

NAG2-1007, NRA 93-OLMSA-06
Avian Blood-Vessel Formation in Space
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Final Report

I. Introduction

A: Hypothesis

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The area vasculosa, the region of blood island formation and the forerunner of the chorioallantoic membrane, was reportedly deformed in some quail embryos that had developed during space flight. Also, other studies have shown that specific cellular events which may be key to neovascularization, such as directed cell migration, homing, intracellular signal transduction, enzymatic activities and the metabolism of extracellular matrix proteins seem to be affected by microgravity. Based on these studies, we hypothesized that the developmental anomalies observed in the past might be related to or caused by delayed or improper vascular development.

B: Objectives of Experiment

The Objective of our research is to test the hypothesis that exposure to microgravity during space flight cause delayed or improper vascular development during embryogenesis. The effects of microgravity on the time course and extent of avian blood-vessel formation are assessed using two models, one for angiogenesis and one for vasculogenesis. The methodological approach is dictated by the constraints of the tissue preservation method used in space. Thus, both in the chorioallantoic membrane (CAM) and in the adrenal, we will evaluate microscopically the vascular architecture and immunostain endothelial cells with specific antibodies (anti- vWF and QH1). The extent of ECM protein deposition will be assessed by immunohistochemistry and correlated with the degree of vascularization, using computer-based image analysis. Also, the cellular source for ECM proteins will be assessed by in situ hybridization.

If indeed we find significant differences in the pattern of neovascularization between ground and space animals, we hypothesize, that such differences might be related to altered expression of angiogenic/vasculogenic growth factors (e.g. FGF or VEGF) and/or their receptors. If the first hypothesis is verified, we will use the available tissues to probe, by immunohistochemical and molecular biological means, for the expression of aFGF, bFGF, VEGF and their respective receptors.

II: Data Analysis and Observations

In addition to the sample, that we had received at the time of the 1year day report (September 1997), we have obtained some additional 100 samples (CAMS and adrenals) from two additional ground/synchronous controls, which were conducted to assess the effects of incubator temperature and rotation on embryonic development. In the last year of this grant we analyzed all the apparently intact CAMs from the original samples from MIR 18, 19, and 21, as well evaluated, mainly by light microscopy, As before, our initial studies of the new samples included CAMs each from the different sets Synch 2 and Synch 3 and four time points (E 7, E 10, E 14, E 16).

As in the past, we are using those established methods, to obtain quantifiable data: a) en face bright field / fluorescence microscopy to delineate at low power magnification vascular morphology and b) immunohisto-chemistry for highlighting the structure of the blood vessels. Given that the number of processed specimen is still too small, and that not all control studies have been completed, we cannot, as yet, make any conclusive statements. However, we can report the following, preliminary observations regarding the vascular development in the CAM and adrenals:

Mir 18: For both the laboratory and the synchronous controls, we obtained rather satisfactory structural preservation and maintenance of antigenicity (as assessed by staining with endothelial-specific antibodies) of the *formaldehyde-fixed* samples. The state of preservation of the *ethanol-fixed* samples (all of day 16 laboratory controls) allowed for light-microscopic evaluation of the vasculature, but precluded immunohistochemical analysis.

Laboratory controls: Our data suggest normal development of the vascularization in both CAM and adrenal, which is comparable, both in terms of time-course, and arborization, to that observed in the controls maintained in our laboratory.

Synchronous controls: The vasculature in the CAMs from embryos that seemed normal appears affected by the simulated launch conditions. In contrast to the laboratory controls, the development of smaller vessels ($< 35 \mu\text{m}$ in diameter) is impaired after embryonic day 10. No blatant adverse effects are observed in the adrenals.

Flight species: No useful samples were retrieved.

Preliminary Conclusions: The light-microscopic (morphometric and histological) analysis of the vascularization in the CAM and adrenals in the laboratory and synchronous controls from MIR 18 is complete. In view of the lack of useful flight samples, we abandoned our plans to analyze in detail the expression of ECM proteins in the formaldehyde-fixed samples using immunohistochemical approaches.

MIR 19: The only samples that could be analyzed from this mission were the laboratory controls and 2 CAMS from E7 flight animals. The vascular development in the CAMs and adrenals of all the controls seemed normal. The vasculature in the CAMs of the 7 day flight animals appeared rather normal, with some minor distortions observed in terms of the angles of arborization of the smaller vessels at the branching points. However, because of the lack of useful samples that could be retrieved, the sample size $n=2$ is by far too small to make any definitive conclusions. Of concern in this particular experiment is the lack of useful samples from the synchronous controls.

MIR 21: This is by far the most successful and encouraging mission. As reported previously, there is a substantial number of useful samples were returned from the flight. In addition the different laboratory and controls will be helpful for sorting out the contributions, if any, of temperature, rotation and simulated launch conditions. Of concern is the inconsistency in state of preservation in many of the original samples retrieved (Flight, LAB-1, SYNCH-1), but not of the last set of controls.

Flight samples: As reported previously, the development of the vasculature in the CAMs in the flight samples, as inferred from vessel density and vessel size, seems retarded, as compared to the laboratory controls (LAB-1). This includes a both diminished numbers of small vessels for the day 14 and 16 embryos as well as a delay in the time point of the peak of angiogenic activity around day 10. However, given that only 3 time points (days 7, 10, 14) are available for this critical period, no exact time-course can be made, without access to further flight samples. No useful RNA could be retrieved from this set (or any other of the previous) sets of the flight samples.

Synchronous controls: As stated in the 1 year report, there was no profound effect of the "flight-simulating conditions" on the development of the CAM in the synchronous controls (SYNCH-1). In this control, however, the eggs had not been exposed to some of the critical components of a simulated launch, but were incubate at an elevated temperature, comparable to that on board MIR. Our data indicate that the development of the vascular architecture (in terms of vessel number and complexity) in both the CAMs and the adrenals appears to be somewhat accelerated by comparison to that of the laboratory controls.

Analysis of samples from SYNCH-2 and SYNCH-3, in which the eggs were exposed to the mechanical forces (g-load, vibrations, etc.) of a simulated launch, indicates that vascular development in the CAM appears to be impaired, resembling the pattern observed for the synchronous controls of MIR 18. The retardation in the vascular development observed in the samples from SYNCH-2, is probably due to the lack of egg rotation in SYNCH-2 vs. 3 rotations/day in SYNCH-3. The elevated temperature in the LAB-3-HI controls is indeed reflected in an accelerated vascular development. A manuscript, describing the effects of the various physical forces on vascular development in the CAM is in preparation for submission to a peer-reviewed journal.

III. Conclusions and Future Studies

At this time point we have evaluated the CAMs from all the samples from MIR 21 and the diverse controls, according to our original plans. Our analysis indicates a retardation in concomitant with possible impairment of the formation of the vasculature in the CAM of flight samples. Given the inadequate levels of fixation for most flight samples, there remain, however, serious concerns about the quality of the sample, and therefore, of the validity of the data. In addition, the number of samples is insufficient for drawing definitive conclusions. The different laboratory and synchronous controls suggest complex effects of the various parameters on vascular development. Elevated temperature accelerates, lack of rotation retards vascular development in the CAM, but does otherwise not interfere with angiogenesis. By contrast, some of the mechanical forces to which the eggs are exposed during the simulated launch seem to impede the regular development of blood vessels at the time of the "angiogenic burst" and hence may be related to the premature deaths of many embryos, observed on MIR 21 and some previous missions.

Given the large number of control samples, most of the original resources allocated to this grant were devoted to analyzing the primary goal of his study, namely to evaluate the effects of space flight on blood vessel formation in the chorioallantoic membrane. The detailed assessment of the vascularization in the adrenals will depend on availability of further funds.

Students involved in this study: 3 undergraduate students

Publications resulting from this grant:

a) *peer-reviewed:*

Henry, M.K., Unsworth, B.R., Sychev, V., Gureyeva, T.S., Dadasheva, O.A., Piert, S.J., Lagel, K.E., Dubrovin, L.C., Jahns, G.C., Boda, K., Sabo, Samet, M.M., and Lelkes, P.I. (1998) Launch conditions might affect the formation of blood vessels in the quail chorioallantoic membrane, *Folia Veterinaria*, 42: S-25-S31.

b) *abstract of presentation:*

Henry, M.K., Unsworth, B.R., Sychev, V., Gurieva, T.S., Dadasheva, O., Piert, S.J., Lagel, K.E., Dubrovin, L., Jahns, G.C., Samet, M.M., and Lelkes, P.I. (1995) Launch conditions might affect the formation of blood vessels in the quail chorioallantoic membrane, *Aerospace Medical Association, 67th Annual Scientific Meeting, Atlanta, GA, May 5-9, 1996.*