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## Final Report

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### SPACEFLIGHT AND DEVELOPMENT OF IMMUNE RESPONSES

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## INTRODUCTION

Evidence from both human and rodent studies has indicated that alterations in immunological parameters occur after space flight [1]. The number of flight experiments has been small, and the full breadth of immunological alterations occurring after space flight remains to be established. Among the major effects on immune responses after space flight that have been reported are: alterations in lymphocyte blastogenesis and natural killer cell activity [1-3], alterations in production of cytokines [4,5], changes in leukocyte sub-population distribution [6], and decreases in the ability of bone marrow cells to respond to colony stimulating factors [6]. Changes have been reported in immunological parameters of both humans and rodents [1]. The significance of these alterations in relation to resistance to infection remains to be established.

The objective of the studies contained in this project was to determine the effects of space flight on immune responses of pregnant rats and their offspring. The hypothesis was that space flight and the attendant period of microgravity will result in alteration of immunological parameters of both the pregnant rats as well as their offspring carried *in utero* during the flight. The parameters tested included: production of cytokines, composition of leukocyte sub-populations, response of bone marrow/liver cells to granulocyte/monocyte colony stimulating factor, and leukocyte blastogenesis. Changes in immune responses that could yield alterations in resistance to infection were determined. This yielded useful information for planning studies that could contribute to crew health. Additional information that could eventually prove useful to determine the potential for establishment of a permanent colony in space was obtained.

## METHODS

The methods used in the study involved the determination of leukocyte sub-populations and the production of cytokines. Tissue samples were obtained from both the parental dams and the fetuses/pups. The tissue were obtained from the flight animals after flight, as well as from ground control animals. For the leukocyte sub-population analysis, whole blood was taken using heparin as the anticoagulant or spleen cells were harvested. Samples of whole blood or bone marrow cells were stained with monoclonal antibodies directed against cell surface antigens. Cells will then analyzed for fluorescence using a Facstar flow cytometer [7].

The spleen and thymus tissues were treated with either phytohemagglutinin-P/concanavalin-A or polyribosinic-polyribocytidyllic acid. After the appropriate incubation period at 37°C, the culture supernatant fluids were harvested and stored at -70°C [8]. The supernatant fluids were assayed by ELISA assays for interferon-alpha and -gamma production as well as interleukin production [8].

The ability of bone marrow cells (from dams) or liver cells (from fetuses/pups) to respond to exogenous granulocyte/monocyte colony stimulating factor was determined by placing the cells in methylcellulose with medium and waiting one week for the development of colonies of 50 cells or more.

## RESULTS AND DISCUSSION

Pregnant rats were flown on the Space Shuttle, and pregnant control rats were maintained in the animal enclosure module (AEM) on the ground. Additional control rats were maintained in standard vivarium housing. Experiments were carried out to determine the effects of flight on immunological parameters of dams, fetuses and pups. The ability of bone marrow cells of the dams to form colonies in response to granulocyte-macrophage colony stimulating factor was inhibited after space flight, but the colony forming cell response of fetus and pup liver cells was not inhibited after flight. Proliferation of spleen cells in response to mitogens was inhibited in flown adult animals compared to AEM controls but was not inhibited compared to AEM controls in cells obtained from fetuses and pups. Previous spaceflight studies indicated alterations in leukocyte subset distribution in adult rats. Preliminary analysis of the results of this study suggest that alterations in leukocyte subset distribution similarly occur in fetuses and pups. Additional analysis of the data is continuing. Cytokine production of dams was reduced after flight, as expected. Cytokines production of pups showed a trend toward reduction after flight, but differences were not statistically significant. The results of this study indicate that some spaceflight-induced alterations in immune responses that occur in adults also occur in fetuses and pups, but others that are induced in adults are not induced in fetuses and pups.

This study has been designed to determine the effects of spaceflight on development of immune responses in offspring of flown pregnant rats. It should provide new information regarding the normal development of the immune response. This information could prove useful in enhancing the understanding of the development of the immune response in humans. Such understanding could provide new information that may be potentially applicable to understanding the mechanism of and treatment of human childhood immunological disorders.

**PUBLICATIONS**

1. Sonnenfeld, G., Miller, E.S., Jr., Mattei, M., Morton, D., Bailliard, F., Fowler, N.A., Swiggett, J.P., Hakenwirth, A.M., Bates, R., and Morris, V. Spaceflight and development of immune responses. *American Society for Gravitational and Space Biology Bulletin*, 9:97, 1995.

This was presented at the annual meeting of the American Society for Gravitational and Space Biology, October, 1995

A manuscript including these results is being prepared for publications. Copies will be forwarded when it is ready.

**REFERENCES**

1. Barone, RP, Caren, LD. Aviat Space Environ Med, 55:1063, 1984.
2. Konstantinova, IV, et al. Vestnik Akad Med Nauk CCCP, No. 8:51, 1985.
3. Taylor, GR, Neal, LS, Dardano, JR. Aviat Space Environ Med, 57:213, 1986.
4. Talas, M, et al. Acta Microbiologica Hungarica, 30:53, 1983.
5. Gould, CL, Lyte, M, Williams, JA, Mandel, AD, Sonnenfeld, G. Aviat Space Environ Med, 58:983, 1987.
6. Sonnenfeld, G, Mandel, AD, Konstantinova, IV, Taylor, GR, Berry, WD, Wellhausen, SR, Lesnyak, AT, Fuchs, BB. Aviat Space Environ Med, 61:648, 1990.
7. Jackson, AL, Warner, NL. In: Rose, NK, Friedman, H, Fahey, JL, eds. Manual of Clin Lab Immunol, 3rd ed, Wash DC, Amer Soc for Microbiol, p. 226, 1986.
8. Kirchner, H, Kleincke, CH, Digel, W. J. Immunol. Methods, 38:213, 1982.

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**UTRICULAR AND SACCULAR PROJECTIONS OF FETAL RATS RAISED IN NORMAL GRAVITY AND MICROGRAVITY.** B. Fritzsch and L.L. Bruce, Dept. of Biomedical Sciences, Creighton University, Omaha, NE.

Deficits in behavioral orientation have been observed in chicks and rats reared in microgravity, suggesting that microgravity may induce the growth of anomalous neuronal connections between the vestibular sensory epithelium and the motor system. We are currently analyzing the projections from the gravistatic sensory epithelia of the inner ear, the utricle and saccule, to the central nervous system in rats flown from gestation day 9 to 20 on the STS-66 flight. Thus far we have analyzed 4 synchronous control- and 4 microgravity ( $\mu\text{g}$ ) exposed fetal rats. Small amounts of the lipophilic dye, Dil, were inserted into the utricle (left side) and saccule (right side). Analysis of the projection patterns yielded 2 interesting results. First, in the  $\mu\text{g}$ -exposed fetuses, the facial sensory neurons had exuberant branches to the utricle that were virtually absent in the controls. Examination of controls revealed that one fetus had 3 labeled facial neurons and the other 3 had none. However, between 7 and 15 neurons were labeled in the facial sensory ganglion of each of the  $\mu\text{g}$ -exposed fetuses. Second, the utricular and saccular axons of controls had elaborate branching patterns throughout all vestibular nuclei, characterized by axons with multiple branches, short side branches and synapse-like swellings. In contrast, axons in 3 of 4  $\mu\text{g}$ -exposed fetuses were largely unbranched, generally ending in growth cones. Projections to rostral targets (e.g., superior vestibular nucleus and cerebellum) covered smaller areas than those of the controls. The projections of the fourth  $\mu\text{g}$ -exposed fetus could not be distinguished from controls.

This preliminary data suggests that gravity may play an important role in establishing functional connections in the vestibular system; its absence may cause a delay in the vestibular development. However, this data may also reflect random variation and further analysis is necessary before reaching any conclusions. (Supported by NASA: NCC 2-861)

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**EFFECTS OF SPACEFLIGHT ON THE ATTACHMENT OF TENDONS TO BONE.** R.B. Johnson<sup>1</sup>, A.K. Tsao<sup>2</sup>, K.R. St. John<sup>2</sup>, R.A. Belcher<sup>2</sup>, M.A. Tucci<sup>2</sup>, D. Parsell<sup>1</sup>, N. Mushell<sup>3</sup>, L.D. Zardiackas<sup>3</sup>, and H.A. Benguzzzi<sup>4</sup>. <sup>1</sup>Schools of Dentistry, <sup>2</sup>Medicine, and <sup>3</sup>Health Related Professions, University of Mississippi Medical Center, Jackson, MS, and <sup>4</sup>NASA-Ames Research Center, Moffett Field, CA.

The objective of this study was to study the structure of the tendon-bone junction in the hindlimbs of pregnant Sprague-Dawley rats flown in the NIH.R1 mission. Changes in the structure of these attachments during spaceflight could predispose the animal to injury following return to gravity. The tendon insertions on the femur, tibia and fibula were studied by light and scanning electron microscopy. Tendon and Sharpey fiber diameters, Sharpey fiber density, cortical porosity, trabecular density and proportional resorption areas were measured using histomorphometric techniques. Changes in mineralization were determined by bone ash and by X-ray diffraction of pulverized bones. Mean parameters were calculated for flight and flight simulation and vivarium controls and were compared by factorial analysis of variance and a post-hoc Tukey's test. Differences in means were considered significant when  $p < 0.05$ . There were significant increases in cortical porosity and periosteal resorption throughout the bones of the flight animals; resorption at the tendon insertion sites significantly reduced Sharpey fiber density. Significant endosteal resorption and decreased trabecular volume was also evident. The patellar tendon of flight animals was reduced in circumference and the patellar fat pad increased in size. The data suggests that spaceflight results in decreased diameters of tendons and weakened tendon-bone attachments, which could make them more prone to displacement following return to Earth. (Supported by NASA: NCC 2-863).

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**NIH.R1: THE EFFECT OF MICROGRAVITY ON EMBRYONIC ELECTROLYTES AND SKELETON.** L.V. Serova<sup>1</sup>, A.M. Nosovsky<sup>1</sup>, Yu. V. Natochin<sup>2</sup>, E.I. Shakhmatova<sup>2</sup>, S.V. Savelyev<sup>3</sup>, N.V. Besov<sup>3</sup>, and T. Fast<sup>4</sup>. <sup>1</sup>Inst. of Biomedical Problems, Moscow, Russia, <sup>2</sup>Inst. of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia, <sup>3</sup>Inst. of Human Morphology, Moscow, Russia, and <sup>4</sup>Lockheed Engineering & Sciences Co., Moffett Field, CA

Fetuses and placentas of female rats exposed to microgravity during gestation days 9 through 20 were dissected and used to investigate water and electrolytes, skeletal development and developmental abnormalities. Identical biosamples were obtained from synchronous and vivarium controls. No significant changes in the content of water, sodium, potassium, calcium, magnesium, iron or copper, which could be unambiguously associated with the effect of microgravity, were detected. Changes in the parameters seen in the flight rats were also observed in the synchronous controls. No significant differences in the ossified areas of the axial skeleton, fore- and hindlimbs, or the skull of the flight and synchronous control rats were found. Vivarium controls were lagging behind the flight and synchronous control rats in terms of these parameters. The paper will also include data concerning developmental abnormalities in the flight and control rats which are currently being processed.

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**SPACEFLIGHT AND DEVELOPMENT OF IMMUNE RESPONSES.** G. Sonnenfeld<sup>1</sup>, E.S. Miller, Jr.<sup>2</sup>, M. Mattei<sup>1</sup>, D. Morton<sup>1</sup>, F. Bailliard<sup>1</sup>, N.A. Fowler<sup>1</sup>, J.P. Swiggett<sup>1</sup>, A.M. Hakeneverth<sup>1</sup>, R. Bates<sup>2</sup>, and V. Morris<sup>2</sup>. <sup>1</sup>Dept. of General Surgery, Carolinas Med. Center, Charlotte, NC., & <sup>2</sup>Medical Immunotherapy Lab., Harrington Cancer Ctr., Amarillo, TX.

The NIH.R1 Space Shuttle experiment was designed to study the effects of spaceflight on rodent development. In this experiment, pregnant rats were flown on the Space Shuttle, and pregnant control rats were maintained in animal enclosure modules (AEM) in a ground-based environmental chamber under conditions approximating those occurring in flight. Additional pregnant control rats were maintained in standard vivarium housing. Experiments were carried out to determine the effects of the flight on immunological parameters of dams, fetuses, and pups. The response of bone marrow cells to colony stimulating factor-macrophage was inhibited after spaceflight in dams, but the response of fetus and pup liver cells was not inhibited by spaceflight. Proliferation of spleen cells in response to mitogens was inhibited in flown adult animals compared to AEM controls, but was not inhibited compared to AEM controls in cells obtained from fetuses and pups. Previous spaceflight studies reported alterations in leukocyte subset distribution profiles in adult rats. Preliminary analysis of the results of this study suggest that alterations in leukocyte subset distribution similarly occur in fetuses and pups. Leukocyte subset distribution will require further evaluation for confirmation because of the low number of cells that were available for analysis. Cytokine production profiles and immunoglobulin levels are currently undergoing analysis. The results of this study suggest that certain spaceflight-induced alterations in immune responses that occur in adults also occur in fetuses and pups, but others that are induced in adults are not induced in fetuses and pups. Funded by NASA grant NCC2-859.