

February 27, 1996

Progress Report - Year 01
and Request for Continued Funding (Years 02 and 03)

Principal Investigator: Gary W. Conrad
Title: Effects of Microgravity on Quail Eye Development ✓
Solicitation No.: 93-OLMSA-06
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Kansas State University Account No.: 5-30679 (Year 01 only)

Dates: Year 01: 05/01/95 - 04/30/96
[Year 02: 05/01/96 - 04/30/97]
[Year 03: 05/01/97 - 04/30/98]

Abstract:

During embryonic development, the most exposed tissue of the eye, the cornea, becomes differentially bulged outward because of constant intraocular pressure (IOP). The component cells of the cornea secrete a unique, paracrystalline extracellular matrix (the stroma) composed of orthogonal plies of collagen fibrils and proteoglycans. The cornea remains avascular, becomes transparent, and becomes more densely innervated than any other region on the surface of the body. Corneas from chicken embryos that flew on STS-47 contain many more cellular processes in the outermost region of the stroma (Bowman's Layer) than any corresponding region of control corneas. These processes appear to be cross-sections of cytoplasmic extensions of cells and are found in that region of Bowman's Layer immediately beneath the basal lamina of the corneal epithelium. Here, we propose to compare corneas of quail that flew in space on Mir-1 with those of ground controls to determine if the same unusual cellular processes are seen as in the space-flown chicken corneas. In the central regions of such space-flown corneas, the processes appear to be either portions of basal epithelial cells whose pseudopodial extensions have migrated down through their own basal lamina into the stroma, or corneal nerves that have innervated the corneal stroma in an unusual manner. Eyeballs of embryos fixed on Mir-1, control embryos fixed at KSC and clinostated embryos fixed at KSU, will provide corneas for this study. Electron microscopy will be used to assess the distribution of the cellular processes in Bowman's Layer in the central region of each cornea. Attempts also will be made to determine the relative glycosaminoglycan distributions in the corneal stromas by indirect immunofluorescence and to record whole-mount staining patterns of the corneal nerves.

Progress Report for Year 01

What has been accomplished thus far?

On two separate flights to the Mir space station on which quail eggs were incubated in a Flight incubator (Mir-18/STS-71 and Mir-19/STS-74), virtually all of the embryos died after 4-5 days of incubation. Because the same pattern of early mortality was observed when quail embryos from the same batch of eggs were incubated in an identical Flight-model incubator on Earth, and yet because embryos from the same batch of eggs developed normally when incubated in a standard laboratory-model incubator, it appears that the design of the Flight-model incubator must be changed before the Flight experiment can really be performed.

Despite this disappointment with the equipment, some information has been obtained:

1. Quail embryos, particularly from the breed developed by the Russian space agency, appear to be quite hardy, able to withstand the stresses of transport to the launch pad, the launch itself, and incubation on Mir. In addition, it appears that the cosmonauts did an excellent job of transferring the quail eggs from the incubator to the ziplock bags of fixative solution and breaking the shells gently to allow fixative penetration, all without release of fixative solution into the cabin. Return of the fixed eggs from Mir to the U.S.-Shuttle, and from the U.S. landing site to NASA-Ames Research Center, all occurred as planned, as did matching fixations performed on Earth on the two control groups of embryos - those incubated in the Flight-model incubator and in the laboratory-model incubator.

2. In our own work, we have determined that the corneas of embryos stored for at least 4 wks at room temperature in the same type of 4% paraformaldehyde solution as used on the Flight and control embryos, when followed by further fixation in glutaraldehyde and osmium tetroxide, show excellent preservation of ultrastructure in the extracellular matrix and intracellularly. Thus, we are confident that we will be able to compare the ultrastructure of corneas from Flight, Synchronous Ground Controls (Flight-model incubator), and Ground (Laboratory-model incubator) Controls.

3. In addition to recording eyeball weight, volume, and diameter, and cornea diameter, transparency, and ultrastructure, we appear to be close to having an operational method that will allow us to perform whole-mount immunostaining of the nerve patterns of corneas, even after long-term storage at room temp. in paraformaldehyde.

What questions have been answered?

1. Flight-model incubator hardware is not designed optimally.
2. Quail embryos are excellent models for this experiment.
3. Russian and U.S. flight and ground personnel can perform all operations as planned, both in flight and on the ground.
4. Fixation protocol produces eye tissues fixed acceptably for our analyses.

5. An alternative fixative solution (85% ethanol/15% glycerol) was tested on Flight and control embryos and shown to give tissue fixation that was very poor, in comparison with fixation by 4% paraformaldehyde.

What new questions have arisen?

1. Could an already-Flight-tested type of avian egg incubator be substituted for the current Flight-model incubator on the Mir station? For example, such an incubator is available from SHOT, Inc. (Space Hardware Optimization Technology, Inc./P.O. Box 351, Floyd Knobs, IN 47119/Vice-President: John C. Vellinger/Tel: (812)-923-9591/FAX (812)-923-9598). This model incubator was used successfully for incubating chicken eggs on STS-29, and chicken eggs are much larger, and encased in more brittle shells than those of quail. Retro-fitting this incubator for incubating quail eggs would not be difficult mechanically. Whether it could fit into the space currently occupied by the quail egg incubator on Mir, and operate on the electrical power source available there, would have to be discussed by Russian and U.S. engineers.

2. A method is needed for providing a continuous, reliable recording and print-out of incubator temperature and relative humidity during the entire incubation period in the Flight incubator, and in both types of control incubators on Earth as well. A comparable system also should be devised for monitoring temperature and relative humidity during pre-launch storage and transport, as well as during egg storage during launch and transport up to Mir. As I indicated in a FAX to Dr. Laurie Dubrovin (02/13/96), an inexpensive device for measuring these parameters is now available from PGC Scientifics (\$29.95).

3. We would like to devise a technique for monitoring the intraocular pressure of embryonic quail eyes during incubation in microgravity, in comparison with incubation on Earth. A device for measuring the intraocular pressure within the globes of even very tiny quail or chick embryos is now available and I purchased one to measure IOP throughout quail and chicken embryogenesis

How does this year's progress affect future work on this task?

We will not be able to perform this experiment unless a Flight-model incubator, suitable for use on Mir, can be designed quickly and be demonstrated to provide as optimal an environment for incubating quail eggs as provided by currently available laboratory-model incubators. Such comparisons should be done first on Earth, repeatedly, to demonstrate the long-term reliability of the new Flight-model hardware. For comparison, it is worth noting that many developmental biologists have simple, laboratory-model avian egg incubators that have been running continuously in their labs for periods exceeding 25 yrs, serviced only by a monthly drop of oil! Such incubators can be used for incubating chick and quail eggs simultaneously.

Until new Flight-model incubator hardware is assembled and tested successfully on Earth, justifying further attempted incubations on Mir, we will use the time and resources available to

try to increase the amount of information that we will be able to obtain from eyes of quail embryos stored in paraformaldehyde fixative solutions at room temperature. For example, these efforts have already provided us with an exciting new method for visualizing the whole-mount nerve patterns of entire quail corneas. Corneas are the most highly innervated structures on the surfaces of vertebrate bodies, so being able to visualize their nerve patterns easily in heavily-fixed tissue is a major accomplishment.

Plans for Year 02

A. As proposed in the original application, Year 02 will be spent analyzing the eyes and corneas from the Flight and control groups from the Mir-1 project and preparing the report for NASA. Physical measurements will be made of eyeball weight (volume), and eyeball and cornea diameter, as well as corneal transparency, scleral ossicle number and shape, and corneal ultrastructure.

B. While awaiting a successful incubation of fertilized quail eggs on Mir-1, we will continue further efforts to develop a method for whole-mount staining of nerves in the corneas of eyes that have been stored in the paraformaldehyde fixative for long periods of time. The purpose of this work is to devise a technique that will allow visualization of the entire pattern of nerves that innervate the cornea. Such nerves have been shown previously to occupy very specific positions in normal corneas. It will be of great interest to determine if embryonic development in microgravity will yield an eyeball in which overall tissue morphogenesis occurs normally, including formation of normal patterns of sensory innervation of the cornea .

C. We will subject fertilized quail eggs to clinostat rotation around both vertical and horizontal axes during incubation in our laboratory on Earth. This will allow us to compile data on the response of the developing quail eye to simulated microgravity conditions and give a basis for comparison with embryonic development during genuine microgravity conditions on Mir-1.

Significance of this research [FY95 Earth Benefits]

The eye is dynamic in at least two respects. First, the eyeball itself develops during embryogenesis much like a balloon or an automobile tire inner tube in that it inflates/enlarges under pressure from within itself. Second, again like a balloon or a tire, it maintains its structure during adulthood by continuous maintenance of pressure within itself; it does not just inflate itself once and simply become rigid. The fact that the cornea of the eye bulges outward more acutely than the curvature of the rest of the eyeball offers yet another analogy: to that of a defective automobile tire or inner tube in which a weak spot in one wall leads to the formation of an acute bulge. In fact, the cornea develops its differentially acute curvature during embryogenesis specifically because of intraocular pressure and maintains normal structure only so long as that internal pressure is maintained.

When we lean over and put our head between our legs, a lot of fluid shifts from the middle of our body to our head, including the region around the eyes, essentially squeezing the eyeballs from outside, and raising intraocular pressure to an average of 30 % above resting levels within 20 sec, rising to levels that significantly overlap those associated with clinical symptoms of glaucoma. When pilots go into microgravity for periods of ~ 20 sec during parabolic flight, a similar shift of fluid occurs from the lower body toward the head and results in an increase in intraocular pressure averaging 58 %; this increase occurs each time microgravity is encountered during a linked series of parabolic maneuvers. No data have been published yet about the degree to which the intraocular pressure of astronaut eyes increases in response to entering an environment of microgravity, e.g., on the U.S. Shuttle or on Mir, nor whether the initial, expected increase in that pressure is maintained for long periods of time. If such high intraocular pressures are maintained in astronaut eyes, the chances of developing glaucoma during long missions in space might be substantial. In addition, because the normal embryonic development of the eyeball and cornea depend on the formation of certain levels of intraocular pressures, it will be important to determine whether these structures will be able to develop normally at all in sustained microgravity. If quail are to be used as a renewable food source for long space missions (e.g., Mars), it will be important to determine whether eye and cornea development will be compromised if quail embryos undergo all development in microgravity, as on the Mir station. Our experiments will determine the extent to which the major steps in the embryogenesis of the eyeball in general, and the cornea in particular, can occur normally in microgravity.

On Earth, it is important to learn more about the basic mechanisms of eye development. To be able to study how the developing eye and cornea respond to a new type of physical environment offers a rich array of opportunities for learning more about the eye, those of humans, as well as those of quail.

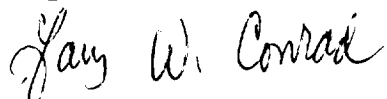
Publications [FY95 Bibliography]:

None

Budget proposed for Year 02:

Same as originally proposed for Yr 02 (see proposal p. 26) -
copy enclosed.

Respectfully Submitted,



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Professor