engineering Research Council.

(GR/E/68549 to LGB and GR/H25195 to EPM.)

[147]

A CRITICAL PERIOD FOR GRAVITATIONAL AFFECTS ON THE FORMATION OF OTOLITHS IN SWORDTAIL FISH.

M.L. Wiederhold, J.L. Harrison and K.A. Parker, Dept. Otolaryngology – Head and Neck Surgery, Univ. Texas Hlth. Sci. Ctr., San Antonio

Gravity and linear acceleration are sensed in fish by specialized organs in which a dense otolith is loosely coupled to mechanoreceptor hair cells, which are, in turn, are tightly coupled to the animal's head. The pull of gravity or linear acceleration on the otolith moves sensitive hairs on the hair cells, causing them to increase or decrease the release of excitatory neurotransmitter from the hair cell to the terminals of the vestibular nerve fibers, sending a coded representation of the stimulus to the central nervous system. Previous experiments in which eggs or larvae of a marine mollusk (the sea hare *Aplysia*) or fish larvae were raised on a centrifuge, demonstrated that the size of the otolith or statoconia (in *Aplysia*) were reduced, in a graded manner, as the g-field was increased, suggesting that some control mechanism was acting to "normalize" the weight of the mass. In the experiments described here, pre-mated adult female swordtail fish (*Xiphophorus helleri*) were flown in the CEBAS aquarium system on STS-89 and 90 (NeuroLab). Developing larvae were removed from the adult ovaries after the shuttle landed. The size of otoliths was compared between ground- and flight-reared larvae of the same size. For later-stage swordtail larvae, with spine lengths from 3 to 6 mm from STS-90 (16 days), the growth of the otolith with increasing spine length was significantly greater in the flight-reared fish for all three otoliths, from the saccule (saggita), utricle (lapillus) and lagena (astericus). However, juvenile fish, 1 cm long at launch, showed no significant difference in otolith size between flight- and ground-reared animals. In very early stage larvae from STS-89 (9 days), with spine length of 1.5 to 3.5 mm, the utricular and saccular otoliths were actually larger in the ground-reared larvae. Thus, it appears that late-stage fish larvae reared in space do produce larger-than-normal otoliths, apparently in an attempt to compensate for the reduced weight of the test mass in space. However, the results from very early-stage larvae and juvenile fish suggest that there is a fairly short critical period during which altered gravity can affect the size of the test mass.

(Supported by NASA: NAG2-952 and NSF: IBN-9529136.)

[146]

THE EFFECTS OF MICROGRAVITY ON BONE MARROW STROMAL CELL CULTURES IN VITRO. P.M. Loomer¹, B. Sukhu², M. Grynbas² and H.C. Tenenbaum^{2,3}. University of California, San Francisco CA, ²Mt. Sinai Hospital and ³MRC Group in Periodontal Physiology, University of Toronto, Toronto ON.

Exposure to microgravity has been associated with several physiological changes including osteoporosis-like loss of bone mass. This study was undertaken to examine the effects of microgravity on bone formation *in vitro* using the rat bone marrow stromal cell model system. Cells were cultured onto calcium phosphate coated chamber slides (2000 cells per well) and grown at 37°C in an atmosphere of 5% CO₂ in air for 3, 5, 7 or 9 days in a-MEM medium containing dexamethosone, B-glycerophosphate and ascorbic acid. Cell cultures were subsequently transferred to a closed system in-flight cell culture platform (Osteo™) and grown under the same conditions with the exception of an air atmosphere. Cells were cultured for a further 9 days in microgravity aboard the STS-95 shuttle flight, and fixed prior to re-entry. In-flight cultures were compared to ground controls grown in the closed system platform as well as regular cultures grown in 5% CO₂ in air. The cultures were analyzed for bone sialoprotein (BSP) and osteopontin (OP) levels using immunohistochemistry. The results revealed increased levels of staining of both BSP and OP for day 5 and 7 in-flight cultures in comparison to ground controls. Day 3 cultures exhibited poor growth for both in-flight and ground controls, while no differences were seen for day 9 cultures. Histological analysis revealed increased mineralization in day 5 cultures, while decreased mineralization was observed in day 7 cultures, in comparison to closed-system ground controls. Day 9 cultures showed similar levels of mineralization as controls. However, all measured parameters of osteogenesis were less for both in-flight and closed-system ground controls cultures than those observed in regular cultures. The results from this study suggest that the cellular responses of rat bone marrow stromal cell cultures to exposure to microgravity may depend upon the degree of maturity of the cultures at time of exposure.

(Supported by Canadian Space Agency.)

[148]

HUMAN PARATHYROID HORMONE (1-84) STIMULATES BONE FORMATION IN RAT BONE MARROW CULTURES DURING SPACEFLIGHT. D. R. Sindrey, D. Kusljic, P. C. Kwong. Allelix Biopharmaceuticals Inc., Miss., Ontario, Canada.

Astronauts suffer from a space-induced bone-loss similar to the effects seen in osteoporosis suffers on earth. We tested the ability of recombinant human parathyroid hormone (PTH1-84) to stimulate osteoblast bone formation in rat bone marrow stromal cultures in microgravity as a possible pharmacological treatment for bone loss in space. Experiments were part of the OSTEO (Osteoporosis Experiments in Orbit) payload, in collaboration with the Canadian Space Agency and were flown aboard the NASA shuttle Discovery STS-95 (October 1998). Cells were sub-cultured 6 days after isolation prior to plating onto 16-well CaP treated Osteologic™ slides from Millenium Biologix. Tetracycline was added to the media as a fluorescent indicator to quantify bone formation. Cells were cultured for 9 days during the STS-95 shuttle flight with media changes on day 1 and every second day. One module was dosed with 2x10⁻¹²M PTH continuously during the flight. A second module received pulsatile doses (1 hr) of 2x10⁻¹²M PTH at each media change. The third module received media only as a control. Two payloads were flown with replicate ground units. Pulse-treated cultures in flight produced 23.7% less than corresponding ground cultures, as measured by tetracycline fluorescence, but produced 55% more mineralized matrix than untreated flight controls. Ground cultures continuously dosed with PTH produced only slightly more mineralized matrix than ground controls (14.9%). However, corresponding flight cultures produced 56.3% less than ground. These results indicate that microgravity decreases osteoblasts response to the bone stimulating effects of pulsatile PTH treatment. However, sufficient responsiveness remains to generate new bone in orbit, suggesting that PTH may be useful as a treatment for bone loss during extended space flights.

**Concurrent Posters
IV–H
Spaceflight Physiology and Medicine**

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EFFECTS OF CONFINEMENT ON *IN VITRO* IMMUNE REACTIONS AND ITS ENDOCRINE REGULATION. P.N. Uchakin¹, B.W. Tobin¹, C.F. Sams², B.E. Crucian³ and I.M. Larina⁴. ¹Mercer University School of Medicine, Macon GA, ²NASA/JSC, ³Wyle Laboratories, Houston TX, USA, ⁴Institute of Biomedical Problems, Moscow, Russia.

Confinement is a stress factor, which may involve the immune system and affect the crews of manned space crafts. It is one of the major provoking factors of eminent microbiological contamination and has a significant psychological impact on crewmates as well. Along with its protective role, the immune and the neuro-endocrine systems play a significant regulative role in homeostatic reactions. It is established that stress induced immune alterations are endocrine regulated. Objectives for this study were to investigate effects of confinement on immune responsiveness *in vitro* and its modulation by corticosteroids. We hypothesized that confinement may affect the immune system *in vivo* and change *in vitro* immune response to mitogen challenge in the presence of stress hormones such as cortisol. Four volunteers spent 240 days and 8 volunteers spent 120 days in a closed habitat chamber as a part of the SFINCSS project. Blood samples were collected before confinement (PRE), 24 hours (POST 24h), 7 days (POST 7d), and 2 weeks (POST 14d) following confinement. We analyzed 1) lymphocyte subset distribution in the whole blood, and 2) secretion of IL-2, IFN γ , IL-4, IL-10, and sIL-2R α in non-treated (Ctrl) and Hydrocortisone-treated (Hcs) mitogen induced cell cultures. The data indicate a significant elevation of T helpers and a decrease of NK cells observed on POST 7d. Secretion of IL-2 significantly declined in both Ctrl and Hcs culture, while IFN γ , and IL-4 declined in Ctrl culture only. Additionally, secretion of IL-10 was significantly elevated in Hcs but not Ctrl culture following confinement. We conclude that confinement is associated with an altered immune responsiveness and cortisol may be involved in these alterations.

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EFFECTS OF LOWER BODY SUCTION (LBNP) WITH SYNCHRONOUS GRADED HEAD-DOWN TILTING. H.G. Hinghofer-Szalkay, B. Haditsch, and P. Pilz. Institute for Adaptive and Spaceflight Physiology, Austrian Society for Aerospace Medicine, A-8010 Graz, Austria (<http://www.asm.at/iap>).

This study applied various degrees of head-down tilt combined with -35mmHg LBNP and measured variables involved in cardiovascular regulation. We developed a computer-controlled test device for combined change of pitch and LBNP. In 10 healthy, middle-aged, male subjects, we determined basic thoracic electrical impedance (Z_0) and cardiac indices; heart rate; blood pressure; hematocrit; plasma mass density (PD); and plasma hormone concentrations (atrial natriuretic factor, aldosterone, catecholamines, renin activity). We combined LBNP with 0, 6, 12, 18, and 24° head-down tilt (HDT) for 30 min each. The LBNP-induced rise of Z_0 , hematocrit and plasma density decreased with increasing degree of HDT but was not fully compensated by -24°HDT. In contrast, cardiac index and heart rate reached supine control values. Similar patterns emerged for endocrine changes. We conclude that certain combinations of HDT and LBNP can produce different effects within hemodynamic and volume dependent variables or parameters, and specific 'equilibration points' are different for distinct subsystems of cardiovascular regulation. We plan further investigations to identify neutralizing stimulus combinations for each subsystem involved by varying LBNP intensity as well.

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IMMUNOPHENOTYPE OF LYMPHOCYTES IN PERIPHERAL BLOOD OF APHERESIS DONORS MOBILIZED BY GRANULOCYTE COLONY-STIMULATING-FACTOR (G-CSF). J.M. Reuben¹, B.-N. Lee¹, M. Korbling² and W.T. Shearer³. Depts of ¹Laboratory Medicine and ²Blood and Marrow Transplantation, Univ. of Texas M. D. Anderson Cancer Center and ³Dept. of Pediatrics, Baylor College of Medicine, Houston TX.

Astronauts on protracted missions to Mars may experience marrow failure due to exposure from space-related radiation. Transplantation of peripheral blood stem cells (PBSC) mobilized by granulocyte colony-stimulating factor (G-CSF) or other cytokines and harvested by apheresis is an effective countermeasure for marrow aplasia; however, little is known of the immunophenotype of the recruited lymphocytes in G-CSF mobilized PBSC preparations. We determined the immunophenotype of lymphocytes obtained from the peripheral blood of 8 normal donors before receiving G-CSF (Pre-GCSF) and after (Post-GCSF) receiving 6 μ g/kg body weight G-CSF subcutaneously every 12 h for 5 days. Compared with Pre-GCSF, Post-GCSF preparations contained significantly higher percentages of CD4+ T-helper cells (P = 0.0027) and CD19+ B cells (P = 0.0011) but significantly lower percentages of CD62L+ naive (P = 0.0206) and CD56+ CD16+ natural killer (P = 0.05) cells. Mobilization of PBSC with G-CSF did not alter the distribution of suppressor (CD8+), cytotoxic (CD8+ CD28+), and memory (CD45RO+) T cells. Additional studies are in progress to determine whether mobilization of PBSC with other cytokines will yield lymphocytes with immunophenotype distinct from those mobilized by G-CSF.

**Minisymposium
Current Ground-Based Models**

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CLINOSTATS AND BIOREACTORS. D.M. Klaus. BioServe Space Technologies, Aerospace Engineering Sciences Dept., University of Colorado, Boulder.

The environment created within a clinostat or rotating wall bioreactor used on Earth is often referred to as "simulated microgravity". Both devices utilize constant reorientation to effectively nullify cumulative sedimentation of particles. Neither, however, can fully reproduce a concurrent lack of structural deformation, displacement of intercellular components and/or reduced mass transfer in the extracellular fluid. Parameters including density, viscosity, and container geometry all play a role and must be mathematically assessed to determine the overall gravity-dependent effects produced by either a clinostat or a rotating wall bioreactor. In addition, the intended optimal application of these two devices differs considerably. A state of particle "motionlessness" relative to its surrounding bulk fluid can theoretically be achieved through clinorotation, which is nearly analogous to being in true weightlessness. The rotating wall bioreactor, on the other hand, while similarly maintaining a culture in suspension under minimal shear stress, purposefully induces a perfusion of nutrients and waste products throughout the fluid environment. A clinostat, therefore, can more nearly reproduce the quiescent, unstirred fluid conditions achievable on orbit, while the rotating wall bioreactor creates a low shear, but mixed, fluid environment optimized for suspension culture growth. Other techniques for exploring altered inertial environments, such as, free fall, neutral buoyancy and electromagnetic levitation may also provide unique insight into the role gravity plays in biological systems. Ultimately, all underlying biophysical principles thought to give rise to gravity-dependent physiological responses must be identified and thoroughly examined in order to accurately interpret data from flight experiments or ground-based analogs.

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HUMAN AND RODENT MODELS. D.A. Schmitt, Head Life Sciences, ESA-ESTEC, Noordwijk, NL.

"Ground-based research" has not the same meaning in all space agencies. Ground-based research has been extensively sponsored in the US because it has always been an integral part of any space flown experiment. The same has been true in the Russian programme but in a different context. For several reasons other space agencies, have been slow in recognising that more emphasis should be put on ground-based research.

Research using Humans as test subjects in preparation of space flight investigations has been done in many different and complementary situations. These are called analogue situations such as hyper-gravity, confinement/isolation, bed rest or water immersion. Isolation and confinement has essentially been used in Russia and in Europe in order to study psychological adaptation to closed environments. As shown recently this type of studies is essential to predict and may be to prevent intercultural/interpersonal conflicts on ISS. This is even more true for interplanetary missions. Bed rest studies are also very essential to study the effects of hypokinesia from a fundamental point of view but also from a countermeasure validation point of view. Such bed rest studies of long duration (up to 3 months) have now also been initiated in Europe in co-operation with NASDA. In addition to the usefulness of this model, it makes no doubt that such large scale clinical studies attract new scientists because bed rest for example is at the cross-road of space and purely "terrestrial" research.

Rodents in suspension models as space flight analogue have been used efficiently since about two decades. This model is now widespread and even used outside the space flight research area. Because of the growing availability of genetically modified strains, mainly for mice, this model will be even more used in the coming years. Rodents are not only a good model to address classical questions such as space flight-induced osteoporosis or muscle atrophy but are also essential to address issues in neuroscience such as the effect of gravity on post-natal development. Ground-based research in that respect is important to understand the effects of hyper-gravity on these mechanisms and to design "intelligent" habitats to be used on ISS.

**ABSTRACT
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