Investigation Title: Cellular Mechanisms of Space Flight-Specific Stress to Plants Experiment

Principal Investigator: Abraham D. Krikorian, State University of New York at Stony Brook

INVESTIGATION OBJECTIVES

1. To use discrete (uniform) somatic embryo fractions at different stages of development to test whether there is a level of development/stage-related element contribution to the observed (chromosome-based) effects.

2. To use somatic embryos which are at the same stage of development but which are of different size and mass to test whether there is a size/mass-related element contribution to the observed (chromosome-based) effects.

3. To use somatic embryos which are cultured on semi-solid medium which is well-drained (dry) versus a more wet semi-solid medium to test whether the water relations of the cells as affected by the prevalence of free water in their immediate vicinity affects the chromosomal (nuclear) responses.

The primary hypothesis for this experiment was that microgravity alters the water and gaseous relations of developing cells that are exposed on nutrient substrates with specific characteristics and water potential in space because the availability and behavior of water in microgravity is significantly different from what it is on Earth. Excess water and accumulation of respiratory gases like carbon dioxide induce a stress in the developing cells and in so doing, aberrant nuclear responses occur as the cell cycle attempts to progress. Persisting changes in plasma membrane-cytoskeleton interface relations is but one aspect of the stress brought on by altered water relations and the resulting hypothesized failure to communicate effectively between cells in developing systems is reflected in many changes, including imperfections in chromosomal movement and division.

What this amounts to is the following: A picture is emerging that a space environment-specific stress can be delivered to plant cells and tissues. The stress is most severely experienced by cells and tissues that are vulnerable due to their size, stage of development and growing environment. On this view, smaller and less developed embryogenic units are more vulnerable than those that are larger. Units that are more larger and more advanced in terms of their progression are less sensitive than those that are just beginning progression of their development. Embryogenic units that are in an optimal growing environment so far as their continued development is concerned are less perturbed than those than are in a less optimal environment.

All this may be reduced to 'Space-specific stress as related to environmental parameters and cell and developmental complexity" hypothesis

Stated yet another way, the main objective of the work detailed in this report was: (1) to characterize and refine our understanding of stress to plant cells in the space environment in the novel context of its relationship to, indeed the very basis of the expression and progression of somatic embryogenesis in totipotent cultured plant cells.

Equally important, and indeed more important in the long run was to ascertain whether the kinds of abnormalities of cytology encountered in other missions have biological significance. That is to say, if chromosomal abnormalities are encountered, do they persist, and if so, what is the consequence of their persistence.

A well-defined somatic cell system from daylily provided the test materials.

PHASE 1 MISSIONS

NASA 4

OPERATIONAL ACTIVITIES

Not provided by PI.

RESULTS

Poor growth and nuclear abnormalities observable in some space-grown plants has been hypothesized as due to a combination of factors such as biological status, the specific way they are grown and the way they experience multiple stresses, some of which are space-specific. Data from a 132 day experiment on 'Mir' using embryogenic cell cultures of daylily allows us to harmonize seemingly contradictory evidence.- a) the more developed an embryo the less likely it is to suffer catastrophic cell stress during growth; the less developed, the greater its vulnerability; (b) the

extent to which the stress becomes manifest is also dependent on the extent of pre-existing stresses imposed by suboptimal growing conditions; (c) an appropriate albeit undesirable "stress match" with other non-equilibrium determinants, much like a 'tug of war', can result in genomic variations in space.

CONCLUSIONS

Fastidiously-controlled growing environments for plant cells must be devised if one is to resolve the matter of direct versus indirect effects of space. Acces to 1 G centrifuges must be an important parameter in these experiments. On a practical level, it is predicted that adapting plant biotechnologies to space conditions will not be a casual matter.

PUBLICATIONS

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3. Levine, H. G., Anderson, K. F. and Krikorian, A. D. (1998) Characterization of the physical environment within BRIC-100VC canisters flown on 'Mir' with embryogenic daylily cell cultures. In: 32nd Scientific Assembly of COSPAR 12-19 July 1998. Nagoya, Japan. p. 449.
4. Levine, H. G., Anderson, K. F. and Krikorian, A. D. (199?) The 'gaseous' environment in sealed BRIC-100VC canisters flown on 'Mir' with embryogenic daylily cell cultures. 32nd COSPAR Scientific Assembly (Nagoya, Japan) (accepted)
5. Krikorian, A. D. 1998. Plant somatic embryos in space. In: 32nd Scientific Assembly of COSPAR 12-19 July 1998. Nagoya, Japan. p. 380.
6. Krikorian, A. D. (199?) Somatic embryos of daylily in space. 32nd COSPAR Scientific Assembly (Nagoya, Japan) (accepted)

Investigation Title: Developmental Analysis of Seeds Grown on Mir

Principal Investigator(s): Mary E. Musgrave, Ph.D., Louisiana State University; Dr. Rita Levinskih, Institute of Biomedical Problems

Additional Investigators: Dr. Gail Bingham and Dr. Greg Briarty

INVESTIGATION OBJECTIVES

1. Analyze space flight effects on plant growth and development processes throughout the life cycle through inflight observations, video recording, gas exchange measurements, and postflight analysis of plant material harvested and fixed, dried or frozen on orbit.

2. Collect seed produced on orbit and grow this seed to produce new plants, comparing the production of seeds by plants from space-produced seeds to that of plants from ground-produced seeds. Seed will also be studied extensively postflight and compared with ground controls and with seed produced during space flight by the plants grown from space-produced seeds.

3. Analyze floral initiation and early reproductive development during space flight, comparing plants from seeds produced on the ground, in space, or by plants grown from seeds produced in space.

4. Evaluate the extent of space flight influence on cell shape in plant organs, and assess the implications on overall metabolism.

5. Determine the extent of space flight-mediated changes in cell structure, organization and physiology.

6. Analysis of the root medium in the Svet Root Modules.

7. Analysis of the microbial organisms present on plant tissue and in the root medium.

PHASE 1 MISSIONS

Mir 24/NASA 5

OPERATIONAL ACTIVITIES

This experiment addressed the problem of seed-to-seed cycling in microgravity, using the species Brassica rapa, a mustard plant with a very short life cycle. The experiment called for 3 consecutive plantings of Brassica, beginning with seeds launched from the ground on STS-84. These were the so-called "Earth seeds", or E1 seeds. Seeds produced in space from this first planting on Mir were collected, dried, and used in the second growth cycle. This second set of seeds was called "Space 1", or S1 seeds. Seeds generated in space from the second planting on Mir, the S2 seeds, were collected and planted in the third planting in space, along with Earth seeds and S1 seeds. Surplus seeds produced by the plants during the first two growing cycles were used to supply dried material for postflight analysis, as well as seeds for subsequent planting in Svet. Additional developmental and physiological information was obtained from fixed plant samples obtained during the growing cycles, from dried plants taken at final harvest stages, and from plants freshly harvested and frozen at the end of the third planting.

RESULTS

This investigation was the first to successfully investigate growth of plants over multiple generations in space. It showed that for the model plant (Brassica rapa):

1. Vegetative growth, flowering, and reproductive development was comparable in microgravity to that observed in the ground control. No stage of the plant life cycle is dependent upon gravity for its completion. Seeds were produced in space, and these seeds were re-planted and grew into new plants.

2. The growth characteristics of the plants in microgravity were highly reproducible, resulting in comparably sized plants in the three growth cycles. Reproducing aspects of the space flight environment in the ground control (carbon dioxide, ethylene, humidity, light, temperature) resulted in plants that did not differ significantly in size and developmental rate from those grown on orbit.

3. The quality of the seeds produced in space was lower than in the ground control. This led to smaller second generation plants in the space flight treatment.

4. The ripening process inside maturing seed pods occurs differently in microgravity, probably due to indirect effects of microgravity on the gaseous microenvironment around the developing seeds.

5. The ability of plants to grow and reproduce in microgravity has been confirmed in this experiment. Further studies with different species will be necessary to determine if reduced seed quality would be a general concern during space flight, or is a factor associated only with this particular plant type and seed pod architecture.

Status of Data Received/Analyzed

Initial analysis of data collected on dried and fixed material during postflight procedures has been completed and a preliminary report has been submitted to a scientific journal for review. The postflight ground control is complete. Processing of both sets of samples is proceeding.

CONCLUSIONS

The Greenhouse 3 experiment has provided a wealth of information and material for future study. The experiment design allowed us to depart from the past practice of comparing space flight plants only to ground-based controls, since reference plants (produced from seed that had been brought from Earth) were grown alongside the second generation space plants for comparison purposes. Growth and reproductive effort by reference plants grown from Earth seeds was comparable in the three growth cycles. In the two growth cycles utilizing first generation space seeds, plants were smaller and produced fewer flower buds than reference plants grown from Earth seed on orbit at the same time.

Following the completion of the high-fidelity postflight ground control, we have understood that ethylene gas on the Mir station was a primary determinant of plant size. The results of analyses completed to date indicate no significant differences between space flight and ground control material in first generation plants. However, second generation space plants were significantly smaller than second generation ground control plants. Our data indicate that the quality of seeds produced on orbit is lower than that of seeds produced in the ground control, thus leading to the smaller second generation plant size. The weight per seed of seeds produced on orbit was significantly lower than that of seeds produced in the postflight ground control. We believe that this diminished seed quality is due to different ripening kinetics inside the seed pod in microgravity. Further investigations with the dried seeds as well as the fixed and frozen plant material may provide additional information about the nature of reduced growth by the plants from first generation space seeds.

PUBLICATIONS

1. Musgrave, M. E., A. Kuang, Y. Xiao, G. E. Hingham, L.G. Briarty, M.A. Levinski, V. N. Sychev and I. G. Podolski. 1998. Repeated seed-to-seed experiments with Brassica rapa on the Mir Space Station. Gravitational and Space Biology Bulletin 12: 56.

2. Bingham, G. E., S. B. Jones, D. Or, I. Podolsky, V. Sytchev. 1998. Water management lessons from plant full life cycle experiments on Mir. Gravitational and Space Biology Bulletin 12: 56.

Investigation Title: Effective Dose Measurements at EVA

Principal Investigators: Sandor Deme, Ph.D., KFKI Atomic Energy Research Institute; Yuri A. Akatov

Additional Investigators: Istvan Apathy and Istvan Hejja

INVESTIGATION OBJECTIVES

Development of an on-board TLD (thermoluminescent dosimeter) system to provide:

1. High sensitivity dose measurement to gain information on extra doses during extravehicular activity.

2. Measurement of ratio of low LET (linear energy transfer) to high LET dose components inside the Mir Space Station.

PHASE 1 MISSION

NASA 4, NASA 5

OPERATIONAL ACTIVITIES

Dislocation of TL (thermoluminescent) dosimeters inside Mir and periodical their readouts by US astronaut. One dosimeter was used as a personal dosimeter, two dosimeters were used during EVA

RESULTS

The dose rate range inside Mir measured by TLD method was in range 9.3...18.3 μGy/h, the EVA dose rate was about 3 times higher then the mean dose rate inside Mir. The ratio of high LET radiation induced TLD peak was about 1.4 times higher than for calibration gamma-radiation source

CONCLUSIONS

The dose rate measured with the individual dosimeter of the astronauts was significantly lower than the mean value of other three dosimeters located in the working area and sleeping cabin.

PUBLICATIONS

1. S. Deme, I. Apathy, I. Hejja, E. Lang and I. Feher: Extra Dose due to EVA during the NASA4 Mission, Measured by an On-Board TLD System. To be published in Radiation Protection Dosimetry in 1999.

Investigation Title: Effects of Gravity on Insect Circadian Rhythmicity

Principal Investigators: Tana M. Hoban-Higgins, Ph.D., University of California at Davis; Alexei M. Alpatov, Institute of Biomedical Problems

Additional Investigators: Gary T. Wassmer and Charles A. Fuller

INVESTIGATION OBJECTIVES

1. To study the long-term effects of altered gravitational environments on the Circadian Timing System of insects

PHASE 1 MISSIONS

NASA 5

OPERATIONAL ACTIVITIES

Collection of activity data from 64 individual beetles under different lighting conditions.

RESULTS

Entrainment (synchronization) of the activity rhythms to light-dark cycles. Free-running activity rhythms in both constant light and constant darkness. Resetting of the circadian clock by light pulses against a background of constant darkness.

CONCLUSIONS

It is possible to induce both phase advances and delays in the microgravity space environment. Light intensity affects the period of the circadian clock in the microgravity space flight environment.

PUBLICATIONS

1. Hoban-Higgins, T. M., A. M. Alpatov, T. Schnepp, P. Savage, E. Hayward, G. Fenton, M. Hale, J. Higgins, S. Piert, G. T. Wassmer and C. A. Fuller. Beetles in space

long-term monitoring of insect circadian rhythms on NASA-Mir. ASGSB Bulletin 10 (1): 66, 1996.

2. Hoban-Higgins, T. M. Body Clocks in Space. Invited talk at American River College for National Science and Technology Week. April, 1996.
3. Hoban-Higgins, T. M., A. M. Alpatov, G. T. Wassmer, W. J. Reitveld and C. A. Fuller. Response of insect activity rhythms to altered gravitational environments. J. Grav. Physiol 4(2): 109-110, 1997.
4. Hoban-Higgins, T. M. Gravitational biology on Mir. J. Grav. Physiol., Invited paper, in press, 1998.
5. Alpatov, A. M., T. M. Hoban-Higgins, C. A. Fuller, A. O. Lazarev, W. J. Rietveld, V. B. Tschernyshev, E. G. Tumurova, G. Wassmer, V. A. Zotov. Effects of microgravity on circadian rhythms in insects. J. Grav. Physiol., Invited paper, in press, 1998.

Investigation Title: Environmental Radiation Measurements on Mir Space Station

Principal Investigator: Eugene V. Benton, Ph.D., University of San Francisco

Additional Investigators: Eric Benton, Allen Frank, and Vladislav Petrov

INVESTIGATION OBJECTIVES

Not provided by PI.

PHASE 1 MISSIONS

NASA 2 - NASA 5

OPERATIONAL ACTIVITIES

Deployment and retrieval of internal Area Passive Dosimeters.

Deployment and retrieval of External Dosimeter Array.

RESULTS

40% Variation in Dose measured as a function of location/shielding inside Core Module.

Three order of magnitude decrease in dose as a function of shielding within the first g/cm^2 as measured on the outside of Mir.

Significant contribution to LET spectra >5 keVμm from proton-induced target fragment secondaries.

CONCLUSIONS

Dose varies significantly as a function of shielding within spacecraft.

Astronauts will receive significantly greater dose during EVA inside the South Atlantic Anomaly.

Proton-induced target fragmentation must be considered as one of the principle sources of dose and dose equivalent to astronauts inside spacecraft.

PUBLICATIONS

Currently being written.

Investigation Title: Greenhouse - Integrated Plant Experiments on Mir

Principal Investigator(s): Frank B. Salisbury, Ph.D., Utah State University; Margarita Levinskikh, Ph.D., Institute of Biomedical Problems

Additional Investigators: Dr. Gail Bingham, Dr. William F. Campbell, Dr. John G. Carman, and Dr. David Bubenheim

INVESTIGATION OBJECTIVES

1. To investigate the effects of microgravity on the productivity of a crop plant, specifically dwarf wheat.
2. To identify the chemical, biochemical, and structural changes in plant tissues induced by microgravity.
3. To determine microgravity's effect on plant processes, such as photosynthesis and water use.
4. To evaluate current facilities for plant growth aboard the Mir.

PHASE 1 MISSIONS

Mir 19, Mir 20, Mir 21/NASA 2, Mir 22/NASA 3

OPERATIONAL ACTIVITIES

The first two seed plantings of wheat occurred during Mir 19. Plant development was monitored by daily observations and photographs taken by the Mir 19 crew. Although the plants grew for almost the entire 90 days of the experiment, failure of four of the six fluorescent lamp sets resulted in low lighting. The low lighting levels and low moisture content in the root module resulted in poor growth of the plants. However, plant samples and equipment were transferred to and brought back to Kennedy Space Center by STS-74, where they were divided among U.S. and Russian investigators for further analysis. A new set of wheat seeds was planted about midway through the Mir21/NASA2 mission. Plant development was monitored by daily observations, photographs and video taken by the crew. The final harvest of the plants that were planted during Mir 21 (known as the seed-to-seed experiment) occurred during the docked phase of the STS-81 mission. The second crop planting, which was originally scheduled to occur during the Mir22/NASA3 mission, was not performed. Fixed samples from the seed-to-seed experiment were returned to Earth for analysis on the STS-81 flight. The Svet/Greenhouse hardware was dismantled and stowed on the Mir until its next usage during the NASA 5 mission.

RESULTS

The overall goal of this work has been to understand the nature of the disruption of reproductive events by microgravity. During this study, we have greatly advanced the understanding of this problem. Specifically, we have shown that properly designed instrumentation and experiment management can provide the environmental data necessary to document the stresses experienced by the experimental crop. These data can exclude many of the possible causes of differences observed between ground and space grown plants. Second, we have shown that we can measure real-time plant transpiration and gas exchange. These data, telemetered to Earth on a daily basis could allow experiment managers to monitor nondestructively (no harvests) the development of plants growing in microgravity on a daily basis. This is the first time that such data have ever been collected in space. Third, we have shown that significant differences exist between the water relations of wet porous substrates in microgravity and on Earth. These differences must be monitored and accounted for to produce healthy plants. The Greenhouse IIb experiments demonstrate that we now have both the knowledge and instrumentation to provide a good plant root environment. Without the models and instrumenta-tion developed in this experiment, this would not be possible.

Status of Data Received/Analyzed

Plant and substrate samples were shipped postflight to US and Russian Investigators. All samples were received in the PI Laboratories in excellent condition. Approximately 70% of the light (LM), SEM, and confocal laser scanning optical microscopic (CLSOM) analyses are complete. However, transmission electron microscopy (TEM) is only about 20% completed. It is anticipated that 90% of the TEM work will be completed by December 31, 1998. Data analysis to date shows that it is very important to conduct a gas exchange ground experiment to provide a comparison with the crop canopy uptake and the old crop root respiration rates that we have measured in space. An

experiment to measure these rates is now being prepared. Once these data have been collected, the gas exchange data will be ready to publish.

CONCLUSIONS

There were several potentially important results from the NASA 3 Greenhouse 2 experiment. For example, biomass production during the 123-d growth period far exceeded that of any other comparable experiment with plants in space, which suggests that plant growth is not adversely affected by microgravity. This phenomenon was again indicated in the second planting, which was harvested after 30 d and returned in the GN2 freezer. Upon final harvest and return to Earth, the most interesting observation was the lack of seeds in the many wheat heads that were produced; we experienced 100% floret sterility.

All the data received from the NASA 3 Greenhouse 2 experiment have been reviewed. The quality of most of the data collected on the hard drives is excellent. This is the first mission where appreciable downlinking support from MIPS was available. Some data files that can be used to calculate photosynthesis were collected. Two data files that will be used to determine respiration in darkness were also collected. Soil moisture data collected during the mission is of good quality and will provide an excellent basis for a paper about the problems encountered with porous substrates in microgravity.

PUBLICATIONS

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2. Salisbury, F. B., G. E. Bingham, W. F. Campbell, J. G. Carman, D. L. Bubenheim, B. Yendler and G. Jahns. 1995. Growing super-dwarf wheat in Svet on Mir. Life Support and Biosphere Science 2:31-39.
3. Bingham, G. E., S. B. Brown, F. B. Salisbury, W. F. Campbell, J. G. Carman, G. Jahns, D. Pletcher, D. B. Bubenheim, B. Yendler, V. Sytchev, M. A. Levinskikh, I. Podolsky, I. Ivanova, P. Kosgtov and S. Sapunovca. 1996. Environmental measurements observed during the greenhouse-2 experiment on the Mir orbital station. COSPAR 31:364.
4. Bingham, G., F. Salisbury, W. Campbell, J. Carman, B. Y. Yendler, V. S. Sytchev, Y. B. Berkovich, M. A. Levinskikh and I. Podolsky. 1996. The spacelab-Mir-1 "Greenhouse-2" experiment. Adv. Space Res. 18:225-232.
5. Salisbury, F. B., W. F. Campbell, J. Carman, G. Bingham, D. L. Bubenheim, B. Yendler, V. Sytchev, M. A. Levinskikh, I. Ivanova, L. Chernova and I. Podolsky. 1996. Plant growth during the greenhouse II experiment on the Mir orbital station. COSPAR 31:364.
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7. Salisbury, F. B., G. E. Bingham, W. F. Campbell, J. G. Carman, P. Hole, L. S. Gillespie, V. N. Sytchev, I. B. Podolsky, M. Levinskikh, D. L. Bubenheim and B. Yendler. 1996. Experiments with Super-Dwarf wheat in Space Station Mir. ASGSB Bulletin 10:34.
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Investigation Title: Incubator - Effects of Weightlessness on the Avian Visuo-Vestibular System: Immunohistochemical Analysis

Principal Investigator: Toru Shimizu, Ph.D., University of South Florida

INVESTIGATION OBJECTIVES

The purpose was to study the fundamental effects of gravity deprivation on the visuo-vestibular system of Japanese quail Coturnix coturnix japonica. In particular, the distributions of various neurochemicals during development were analyzed by using immunohistochemical techniques. Development of the visual brain was also studied by measuring the volumes of the structure called the optic tectum.

PHASE 1 MISSIONS

NASA 2

OPERATIONAL ACTIVITIES

Quail eggs were launched on a Space Shuttle (STS-76) and placed into the on-board Mir incubator. On specified days, eggs were removed from the incubator and shells were cracked in a plastic bag filled with a fixative solution of 4% paraformaldehyde. All bags were stored at ambient temperature until returned on a Space Shuttle (STS-79). The fixed eggs (embryos) were shipped to Ames Research Center for dissection by the Russian and U.S. PIs.

The PI analyzed the forebrains of 4 embryos from the flight group (2-E16s and 2-E14s) and compared them with samples from the ground-based control group. Tissues were frozen, cut in a cryostat, and processed with antibodies against 14 neurochemicals, which are known to exist in the avian and mammalian visuo-vestibular systems.

RESULTS

1) For the samples which had adequate fixation, the results showed relatively consistent staining patterns for several neurochemicals, such as a calcium-binding protein (CB) and an enzyme for acetylcholine, which are important markers for avian sensory development. Although the positive staining was already visible in the visuo-vestibular system of the E14s and clearly detected in the E16s of the flight group, the number of stained cells appeared to be fewer, and the staining was more faint, than stained cells in the control group. 2) The avian optic tectum is the major retinorecipient structure with well-developed laminations. These layers were clearly stained with Nissl staining in all groups. Portions of the optic tectum of some samples were either damaged or detached due to an incomplete fixation, and thus no accurate measurement was possible for these samples. With the collaboration of Dr. Dmitri Lytchakov (Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences), a few cases were observed with an abnormal development of the eyes and optic tectum under the microgravity condition. For instance, there were cases with retarded or asymmetrical development of the eyes and tectum.

CONCLUSIONS

These results indicate that microgravity significantly affects the embryogenesis of the avian brain in terms of morphology and chemistry. The number of subjects and tissue fixation methods need to be improved to confirm these observations.

PUBLICATIONS

1. Shimizu, T. (1997). Effects of weightlessness on the avian visuo-vestibular system: Immunohistochemical analysis. NASA Technical Memorandum, 66-67.
2. Bower, A. N. & Shimizu, T. (1998). Effects of weightlessness on the avian visuo-vestibular system: Immunohistochemical analysis. NASA 1st Annual Partners in Research and Education Conference.

Investigation Title: Incubator - Effects of Weightlessness on Vestibular Development of Quail

Principal Investigator: Bernd Fritzsch, Ph.D., Creighton University

Additional Investigators: Laura L. Bruce, Ph.D.

INVESTIGATION OBJECTIVES

1. To examine the central projection from gravistatic receptors (utricle and saccule) into the brainstem in quail raised in zero gravity from egg laying to approximately hatching (17 days) and to compare these results with those from synchronously incubate eggs and control groups.

2. To examine the peripheral termination of vestibular fibers at the gravistatic receptors within the sensory epithelia and to compare these results with those from control quail.

PHASE 1 MISSIONS

Flown on Mir, total of three flights, only one flight (Atlantis) resulted in some successfully incubated quail eggs.

OPERATIONAL ACTIVITIES

Not provided by PI.

RESULTS

In the absence of a fixation suitable for DiI tracing, we tried to analyze the ears using immunohistochemical techniques. Initial stains indicated in control quail that we would be able to label the nerve fibers using an antibody against β-acteylated tubulin. However, using this antibody we did not get any staining in the microgravity expose ears. Again, the insufficient fixation is likely to blame. As a last resort we embedded the ears in plastic for a thick section analysis of hair cell numbers and degree of maturation. Unfortunately even this rather simple issue could not be analyzed in the microgravity-exposed chicken due to inadequate fixation. Both the incubator and the fixation technique are currently being revised by NASA.

CONCLUSIONS

The data confirm previous findings that quail embryos can, under proper circumstances, develop until hatching in microgravity. There were no gross abnormalities in the few ears of the late embryos (we received 3 ears at E14.5 and 4 ears at E16.5). Due to inadequate numbers of samples returned and their fully insufficient fixation, no conclusions could be reached that warrant any publications.

PUBLICATIONS

1. Bruce, L.L. and Fritzsch, B. (1997) The Development of Vestibular Connections in Rat Embryos in Microgravity. J. Gravit. Physiol. Vol. 4: 59-62.

2. Fritzsch, B. (1998) Evolution of the Vestibulo-Ocular system. Otolaryngology - Head and Neck Surgery, 119: 182-196.

3. Fritzsch, B. (1998) Of mice and genes: Evolution of vertebrate brain development. Brain, Behav. Evol., 52: 207-217.

Investigation Title: Incubator - Expression of Contractile Proteins in Microgravity

Principal Investigator: Page A. Anderson, M.D., Duke University Medical Center

INVESTIGATION OBJECTIVES

The study of the effects of microgravity on troponin T and troponin I isoform expression in the quail.

PHASE 1 MISSIONS

SLM-1 Incubator II

OPERATIONAL ACTIVITIES

Dissected fixed hearts from embryonic quail of a range of developmental stages in ovo from control groups and flight groups. Harvested RNA from the hearts. Performed RT-PCR to assess ability to obtain cDNA from fixed tissue and to develop primers to examine relative expression of two troponin T isoforms expressed in the quail in ovo.

RESULTS

We successfully purified RNA from fixed embryonic cardiac tissue in amounts sufficient for us to perform RT-PCR experiments. We obtained two PCR products using primers based on rabbit cardiac troponin T (cTnT) cDNA. The sequences from an alternatively spliced region and that from a central highly conserved region were of the appropriate size. One of the two RT-PCR products was a quail sequence. We have subsequently used primers from highly conserved regions of cardiac troponin T cDNA. These primers were successfully used to obtain a quail cardiac troponin T cDNA sequence that lacks the 5' region. This quail cardiac troponin T sequence has not been previously described. Rare cDNA from the human heart suggested the potential for splicing in the central highly conserved region of the molecule. The cDNA, we obtained from the quail heart, supports the presence of such splicing.

CONCLUSIONS

Our modification of previously published methods for obtaining RNA from fixed tissues results in a markedly greater yield of transcript. RT-PCR of quail cardiac RNA has yielded a novel product. It lacks sequences from two central exons, similar to a rare human cardiac troponin T cDNA. The new splicing pattern may have functional consequences. If such cDNA are translated, the resultant proteins would lack regions thought to be important in the interaction of the proteins that regulate cardiac contractions. The protocols developed in this investigation will prove useful in studying gene regulation in microgravity. The stability of RNA in fixed tissues allows fixing of solid tissue and blood in microgravity and subsequent analysis many months and potentially years after the tissue was harvested. Thus, gene expression and its modification by microgravity can be examined over time in the adult and during development in the embryo and fetus. Our findings may be important in understanding protein-protein interaction that regulates cardiac contraction.

PUBLICATIONS

Not provide by PI.

Investigation Title: Incubator - Hypogravity's Effect on the Life Cycle of Japanese Quail

Principal Investigator: Patricia Y. Hester, Ph.D., Purdue University

Additional Investigators: Joseph I. Orban, Steven J. Piert, and Tamara Guryeva

INVESTIGATION OBJECTIVES

A series of studies were conducted to determine the effect of activities preceding space flight and during space flight on the use of calcium from the shell of developing quail.

PHASE 1 MISSIONS

NASA 2

OPERATIONAL ACTIVITIES

Quail eggs were subjected to preflight dynamics, centrifugation, vibration, or a combination of vibration & centrifugation prior to incubation.

Quail eggs (48) were launched on the Shuttle and incubated in a Slovakian incubator on Mir.

Development was stopped on 3, 7, 10, 14, & 16 d of incubation.

RESULTS

Preflight activities & dynamics had no effect on the quail embryo's survivability or their ability to utilize calcium from the shell.

Calcium utilization was impaired in embryos incubated in microgravity when compared to ground laboratory controls.

CONCLUSIONS

Calcium utilization was impaired in quail embryos incubated in microgravity. It was not clear if this impairment was due to factors other than microgravity.

PUBLICATIONS

Abstracts

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2. Orban, J. I., and P. Y. Hester, 1998. Calcium uptake by quail embryos incubated in space. Am. Soc. Gravitat. Space Biol. 12: 48.
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Journal Articles

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Investigation Title: Incubator - Skeletal Development in Long-Duration Space Flight

Principal Investigator: Stephen B. Doty, Ph.D., Hospital for Special Surgery

INVESTIGATIVE OBJECTIVES

This flight experiment was to study the bone formation and skeletal development during embryogenesis which was occurring in space. This would permit distinctions between purely genetically driven processes (e.g. Mesenchymal condensations, cartilage analage formation, etc.) and mechanically or environmentally driven events (e.g. Bone remodeling, cell replacement, etc.). The Specific Objectives were: (1) Use physical measurements of wing and leg size to determine gross limb and skeletal development. (2) Measure areas of mineralization by image analysis and x-ray microanalysis in limbs and flat bones during different stages of development. (3) Use electron microscopy to evaluate mineral deposition within collagen matrix and compare this to the distribution of alkaline phosphatase activity (an enzyme necessary for mineralization to occur). (4) Use immunocytochemistry to localize and compare the different distribution of collagen types in cartilage and bone. (5) Compare the development of flat bones with the long bone development since they are formed by different mechanisms and may be affected differently by space flight.

PHASE 1 MISSIONS

NASA 2

OPERATIONAL ACTIVITIES

Random bred quail eggs (Coturnix coturnix japonica) were obtained from University of Wisconsin (Dr. Wentworth) and shipped to KSC where they were weighed, numbered and candled. They were placed into the RSKE with an ATR-4 to monitor temperature. The RSKE was placed into the CRIM and monitored until turnover. Launch was on 3/22/96 (STS-76) to carry the eggs to Mir. Inflight fixations occurred on Mir, corresponding to embryonic days 0, 3, 7, 10, 14, and 16. Fixed eggs were returned on STS-79, and Russian and US PIs carried out tissue dissections on these embryos at ARC. There were appropriate Synchronous and Laboratory Controls available for dissection at the same time.

RESULTS

There was no measurable difference in leg length or wing length at any embryonic age, comparing Flight and Controls. Morphometric measurements of cartilage and bone content of the long bones does suggest a delay in conversion of cartilage to bone in flight animals at embryonic day 10, but this was not evident at later development times. In the mandible, x-ray microanalysis of Ca and P content of the bone matrix showed a reduced content of these bone minerals at day 10, but no differences at later embryonic age. Electron microscopy showed a reduced bone matrix and reduced mineralization in the earliest developing embryos (embryonic days 7 & 10) at the cartilage/bone interface of the tibias. But the fixation of the older embryos was too poor to permit any meaningful electron microscopy. Staining for matrix proteins has not been helpful because all samples indicate a normal distribution of matrix (e.g. Collagen type I, II, and X; proteoglycans and fibronectin). We have started staining for Proliferating Cell Nuclear Antigen, cyclin D, and ubiquitin as indicator of changes in cell cycle or cell proliferation in cartilage and bone. This latter procedure was not included in the original grant but during this study, it became apparent that any changes during embryogenesis will have to occur very early in development and therefore cell differentiation, proliferation and cellular apoptosis may be better areas of study.

CONCLUSIONS

We have found good evidence that very early bone formation and mineralization appear to be delayed during embryogenesis. This occurred in the flat bones (mandible) and long bones (tibia) so the effect due to space flight is not confined to endochondral bone formation (i.e. conversion of cartilage to bone). However, it becomes very difficult in studies of this type to determine whether the bone cartilage in these older embryos and visually it becomes impossible to determine whether any of these processes have been slowed. In the early embryos, the appearance or lack of appearance of mineral is easily determined. Also, the fixation of the older embryos was quite poor, probably due to the thickened epidermis and feathers which inhibit penetration of fixative into the deep tissues,

such as bone. This precluded any electron microscopy which could have provided more detailed analysis of the collagen matrix and its mineralization. In like manner, the immunostaining of matrix proteins by light microscopy is not sensitive enough to determine whether very slight changes in matrix have occurred, which is probably the case for this study. With our ongoing analysis of looking for proliferating cells (stained for proliferating cell nuclear antigen), cell apoptosis and cell cycling, we may find changes in cellular differentiation which will provide clues as to how and where cell activity was decreased during space flight.

PUBLICATIONS

Not provided by PI.

Investigation Title: Standard Interface Glovebox Hardware Verification

Principal Investigator(s): Paul D. Savage, NASA/Ames Research Center

Additional Investigators: N/A

INVESTIGATION OBJECTIVES

Perform functional verification procedures to ensure proper containment for investigations utilizing the Standard Interface Glovebox Operations.

The objectives of the experiments/investigations will be reported in their own individual reports. The MGBX project does not attempt to report on the science objectives associated with the different investigation conducted in the facility. However, the objective for the MGBX facility is to provide a common facility that represents one or two levels of containment, so that the investigation teams can focus on development of flight hardware to obtain the desired science and minimize the need to meet the containment issues associated with manned space flight.

PHASE 1 MISSIONS

NASA 2 - NASA 5

OPERATIONAL ACTIVITIES

The SIGB was successfully installed in its location in Priroda. Following the performance of the functional verification procedure, the SIGB was found to be fully operational.

RESULTS

The MGBX facility has operated flawlessly during the 290 hours of accumulated time through NASA 4. The facility is working as designed.

Status of Data Received/Analyzed

The NASA 4 investigation data has been reduced and supplied to the respective teams.

CONCLUSIONS

The Mir Glovebox (MGBX) for Microgravity Investigations is a facility located in the Priroda module of the Mir Space Station. The facility provides a work area for microgravity investigations that can be physically isolated from the manned environment. In addition, the facility can provide a negative pressure in the physically isolated work area with respect to the manned environment using an air filtration system that is closed (isolated from the manned environment) as long as no leaks occur in the physical isolation system. This air circulation system represents a second level of containment in that nothing can get from the work area into the manned environment without passing through two separate banks of filters. The filters are capable of capturing all solid particulate (>3 microns in diameter) and liquids.

PUBLICATIONS

N/A