AMPHIBIAN FERTILIZATION AND DEVELOPMENT IN MICROGRAVITY

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Introduction

The frog egg is a small 1-2 mm spherical cell surrounded by several gelatinous layers which swell when they contact water. The egg is divided into hemispheres, one of which is lightly pigmented and contains a high concentration of yolk platelets; the other hemisphere is darkly pigmented and is filled with cytoplasm of a lighter density. When the female frog sheds her eggs into the aquatic medium, the eggs are randomly oriented with respect to the gravity vector. Once fertilized, the eggs rotate such that the heavy lightly pigmented hemisphere is down and the opposite hemisphere is up.

Is the geotropic response exhibited by fertilized frog eggs required for normal amphibian development? Over the past century, many developmental biologists attempted to answer this question using a variety of approaches, including forcibly inverting the eggs between glass slides, tumbling them in streams of water, rotating them on clinostats and centrifuges, and using a varity of immobilizing agents like agar and gelatin to hold them in place (1-9). These experiments indicated that gravity and centrifugal force can definitely affect amphibian development. For example, eggs immobilized and held in a position 90° inclined from the gravity vector will form the dorsal structures of the body axis from the uppermost side of the egg (2). This topography is seen even if the point on the egg where the sperm penetrated (Sperm Entry Point, SEP), the prospective ventral side of the egg, is held uppermost. Thus gravity can be used to ex-

perimentally override the normal mechanisms that specify the position of the dorsal structures relative to the SEP. However, in spite of a plethora of ground-based research, it is not known if gravity has a role in the development of a normal egg, i.e., an egg that is free to rotate inside its fluid filled membrane and gelatinous coating. The SL-J Frog Embryology Experiment (FEE) will attempt to conclusively answer the question, "Is gravity required for normal amphibian development?"

Experimental Design

During the year before launch, female frogs will be tested every 3 months for the quantity and quality of eggs produced. Two weeks or more prior to launch, male and female frogs will be transported to the John F. Kennedy Space Center (KSC). During the few weeks before launch, groups will be periodically tested for egg quality to assure that the frogs have adapted to the KSC laboratory environment.

About 27 hours before launch, four females will be placed in a damp foam-lined box, called the Adult Frog Box (AFB), through which 100 cc/min of air will be circulated. The AFB will be lowered into the Spacelab and loaded into the Frog Environmental Unit (FEU) (Figure 1) during the final pre-launch preparations. A sperm suspension, for use in flight to fertilize the eggs, will also be prepared and loaded during the pre-launch period. The sperm suspension, together with a kit of syringes containing Human Chorionic Gonadotropin (HCG), will be stored in a refrigerator aboard the shuttle until needed in flight.

On the first day of flight, the AFB will be transferred from the FEU to the General Purpose Work Station (GPWS), which is a type of glove box specially designed to allow the crew to use chemicals and biological materials during the flight without contaminating the shuttle/Spacelab environment. Inside the GPWS the four adult frogs will be injected with the HCG hormone and returned to the FEU. Approximately 16 hours after injection, ovulation should have taken place and 15 to 20 eggs from each frog will be placed on each of two egg baskets and covered with sperm for 10 minutes. The egg baskets are inserted into acrylic egg chambers and 50 ml of "pond water" (20% strength Modified Ringers solution) is added. One of the chambers from each frog will be placed on a centrifuge within the FEU and rotated to simulate normal terrestrial gravity (1 g). The remaining chambers are incubated under microgravity conditions within the FEU. Forty minutes after fertilization, the four chambers exposed to 1 g will be removed and observed within the GPWS using a dissecting microscope and camera system. On the basis of egg rotation and egg appearance, the "best" and "second best" frogs will be selected to contribute additional eggs.

Using the two best frogs, 22 egg chambers will be loaded with eggs and fertilized. Eleven chambers will be incubated at microgravity and eleven will be incubated on the centrifuge. At various times during the flight, chambers will be removed from the FEU and transferred to the GPWS where a formaldehyde-based fixative will be injected in order to preserve important developmental stages for in depth study following the flight.

Five of the 22 chambers plus the eight fertilization test chambers will be returned to Earth with live tadpoles. The swimming behavior of these free swimming tadpoles will be examined

within several hours of landing and some will be fixed for a detailed analysis of their inner ear, the otolith, the animals "balance system." Lychakov and Vinikov (10,11) reported an increase in otolith size in tadpoles that developed (but were not fertilized) in space. Live tadpoles from the SL-J flight will be raised through metamorphosis for studies of maturation including an analysis of the effects of space flight on their ability to produce normal progency.

Following the flight, the fixed embryos will be serially sectioned and stained using procedures that were developed for this experiment. The embryos fixed at the 2- to 4-cell stage will be examined for the distribution of cytoplasmic contents, including the various classes of yolk platelets, and the location and shape of the cleavage furrows. The gastrulae will be assessed for the normality of the complex cellular rearrangements that constitute blastopore formation and involution, i.e., the differentiation of the embryo into its specialized body parts. Using a new procedure, the gastrulae will be bleached which will enable the initial sperm entry point (SEP) to be located. The correlation between the SEP and the dorsal lip of the blastopore will be determined. Under normal terrestrial conditions it has been shown that the SEP typically is located on the side of the egg opposite the future dorsal side of the embryo (2). The neurulae will be examined for the normality and completeness of the neural plate and archenteron expansion. The tadpole stages will be used to study the allometry and morphology of the various organ systems.

Expected Results

We expect that the amphibian embryos developing at microgravity will have essentially normal morphology. This expectation is based on the results of previous space flight data (albeit

from embryos fertilized at 1 g and launched at various stages of development) (12,13), random motion experiments (4), and clinostat experiments (7,9) in which most embryos developed normally. Moreover, a recent IML-1 experiment has exposed amphibian embryos to microgravity during the gravity-sensitive period between fertilization and first cell division and most embryos appear normal although no embryos were fixed after gastrulation and none were allowed to develop to hatching due to the constraints of the hardware (G. Ubbels, personal communication). The SL-J frog embryology experiment will assess the normality of the complete ontogeny of the organism including behavioral analyses.

Swimming behavior of tadpoles fertilized on the ground and launched prior to the formation of their otolith systems are expected to exhibit abnormal behavior in microgravity based on our experiments with short-term exposure to microgravity during parabolic flights on NASA's KC-135 airplane (14) and previous experiments with tadpoles and fish aboard the Skylab and Apollo spacecraft. We are far less certain that this behavior will persist throughout the 7-day flight and for how long it may persist post-flight. Similarly, we are less certain about the swimming behavior of tadpoles which were fertilized and developed at microgravity. Never having been exposed to gravity, their swimming behavior may be quite different than normal tadpoles, both at microgravity and at 1 g post-flight.

Regarding the SEP-dorsal axis topography, we expect that the correlation will be normal; i.e., the dorsal structures will form on the side of the egg opposite the SEP. We base this assessment on recent findings that the normal topography depends on cytoplasmic rearrangement that can actually work against gravity in an experimental situation. However, other scientists predict

a disruption of the normal cytoplasmic rearrangements (7). If they are correct, a randomization of the SEP-dorsal structures relationship will occur. The Spacelab J Frog Embryology Experiment should resolve this issue.

References

1. Ancel, P., and P. Vintemberger (1948) Bull. Biol. Suppl., 31, 1.
2. Black, S., and J. Gerhart, (1985) Dev. Biol., 108, 310.
3. Born, G. (1885) Arch. Mikrosk. Avat., 24, 475.
4. Morgan, T. (1904) Anat. Anz., 25, 94.
5. Pasteels, J. (1964) Adv. Morphog., 3, 363.
6. Neff, A., M. Wakahara, A. Juraud, and G. Malacinski (1983) J. Embryol. Exp. Morph., 80, 197.
7. Neff, A., G. Malacinski, and H.-M. Chung (1985) J. Embryol. Exp. Morph., 89, 259.
8. Pfluger, E. (1883) Pflugers Arch., 32, 1.
9. Tremor, J., and K. Souza (1972) Space Life Sci., 3, 179.
10. Lychakov, D., and Y. Lavrova (1985) Sp. Biol. Aerosp. Med., 19(3), 70.
11. Vinikov, Ya., O. G. Gazenko, D. V. Lychakov, and L. R. Palmbakh (1974) Zhurnal Obshchey Biologii, 44(2), 147.
12. Young, R., and J. Tremor (1968) Bioscience, 18, 609.
13. Souza, K. A. (1987) Biological Sciences in Space, International Symposium on Biological Sciences in Space, Nagoya, Japan. Editors: Watanabe, S., G. Mitarai, and S. Mori, MYU Research, Tokyo.
14. Wassersug, R. J., and K. A. Souza (1990) Naturwissenshaften 77, 443.

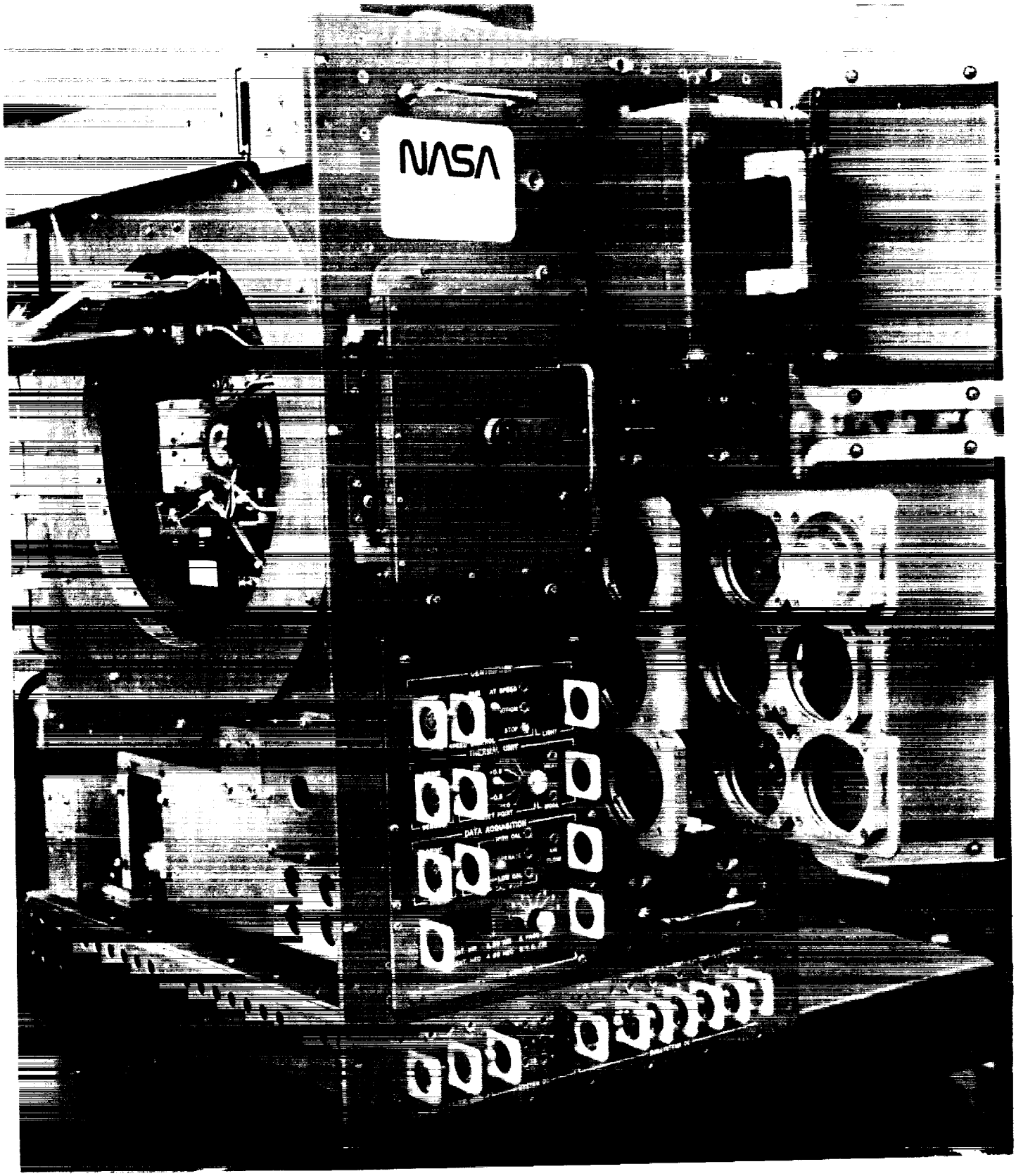

Figure 1. Frog environmental unit.

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