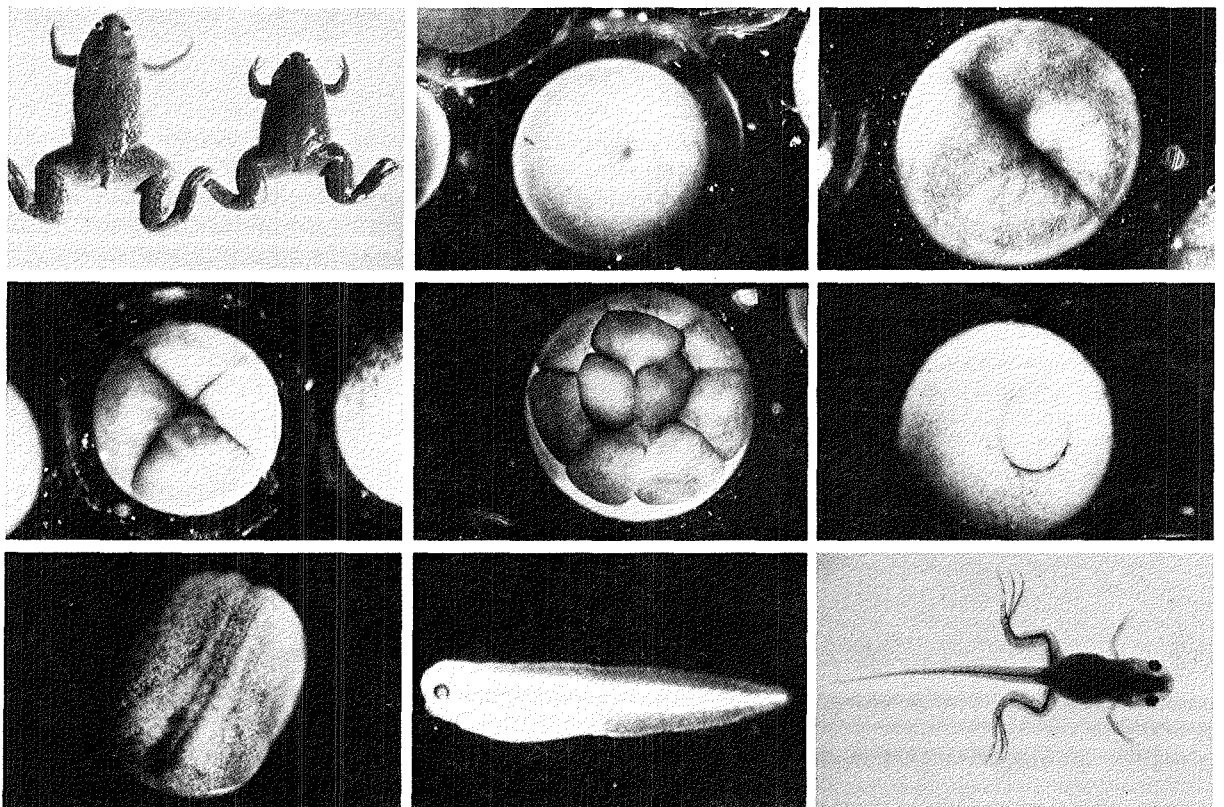


# NASA Developmental Biology Workshop Arlington, Virginia, May 1984



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# **NASA Developmental Biology Workshop Arlington, Virginia, May 1984**

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195-35578 #

Cover: Cover artwork of amphibian development was derived from photographs provided by Carolina Biological Supply Company, Burlington, North Carolina.

## PREFACE

The Life Sciences Division of the National Aeronautics and Space Administration (NASA) as part of its continuing assessment of its research program, convened a workshop on Developmental Biology to determine whether there are important scientific studies in this area which warrant continued or expanded NASA support. The workshop, convened on May 2-4, 1984, in Arlington, Virginia, consisted of six panels, each of which focused on a single major phylogenetic group. The objectives of each panel were (1) to determine whether gravity plays a role in the ontogeny of their subject group; (2) to determine whether the microgravity of spaceflight can be used to help understand fundamental problems in developmental biology; (3) to develop the rationale and hypotheses for conducting NASA-relevant research in developmental biology both on the ground and in space; and (4) to identify any unique equipment and facilities that would be required to support both ground-based and spaceflight experiments.

**Acknowledgment:** The editors wish to express their appreciation to Dr. Donald Beem and Ms. Lou Salmon of the American Institute for Biological Sciences for their assistance in the organization and conduct of the workshop.

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## EXECUTIVE SUMMARY

During the 3-1/2 billion years that life has been evolving on our planet, the force of gravity has been relatively constant. As such, gravity has been an environmental factor with which all living things have had to deal. Some species evolved skeletal systems to provide structural support for increased body size and enhanced locomotion. Gravity-sensing systems were also evolved to provide the organism with gravity vector information and thus aid the organism in directed growth and movement. While it is easy to understand why such features evolved in the omnipresence of gravity, it is not at all easy to understand what may happen to such systems in its absence. For example, will a mammalian embryo fertilized and grown in microgravity form a normal calcified skeleton and vestibular system? Will the amphibian egg fertilized in orbital spaceflight follow its normal pattern of differentiation and development? Or, more simply, can organisms survive through multiple generations in the absence of gravity?

The six panel reports contained in this volume are the results of the first in a series of workshops designed to explore the role and influence of gravity in the ontogeny of living systems. Each panel was asked to identify instances of gravitational influence or response in normal reproductive and developmental processes. For identified cases of gravity sensitivity, panelists were also asked to develop scientific hypotheses which ultimately required the use of microgravity to test. In addition, they were requested to determine what specialized types of ground-based or spaceflight equipment would be required to test the hypotheses. The following sections briefly summarize the most significant recommendations and comments of each panel.

### I. Mammalian Development Working Group

Chairman: J. R. Keefe, Case Western Reserve University

Very little is known about the effects of gravity on early mammalian development and about the influence of microgravity. However, based on the substantial spaceflight data documenting significant changes in the physiology of male humans, nonhuman primates, and rodents, the panel believed that the normal physiology of a female animal would be significantly altered in microgravity such that, "The potential for an indirect influence . . . acting upon the developing or nurturing subject via modifications of the maternal system operant at that time is greater than the potential for direct gravity action at many, if not most, developmental stages." However, because in mammals the maternal/fetal unit is inseparable, the differentiation of direct from indirect effects may be difficult. In vitro fertilization and culture methods, and the marsupial model should help us to understand transplacental effects on development induced by microgravity. Developmental stages which are known to be sensitive to maternal systemic changes and thus potentially affected by the stress of spaceflight and/or microgravity are copulation, insemination, fertilization, cleavage, blastulation, implantation, embryogenesis, placentation, and early postnatal development (due to potential altered lactation). Developmental processes that are expected to show direct responses to altered gravity include:

- a. Maturation of the musculoskeletal system
- b. Allometric changes in morphology and body composition

- c. Development of vestibular and proprioceptive systems
- d. Development of the cardiovascular system
- e. Matrix patterning and composition of connective tissue
- f. Development and control of metabolic rate
- g. Behavior (e.g., mating, birthing, nursing)
- h. Sexual maturation
- i. Development of normal circadian rhythms

To develop a firm foundation upon which to build significant scientific hypotheses regarding the role and influence of gravity on developmental processes, the panel recommended a strong ground-based research program be conducted. Using such tools as the clinostat and centrifuge, animal responses to altered gravitational vectors can be determined and sensitive developmental stages and processes identified. However, ground-based studies must be conducted in parallel with spaceflight experiments to provide the full range of gravitational input, i.e., from micro to hypergravity, and also to validate the utility of ground-based tools and models.

The panel noted that access to long-term exposure to microgravity, i.e., 130 days or longer, is essential to further our understanding of how gravity has influenced and shaped life on this planet. Short-term spaceflight, i.e., 7-30 days can also be useful to the Developmental Biology community by providing a means of developing and testing hypotheses, model systems, and equipment. In this latter case the panel identified the following "highly desirable spaceflight equipment":

1. Highly flexible (not species specific) Shuttle Middeck animal housing facility with video monitoring
2. Centrifuge for variable gravity studies and for an artificial gravity control environment
3. Small animal mass measuring device

In summary, the panel believed that, in general, reproduction and development of mammals are influenced by and responsive to gravity. It also felt that the use of spaceflight, especially long-term spaceflight, is required to understand the role gravity has played and continues to play in the evolution of species and to understand how the reproduction and the development of living systems respond to gravity and the lack thereof.

## II. Avian Development Working Group

Chairman: J. Fallon, University of Wisconsin

With the exception of a few sketchy Soviet reports, no scientific data exist pertaining to the effects of microgravity on early avian development. However, there are experimental data which indicate that gravity has a role in antero-posterior axis formation in the developing embryo. The anteroposterior axis of the

avian embryo is established before the egg is laid. Baer's rule states that the cephalic end of the avian embryo will be away from the observer when the pointed end of the shell is at the observer's right. In vitro studies with chickens showed that axis formation is labile during the first 14 hr in utero, gradually becomes fixed during the next 2 hr, and is fixed by the last 4 hr in utero. It is assumed that gravity is the determining factor in the anteroposterior axis formation. The Working Group proposed to test this assumption and other hypotheses by flying the following experiments:

1. Expose artificially inseminated hens to microgravity and examine subsequent embryonic development including musculoskeletal development and the formation of the chorioallantoic membrane.

2. Expose freshly laid duck eggs to microgravity and inject fixative on days 1, 2, 3, 4, and 10 of flight. In the duck embryo, hypoblast formation and anteroposterior determination are separate events. Would the hypoblast form normally in microgravity?

3. Compare altricial (e.g., parakeet) with precocial (e.g., chicken, duck, quail) avian development in microgravity.

4. Compare the number and type of embryonic movements at normal and microgravity and determine if avian circadian rhythms are altered in embryos which developed and hatched in microgravity.

To conduct these experiments in space, specialized equipment requirements include: an avian incubator, a centrifuge to provide artificial gravity for control specimens, and video capabilities to monitor development and behavior.

### III. Amphibian Development Working Group

Chairman: George Malacinski, Indiana University

This Working Group considered "development" as a series of phases or stages, beginning with pattern specification followed by differentiation, and culminating with growth (i.e., an increase in mass). Amphibian embryos are particularly well suited for studies on pattern specification and have been shown to display an unequivocal response to gravity: upon sperm penetration the spherical egg rotates so that the darkly pigmented "animal hemisphere" opposes gravity. Upon sperm penetration radial symmetry is lost and the future dorsal/ventral axis of the embryo is established (i.e., the future dorsal side of the embryo forms opposite the site of sperm penetration). Results of recent ground-based experiments suggest that anuran amphibian eggs will display an uncoupling of dorsal/ventral polarity from the sperm entrance point in microgravity and, despite this uncoupling, normal development will still occur. Therefore, the following two hypotheses should be tested in a microgravity environment:

1. In the amphibian embryo, establishment of dorsal/ventral polarity is driven by internal events (e.g., cytoskeleton formation) rather than external forces (e.g., gravity).

2. Anuran amphibian eggs will develop normally to at least the early tadpole stage in microgravity.

Additional hypotheses to be tested in space are:

3. Aquatic organisms do not display the response to microgravity (e.g., calcium loss) that terrestrial organisms experience.

4. Microgravity accelerates mitosis in epimorphic (growth-dependent) appendage regeneration. And, as a corollary, wound healing (tissue regeneration) is accelerated in microgravity.

5. Previous attempts to fertilize fish eggs (guppies) in space have failed, although it is not clear why. Therefore, the hypothesis, "guppy fertilization or early symmetrization requires normal terrestrial gravity" should be tested.

The Working Group also identified the need of additional ground-based research to complement the spaceflight research and facilitate the interpretation of flight data. Two hypotheses which can be addressed on the ground are:

1. Normal pattern specification in the anuran amphibian embryo requires proper internal cytoplasmic arrangements.

2. The egg of Xenopus laevis is a suitable paradigm for all amphibian and many fish eggs.

Virtually all of the above hypotheses can be tested with spaceflight hardware already under development for Spacelab experiments. However, the Working Group recommended that the Amphibian Embryology experiment scheduled for a 1988 flight be completed before further amphibian flight experiments are designed. Armed with flight data and the appropriate ground-based data, scientists should easily generate an answer to the basic issue, "Is Xenopus a suitable model for generalizing amphibian/fish/reptile gravity effects?"

#### IV. Insect Development Working Group

Chairman: Morris Rockstein, Coral Gables, Florida

In light of the vast quantity of research conducted with the fruit fly, Drosophila melanogaster, the Working Group recommends that this species be used as the insect model of choice for the initial phases of microgravity research. Although relatively few species have been exposed to the microgravity of orbital spaceflight, a substantial number of spaceflight experiments have been conducted by both U.S. and Soviet investigators using D. melanogaster. From these studies and the results obtained, the Working Group proposed that the following hypotheses be tested during spaceflight:

1. There is a decrease in successful mating competency in space.

2. There is a reduction in female fecundity in space.

3. Insects hatched in space will be more adept in controlling flight activities in microgravity and will be more successful in mating in space than those subjected to spaceflight after eclosure and early flight experience on Earth.

4. Insects born and raised in microgravity will experience behavioral and metabolic changes when returned to normal terrestrial gravity.

All but a few of the above hypotheses require microgravity of more than 2 weeks for satisfactory testing. With longer microgravity exposures, sufficient to provide complete life cycle studies, one could address such key questions as "Are differentiation processes dependent on gravity for proper expression?" Or, "Will the genetic program govern development independent of gravity?"

In order to properly test the above hypotheses, the Working Group identified several key pieces of flight equipment: temperature and humidity controlled incubators, a variable-speed centrifuge for a 1 x g control environment and gravity threshold studies, and appropriate video systems to record developmental processes and adult behavior.

#### V. Aquatic Invertebrate Development Working Group

Chairman: D. Meyers, Milsap College

There is little definitive evidence (owing principally to the lack of research in this area) that gravity plays a significant role in the embryogenesis of aquatic invertebrates. However, the buoyant nature of the aquatic environment and the small size of most invertebrate eggs may render gravitational force and the gravity vector relatively inconsequential during the early development of aquatic invertebrates. To properly address the role of gravity in the development of these organisms, the following research topics should be pursued:

##### Reproduction, growth and development in microgravity

1. Geotropisms and their role in reproduction and development.
2. Structure and mode of action of gravity receptors.
3. Cytoplasmic localization in relation to egg polarity and embryonic organization.
4. Influence of gravity and the lack thereof on the calcification process, particularly during early development.

Many of these topics could be addressed on 7- to 10-day orbital flights, and initial studies could be conducted with minimal crew involvement and within the confines of the Space Shuttle middeck.

#### VI. Microbial Development Working Group

Chairman: G. Nelson, Jet Propulsion Laboratory, California Institute of Technology

A major advantage in using microorganisms for scientific studies is that one can use large numbers of identical specimens in a rigorously defined environment in a small volume. In addition, generation times are relatively short, which enables investigators to perform complete life-cycle studies within the 1- to 2-wk microgravity exposures currently offered by the Space Shuttle. In spite of these

advantages, very few microbial experiments conducted in space to date can be considered definitive. Either the experiments were poorly controlled or designed, or equipment problems compromised the experiments in some way. While it is well known that many microorganisms exhibit geotropisms, it is not known what role, if any, gravity plays in normal microbial growth and reproduction. The Working Group considered that the most important question before microbiologists relating to gravity is, "Can a generalized eukaryotic cell detect and use gravity?"

The Working Group proposed several research questions as valuable first steps in acquiring an understanding of gravitational effects on microbial development:

1. Can changes in the gravitational vector induce "stress-protein" formation, as stress-protein synthesis is a general and sensitive response to changes in cellular environment. If so, what is the level of sensitivity of the organism(s) to changes in gravity and how do the organisms "sense" gravity?
2. Is gravity required for establishing cellular asymmetry or polarity?
3. Are growth rate and energy consumption significantly affected by the presence or absence of gravitational force?

To conduct these and other experiments, the Working Group identified the need for a variable-speed centrifuge, appropriate video/microscope systems, and temperature/humidity-controlled incubators.

In summary, the panels consistently found that little is known about the influence of gravity in reproduction, growth, and development; consequently, they found it difficult to develop strong hypotheses when so few observations have been made. Nearly every panel advocated conducting a general broad-based spaceflight experiment as a first step in determining what, if any, effects the absence of gravity may have on development. In parallel, ground-based studies utilizing hypergravity and clinostats were recommended to increase the data base from which sound hypotheses could be developed and tested. Very few specialized pieces of flight equipment are required to support the majority of developmental biology experiments presented in this report. However, it is noteworthy that all panels identified the need for a variable speed centrifuge to provide a controlled  $1 \times g$  environment in space and also to provide a tool for gravity threshold research.



# EFFECTS OF MICROGRAVITY AND HYPERGRAVITY ON INVERTEBRATE DEVELOPMENT

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## SUMMARY

Invertebrates, and especially insects, have often been used in research on the biological effects of microgravity and hypergravity, both in the United States and in the Soviet Union.

The data suggest that abnormal gravity loads do not increase the rate of mutations in these lower animals. Moreover, insects such as Drosophila melanogaster and Tribolium confusum have been able to reproduce aboard unmanned and manned space satellites, though no precise quantitative data have been obtained on mating competence and various aspects of development.

Our own research with Drosophila flown on Soviet Cosmos spacecraft suggests that flight behavior is seriously disturbed in insects exposed to microgravity, which is reflected in increased oxygen utilization and concomitant life shortening. The decrease in longevity was less striking when the flies eclosed in space, which suggests that they could adapt to the altered gravitational environment when maturation of flight behavior took place in microgravity.

The reviewed data suggest that further research on the development of invertebrates in space is in order for clarification of the metabolic and behavioral effects of microgravity and of the development and function of the orientation and gravity-sensing mechanisms of lower animals.

## INTRODUCTION

Like all other animals, invertebrates have evolved under the influence of the Earth's gravitational field. Therefore, it is reasonable to assume that gravity (g) has played a key role in shaping their structure and function, and that exposure to hypergravity or microgravity must result in a variety of cellular and physiological reactions. This has been recognized over the years by NASA advisory committees, which have specifically recommended the implementation of space-biological research on invertebrates. The 1970 Summer Study of the National Academy of Sciences pointed out that the exposure of invertebrates to near weightlessness may be useful for the study of the statolith mechanisms which play a role in orientation (ref. 1). In a more recent report, the Space Sciences Board of the National Academy of Sciences (ref. 2) notes that, "In the fields of cell, plant and invertebrate biology, the biological questions that may be best examined with space techniques relate to the responses of organisms to gravity by their growth, form and orientation." Further, according to the report, "As with plants, the influences of gravitational acceleration on invertebrates may be considered under the two broad categories of effects mediated by (i) specialized receptors such as proprioceptors, acceleration receptors, and organs containing statoliths and (ii) small shifts in the distribution of organelles or similarly non-specific mechanisms."

To my knowledge, the first studies on invertebrates exposed to hypergravity were performed by Wunder and Luther (ref. 3), who showed that when Drosophila larvae were centrifuged at 5000 g, their rate of growth and final size attained were lower than those found in noncentrifuged controls.

Because of their small size and simple housing and feeding requirements, many invertebrates are ideal experimental animals for spaceflight research. This has been recognized by the Soviet space biologists who have often used the most thoroughly investigated invertebrate, i.e., Drosophila melanogaster, in their systematic program of research on board unmanned and manned satellites (refs. 4 and 5). As far back as 1960, fruit flies were exposed to the space environment for 24 hr in an unmanned Soviet "Spaceship-Satellite" and, in 1961, larval cultures and imagoes of D. melanogaster accompanied the first cosmonaut Yuri Gagarin in his orbital flight on board "Vostok" 1. Since that historical mission, fruit flies have logged the impressive total of about 2500 hr of exposure to near weightlessness on U.S.S.R. unmanned satellites and 4000 hr on manned spacecraft (tables 1 and 2). By contrast, the corresponding figures of exposure of D. melanogaster to the space environment on American satellites are only 48 hr for unmanned biosatellite flight and 0 hr for experiments performed on manned vehicles, although a variety of other invertebrate species did receive some attention (table 3). Unfortunately the majority of U.S. spaceflight studies on invertebrates used them as biological radiation dosimeters, rather than as experimental subjects for developmental or other non-radiobiological studies.

The availability of the Shuttle Spacelab will allow the realization of further invertebrate research in space. This research should be encouraged not only because it is technically uncomplicated, but also because it will provide important scientific data. It is true that numerous observations, including NASA's collaborative research with the Soviet geneticist G. P. Parfenov (refs. 6-9), suggest that exposure to weightlessness does not influence the fundamental processes involved in growth and morphogenesis. Moreover, since weightlessness per se does not have a mutagenic effect, it may seem pointless to perform further flight experiments on invertebrates. However, it should be stressed that most space-biological research (except for the thorough studies of Parfenov on Drosophila genetics, ref. 10) has been poorly controlled; therefore, the possibility exists that the more rigorous experiments feasible in space laboratories may detect quantitative changes in developmental parameters which have thus far escaped detection. Moreover, we should keep in mind that many invertebrates, and particularly the highly evolved insects (figs. 1 and 2), have gravity-sensing mechanisms which rival in their sensitivity and sophistication those of mammals. It has been pointed out that insects may have been the first animals which left the sea environment (where the gravitational pull of the Earth is counteracted by buoyancy) to successfully adapt to life on solid ground and even to master controlled flight (ref. 3). This expansion of the ecological niche of invertebrates was accompanied by the development of the extremities needed to support motion both on the ground and in air (figs. 3-7), and position and acceleration sensing devices such as the bristle fields found at the insect body joints and the flight-supporting halteres (figs. 7-9). Obviously, the sensory deprivation associated with the lack of gravity in space must result in a host of behavioral reactions which so far have not been satisfactorily observed or quantified. Furthermore the development of these gravity-sensing systems may be altered in microgravity or may not even be initiated.

If an excessive caution may prevent the realization of interesting flight experiments on invertebrates, the naive attitude that, since gravity is an important

component of the environment, microgravity may induce a garden variety of changes at practically every level of biological organization must also be avoided. As often stressed by the Space Sciences Advisory Boards (refs. 1 and 2), spaceflight experiments are very expensive and "fishing trips" in search of ill-defined responses to weightlessness are not justifiable. It is the feeling of the NASA Advisory Committee that, after nearly a quarter century of biological spaceflight both by humans and lower animals, the era of the exploratory flight experiments should come to an end and a second generation of studies (to be performed by scientist-astronauts aboard the Shuttle Spacelab) should deal with testable hypotheses on gravity-dependent processes, which are strongly supported by the data from previous spaceflights and the results obtained in ground-based laboratories.

For biologists interested in flight experiments, the remainder of this paper will focus on the main data on invertebrates, many of which were published in Russian reports not easily accessible to Western scientists. A brief discussion of the scientific relevance of these data and some ideas about working hypotheses worth testing in future flight experiments are presented.

## EXPERIMENTAL DATA

### Spaceflight Research on Drosophila

It is not surprising that, since Drosophila enjoys a well-deserved popularity as an excellent model for genetic studies, research on the mutational effects of altered-gravity loads has focused on this insect.

Early spaceflight research by both Soviet (ref. 4) and American geneticists (refs. 13-16) suggested that microgravity or cosmic radiation, or an interaction of both, caused an increase in mutations. However, a more critical analysis of the data as well as the results of many other flight experiments from the Soviet Union (tables 1 and 2) showed unequivocally that microgravity is not mutagenic. Similarly, though there have been reports of morphological abnormalities in fruit flies which had developed in space, it could not be established that these abnormalities were the result of microgravity instead of vibration, abnormal temperature, faulty nutrition, or other poorly controlled factors present during spaceflight (ref. 4). Our own observations on fruit flies which were conceived, developed, and eclosed in space aboard the Cosmos 936 biosatellite support the view that microgravity does not exert a detrimental effect on the processes of cell growth, division, and differentiation which are involved in normal morphogenesis (figs. 2-7).

The first experiments ever performed on animal reproduction in space took place in 1962 aboard the manned satellites Vostok 3 and 4, with the assistance of cosmonauts A. G. Nikolaiev, P. R. Popovich, and V. F. Bykovsky. In their report of that pioneering experiment, Antipov *et al.* (ref. 17) comment that, "While planning these experiments one could expect that weightlessness would affect processes of copulation and laying of eggs." The fruit flies proved to be more adaptable than expected since they were able to mate and lay eggs in the microgravity environment. This interesting result does not rule out the working hypothesis that microgravity may exert a negative influence on reproduction. As pointed out by Antipov *et al.*, these experiments have shown that fecundation, laying of eggs, and development of Drosophila under conditions of microgravity with a duration of up to 4 days can proceed normally. However, the data obtained were only of a qualitative

nature and do not allow us to determine quantitatively changes in mating behavior or in the viability of embryos, larvae, etc.

In contrast to the early stages of the Drosophila life cycle, which are not grossly altered by microgravity, the last stage, namely the aging process, probably is altered by abnormal gravity loads (refs. 6-9). This effect is suggested by our preliminary finding that fruit flies which were exposed as young adults to microgravity for nearly 20 days showed a strikingly reduced life span when allowed to age normally upon return to our NASA laboratory (fig. 10). Our working hypothesis is that flies which are exposed to microgravity after completing maturation on the ground cannot control their flying behavior in the absence of the usual gravitational cues. This inability, in turn, results in a disordered motor activity, with concomitant increase in oxygen utilization and (in agreement with the predictions of the rate-of-living and free radical theories of aging (refs. 6-9). We further assume that imagoes eclosed in space adapt or learn to control flight in microgravity during the first hours of adult life, when the insects may be more receptive to the function-molding inputs of the environment. This adaptation must result in a less wasteful utilization of oxygen and, therefore, in a life span at microgravity similar to that found under normal 1-g conditions.

As will be noted below, our interpretation of the Cosmos data is supported by the finding that exposure of fruit flies to continuous rotation in horizontal clinostats results in both an increased level of activity and a significant shortening of life span.

#### Effects of Hypergravity and Clinostat Rotation on Drosophila

A complete understanding of the role of gravity in invertebrate biology cannot be obtained without exposure of these animals to high gravitational fields. Again, the most comprehensive studies on the effects of high-gravity loads on growth and development have been carried out on D. melanogaster because Drosophila larvae are ideally suited for refined quantitative determination of the efficiency of growth, since they show an almost perfect linear correlation between the logarithm of their volume and the passage of time (ref. 18). Wunder et al. (ref. 18) took advantage of this for demonstration that, though growth was possible in fields as intense as  $5000 \times g$ , both the rate of growth and the final size attained were below the values seen in larvae maintained at  $1 \times g$ . The growth rate decreases as the field intensity increases beyond  $1000 \times g$ . Paradoxically, at fields of  $500 \times g$  there is a 25% increase in growth rate. As in previous experiments on wheat seedlings (ref. 19), the larvae showed a faster than normal growth upon removal from the centrifuge. This interesting phenomenon was accompanied by a decreased oxygen utilization, which suggests that, following exposure to high-gravity loads, the bioenergetic processes supporting growth became more efficient (ref. 20). This metabolic effect of exposure to hypergravity appears to be a universal phenomenon, since an increased rate of growth has been observed in turtles exposed to  $5 \times g$  and mice kept at  $1.5 \times g$ . On the other hand, mice centrifuged at  $4 \times g$  grew at a slower rate than "pair fed" controls (ref. 3). This last finding results from the fact that an animal must dissipate energy in direct proportion to its weight to maintain body position and locomotion against the pull of gravity. Small increases in body weight resulting from exposure to moderately raised gravity fields in the centrifuge may be counteracted without an effect on growth by increasing food intake and metabolic efficiency. However, there is a limit to the amount of food that an animal can ingest; therefore, centrifugation at high gravity levels must result in

the diversion of a considerable amount of energy to be wasted counteracting the pull of gravity, and a concomitant reduction in the amount of nutrients available for cell growth and replication (ref. 20).

Preliminary research on the effects of Drosophila rotation in clinostats has yielded interesting results. It should be noted that clinostats do not provide an exact replica of the microgravity environment of space. Nevertheless, we are of the opinion that rotation about the horizontal axis using clinostats, with continuous change in the direction of the gravity vector, may result in disorientation phenomena akin to those occurring in microgravity. This view is supported by the finding that, though the development of Drosophila was uninfluenced by clinostat rotation, flies showed an increased locomotion and decreased life span when kept in the clinostat from eclosion to death (ref. 21).

### Tribolium Experiments

After Drosophila, the flour beetle Tribolium confusum is the most often used invertebrate in the biological flight experiments of both the United States and the Soviet Union. A pioneering experiment by Buckhold et al. (ref. 13) dealt with the effects of microgravity and the combined effects of microgravity and gamma radiation on mutations and wing development in beetles exposed to the space environment aboard the U.S. spacecraft Biosatellite II. The beetles were in the pupal stage at the start of their 2-day exposure to spaceflight. The results suggested that microgravity did not affect survival of the insects. On the other hand, pupal period, wing abnormalities, and mutations were significantly increased. The conclusion was that some factor in spaceflight, probably microgravity, was responsible for the effects observed, though a temperature drop occurring before retrieval of the flight capsule could have played a role.

More recently, flour-beetle eggs have been used in the radiobiological experiments performed aboard Apollo 17 and during the Apollo-Soyuz mission (ref. 16). apparently the eggs were extremely sensitive to the high-energy particles of the cosmic flux. Hatching frequency was significantly reduced, and after hatching there was a high mortality at the larval stage. Developmental abnormalities (e.g., a curved abdomen, fusion of body segments, and splitting or shortening of elytra) showed a striking increase from 2.5% in the controls to 48% in the space beetles.

Another investigation on flour-beetle development in space has been performed by G. P. Parfenov on T. castaneum exposed to weightlessness aboard the unmanned biosatellite Cosmos 605 (ref. 10). There was not enough time for the complete cycle of development of the beetles to be completed during the flight, which lasted about 20 days. The specimens placed aboard were at the embryo, larva, and pupa stages. The data showed no detrimental effect of weightlessness on the processes of hatching of larvae, pupation, or eclosion. Moreover, the survival rate of the specimens at all stages of development was about the same as for controls.

Further research on the effects of microgravity on the biology of Tribolium was performed on the beetles flown in the manned space station Salyut 6. In this long-duration experiment, T. castaneum completed its developmental cycle, from fertilization to the eclosion of the mature imagoes of the next generation, in a normal way. There were no significant genetic changes (ref. 22). This lack of response to altered gravity is in agreement with a clinostat study showing that rotation on this instrument did not result in genetic changes or abnormal

development in T. confusum and T. castaneum, even though the insects were exposed to an abnormal gravity vector during eight consecutive generations (ref. 23).

### Flying Behavior in Microgravity

In 1982 the Shuttle STS-3 carried an insect flight experiment designed by Todd E. Nelson, an 18-yr old high school senior from the Southland 500 Schools in Adams, Minnesota (Philpott, D. E. and Miquel, J., unpublished observations). The experiment exposed three species of insects to microgravity to explore the disturbances in flight behavior. Apparently the lack of gravity did not significantly interfere with the flight of Velvet Bean Caterpillar moths (Anticarsia gemmatalis). On the other hand, as viewed on videotape, worker bees (Apis mellifera) were unable to sustain a controlled flight. They appeared to float in the container throughout the Shuttle mission and occasionally tumbled when they used their wings, rather than exhibited normal directional flight. The center of gravity of bees is located dorsally behind the wings, which may be responsible for the observed pitching, since the lack of a counterweight at microgravity probably results in a loss of balance. House flies (Musca domestica) seemed more successful than bees in establishing normal flight patterns in microgravity.

Insects differ in the degree of sophistication of their flight mechanisms. Thus, bees have two pairs of wings and lack halteres (figs. 5 and 6), while house flies and other diptera have only one pair of wings (fig. 7), with the other pair transformed into gyrostat-like halteres (figs. 7-9). This refinement of the flight apparatus may give the flies a much more accurate perception of the body accelerations sustained during flight (and a better postural control) than is available to bees and other four-winged insects. In the case of moths, the large ratio of wing area to body weight may play a role in the preservation of flying ability in weightlessness.

Besides the striking differences in flying behavior between house flies and bees, exposure to microgravity also resulted in another interesting effect on locomotion. During spaceflight bees were not able to crawl on the walls of their housing unit as were flies. This difference in walking ability between flies and bees during exposure to weightlessness may be related to the different functional design of their feet. Thus, while flies have an adhesive structure (fig. 3), which allows clinging to smooth surfaces, bees have claws surrounding a footpad (fig. 4).

Our electron microscopic study of the Shuttle-flown bees revealed a perfectly normal structure of the exoskeleton, suggesting that despite their difficulty in controlling flight, the bees had not been injured by bumping against the walls of the housing unit.

Although this experiment was not directly concerned with the subject of this review, i.e., development in space, it has clear developmental implications.

In our opinion it is advisable to investigate further the flying behavior of insects which are exposed to microgravity in space after developing on Earth, and of insects which have eclosed in a space laboratory. The hypothesis to be tested is that insects which develop and mature in space may control flight in microgravity better than those exposed to microgravity after completing their development at 1 x g.

## Flight Experiments on Other Invertebrates

Ascaris eggs and Artemia salina cysts have been used in several Soviet space-flights for investigation of radiation effects (ref. 5). Cysts of the crustacean Artemia salina and adult parasitic wasps (Habrobracon juglandis) were also exposed to microgravity in the U.S. Biosatellite II (ref. 16). In these early experiments no conclusive effects on the developmental process could be shown. In better controlled studies, Artemia salina eggs exposed to the space environment during the Apollo-Soyuz mission, Ruether *et al.* (ref. 16) showed a delay in hatching and an increase in developmental anomalies, especially in the extremities and the abdomen. Further research on the larvae of nematodes (Nematospiroides dubius) and grasshopper eggs (Carausius morosus) flown aboard Apollo 16 and during the Apollo-Soyuz mission, respectively, failed to show a significant effect on development (ref. 16).

An earlier experiment on sea urchin eggs (Arbacia punctulata) was flown in the Gemini 3 mission, but no data were obtained because of a technical failure of the housing unit (ref. 16).

Only one flight experiment on Arachnida has been performed to date, that of the high school student Judith Miles, showing that cross spiders (Araneus diadematus) spin finer threads at microgravity (in Skylab 3) than on the ground (ref. 16). As in the case for the flying behavior of insects, the net-webbing of spiders may be a behavior worth studying on animals developed in space.

## CONCLUSIONS

The reviewed data support the concept that the cellular processes involved in genetic change, mitosis, and differentiation are insensitive both to microgravity and hypergravity, as well as to rotation in horizontal clinostats. Thus the experimental gravitational research on invertebrates supports the early views of Morrison (ref. 24), according to which, "DNA processes will not be affected by zero gravity." The probable reason is that biochemical reactions are controlled by electromagnetic forces which are much more intense than gravitational fields (ref. 25).

At a higher level of organization (i.e., that of the subcellular organelles), invertebrate response seems to differ from that of plants exposed to abnormal gravity loads. In plants exposed to microgravity aboard Soviet satellites, the starch granules (statoliths) were distributed uniformly throughout the cytoplasm, whereas in ground-based controls the starch accumulated at the bottom of the cells (ref. 25). No such changes in distribution of subcellular particles or organelles have been seen in invertebrate cells exposed to microgravity or hypergravity; however, no one has looked at the statolith-like structures located in the halteres of insects fixed before reentry to a normal gravitational environment. Perhaps the high viscosity of the cytoplasm of animal cells and their cytoskeletons prevent a redistribution of their structures when exposed to microgravity.

At the physiological and behavioral levels, it seems well documented by numerous flight experiments that insect reproduction can take place in microgravity. However, there is a lack of precise quantitative data on such important parameters as duration of development from fertilization to eclosion, and number and success of copulations in comparison to the values obtained in ground-based laboratories. As we just discussed, previous research from the U.S. and the Soviet Union has

emphasized radiation biology and genetics with an almost total neglect of the physiology and behavior of invertebrates exposed to microgravity. This, in my opinion, justifies the experimental testing of hypotheses, such as the following:

1. Development may be slowed down in microgravity because of disturbed larval behavior with decreased food intake.
2. There is a decrease in male mating competence and female fertility in space.
3. Insects endowed with halteres can better control flying behavior in microgravity than can other insects.
4. Insects eclosed in space will be more adept at controlling flight and performing mating than those tested in space after eclosing on the ground because of adaptation to microgravity during the first hours of their imaginal life.
5. Drosophila strains showing geotactic responses may lose these responses following development and eclosion in space.
6. Invertebrates which have developed in microgravity may show altered behavior (locomotion, flight, taxis, and mating) when tested afterward in Earth-based laboratories.
7. Development of invertebrate gravity-sensing systems will not occur normally in microgravity.

The preliminary observations on irregular flight and net-webbing in insects and spiders exposed to microgravity suggest that further behavioral research in a broad selection of invertebrates may prove very rewarding in future flight experiments. As pointed out earlier, a comparative study of the effects of development on flying behavior of insects endowed with different flying mechanisms seems worth doing. This area of research is in agreement with the recommendations of the National Academy of Sciences Advisory Board (ref. 1), according to which, "Long-term exposures to low-g conditions probably will induce behavioral changes which are qualitatively different from those resulting from short term exposures during which significant adaptations do not occur. Such changes will prove to have both theoretical and practical interest." The Advisory Board further notes that, "One of the fundamental questions involved in future spaceflights is whether animals born and reared in a gravity-free environment exhibit normal anatomic, physiological and behavioral patterns upon return to earth."

In addition to the research on flight mechanisms and geotaxis (fig. 11), it is advisable to perform well-controlled experiments aboard the Spacelab on the quantitative aspects of mating. Among all components of reproduction, only mating may show a clear gravity-dependence, because it requires a high degree of neuromuscular coordination and postural control. Thus, a comparative study of the mating performance of insects showing complex courtship behaviors may provide valuable information both for understanding of invertebrate reproduction and of gravity-sensing mechanisms. Small insects could be conveniently housed aboard Spacelab in an escape-proof unit designed in our NASA laboratory (fig. 12).

In view of the genetic bias of most space biologists, it is not surprising that Drosophila has been the most widely used invertebrate in spaceflight research.



There is an obvious need for broadening the studies of development in space to other insect species and to lower invertebrates. It is doubtful that their embryonic development will be influenced by microgravity, in view of the negative results obtained on Drosophila and Tribolium. Nevertheless, gravitational research on such diverse postural perception mechanisms as the lyriform organ of the spider (ref. 26) and the antennal-socket setae of the microcrustacean water flea (ref. 27) may contribute to the understanding of the orientation mechanisms of lower animals.

The ultimate goal of research in gravitational biology is to provide a unifying view of the role of gravity in shaping structure and function. The present literature survey suggests that, largely because of the extant invertebrate research, the concept that abnormal gravity loads may influence cell genetics has fallen into disrepute (ref. 28). On the other hand, the invertebrate data lend support to the interesting hypothesis that, though altered gravity is not mutagenic, both hypergravity and microgravity trigger interesting somatic responses because of their effects on metabolic rate (refs. 29-34). In addition, microgravity has a disorienting effect that influences energy metabolism indirectly because of its effects on locomotion and muscle workload (figs. 13).

Because of the richness and predictability of invertebrate behavior and the ease with which these animals can be incorporated into flight experiments, we believe that future research on these animals in space will contribute much to our understanding of the influence of gravity and the absence thereof on invertebrate behavior and development.

#### ACKNOWLEDGMENTS

I wish to express my appreciation to Kenneth A. Souza and Norman V. Martello for their support of my work and stimulating scientific discussions.

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TABLE I.- DROSOPHILA FLIGHT EXPERIMENTS FROM  
THE SOVIET UNION (ref. 5).

Year	Mission	Flight duration (days)
1960	Spaceship-Satellite 2	1
1961	Spaceship-Satellite 4	0.06
1968	Zond 5	7
1968	Zond 6	7
1970	Zond 8	7
1973	Cosmos 605	21
1974	Cosmos 690	21
1975	Cosmos 782	19.4
1977	Cosmos 936	21
1961	Vostok 1	0.08
1961	Vostok 2	1
1962	Vostok 3	4
1962	Vostok 4	3
1963	Vostok 5	5
1963	Vostok 6	3
1964	Voskhod 1	1
1965	Voskhod 2	1
1971	Soyuz 10	2
1973	Soyuz 17	29.5
1975	Soyuz 18	63
1976	Soyuz 21	49
1978	Soyuz 27	5

The Spaceship-Satellites Zond and Cosmos were unmanned biosatellites. In the Vostok, Voskhod, and Soyuz the cosmonauts assisted in the implementation of the experiments during spaceflight.

TABLE II.- OTHER SOVIET FLIGHT EXPERIMENTS  
ON INVERTEBRATES (ref. 5).

Year	Mission	Organism
1962	Vostok 3	<u>Ascaris</u> (eggs)
1962	Vostok	
1970	Zond 8	<u>Tribolium castaneum</u>
1973	Cosmos 605	
1975	Soyuz 18	
1975	Cosmos 782	<u>Artemia salina</u> (cysts)
1977	Cosmos 936	

TABLE III.- ORGANISMS USED IN U.S. BIOLOGICAL EXPERIMENTS IN SPACE.

Year	Mission	Flight duration (days)	Organism
1967	Biosatellite 2	2	<u>Artemia salina</u> (cysts)
1967	Biosatellite 2	2	<u>Tribolium confusum</u> (pupae)
1967	Biosatellite 2	2	<u>Drosophila melanogaster</u> (larvae)
1967	Biosatellite 2	2	<u>D. melanogaster</u> (pupae and adults)
1967	Biosatellite 2	2	<u>Habrobracon juglandis</u>
1972	Apollo 16	11.1	<u>A. salina</u> (eggs)
1972	Apollo 16	11.1	<u>Nematosporoides dubius</u> (larvae)
1972	Apollo 17	12.6	<u>A. salina</u> (eggs)
1972	Apollo 17	12.6	<u>Carausius morosus</u> (eggs)
1972	Apollo 17	12.6	<u>T. confusum</u> (eggs)
1973	Skylab 3	59	<u>Araneous diadematus</u> (adult)
1975	Apollo-Soyuz	9	<u>A. salina</u> (eggs)
1975	Apollo-Soyuz	9	<u>T. confusum</u> (eggs)
1975	Apollo-Soyuz	9	<u>C. morosus</u> (eggs)
1975	Cosmos 782	19.5	<u>D. melanogaster</u> (larvae, pupae, adult)
1976	Cosmos 936	18.5	<u>D. melanogaster</u> (eggs, larvae, pupae, adults)
1982	Shuttle STS 3	5	<u>Apis mellifera</u> (adults)
1982	Shuttle STS 3	5	<u>Anticarsis gemmatalis</u>
1982	Shuttle STS 3	5	<u>Musca domestica</u>

(Modified from ref. 29)

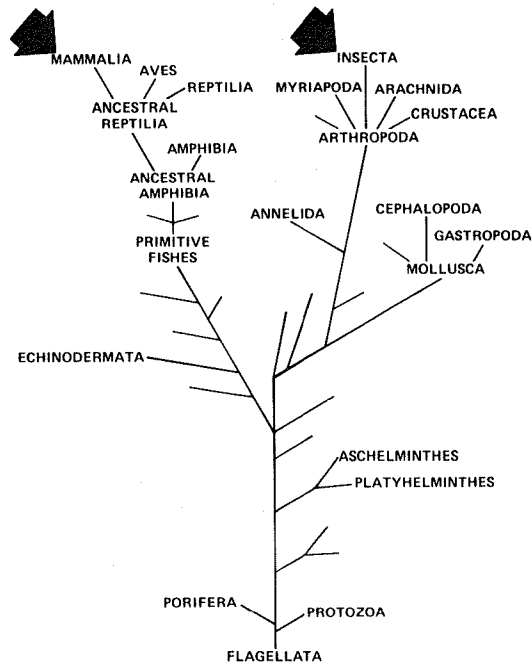


Figure 1.- This evolutionary tree shows the two diverging branches of animals leading to mammals (among the vertebrates) and insects, which are the most abundant and evolved invertebrates. Insects have been often used in the spaceflight programs of both the United States and the Soviet Union; it can be expected that, because of the richness and predictability of their stereotyped behavior, they will remain favorite experimental animals in future spaceflight research (from refs. 11 and 12).



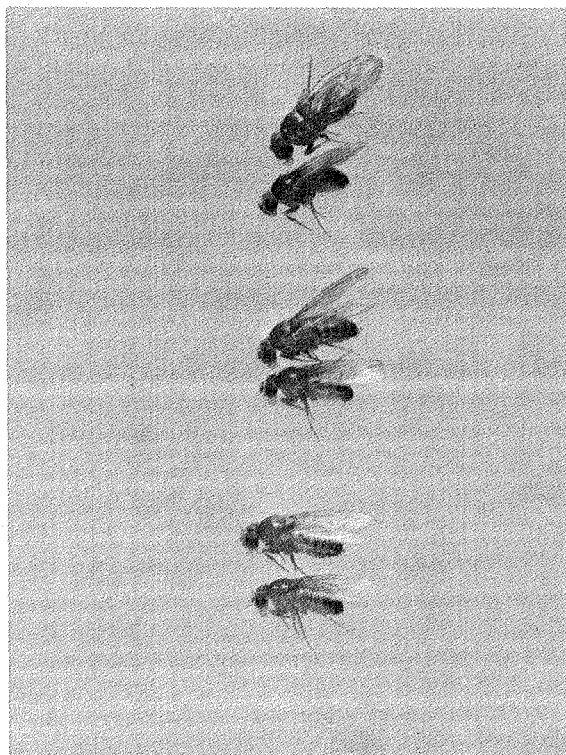


Figure 2.- *Drosophila melanogaster* has been used for extensive studies on the effects of microgravity on mutations. Moreover, in two collaborative U.S.-Soviet experiments flown in Cosmos biosatellites, other biological parameters such as development and aging were also investigated. In this illustration, we show male and female flies which developed on the ground (upper couple), at  $0 \times g$  (middle couple), and in the satellite in a centrifuge at near  $1 \times g$  (bottom). It is apparent that microgravity did not result in abnormal morphogenesis (ref. 7) (magnified 12 times).

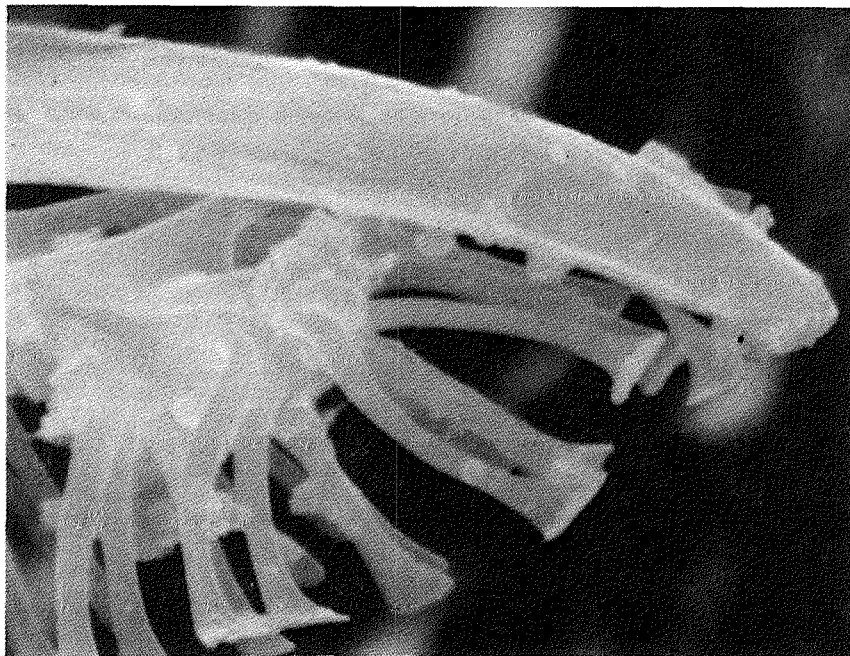
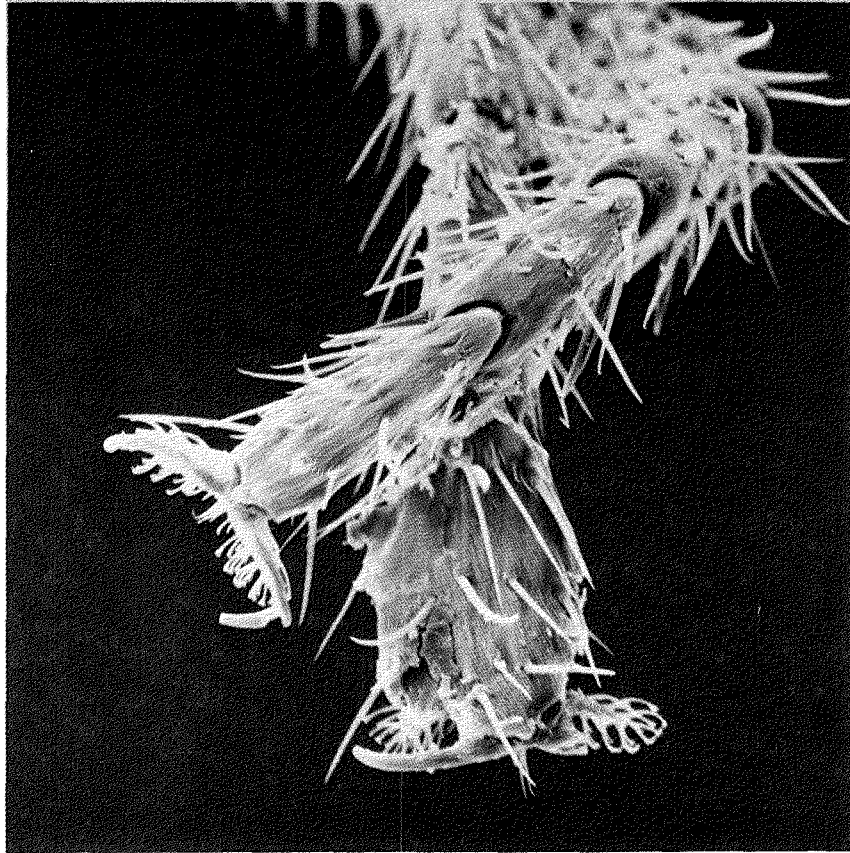


Figure 3.- Scanning electron micrographs of legs and feet of D. melanogaster, showing nails and adhesive structures (ref. 8) (magnified 500 and 7000 times).

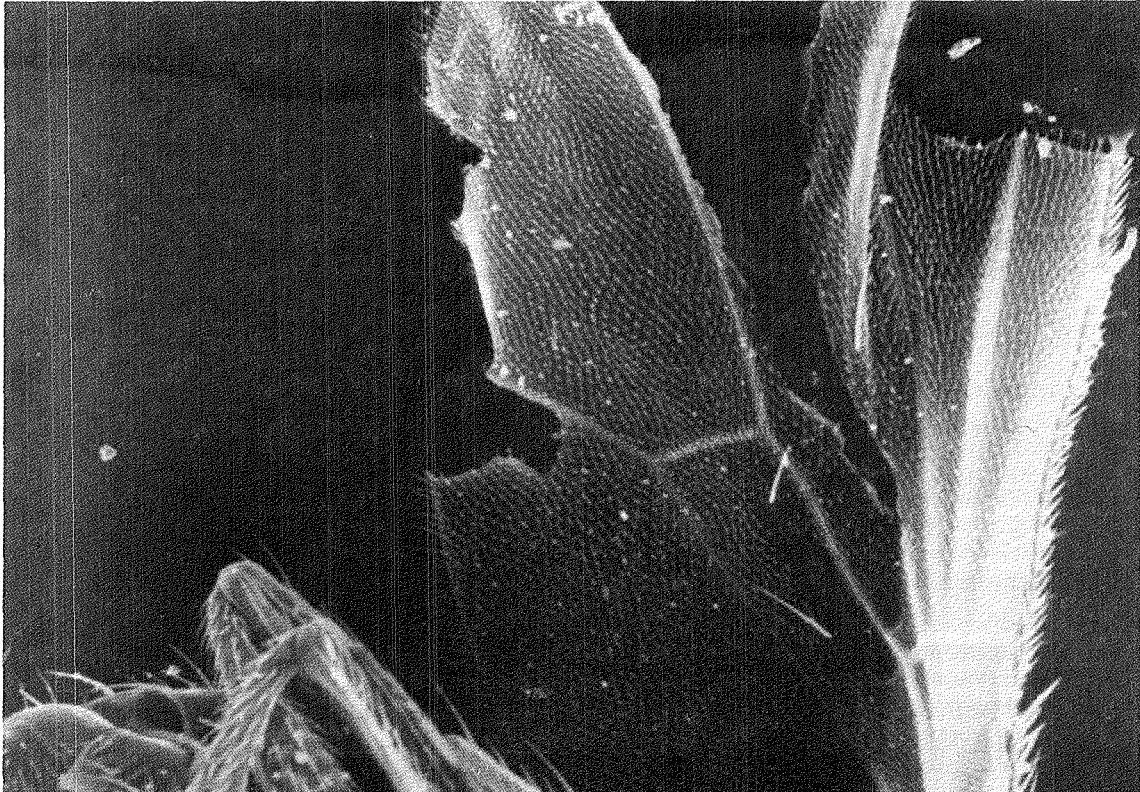
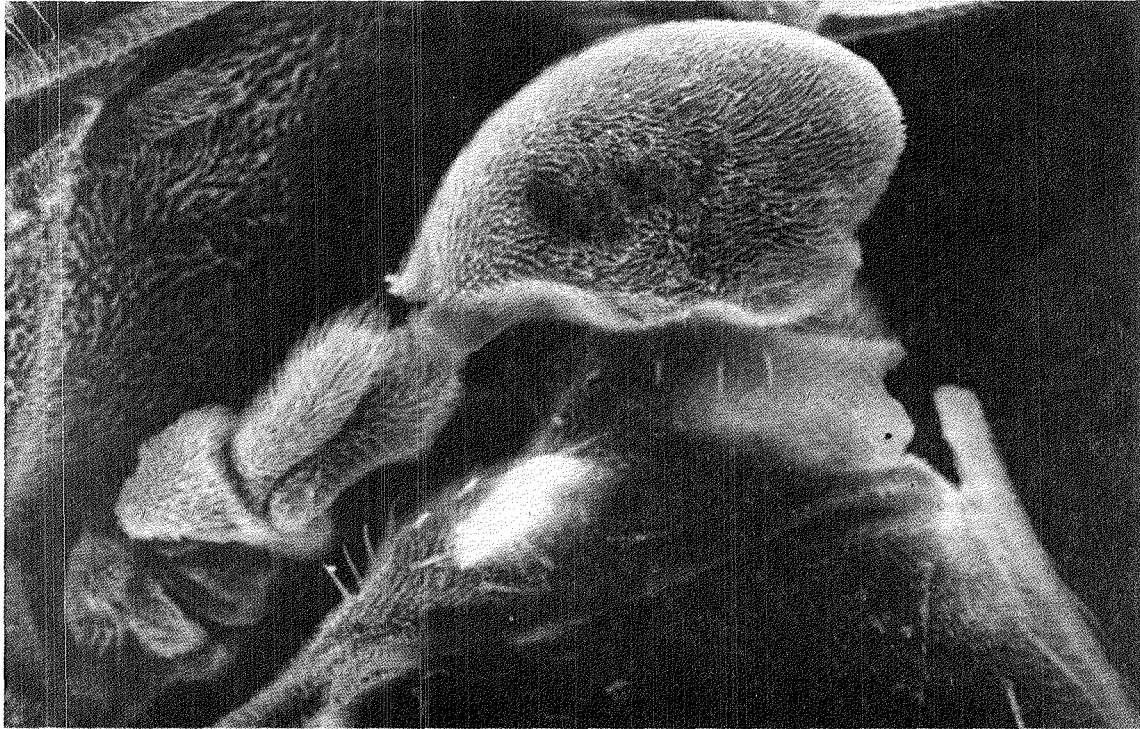


Figure 4.- Gyroscope-like haltere of Drosophila which allows a very precise control of pitch and roll during flight. Broken wing in a fly exposed to microgravity in space (ref. 8) (magnified 300 times).

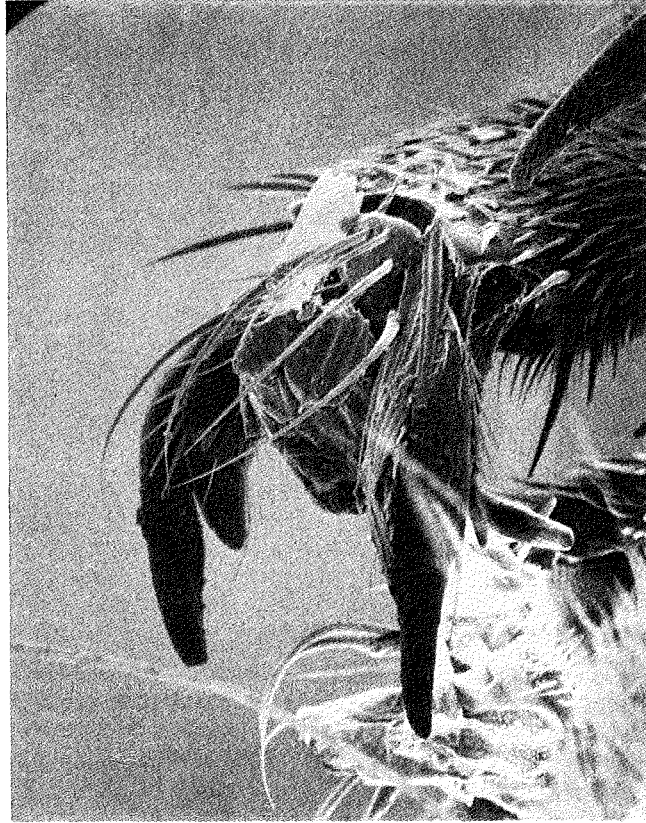


Figure 5.- Foot of a worker honey bee which was flown aboard the Shuttle (ref. 24)  
(magnified 3000 times).

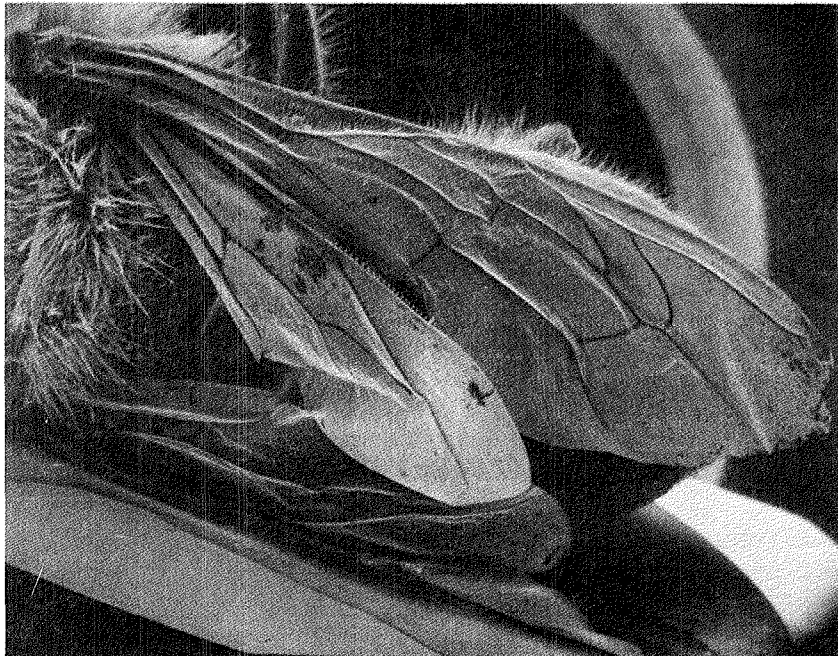


Figure 6.- Double wing of space-flown bee (ref. 24) (magnified 20 times).

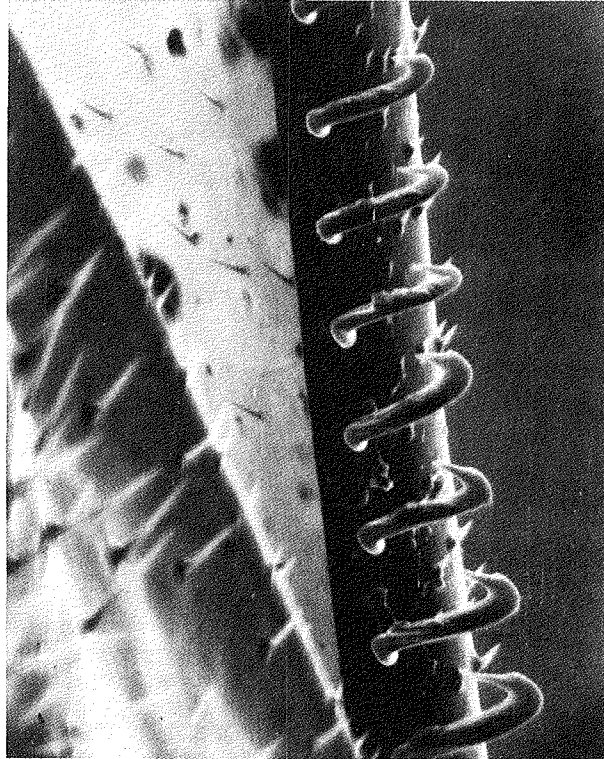


Figure 7.- Hooks present at the edge of the posterior bee wing. These hooks attach to a ridge on the anterior wing for facilitating synchronous movement of the wings during flight (ref. 24) (magnified 625 times).

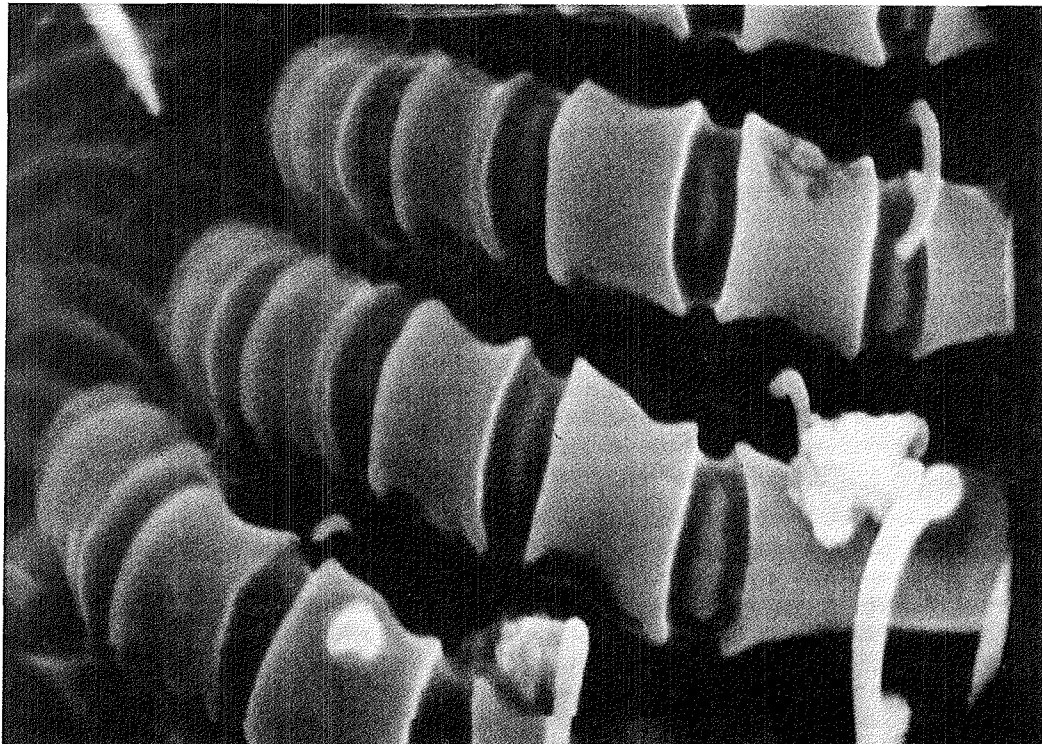
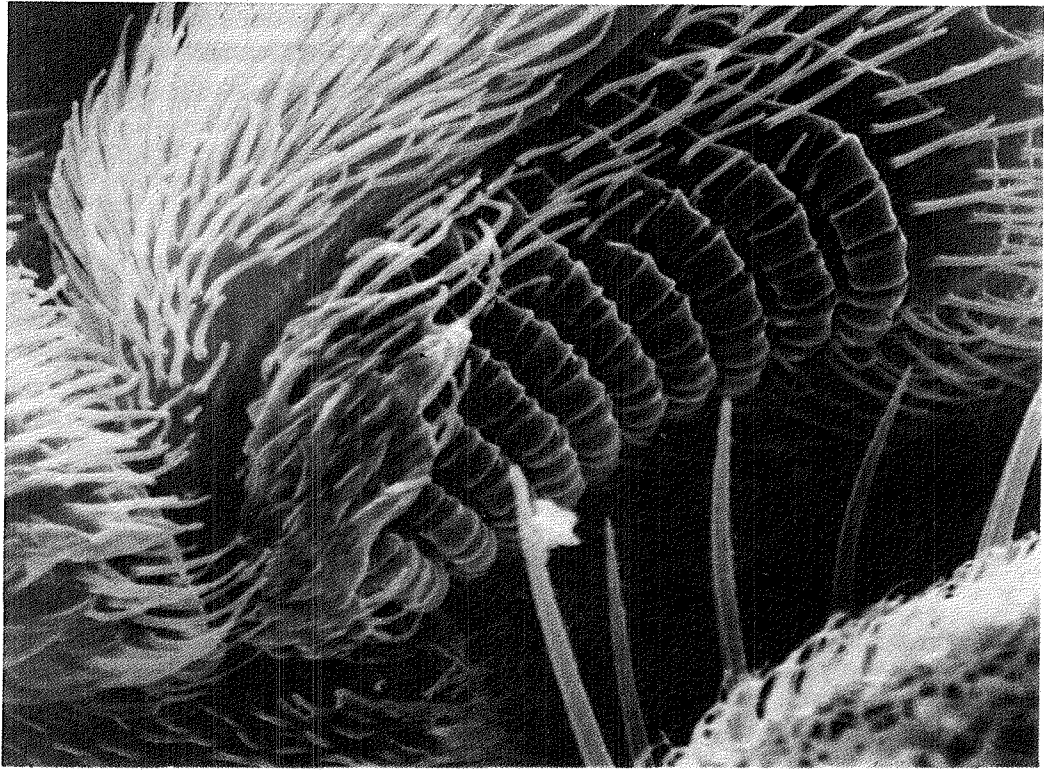


Figure 8.- High-power scanning electron micrographs of the differentiated chitinous structures of the Drosophila halteres (magnified 3000 and 7000 times).

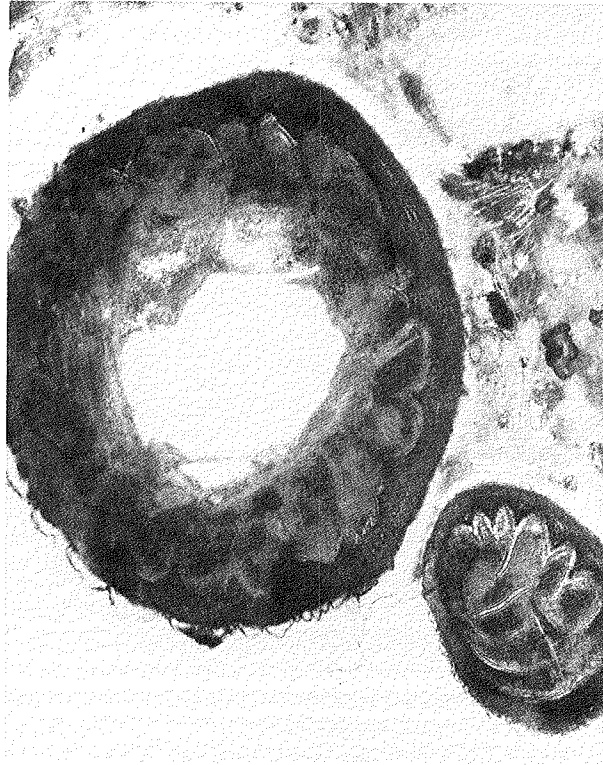


Figure 9.- Electron micrograph of the statolith-like structures found inside the halteres of Drosophila. Like the otoliths of the mammalian inner ear, these insect structures play a role in gravity and body-weight perception (magnified 12,000 times).



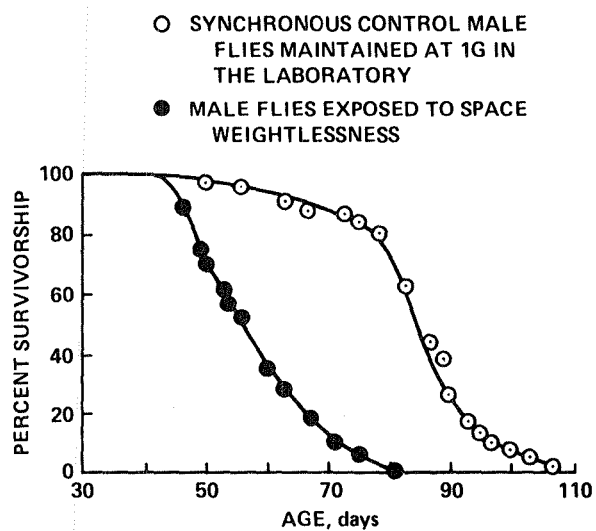


Figure 10.- When imagoes of D. melanogaster Oregon R were exposed to microgravity during about 19 days, there was a considerable life-shortening, probably because of increased oxygen utilization caused by disturbed flying behavior.

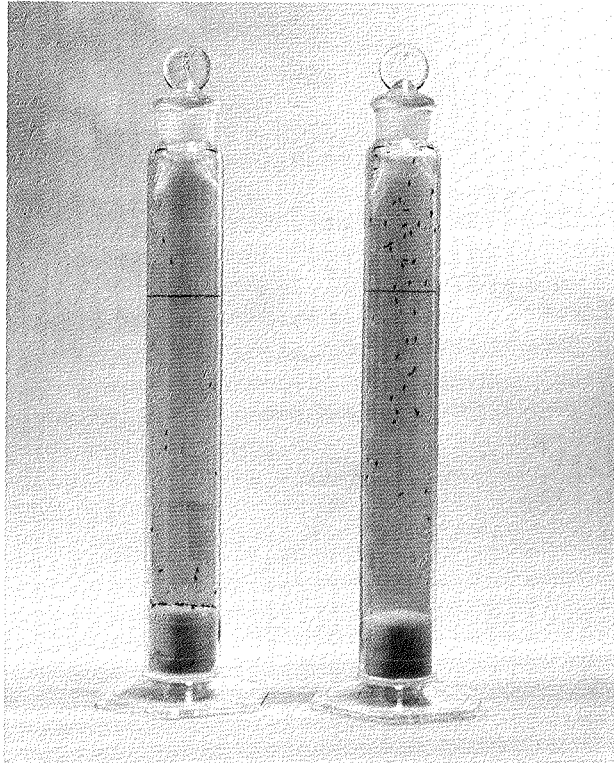


Figure 11.- Many insects show tropisms or taxis such as exemplified here (old fruit flies, left; old fruit flies, right). Some of these behavioral traits may be abolished or modified in intensity by development in microgravity.

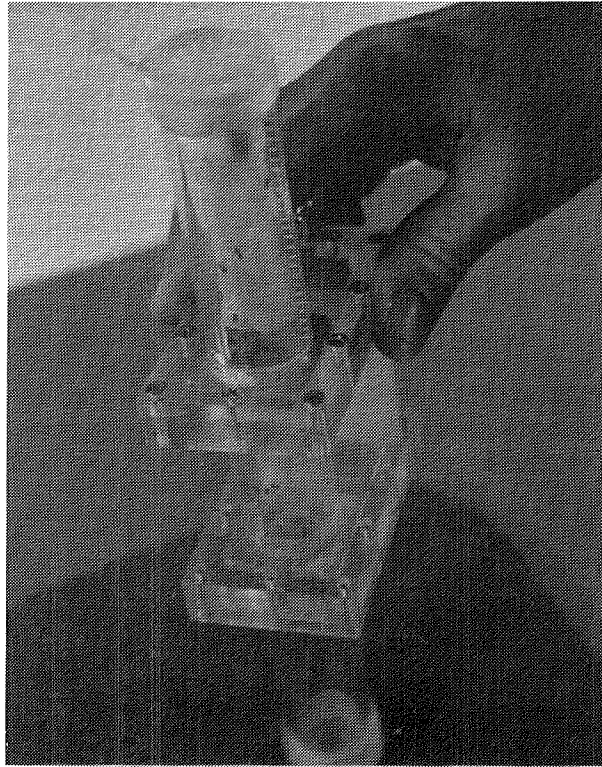


Figure 12.- Escape-proof housing unit for insect reproduction studies which has been designed in our laboratory and could profitably be used in the Shuttle Spacelab. Males are kept in the upper compartment and females in the middle compartment. After 24 hr of spaceflight the interconnecting door is opened and the flies are allowed to mate and lay eggs for 24 hr. Then the other door is opened and all adult flies are shaken and segregated into the compartment devoid of food. The eggs remain on the food and the developmental process can be studied, rendering attention to the quantitative parameters, which have been neglected in most developmental studies performed in space thus far.

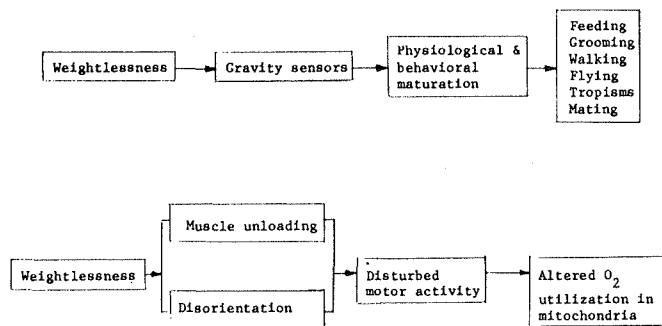


Figure 13.- The experimental data suggest that microgravity does not exert a direct effect on all invertebrate cells, but only on those developing cells which specialize in gravity sensing. This postulated effect of microgravity may affect the behavioral maturation of the animals, with concomitant alterations in metabolism and behavior.

## VERTEBRATE DEVELOPMENT IN SPACE

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### GRAVITY IS A DRAG (and has been for eons and eons!)

The concept that gravity, an omnipresent environmental factor, must have been a significant stressor in the evolution of mammalian developmental sequences from the most subtle to the most major changes can at last be tested under conditions of variable gravities from null to one. To speak of gravitational impacts upon mammalian development, one must also speak of the potential for either direct or indirect effects. If we assume that female mammals demonstrate adaptive physiological responses to null gravity similar to those displayed by flighted males of the species, then the question of indirect, transplacental effects of microgravity exposure becomes highly significant in evaluating the results of developmental studies under altered gravity.

The purpose of the present meeting is to consider means of evaluating both the direct role of gravity on all processes of mammalian reproduction and development as well as defining the means of assessing indirect transplacental aspects. The specification of a "prioritized" sequence of studies designed to maximize the study of the effects of altered gravity on developmental processes in general and its specific impact on mammalian reproductive maintenance should result in optimum utilization of the numerous opportunities for spaceflight experimentation in the next decade. Finally, I would stress that both historically and during the near future we have been and will be dealing with "acute" exposures to spaceflight conditions, representing but brief intervals during the life history of any one mammalian species. It is imperative that we also consider the potential present in the development of a spaceflight system/program specifically designed to provide "chronic" exposure of a representative variety of mammalian species with periodic sampling for multiple generations to fully assess the potential impact of an altered gravitational vector on general mammalian development.

It is seldom that one can totally recall the history of any branch of science. Space Biology, as a relatively recent division of an historically older Gravitational Biology, is, regrettably, so blessed. Although most previous flight projects have been severely limited by preflight operating constraints, relative flight brevity and minimal opportunity for "in-flight" manipulation or sampling of the experimental population, they offer an insight into "windows of opportunity" existent within the broader science of developmental biology. The following brief summary endeavors to capture both the spirit of the individual project and the limiting factors affecting either experiment design or outcome. We are fortunate that within the members of our groups today are many of those directly responsible for the design and implementation of most of these projects.

If gravitational vectors have served as evolutionary drivers in mammalian development, then one should expect such effects to begin with the initial laying down and stabilization of oocyte cytoplasm, with particular reference to cytoskeletal organization and integration. Any subsequent rearrangement of cytoplasm from fertilization through cleavage should also be suspected of gravitational sensitivity. Alterations to the cytoskeleton are likely to continue to be expressed during

development by varying patterns of cell migration, recognition and association, morphogenetic death, and tissue plasticity. Pattern formation developed through cell-cell associations or cell surface features should be susceptible prior to establishing persistent bonding forces exceeding local gravity variations. Finally, the role of intracellular calcium control mechanisms and the potential inherent in the coevolution of calcium regulation (ref. 1) and gravity sensitivity must be evaluated.

#### STUDIES ON EARLY AMPHIBIAN DEVELOPMENT

One of the most widely studied (and contested) effects of gravity upon early vertebrate development has been the rotation of the yolk-laden portion of the fertilized amphibian egg and the establishment of initial axial symmetries (refs. 2-5). Although varying from species to species, the period of maximum gravitational sensitivity appears to become evident during the second-hour post fertilization and to extend into the first cleavage period in some species. Such studies have been further complicated by the variety of experimental manipulations employed and the lack of consistency in their application. Many of the means of restricting rotation are themselves conducive to producing a range of developmental anomalies with severity of the abnormal development dependent upon the scalar range applied.

For example, Tremor and Souza (ref. 6) demonstrated that gravity compensation (vectoral randomization) through horizontal clinostating of unrestrained fertilized eggs of Rana pipiens or Xenopus laevis produced abnormal development only at certain rotational speeds (refs. 6 and 7). Similar observations have been made in horizontally rotated fertilized eggs of Gallus domesticus (M. W. Edgar and J. R. Keefe, unpublished results).

Fundamental to the understanding of the effects of gravity upon early development is the establishment of the structures responsible for detecting imposed gravitational fields. Malacinski (refs. 8 and 9) has recently proposed a "Density Compartment Model" utilizing a variety of cytological approaches. The model proposes compartmentalization of the cytoplasm, with particular emphasis on yolk platelet composition of individual compartments, integrating the results of earlier experimental studies into a gravity-detecting cytoplasmic system capable of being experimentally tested.

It is also important to determine thresholds of gravity sensitivity throughout development. While alterations of the normal 1-g vector during early critical time periods may produce developmental anomalies, reflecting the sensitivity of the organism at that moment, the application of fractional gravity loads over extended periods of development may have a more significant impact on development of the mature organism.

The earliest developmental studies to be undertaken in the U.S. space program were those of Young and Tremor, beginning in the mid-60s (table 1). In what was intended to be a series of studies, the earliest processes of fertilization, initial cleavage, and early embryonic development were to be studied utilizing sea urchin (Arbacia) or frog eggs (Rana pipiens) fertilized in flight. Because of technical restrictions on hardware, flight qualifications, and problems in maintaining viability of unfertilized eggs in vitro over an indeterminate launch-pad hold, these studies had to depend upon ground-based fertilization, with chilling (4°C) of

selected fertilized eggs until the time of launch. Such treatment was demonstrated to have no deleterious effects, although it resulted in delaying the microgravity exposure until just before first cleavage of all eggs.

The sets of studies in table 1 suffered from launch-time limitations requiring ground-based fertilization 12.5 hr before launch, selection of flight group eggs from those first showing fertilization rotation, chilling to 4°C to delay normal developmental processes, and elevation of incubator temperature to 21°C sometime after lift-off.

Since post-fertilization rotation and early cytoplasmic organization could be affected by exposure to normal gravity vectors, in effect providing the fertilized egg with a "gravitational memory," Souza and co-workers (K. A. Souza, unpublished results) are preparing an experiment for Spacelab J (January 1988) in which male and female gametes of Xenopus laevis will be mixed in flight and studied for successful fertilization, egg rotation, development of bilateral symmetry, and growth through blastula and gastrula stages.

#### SOVIET FROG WORK

Similar studies to those amphibian experiments mentioned previously have reportedly been conducted, either jointly or independently, on several Soviet flight projects (e.g., Soyuz 10 (1971 [frog eggs], Soyuz 17/18-Salyut 4 (1975) [frog eggs], Soyuz 20-Salyut 4 (1975), etc.). Our knowledge of these projects is limited to summaries of press-agency releases occurring during the flights since no reports have appeared in the scientific literature.

#### DEVELOPMENT OF Fundulus heteroclitus

A demonstration project carried out by Owen Garriot on Skylab 3, in which 50 fertilized Fundulus eggs were exposed to spaceflight from late gastrula through hatching and early maturity, resulted in normal swimming behavior of the young fry contrary to the disjointed, apparent vestibularly deafferentated swimming of several young adult fish flown. This project was NOT intended as an experiment and no provision for feeding, in-flight sampling, or postflight analysis was provided. Postflight morphological analysis of the poorly preserved remnants suggested that while the sensory maculae of the membranous labyrinth developed adequately, a developmental aberration of the otoconia may have taken place, perhaps as a result of an altered in-flight calcium balance, although no apparent disruption of the calcification process, normal in the developing cartilage of this species, was observed (J. R. Keefe, unpublished results).

Two experiments designed to more properly evaluate the effects of spaceflight upon the early developmental stages of Fundulus were flown as a unique experiment on the joint Apollo-Soyuz Test Project (ASTP) and as a joint project with Soviet coinvestigators on the USSR unmanned biological spaceflight Cosmos 782. In each of these, five developmental stages, chosen for their relevance to vestibular system development in this species, were selected and 100 timed embryos at each stage were packaged in plastic aquaria and flown for either 9 or 19 days, respectively.

The ASTP project (ref. 13) consisted of a single flight package that during the mission was deployed upon a cabin wall, providing a stationary microgravity exposure with normal lighting cycles. Five groups of embryos timed to be at 32-hr (midgastrula), 66-hr (initial statoliths), 128-hr (vestibular development complete), 216-hr (functional vestibular system) and 336-hr (able to hatch) development at the time of lift-off were flown.

The Cosmos 782 experiment (ref. 14) consisted of both microgravity and gravity-compensated flight packages flown in darkness. The latter were mounted upon a flight centrifuge providing an ~0.6-0.8 gravity. The fertilized eggs were prepared in the laboratory at NASA Johnson Space Center, chilled to 10°C, transported to the Moscow laboratories, and subsequently warmed at the suitable preflight times to produce time stages matched to those previously described (32, 66, 128, 216, and 336 hr). The Soviet component of this joint (ref. 15) project consisted of four categories of developing Fundulus from the same population of fertilized, chilled, and transported eggs: a) microgravity; b) in-flight 0.6 × g; c) preflight, clinostat-adapted, in-flight microgravity; and d) preflight, clinostat-adapted, in-flight, 0.6 to 0.8 × g centrifugation.

Results of each of these projects were highly encouraging. Development proceeded normally, if not more rapidly than normal, attributable to the lack of stratification and even dispersal of the gas phase within the aquaria. Hatching rates and normalcy ratios were highest for the space-flown specimens on the ASTP. A nonflight-related contamination problem caused severe developmental abnormalities in the U.S. Cosmos 782 population. No significant deviations in vestibular morphology were reported by the U.S. investigators although their Soviet counterparts claimed marked variations in "otoconial membrane" morphology in certain groups which were attributed to spaceflight exposure.

Preliminary behavioral analyses of the space-flown ASTP specimens suggested unusual diving, stabilizing, and righting responses although postflight testing of space-flown specimens during periods of brief exposure to the microgravity of parabolic flights was unable to confirm this observation. The Soviet investigators on Cosmos 782, utilizing specimens of Fundulus that had been "experienced" to "microgravity" by subjection to preflight clinostatting, reported no significant differences from such preflight exposures with the flighted specimens similar in behavior to those subjected to ground simulation conditions.

In addition to the work described above, Soviet scientists have continued their developmental studies utilizing both Fundulus and other Piscean species on recent Cosmos and Salyut flights.

Of continuing interest is the Soviet work on an internally fertilizing, viviparous guppy (Lebistes reticulatus). The female of this species internalizes and maintains the sperm mass, fertilizing the eggs on a continuing basis and retaining the developing eggs until a later (~21 days) release stage. In the early flight experiment on Cosmos 782, these fish demonstrated normal fertilization throughout the preflight period and in those flighted specimens flown on a 0.6-g centrifuge while the "weightless" variants (microgravity) were reported to have terminated the fertilization process coincident with flight lift-off. No eggs were reported to have been fertilized during the spaceflight period proper while normal fertilization was reported to be restored during the immediate postflight period.



The unidentified Soviet scientists (ref. 16) theorized that, "Evidently, some flight factors inhibit the fertilization of oocytes." While these studies (unpublished) did not present any data on the early stages of embryogenesis in this species under conditions of microgravity, they have raised serious doubts about the ability of normal vertebrate fertilization under spaceflight conditions. It is our understanding that these studies have been privately continued on several recent Cosmos missions and, from brief news releases available, on several recent Soyuz/Salyut missions as well. The results of these studies have not yet appeared in print, although the fact that Soviet scientists continue to pursue such work strongly suggests that fundamental problems in vertebrate fertilization continue to interest them.

#### STUDIES OF DEVELOPMENT ON COSMOS 1129

Three joint U.S./U.S.S.R. experiments relevant to our discussion were performed on the 19-d Cosmos-1129 flight (1979).

Pitts and coworkers (ref. 17) studied the whole-body composition of young adult male rats subjected to the 19-day spaceflight exposure and demonstrated adaptive changes reflective of those previously reported in male flight crews from both countries. Changes of particular relevance to our present discussion included decreased growth, marked diminution of extracellular water fraction, and a sizable reduction in fat-free body fraction representing bone mineral.

These observations, when coupled with other studies on flighted rats and man, suggest that in-flight adaptive changes (fluid electrolyte balances, serum mineral levels, and hormone levels) reflective of physiological compensation for gravity reduction are cause for concern with respect to transplacental effects on normal development. Changes in distribution of body fluids, if they coincide with the vascular development of placentation, may be disruptive of this sensitive developmental stage.

Sixty fertilized Coturnix eggs were flown in a flight incubator which failed to maintain adequate humidification during the latter half of the mission, resulting in the precocious death of the flighted specimens. Although the processes of early development occurred in normal  $1 \times g$  prior to lift-off, the subsequent development of the quail was adjudged by investigators from both countries (ref. 18) to be normal up to the point of incubator failure.

In an attempt to combine in a single longitudinal study the effects of microgravity upon the processes of mammalian copulation, insemination, fertilization, early embryogenesis, implantation, placentation, and early fetogenesis in flighted Wistar rats, five female and two male rats were flown in a divided cage. After the initial 2-day acclimation period, the divider was opened and mating was presumed to have occurred.

While the results of this experiment are still the subject of disagreement along the investigators (refs. 18 and 19), the fact neither the flight nor the synchronous ground-based control groups delivered any litters and no fetuses were recovered from laparotomized flight females suggests that factors affecting this experiment were not directly related to the spaceflight microgravity exposure.

## STUDIES OF MID-FETAL MAMMALIAN DEVELOPMENT ON COSMOS 1514

In December 1983 two groups of five Wistar rats at 12-day gestation were flown for 5 days. One group of pregnant females was sacrificed at the recovery site, and the fetuses were preserved for morphological studies (J. R. Keefe and J. R. Alberts, unpublished results). The second group of five was allowed to proceed to term and four of five delivered litters. A series of tests have been undertaken on both maternal behavior and early postnatal sensory-motor development of the pups (J. R. Alberts and J. R. Keefe, unpublished results).

Although the analysis of the data from this flight experiment is just getting under way, several observations can be made: there is nothing in the spaceflight environment that critically affects normal mid-fetal mammalian development, either directly or transplacentally, and spaceflight exposure during the mid-fetal period of gestation does not adversely affect the development of normal maternal characteristics.

### CONCLUSION

The preceding brief sketches of developmental biology studies during spaceflight are intended to be complete in scope and to provide the reader with an overview of the present status of such studies.

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TABLE I.- STUDIES OF EARLY AMPHIBIAN DEVELOPMENT

Flight	Year	Duration	Specimens/stages
Gemini 3 (ref. 10)	1965	~5 hr	Eight chambers of <u>Arbacia</u> : Fertilize four pre/four in flight. Time-fix samples in flight. Hardware failure.
Gemini 8 (ref. 11)	1966	~11 hr	Twenty <u>Rana</u> eggs/four chambers: Time-fix in flight. Normal early cleavage obtained.
Gemini 12 (ref. 12)	1966	~4 days	Twenty <u>Rana</u> eggs/four chambers: Time fix in flight. Normal embryos, 3/5 recovered live. Live tadpoles died soon after landing--unexplained.
Biosatellite 2 (ref. 5)	1967	~2 days	One hundred and twenty <u>Rana</u> eggs/16 chambers: Time fix at intervals. Range of development; mid-first cleavage to neural plate. Live specimens followed control patterns postflight.



# FINAL REPORT OF THE NASA MAMMALIAN DEVELOPMENTAL BIOLOGY WORKING GROUP

J. R. Keefe, Chairman

## INTRODUCTION

This report covers the initial discussions of the Mammalian Developmental Biology Working Group. We considered "Development" to encompass all aspects of the mammalian life span from initial germ-cell production through the complete life cycle to death of the organism. Thus, gamete production, fertilization, embryogenesis, implantation, fetogenesis, birth, peri- and postnatal maturation, and aging were all considered as stages of a developmental continuum relevant to problems of Space Biology. Unfortunately, owing to the time constraints at the initial Workshop, our deliberations thus far have been limited to stages of the development cycle from fertilization to early postnatal life. Numerous critical topics and developmental stages require further careful attention. For instance, we have not yet addressed important issues related to sexual differentiation, puberty, and the overall endocrinological milieu.

The sequential development of each organism is known to be a matter of critical timing in which each event must occur within a precisely specified time interval to properly influence parallel and subsequent processes. Evolutionary pressures have provided a reserve of plasticity, permitting each organism to respond within a normal range of variation in the functional loading of each developing system. Current ground-based studies of vertebrate ontogenesis would strongly suggest a division of the overall developmental process into a gravity-sensitive early phase (copulation? fertilization? initial cleavages), an orientation-independent phase (embryonic and fetal stages) and a gravitationally dependent phase (postnatal maturation).

It must be understood that these are, at best, artificial divisions with limited experimental material since, in most species of vertebrates, selected stages are either neutrally buoyant in an aqueous environment (and therefore require artificial restriction to control gravity orientation) or are difficult to expose directly to gravitational variation without secondarily involving a complex support system (e.g., placental mammals).

The unique relationships existent between mammalian maternal systems and the developing gamete/embryo/fetus/newborn (the fetoplacental unit) were also examined during our brief initial session. The potential for an indirect influence of altered gravity levels acting upon the developing or nurturing subject via modifications of the maternal system operant at that time is greater than the potential for direct gravity action at many, if not most, developmental stages. But since the maternal-fetal unit is inseparable in mammals, differentiation of direct and indirect effects would be extremely difficult.

The primacy of comparative studies of developmental processes in diverse species particularly suited for the question at hand was stressed by the group. We did not have sufficient time (or the expertise available) during this initial session to extensively consider the full range of alternate species, or the unique attributes that each might contribute to the comparative aspects of developmental studies (e.g., altricial, requiring postnatal nursing care, versus precocial,

postnatally self-active, etc.). For instance, the precocial development of the guinea pig is an example of a species highly suited to studies on the immediate perinatal period, although its elongated gestation period limits the usefulness in spaceflight studies of full development. Marsupials, which represent only 6% of mammalian species, are of significant value in comparative developmental studies since their evolutionary resolution of numerous perinatal developmental processes, while following patterns different from other mammals, have achieved a comparable functioning adult status.

In many instances, selection of a particular species is dependent upon the specific developmental system to be studied. For instance, studies on the onset and maturation of fetal metabolism designed for correlation with human fetal metabolic development would best be served by utilizing Marsupial species. On the other hand, studies designed to reveal the potential for indirect transplacental effects of altered gravity, especially as it might apply to the human system, would best be served by utilizing species with hemochorial placentae such as the rodents (rat and mouse). Certainly, for studies of early embryogenic processes, such as initial development of organ primordia, which are carried out in most mammals during implantation (a process that depends upon a delicately balanced, synergistic interaction of estrogen and progesterone to provide optimal conditions for embryo attachment and embedding in the endometrium), species showing similar processes with respect to timing of relative events, as well as hormonal dependence and balance, should be chosen.

We also did not delve into the question of the effect of variations in gravitational effect upon the development of bipedal versus quadrupedal mammals, although most investigators felt that differences between these two groups would be significant, especially during the postnatal maturation period. For example, the ontogenesis of highly evolved fluid-return mechanisms (both cardiovascular and lymphatic) in bipedal mammals would be expected to be extremely sensitive to functional deviations in gravitational loading.

At every stage of our deliberations about mammalian development in microgravity, we found ourselves approaching a wide range of questions from a precarious, frustrating standpoint: we presently lack the most fundamental data that would guide us in developing and articulating specific, testable hypotheses. Our efforts, then, followed three basic approaches:

1. Extrapolation from the existing database on mammals subjected to altered gravity.
2. Consideration of basic, normative studies.
3. Collective, intuitive assertions.

#### Extrapolation from the Existing Database on Mammals Subjected to Altered Gravity

This subject is devoted almost exclusively to male mammals. Nearly every experimental female mammal to have been in space has been flown on Soviet missions and we are lacking most of the baseline data from them.



Gravitational studies during any period of development, other than those conducted by nature during evolution, are nonexistent for most mammals. A very few species have been subjected to brief periods of hypergravity and even fewer have undergone one or more complete life cycles at an elevated gravity load (refs. 2 and 3). A rat colony consisting of 11 successive generations, conceived and reared at 2.1 g, is currently established at the NASA Ames Research Center Centrifuge facility under the care of Dr. J. Oyama (ref. 3). Simulation studies (head-down tilt to simulate body fluid shifts by suspension of hindquarters) have thus far been restricted to male rats (E. M. Holton, 1984, personal communication; See Shuttle STS-8 and Series STS-41B, table I).

The number of experimental mammals that have spent significant periods of time under conditions of microgravity spaceflight is very small and is summarized in table 1.

#### Consideration of Basic Normative Studies

We have begun to examine the kinds of data that would be feasible to collect on initial flights (shuttle, free-flyer and early-generation Space Station) that would be most productive in establishing a database upon which to build sound programs of basic, scientifically exciting investigations. Several limitations in knowledge were stressed by the group when they considered physiological adaptation to microgravity by female mammals and the potential effect of such exposure on the full range of mammalian developmental processes:

1. There are no data available on the adaptive physiological or behavioral responses to microgravity exposure of female mammals.
2. There are only limited data available on the adaptational changes occurring in experimental male mammals subjected to spaceflight, and most of these data have been gathered in the postflight interval during readaptation to Earth-normal gravity.
3. The maximum length of exposure to microgravity thus far obtained represents but a miniscule fraction of the life span of the individual species. We do not know the effect of exposures for significant portions of the life history of any mammal, including humans.
4. There are no data available on the processes of muscle or nerve regeneration in any species, and the data on cell-cycle patterns under conditions of microgravity are restricted to a single instance of video analysis of cultured cells.
5. There are no instances of in-flight mammalian copulation and studies of vertebrate fertilization are nonexistent. We consider these to be extremely sensitive stages to disruption by altered gravitational vectors.

Faced with the lack of experimental baseline data on female mammalian adaptive responses to gravitational alterations we operated under the following set of assumptions:

1. Adaptive physiological responses to microgravity in female mammals will be at least as significant as those reported for flighted male mammals.

2. Normal female mammal cyclical changes must be superimposed upon the adaptive physiological baseline.

3. Stress-related alterations known to produce effects on normal mammalian developmental processes will not be aggravated or intensified via synergistic interaction with microgravity stressors.

#### Collective, Intuitive Assertions

Most of the members of the working group intuitively felt that gravity has played a prominent role in the evolution of each step of normal development. However, given the absence of experimental data on direct gravitational effects on mammalian gametogenesis, fertilization, cleavage, embryogenesis, or fetal maturation, we felt that we could not positively identify direct responses to gravity in the following stages:

GAMETOGENESIS	BLASTULATION*
COPULATION*	IMPLANTATION*
INSEMINATION*	EMBRYOGENESIS*
FERTILIZATION*	PLACENTATION*
CLEAVAGE*	FETOGENESIS

It is expected that certain of these stages (e.g., fertilization, embryogenesis) should be more sensitive than others to altered gravity loads that produce changes in systemic calcium, fluid electrolyte, and hormonal balances. It is considered possible that gametogenesis and fetogenesis might show direct sensitivity to altered gravitational exposures of longer duration, potentially overcoming any "gravitational memory" of the system.

For example, shifts in fluid electrolyte balance, endocrine (especially stress-related) values, and serum amino acids, and even slight variations in serum calcium levels would be anticipated to have disruptive potential in those stages identified by asterisks as well as in the process of oocyte maturation. All space-flown male mammals have shown significant adaptational shifts in these parameters. If comparable changes or effects were to occur in the maternal (pregnant) animal under microgravity exposure, there is a significant probability that prenatal development would be affected. The nature of these possible effects can be determined only through flight studies. On the other hand, one must accept the real possibility that in spite of marked deviations from normal gravity values in various maternal metabolic parameters, "normal" development may proceed without any significant difference from

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\*The working group would intuitively expect significant indirect (transplacental) responses to altered gravitational fields on these indicated stages. These stages are widely known to be sensitive to maternal systemic variations produced by a broad range of physiological stressors and, even given the lack of baseline data on responses of female mammals to microgravity, there is reason to believe that microgravity stresses will exert a significant effect on development during these stages.

Earth-bound animal development until those stages are reached at which gravity exerts a more direct effect.

## SOME EXAMPLES OF INDIRECT GRAVITATIONAL EFFECTS

### Cellular Level

Every cell maintains its internal compartmentalization and complex interface with the external milieu through a system of cytoskeletal elements. These structural members are responsible for maintaining cell-cell junctions, cytoplasmic transport, and intercellular communication; regulating the surface features of cells for differentiated function; and handling the integrated distribution of metabolites. The cytoskeletal elements determine individual cell shape and motility, acting through cell-cell junctions for the establishment of specifically differentiated tissue and organ shapes. One expects these physical structures to display a plasticity responsive to the sum of forces imposed upon each cell at each point of its life cycle. Several investigators (refs. 12-15) have postulated roles for various cytoplasmic organelles in basic gravity perception--functions that would have to be ultimately expressed through the cytoskeletal system of the cell. All of these earlier cited studies were made before recent additions to our understanding of the fundamental nature of the cytoskeleton and the extent of its interaction, both in regulating cytoplasmic organelle distribution and positioning and in inter- and intracellular force distribution.

The nature of the balance of forces within each cell and at the cell surface interface with the local environment would suggest that modifications in imposed gravity might be expressed by alterations in the normal behavior of the cell. The effects of such alterations, if they exist, have not been demonstrable in previous spaceflight experiments that have examined isolated cells from a variety of sources growing under buoyant tissue-culture conditions. However, subtle modifications have been observed in such systems as immune responsiveness and red-cell aging that might be reflective of alterations in cytoskeletal systems known to be active in the regulation of cell surface receptors (e.g., capping), cell division, and morphology.

We would argue that future microgravity studies of cytoskeletal patterns in cells that are normally anchored in a precise in vivo orientation for optimal physiological function will reveal alterations in the form and substance of cytoskeletal elements. The use of long-term microgravity exposures will allow these modifications to be more reflective of the new balance of internal forces against which they must be arrayed.

The maximal alteration to be anticipated would be in developing systems that are continuously exposed to a microgravity environment and which come to a normal equilibrium with the new balance of local forces. These variations will be reflected in altered surface features (receptors, recognition sites, junctions); subtle changes in intracellular metabolism (polarity, secretion, hormone regulation, membrane flow, energy balance); and potentially significant alterations in overall tissue and organ structure. The latter may require multigenerational exposures to reveal functional adaptation since evolution has provided developing organ systems with structural reinforcements in the developing connective tissue tree and vascularization patterns that would also have to show adaptation.

## Body-Fluid Shifts

Excretion of body fluids, with accompanying shifts in sodium and potassium levels, is a dramatic process induced in male mammals upon entry into microgravity. Adaptational shifts of 1.5-2 l of fluids is typical of space-flown human males. However, body fluid retention is a normal consequence of mammalian pregnancy. Human females show an increase of 1.5-4 l over the entire period of pregnancy. Blood volume increases on average by 20-30% (decreasing in the perinatal period), with a marked retention of sodium. One of the factors responsible for the elevated fluid retention is an alteration in fluid oncotic pressure caused by plasma-protein loss, with a sharp decrease in total plasma proteins during the first trimester of pregnancy. This shift in plasma proteins also leads to modifications in renal clearance factors for a variety of materials caused by an alteration of hormonal control features. Marked changes in plasma proteins and renal processing have also been noted in space-flown male mammals.

Bipedal mammals (such as human males) show significant fluid and electrolyte responses to gravity-unloading as a consequence of their body size, extensive fluid compartmentation, and concomitantly large hydrostatic pressure gradients with effective antigravity return mechanisms. A reasonable scientific question is whether smaller, particularly quadrupedal, mammals such as the rat and mouse would exhibit levels of dynamic fluid alterations in response to gravity-unloading sufficient to affect prenatal developmental processes. In addition, certain experimental species, such as Gerbilline rodents, are renowned for their highly differentiated fluid-handling capability and would be expected to present markedly different space-adaptation parameters. Ground-based modeling studies should be used to determine the suitability of these individual species for flight studies.

Of particular concern here is the rather precise regulation that must occur across the feto-placental unit. Alterations of any serum-borne metabolite have the potential for severely affecting the rapidly developing embryo/fetus. The most sensitive period for disruption would appear to be the initial embryonic period immediately following placentation.

## Mineral Metabolism

We are especially concerned that maternal calcium (and phosphorus) stores may be depleted by microgravity adaptation beyond that required for normal pregnancy and lactation support. There is a six-fold increase in fetal fat-free body concentration of calcium and a three-fold increase in phosphorus during the human fetal period. The human fetal requirement per hour during the last 3 mo of gestation, expressed as percent amount in maternal plasma, is 5% of the maternal calcium (13 mg/hr) and 10% of phosphorus (7.4 mg/hr) (assuming maternal plasma volume of 2.5 l). Atkinson and West (ref. 16) have shown that healthy lactating women mobilize about 2.2% of femoral bone mineral in 100 days. Assuming loss applying to total body skeleton in equal amounts (body = 1.2 kg), daily mobilization of calcium would be estimated at 250 mg/day.

If such a mobilization is superimposed upon the continuing loss of body calcium stores occurring during adaptation to the microgravity unloading displayed by all astronauts, then a serious hazard exists for both maternal and developing systems. The rather interesting scientific question here is whether skeletal system development in the fetus and newborn animal under microgravity exposure is affected in such

a manner (i.e., decreased calcium) that maternal calcium requirements would be reduced concomitantly. A simultaneous question is whether such a "calcium-depleted" newborn individual could survive the rigors of early postnatal maturation.

The calcium ion plays a significant role in many cell-mediated processes and is the most prominent example of a highly conserved ionic species that has been selected by evolutionary pressure under constant 1-g conditions (ref. 17). It is significantly involved in cellular motility, cell-cell interactions, and in cellular polarization, as well as being the primary regulatory ionic species involved in all forms of biological contraction and motility. The calcium ion is of major importance in the transduction mechanisms of nerve processes and of a variety of hormones and serves as an interactive intermediary with other cations in fluid/electrolyte balances. Calcium is a prime regulatory factor in the most fundamental processes of mitosis and meiosis and has had an elaborate systemic regulatory mechanism evolved to precisely control its concentrations at all levels with readily available body reserves. Our understanding of the regulation of this single ionic species at the subcellular, cellular, tissue, and organismic level is, as yet, primitive and we can fully expect that studies carried out at fractional gravity loads will markedly enhance our understanding of the basic mechanisms and their significance for each of the fundamental biological processes involved (ref. 18).

The utilization of calcium-related structures (statocysts, otoconia) for perception of gravity is reflective of the evolutionary dependency of all species on this ion. All spaceflight experiments to date must be considered to have been of minimal flight exposure duration with respect to this ion and its regulatory processes. The study of calcium regulation at all levels ultimately requires multigenerational exposure of the experimental species to near-null gravity with multiple sampling intervals and close control of any other physical factor with potential for physical transduction phenomena (vibration, sound, convective currents), a capability only possible with a Space Station or other free flyer.

#### DEVELOPMENTAL PROCESSES DIRECTLY AFFECTED BY GRAVITY

One area that the working group was confident would show direct responses to altered gravity-loading was the peri- and postnatal maturation period. A wide variety of developmental processes, which usually are time- and sequence-critical, but in which the full range of responsive plasticity to imposed functional loading is yet unknown, would be expected to be directly affected by exposure to microgravity. A few of the more sensitive developmental parameters discussed were:

1. Musculoskeletal system, especially weight-bearing long bones, joints, and the vertebral column. Of special concern in this regard is the primary role of gravity as a calcium regulator; the time and/or the order of ossification during fetal and neonatal life; and, the pattern of chondro-/osteogenesis. Long antigravity musculature and its associated proprioceptive mechanisms would also suffer development modulation in an altered gravity environment.

2. Allometric changes in morphology and/or body composition and organ size relationships. The definable quantitative features of the organism may change when the tonic, "shaping" effects of gravity are removed during development.

3. Vestibular system, especially persistence and maturation of central projections and the proper integration of proprioceptive-vestibular functions. This system is closely related to the next parameter.

4. Proprioceptive system with emphasis upon maturation of muscle-joint pathways and cerebellar integrative circuits. While this would seem obvious for the integration of primary antigravity muscles, we also anticipate marked deviation during postnatal maturation of extraocular and head-neck proprioceptive integration in all mammals, because these systems have evolved to accommodate precise sensory integration (ocular gaze and head position) during variations in gravitational (and rotational) stresses.

5. Cardiovascular system, particularly the development of functionally sufficient venous and lymphatic return mechanisms. We anticipate this to be a very sensitive developmental system, because these systems develop in direct response to imposed functional loading.

6. Interstitial connective tissue, in which matrix patterning and composition would be expected to respond to altered gravity loads. Every multicellular organism has evolved a complex tissue of cells and interstitial matrices to facilitate the movement of metabolites, diffusion of gases and maintenance of localized differentiated fluid balances.

This connective-tissue system of variably hydrated proteoglycans, glycoproteins, tissue-specific elastic and collagen fibers, and a wide range of cell types (many with patterned intercellular communication) is involved in tissue maintenance and homeostasis and also serves as the primary regulator of foreign substances that have gained entrance to the internal environment. The matrix retards and isolates these invading substances, facilitating the cellular responses of recognition and destruction through either local or systemic action. A "corporate memory" of prior immunological "close encounters" is maintained by several varieties of connective tissue cells for rapid responses to subsequent encounters.

Such a multifaceted system has obviously undergone major evolutionary adaptations in each of its functional roles. The all-pervasive nature of the matrix and the fibrous elements, acting as the primary interface between and among all tissue/organ systems, as well as serving as the force-transduction mechanism harnessing each muscle cell of the body and being continuous with the ground matrix of all cartilage and bone (which are highly differentiated states of this system), provides an excellent candidate for an overall systemic accelerometer. This system shows local specialization in its response to imposed physical stress (gravity loads) by varying the physical nature and local density/orientation of its matrix and fibrous elements. Current evidence indicates that presentation times (exposure duration required to produce observable change) are less than have been experimentally determined (limited by available techniques) during acute stress exposures.

The orientation and maintenance of the matrix and fibrous elements of the connective tissue are primary functions of the fibroblasts. These cells show polarized secretion of matrix/fiber pro/precursor molecules dependent upon their orientation to imposed directional stresses. Each fibroblast is a component element of an extensive communicating cellular network. We need to know where and/or what is the central integrative switchboard of this network. Is this network ultimately coupled through tendons, ligaments, and stretch-receptors to the central proprioceptive mechanisms of the organism? Does the individual fibroblast serve as

a stress sensor and, if so, what is the actual receptive mechanism (is it intra-/extracellular)?

Fibroblasts have been primary candidates for spaceflight studies of cells in culture. The experiments have been primarily designed to study the culture system and to evaluate "single" cell responsiveness under conditions of microgravity. These studies have demonstrated the capability of individual fibroblast cells to undergo continued growth and maintain their phenotypic expression although the quality or orientation patterns of the newly formed cellular products (interstitial matrix and fibers) have not been studied.

Our concern is that such studies have not dealt with the fibroblast in its accustomed cell-cell interaction within a differentiated microcosm of stressed local complexes of oriented and hydrated matrix and fibers. While the isolated, cultured fibroblast responds as any cultured cell would under conditions of microgravity, the more fundamental and interesting basic questions have not been approached.

Of greater significance to developmental biologists is the role of the primitive connective tissue in the entire series of complex epithelio-mesenchymal interactions that are required to properly establish most organ systems. These interactions primarily occur during early embryogenesis and are experimentally approachable on STS-duration spaceflights. Further developmental concern is focused upon the postnatal maturation of the connective tissue in its primary role of vascular channel support and structural reinforcement of newly established tissue and organ systems.

While numerous aspects of connective tissue development and maturation are approachable on normal-duration STS flights, we look forward to the capability to apply longer-term longitudinal studies of the in vivo connective tissue under varying gravity loads; this is an approach that ultimately requires the facilities of the Space Station.

7. Metabolic rates for which the "cost" of locomotor activity as well as "vegetative functions" will be substantially altered in a microgravity environment. Our primary developmental concerns are that an altered maternal metabolism may be reflected in deviations of circulating metabolites beyond the normal ranges capable of being accommodated by placental transport mechanisms and that maternal energy requirements may diminish reserves available for fetal development.

8. Behavioral modifications, particularly in systems that are established by social contact and patterned interactions. Generally speaking, mammalian behavior is heavily influenced by the quantity and quality of early stimulation. Development during weightlessness could produce changes in patterns of behavior within and between individuals. Copulation and birthing are two such potentially sensitive functions.

9. Sexual maturation. As yet a poorly understood process at Earth-normal gravity (especially during the prenatal phase), this aspect would be expected to show serious modifications during both prenatal and postnatal phases. For example, the ability of sexual development and behavior patterns (e.g., aggression) to be markedly influenced by the genetic sex of adjacent in utero conceptuses (fetal neighborhood environment) reflects the developmental sensitivity of this system.

10. Circadian rhythmicity. Such rhythms have been shown to become entrained to the maternal environmental lighting pattern during the midfetal period in rats, and which have been shown to be affected by spaceflight in male mammals, regardless of the environmental lighting paradigm, would also be expected to be affected. The development of rhythmic functions, while yet at an early research stage, has demonstrated the highly responsive nature of this system to a wide variety of "zeitgebers" during both prenatal and early postnatal periods.

Organismic regulation and systemic integration of gravity responsiveness in most of these processes is poorly understood. A variable-gravity exposure facility with sufficient exposure duration is now required to properly study the full range of both ontogenetic and phylogenetic development and the role of gravity in these processes.

#### NEAR-TERM DATA REQUIRED FOR DEVELOPMENT OF FUTURE FLIGHT EXPERIMENTS

The working group spent a brief summary period discussing which of the myriad pieces of information that were lacking should be first attained. The following section presents a listing of some of the data that the working group felt would be required in the very near term for planning and implementation of future developmental biology projects:

1. Ground-based studies to assist in establishing priorities for future flight projects. For example: (this is an incomplete listing of the studies suggested by various members of the first working group)

- a. Identification/selection of alternative species/housing patterns for flight
- b. Effects of altered gravitational loads on maternal energy metabolism and fluid metabolites (e.g., calcium, electrolytes, amino acids, hormones)
- c. Effect of altered maternal calcium stores on embryogenesis/fetogenesis
- d. Effect of altered gravitational exposures upon differentiation, maturation, and integration of vestibular and proprioceptive systems and the role of functional loading upon the integrated development of these systems
- e. Development of "yoked" in vivo/in vitro studies on a variety of small mammals that are "microgravity-conditioned" (alter fluid electrolytes, hormones, etc., both systemically and on cultures, based upon values derived from earlier flight studies) to establish experimental protocol for future flight studies
- f. Application of clinostat simulation to in vitro processes of mammalian fertilization and early embryogenesis.

CURRENT DATA: Very limited studies in progress.

FUTURE PROSPECTS: Uncertain, but require major commitment.



2. Baseline physiological data collected on a variety of female mammals exposed to variable gravity from 0 to 1.5 g for time periods sufficient to ensure adaptational stability to the new environment.

CURRENT DATA: None available.  
FUTURE PROSPECTS: None known.

3. Baseline data on adaptational change collected in space-flown experimental mammals reflecting behavioral, social, and postural adaptations to microgravity. Requires videotaping of initial and adapted/stable conditions.

CURRENT DATA: Very limited.  
FUTURE PROSPECTS: None known.

4. Baseline data collected on mammalian growth and energy consumption during microgravity acclimatization and in their new steady state. Requires decent metabolic studies with sufficient animal numbers on a variety of species.

CURRENT DATA: Very incomplete (only few male rats).  
FUTURE PROSPECTS: None known.

5. Alternative housing developed for experimental mammals more reflective of the microgravity environment. Utilize the various animal species to create habitats desired for optimal housing of each species. For example, a variety of studies have shown that hypokinetic restraint stress (e.g., small cages, fitted suits, restraint straps) leads to decreased fertility in rodents, with reduced litter sizes and weights. Imposition of immobilization stress on pregnant rodents causes reductions of >50% in implantation, markedly reduces fetal growth, and sharply increases teratology, depending upon the time and duration of stress imposition. These emphasize the potential for stress disruption of fundamental systems required to study the effect of altered g loads.

CURRENT DATA: Utilizing Earth-normal housing.  
FUTURE PROSPECTS: None known.

6. Data on the occurrence of fertilization, as a fundamental vertebrate process, in microgravity.

CURRENT DATA: None available.  
FUTURE PROSPECTS: Shuttle missions SL-J and D-1; in-flight amphibian (Xenopus) fertilization experiments.\*

7. Mechanism developed for selection/conditioning of mammalian experimental flight subjects. We need to establish selection criteria, "training/conditioning" paradigms, and means of qualifying and selecting animal flight candidates.

CURRENT DATA: None.  
FUTURE PROSPECTS: None known.

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\*These experiments are significant and necessary first steps, but their overall applicability to similar processes in mammals is debatable and requires further work with mammalian fertilization.

## NEAR-TERM SPACEFLIGHT PROJECT REQUIREMENTS

While many of the deficiencies in our current understanding of the role of gravity in fundamental developmental processes can be approached, at least on an initial basis, on currently available flight opportunities, the working group wishes to stress that the full study of mammalian development under conditions of microgravity requires new, longer-duration, spaceflight capability.

Current flight opportunities of 5-14 days (STS) or 5-21 days (COSMOS series) are far too short to permit developmental studies from copulation/insemination through parturition in space-acclimated female mammals. We require the capability to fly small mammals for periods of up to 130 days so that the following studies could be accomplished.

<u>Condition to be studied in microgravity</u>	<u>Minimum time required, days</u>
1. Adaptation and achievement of equilibrium baseline	~7-10
2. Breeding, embryogenesis, and parturition	30
3. Sexual maturity	60
4. Second-generation of #2	<u>30</u>
TOTAL	130

We are aware that there are no planned flight opportunities of this magnitude before the deployment of a fully operational Space Station. We therefore urge immediate implementation of the following near-term course:

1. Development of a closely integrated series of flight experiments on selected small mammalian species designed to establish (a) baseline data on microgravity adaptation in female mammals; (b) steady-state physiological (metabolic) conditions achieved by a selected variety of experimental (flight-adapted) small mammals with specific attention to differences between male and female; (c) proper microgravity housing of a variety of small mammals; and (d) mammalian (variety/number very important) capabilities in microgravity.

Requirement (d, above) would, in turn, provide time for the (a) copulation/insemination/fertilization in microgravity of acclimated small mammals (including in-flight retrieval of zygote and transfer to culture (stationary vs clinostated) for subsequent Earth-based analysis); (b) copulation/insemination/fertilization/implantation sequence in microgravity-acclimated species (including sampling of species just prior to return to Earth); and (c) artificial activation of mammalian oocytes under microgravity (need small variable gravity centrifuge and clinostat for in-flight control exposures).

These studies should be accomplished in the mid-deck locker facilities on 8-14 day STS missions (assuming 7-day acclimation period) and would require in-flight monitoring of metabolic activity (growth and energy studies) as well as adaptational and behavioral monitoring via real-time video. The implementation of this series would establish a firm foundation for the further elaboration of studies in mammalian developmental biology.

The members of the working group strongly recommend the formation of a NASA mammalian development task force to assist in the definition, design, and implementation of the initial fundamental studies described above. Among the functions of the taskforce would be

- a. The development of a highly integrated series of flight and ground-based experiments, with precisely defined logic and established scientific priorities.
- b. The definition and selection of suitable experimental species to maximize the flight data derived from each experiment.
- c. The establishment of required numbers of experimental subjects and the design of suitable ground-based and flight-control subjects.
- d. The identification and active recruitment, for participation in each flight project experiment, of the most active current researchers in those fields most relevant to that specific aspect of the entire series.
- e. The coordination and integration of the entire series of experiments.

The mammalian development task force would represent a continuing presence and interface with the developmental biology community, ensuring continuing peer support for the specific program itself as well as for the overall Space Biology program. The task force should be responsible for the generation of a series of status/progress reports, defining the status of various aspects of mammalian developmental biology under conditions of altered gravity. These reports would be presented before national and international meetings of developmental biologists and published in journals devoted to either developmental biology or the specific organ system(s) under study.

Such an approach would ensure the optimization of experimental design and widest possible support from the scientific community.

2. Modification of the LDEF free-flyer concept to support a fully automated vivarium capable of being deployed by STS.

This spacecraft would provide life-support capabilities for a variety of small mammals and plant species selected to demonstrate long-term adaptation to microgravity exposure. The flight project should also include in-flight breeding of small mammals and seed-to-seed capabilities for the plant studies with real-time video capability. The "Space Vivarium" would be periodically sampled by STS crews with preplanned retrieval mechanisms and refurbishment of consumables established that would minimize spurious gravitational vectors during the sampling procedures. Life-support capabilities developed for this project should be directly applicable to future requirements for Space Station development.

Ultimately, the NASA life sciences community must plan for the establishment of a multigenerational capability to be integrated within the initial Space Station deployment (see discussion in ref. 19, pp. 16-20).

One of the desirable outcomes of any meeting is the intellectual stimulation that arises through the synergism of group members. Several members of our group developed specific experimental flight protocols during the meeting and have continued to intellectually explore the novel experimental tool of variable gravity

exposure on the elucidation of mammalian developmental processes that, in our judgment, will be necessary for incisive and conclusive analyses.

### In Vitro Projects Yoked with In Vivo Studies

For instance, we developed ideas for paradigms in which in vivo and in vitro studies would be "yoked" (coupled exposure variables) to each other during space-flight, allowing investigators to produce conditions in vitro that were yoked to the physiological conditions being monitored in a maternal-fetal system--while both the organisms and the dishes were in space. Matched in vitro controls would be maintained on small, variable-gravity centrifuges both in flight and Earth-based. Such a parallel paradigm would be a powerful tool for the separation and precise definition of indirect, maternally mediated effects from the more direct effects of space conditions on basic developmental processes. It is possible that a valid simulation model could evolve that would provide Earth-based researchers with the capability of emulating microgravity adaptive responses in either single metabolic systems or whole animals.

### Organ Cultures of Bone

"Bone is the only tissue in the body of higher vertebrates to differentiate continuously, remodel internally, and regenerate completely after injury" (ref. 17). The use of organ cultures of bone, taken from precisely defined regions of known age and growth characteristic, and subjected to precisely oriented and defined imposed stresses, should allow us to dissect the regulatory factors controlling this complex of fundamental biological processes and to ascertain the gravity-receptive and gravity-responsive control points in this system.

Regulation of systemic calcium requires an active interplay between at least two systemic hormones, at least two bone-cell-derived growth factors, and between several bone-cell types. The most likely scenario consists of a dual regulatory system with immediate (moment-to-moment) control centered upon the reversible deposition/mobilization of bone matrix calcium by the osteocytes proper and stress-imposed chronic remodeling involving both local osteocytic activity as well as recruitment of either osteoclasts (from blood monocyte population) or osteoblasts (from progenitor cell population) for the more gross regulation involved in stress responses in bone.

The design and implementation of organ culture studies in bone under precisely defined environmental parameters should be one of the more important near-term tasks of Gravitational Biology. Longer-duration STS flights (10-14 days) and, ultimately, the Space Station offer unique opportunities to study these processes for extended periods of time with frequent sampling and modulation of stress parameters to clearly define the fundamental regulatory mechanisms in this system.

### Can Mice Reproduce in Space

The project objective is to determine to what extent ovulation, fertilization, implantation, and development to term can occur in superovulated mice exposed to microgravity. (Wiley, L. M., 15 May 1984, personal communication. Dr. Wiley also

included an experimental protocol which hopefully will be developed into a complete flight experiment proposal.) Its specific aims are

1. To determine the effects of spaceflight on ovulation in superovulated mice; this will be done by counting the number of corpora lutea on the ovaries of superovulated mice 24 hr after HCG injection. (Flight duration required: 2-day post-adaptive steady state.)
2. To determine the effects of spaceflight on mating behavior: this will be done by counting the number of copulation plugs 24-hr after HCG injection. (Flight duration required: 2-day postadaptive steady state.)
3. To determine whether fertilization and implantation will occur in microgravity, accomplished by sacrificing mice 7 days after mating and counting number of implantation sites. Using mice with one prior litter can compare old versus new sites to determine efficiency of implantation. (Flight duration required: 7-day postadaptive steady state.)
4. To determine whether mouse embryos conceived in microgravity can develop to term; return mated mice to Earth-normal gravity, and await term delivery. (Flight duration required: 10-14 day postadaptive steady state.)

The above listing is by no means all-inclusive, but it is intended to illustrate the variety of approaches that were considered by various members, either singly or in "synergic scientific collusion."

It should be stressed that all of the working group members felt that significant initial approaches to the definition of baseline data and identification of areas of mammalian development directly responsive to altered gravity states could be achieved on near-term STS- and COSMOS-type flight projects, but that current mechanisms for the generation of flight-project experiments are unlikely to generate the integrated system of studies currently required.

The consensus of the working group was that additional meetings to consider both specific aspects of mammalian developmental biology sensitive to gravitational variation and the design criteria for experimental utilization of the spaceflight environment would be intellectually stimulating and scientifically fruitful. Several members suggested that such sessions might be held in conjunction with regional, national, or international meetings of developmental biology societies to encourage a broader base of participation and scientific support.

#### FLIGHT EQUIPMENT REQUIRED

The working group did not have time to develop an extensive and exhaustive "shopping list" of specialized equipment that would be deemed necessary for the specific experimental efforts envisioned for mammalian developmental biology. However, several items of equipment were frequently cited as highly desirable elements, and they are included here.

1. Highly flexible middeck housing capability for small mammal diversity required for comparative studies described earlier. Must provide for:

- a. Appropriate housing configuration (not open cube).
- b. Complete metabolic studies.
- c. Real-time videotape monitoring.
- d. Small life-science laboratory equipment items.
- e. Small life-sciences work station (modified locker).

2. On-board centrifuge capable of variable-gravity presentations to tissue/organ culture assemblies as well as to small mammals (mouse, rat, gerbil, hamster, guinea pig, armadillo) from 0.1 to 1.5 x g. Should be capable of middeck operation on numerous flights and of normal Earth-based laboratory operation for yoked control studies.

3. Small-animal mass-measuring device capable of measuring a mass range 1.0 to ~400 gm with a precision of ~0.01 gm.

#### ACKNOWLEDGMENT

The chairman and panel wish to thank Dr. P. J. Duke for her review of the panel's report and numerous helpful comments.

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TABLE I.- EXPERIMENTAL MAMMALS FLOWN FOR SIGNIFICANT TIME PERIODS

Flight	Species	Days	No. and sex	Experimental tests	Reference No.
Korabl Sputnik2	Mus mus.	1	Um/1f	Chance pregnancy - age = ?	a
Apollo 17	Perognath.	12.6	4m/1f	HZE radiation study	4
Cosmos 110	Canis fam.	22	2 m	Telemetry/physiology	5
Series 605	Rattus	22	36 m	Postflight analyses	6,b,c
690	Rattus	20.5	35 m	CS <sup>137</sup> radiation	
782	Rattus	19.5	25 m	Postflight organ analyses	7,8
936	Rattus	18.5	10/20 m	1g/0g postflight analyses	9
1129	Rattus	19	30 m	Whole-body analyses	10
1129	Rattus	19	2m/5f	Attempted in-flight breed	11
1514	Rattus	5	10/f	Matern/fetal exposure	d,e
Shuttle STS-8	Rattus	6	6 m	Middeck cage test	f
Series STS-41B	Rattus	8	3 m	Control gnotobiotic	f
	Rattus	8	3 m	Arthritic gnotobiotic	f

U = unknown

<sup>a</sup>G. P. Parfeyenov, 1984, personal communication: Chance pregnancy (stage at lift-off early but not known) in only female in group recovered from this 1960 1-day precursor to manned flight. The litter, born well after recovery, "appeared normal."

<sup>b</sup>Results of Scientific Investigations on KOSMOS 605 Satellite. (NASA "In-House" translation of Rezul'taty Nauchnykh Issledovaniy na Sputnike "Kosmos-605," 1974, 299 pages.)

<sup>c</sup>Final Report on KOSMOS 690. 185 pages. (NASA "In-House" translation of [source document not given] (ref. 6).)

<sup>d</sup>J. R. Keefe, I. B. Krasnov, J. R. Alberts, and L. V. Serova, 1984, COSMOS 1514 rat neuro-ontogenic morphological study, pp. 1-32, Preliminary Results Report, in press.

<sup>e</sup>J. R. Alberts, L. V. Serova, J. R. Keefe, and I. B. Krasnov, 1984, Postnatal neuro-sensory maturation of rats derived from COSMOS 1514, pp. 1-22, Preliminary Results Report, in press.

<sup>f</sup>E. M. Holton, 1984, personal communication.



## REPORT OF THE AVIAN DEVELOPMENT WORKING GROUP

John F. Fallon, Chairman

### INTRODUCTION

We considered all stages of life to be amenable to developmental analysis.

#### Anteroposterior Axis Formation in Birds

Introduction- One particular problem attracted our attention. The anteroposterior axis of the avian embryo is established before it is laid. Baer's rule states that the cephalic end of the avian embryo will be away from the observer when the pointed end of the shell is on the observer's right. There are experimental data available which indicate gravity has a role in the establishment of the anteroposterior axis while the egg is in the uterus; this results in Baer's rule.

The Facts of the Matter- From ovulation to laying takes 25 hr in chickens. Between 0 and 5 hr, an egg is in transit to the uterus; 20 hr are spent in the uterus. In this organ the shell is applied while the egg rotates counterclockwise, with the pointed end toward the cloaca. There are 10-15 rph. This action causes the albumen to rotate within the shell membrane. However, the yolk does not rotate, but is pulled in the direction of the flow such that the multilayered blastodisc assumes an acute angle. The cephalic (anterior) region of the embryo forms closest to gravity. During the fifteenth and sixteenth hours in the uterus, cells are shed from the center of the blastodisc. The cells fall away from the blastodisc beginning with the uppermost cells (future tail). They fall into the space between the embryo and the yolk and accumulate near the future head end of the embryo. The remaining single layer of cells is the hypoblast. It is assumed the shed cells die. However, it is not clear whether shedding and/or where the cells come to rest, has any role in the formation of the anteroposterior axis. Considering these facts, egg transit through the uterus may be expressed in terms of three phases:

Phase 1.- 14 hr, precell shedding

Phase 2.- 2 hr, period of cell shedding

Phase 3.- 4 hr, postcell shedding

It is possible to manually remove eggs from the uterus and reorient the egg (e.g., pointed end toward gravity). Once again the blastodisc will form an acute angle. If this is done during Phase 1, the anterior end of the embryo will form toward the pointed end of the egg. However, when the same experiment is done during Phase 2, the anterior end of the embryo will develop randomly. During Phase 3 the anteroposterior axis cannot be changed and Baer's rule prevails. Thus, the anteroposterior axis is labile during Phase 1, gradually becomes fixed during Phase 2, and is fixed during Phase 3. It is assumed that gravity is the determining factor in anteroposterior axis formation. Experiments to actually test this on Earth are not reasonable, for the slightest tilt will lead to gravitational pull and compromise the experiment. Exactly what role the cell delamination plays is not clear.

Proposed Experiments- The basic question is whether an anteroposterior axis will form in the avian embryo at 0 g. A parallel question is whether the hypoblast formation (cell delamination) will occur.

There are three possible approaches. The first is to send artificially inseminated hens into space, collect the eggs they lay, and look for normal development. Because the ovulatory cycle is regular in hens, it should be known that the embryo did experience 0 g during the critical hours of Phase 2. There are problems with this approach in that chicken hens are notorious for shutting down egg laying when they are disturbed. This problem could be eliminated by rigorous training of the hens before they are put into space. (It is noted that care must be taken in the strain of hen to be flown; the White Leghorn would be unsuitable because it is so easily unsettled.) A second approach is to put the hens in space and manually remove the eggs. A third approach is to send up manually removed eggs. There are severe time constraints with this approach.

We note that the ideal situation is the first approach. By-products of this approach will be a test of avian fertilization in space, and whether hens will lay eggs in space.

This experiment will require that the embryos be fixed for light-microscopy at intervals after laying.

#### Hypoblast Formation in the Duck

Apparently hypoblast and anteroposterior axis determination are separate events in the duck embryo. Cell delamination does not occur until after laying in the duck. Would the hypoblast form normally at microgravity? This experiment requires that freshly laid duck eggs be put at microgravity and fixed for light-microscopy on days 1, 2, 3, 4, and 10. The eggs would be examined for normal development.

#### Avian Organogenesis (Two systems Stressed)

Bone Formation Initiated in Space- Since it appears that bone breakdown, but not bone addition (synthesis), occurs in space, the Avian Group suggests it is reasonable to explore embryonic bone formation at microgravity. The developing limb would be used.

There are at least three resulting possibilities:

1. Bone formation takes place and at a normal rate.
2. Initiation of bone formation takes place, but at a slower than normal rate.
3. Bone formation does not take place.

The associated questions and problems are:

1. Whatever the case with the bone formation, it would be useful to examine the joints, ligaments, tendons, and associated long-bone musculature for normal development. In the case of muscles of the limb, cell migration from somites to the

limb buds is required for normal development. Thus, normal cell migration at microgravity will be necessary for normal limb muscle formation.

2. Since the limb long bones form by endochondral ossification, the results in the limb should be compared with membrane bone (e.g., skull, clavicle).

3. If there is a change in the rate of long-bone development, or in long-bone size, follow-up experiments should be designed to look at matrix components of cartilage and bone.

Development of the Chorioallantoic Membrane- The chorioallantoic membrane is a crucial organ for avian development. The basic question is whether it develops normally at microgravity. Such problems as normal gas exchange and calcium mobilization would be examined.

We stress at this point that Earth-based experiments on the basic biology of calcium mobilization in avian embryos are required. This is a fundamental question with little work being done; little financial support seems in the offing.

#### Other Experiments

How Do Avian Embryos Develop in Space?- Compare altricial (e.g., parakeet) with precocial (e.g., chicken, duck, quail). The parakeet has a short, the quail an intermediate, and the duck a long incubation time.

Compare Development of Weight-bearing Versus Nonweight-bearing Muscles- For example, the anterior latissimus dorsi versus the posterior latissimus dorsi. This could be viewed in two parts:

1. Carry out the typical battery of tests (contractility, histochemical, ultrastructural) during development and after hatching.

2. After hatching, carry out behavioral studies. Examine how the birds adapt at microgravity, bring them to Earth and again study behavior. The physiology and histology of the muscles would be followed over time.

#### Other Behavioral Studies-

1. Compare the number and type of embryonic movements at microgravity. There is extensive literature to compare to  $1 \times g$ . Methods should be adapted so this could be done noninvasively, or using red light for photography.

2. Study circadian rhythms at microgravity. Birds should be hatched in space and a series of rhythms studies; e.g., pineal melatonin enzymes, steroid levels, mitotic rhythms in various organs (e.g., skin), etc. We stress that the avian embryo is ideal for such studies because there will be little maternal influence on the embryo.

#### Controls

Controls are of exceptional importance. Ground-based experiments conducted parallel to Shuttle experiments must be made. Similarly, a  $1 \times g$  control on the

Shuttle would be invaluable. We did not go further with control design because of time constraints.

#### Earth-based Experiments

In some cases, it is possible that clinostat experiments would be useful before the Shuttle is used. However, we did not know enough about this to feel comfortable proposing it.

It was suggested that shell-less cultured embryos might be useful for some of the studies. Equipment needed on Shuttle are:

- hen-holding facility
- 37°C incubator
- egg-hatching facility
- centrifuge
- noninvasive monitor for behavior
- microscope and cinematographic capability
- tissue-fixation capabilities
- space to fly the Avian Advisory Group
- space to fly Committee Chairman's Assistant

## REPORT OF AMPHIBIAN DEVELOPMENT GROUP

G. Malacinski, Chairman

### INTRODUCTION

The Amphibian Development Group (ADG) believes that several research areas exist which warrant a substantial developmental-biology effort by NASA. However, two general points require discussion before specific hypotheses are mentioned, definition and pattern specification.

#### Definition

It is important to define the term "developmental" biology. The ADG believes that more precise (sophisticated) terminology should be employed in place of such catch-all terms as "developmental biology," or "developmental effects." "Development" is best considered as a series of "phases" or "stages."

1. Pattern specification- the establishment of the plan or program of later differentiation; e.g., egg symmetrization, or number of digits in a hand.
2. Differentiation- the accumulation of specialized cell or tissue products in or between cells; e.g., central nervous system responsiveness (firing), or the accumulation of cartilage in digits.
3. Growth- an increase in mass (number of cells or size of cells).

Amphibian and fish embryos are extremely well suited for studies on pattern specification, whereas other systems (e.g., avian or mammalian) might be just as well suited for studies on differentiation or growth.

Those distinctions are important for at least two reasons:

1. More precise focus regarding underlying mechanisms is called for when those distinctions are made. That facilitates the formulation of specific models or hypotheses.
2. Stress effects (i.e., the effects of weightlessness on structures (e.g., bones) which normally bear a load) are distinguished as being indirect, in contrast to direct effects of microgravity, which would be expected to act on pattern specification. That is, direct gravity effects are distinguished from indirect stress effects.

#### Pattern Specification

Only minor microgravity effects are readily apparent, in previous data, on animal pattern specification (refs. 1-3). The ADG is impressed by the lack of major, disruptive effects of microgravity on the animal-model systems which have so far been tested in space. As will be mentioned later, two caveats must be borne in mind:

1. All necessary control experiments have not been performed.
2. The most appropriate test systems have not been employed; e.g., Artemia (brine shrimp) is considered an especially poor model system, for it is famous for its ability to develop under the most severe and extreme environmental conditions.

#### FUTURE DIRECTIONS FOR DEVELOPMENTAL BIOLOGY

Returning to the main issue--future directions for amphibian/fish/reptile "developmental biology"--the ADG offers the following comments.

First, one of the subject species, the amphibian egg, displays a clear-cut, unequivocal response to gravity; upon activation it "rotates" so that the darkly pigmented (animal) hemisphere opposes gravity. Recent ground-based experiments (clinostats) performed by Neff and Malacinski have generated two predictions:

1. Anuran amphibian (e.g., Xenopus) eggs will display an uncoupling of dorsal/ventral (D/V) polarity from the sperm entrance site, in the microgravity of space.
2. Despite that uncoupling, pattern specification will regulate growth and normal tadpoles will develop in space.

Unfortunately, clinostats may not mimic all the features of a microgravity environment. Therefore, hypotheses should be tested in space (preferably in the Spacelab):

1. Establishment of D/V polarity is driven by internal forces (e.g., cytoskeleton), rather than external forces (e.g., gravity).
2. Anuran amphibian (e.g., Xenopus) eggs have the capacity to specify pattern/differentiation to at least the early larval stage in microgravity.

The ADG considers the planned "amphibian-egg experiment," scheduled for the Shuttle-Spacelab J Mission, 1988, to be a valuable experiment. The ADG believes that it is well conceived and properly designed (in contrast to the ESA experiment (Shuttle D-1 Mission, 1985), which has inherent design problems.

Second, ground-based experimentation should proceed to more precisely predict the results of the 1988 space experiment. For example, the following hypothesis, "Normal pattern specification requires proper internal cytoplasmic arrangements and 'density compartment' relationships," can be tested by using ground-based equipment and strategies (e.g., inverted eggs, centrifuged eggs, clinostated eggs) and histological methods (e.g., 5- $\mu$ m plastic sections to examine the five to six major "density compartments" of the Xenopus egg cytoplasm).

With an adequate data base, the 1988 space experiment will help resolve the long-standing question of, "How rigidly organized must the Xenopus egg cytoplasm be, for normal pattern specification?"

Third, further ground-based experimentation should be carried out to test the following hypothesis, "The Xenopus egg is a suitable paradigm for all amphibian and



many fish eggs." Typically, the most convenient laboratory animals; e.g., Xenopus, Fundulus, Artemia, etc., have been employed for microgravity experimentation. However, they may not be the most appropriate choices when the entire range of orders of amphibia and fish is considered. It is not known, for example, whether amphibian eggs laid in nonaquatic environments (e.g., tree-frog eggs) or fish eggs maintained in the uterus, display the typical "rotation response" to gravity exhibited by Xenopus and other laboratory anuran species.

The ADG believes that the "broad vision" required for generalizing based upon Xenopus data has been lacking in space biology (as it has also been lacking in other areas; e.g., molecular biology).

Fourth, since amphibia usually (but by no means always) exist in aquatic or semiaquatic environments, they might cope with microgravity differently than do terrestrial organisms (e.g., mammals). The buoyant environment in which typical aquatic amphibia (Xenopus) live might have generated a novel mechanism for dealing with "reduced weight load." The following hypothesis is suggested: Aquatic organisms do not display the response to microgravity (e.g.,  $Ca^{++}$  loss) that terrestrial organisms experience. Should that hypothesis be proven, the amphibian system could be used to study the phenomenon of  $Ca^{++}$  loss (i.e., a "difference analysis"; compare amphibian with mammal). The axolotl, which exists in the adult form in either completely aquatic or semiterrestrial morphologies, would be ideal for testing that hypothesis.

Fifth, previous space research has suggested that Fundulus development is accelerated in space. The ADG suggests that the data be carefully reexamined, and special attention to the controls be given. If the data appear to be sound, the proposal is offered that microgravity accelerates cell division, cell migration, etc., which is manifested in terms of an overall acceleration in developmental rate. The following hypothesis was formulated to determine whether cell division, in general, is accelerated in microgravity: microgravity accelerates mitosis in epimorphic (growth-dependent) appendage regeneration. If the hypothesis is proven, the generalization will be made that microgravity influences the regulation or mechanics of mitosis. The amphibian (urodele) limb regeneration would be an ideal model system for such a study. Also, wound healing (tissue regeneration) should be tested in space. The hypothesis "wound healing is accelerated in space," if proven correct, would provide basic information about the mechanics of cell division, and its regulation. Should that hypothesis be proven correct, medical treatments (e.g., burn healing) could be contemplated as a possible use for a space lab.

Sixth, another set of data which require review are the data on fertilization in guppies. If the data that fertilization failed in space are sound, the following hypothesis should be tested: Guppy fertilization or early symmetrization requires  $1 \times g$ . If that hypothesis is proven, further examination of the mechanism of guppy fertilization would be encouraged. Guppies offer the advantage that they can be readily artificially inseminated.

Seventh, the ADG was impressed with the broad range of developmental patterns displayed by fish. Virtually all types of morphogenesis known in vertebrates are displayed by one or another type of fish. Also, some species (e.g., zebra fish) develop very rapidly, and would be a useful model system for multigeneration studies. NASA should consider developing, with ground-based work, some fish-model systems for use in future spaceflights.

Finally, reptiles were considered as model systems for gravity effects. No significant advantages to the use of reptiles were perceived. Fish, avian, or amphibian systems are probably more practical for spacecraft studies. No specific hypotheses emerged from a review of the developmental biology of reptiles.

Priorities for the research were established by the ADG:

1. For the time being, ground-based experiments of the type described in this report should be pursued.

2. Once the amphibian spaceflight experiments have been performed (1985-G. Ubbels; 1988-K. Souza), the data should be evaluated before further experiments are designed.

3. By the time the spaceflight data are available, the ground-based data should be ready. Then an answer to the basic issue, "Is Xenopus a suitable model for generalizing amphibian/fish gravity effects?" should be easy to generate.

4. Equipment needed: A 1-g centrifuge--already planned for the 1988 spaceflight experiment--is necessary.

#### CONCLUSION

The external development offered by most amphibian/fish systems is ideal for space biology research. Embryos are relatively large, easy to visualize, and responsive to manipulation (e.g., ligation, rotation, centrifugation, inversion, etc.). Many such embryos are well known for their classical gravity response. Ground-based work should be carried out to collect data which will aid in formulating generations. Finally, distortions between direct gravity effects on pattern specifications versus indirect (stress-related) effects on differentiation or growth should guide all analyses in amphibian/fish space biology.

Members of the ADG, as well as members of other groups (e.g., avian group) were concerned and disappointed with the emphasis placed by the mammalian working group on "transplacental" or "indirect" gravity effects. Many members of various nonmammalian groups expressed the concern that much of the mammalian work falls into the categories of "stress physiology" or "reproductive physiology," rather than into the category of "developmental biology."

The ADG believes it would be useful to learn about the "physics" of gravity responses; i.e., most developmental biologists do not understand the extent to which the buoyant environment of many amphibia cancel or diminish normal gravity forces. An essay on the "physics of reduced gravity" for biologists would be very useful to the ADG members. Likewise, a discussion with an astronaut would also be helpful, so that a more intuitive knowledge of microgravity would be developed by ground-based researchers.

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## REPORT OF THE INSECT DEVELOPMENT GROUP

M. Rockstein, Chairman

### INTRODUCTION

Considering the overwhelmingly voluminous bibliography on all phases of its biology, this group unanimously chooses Drosophila melanogaster as the insect species of choice, in regard to gravity-response experiments involving normal reproduction and development. This selection would include at least three different strains.

At the same time, we recognize the potential for comparable studies in nematodes, yeast, and Neurospora, again because of the extensive knowledge of the development process for these organisms on Earth (i.e., in a normal gravity environment).

The specific gravity responses which might be affected by microgravity and are exhibited in normal reproduction and development include normal flight for courtship, mating and oviposition, tropisms for pupating or emergence of the adult, and crawling for getting food by the larval instars at the organismic level.

At the suborganismic level we believe maturation of developing eggs in the virgin female and embryonic development of the developing egg could be affected by microgravity and warrant study.

Based on the still limited data on this subject, in relation to insects, per se, and speculation based on known geotropically related activities of insects like this species, space experiments might give us a better insight into functional anatomical, morphological, and concomitant biochemical changes which take place from fertilization through final maturation to the adult stage; e.g., would courtship and mating patterns remain unaltered, would normal (1 x g) neuron and striated-muscle juxtaposition, as it appears in the adult, be the same, if the total life cycle from virgin egg to fertilized egg, and to the adult were to be completed in a microgravity state? Are these processes immutable, genetically predetermined, or dependent on gravity for expression and maturation in a normal terrestrial environment?

### HYPOTHESES

Following are several hypotheses that could be tested in microgravity:

1. There is a decrease in successful mating competency in space.
2. There is a reduction in female fecundity in space (number of eggs oviposited).
3. Insects eclosed in space will be more adept at controlling flight activities and will be more successful in mating than those tested in space after eclosure and early flight experience on Earth (i.e., adaptation will occur within the first

few hours in space). earth-flown, normal flies (wild) will show heightened metabolism on flying in space (nature vs nature).

4. Insects completely developed in space may show altered behavior (in locomotion, including flight, taxis and courtship behavior, and mating) following their return to Earth (following such long-term microgravity exposure). This altered behavior may be an outward manifestation of altered biochemical as well as morphological maturation and development.

5. There are gravity receptors in most mobile insect species (proprioceptors, gravity receptors).

#### RECOMMENDATIONS

The panel urges NASA not to undertake spaceflight-testing of the previous hypotheses without first conducting suitable ground-based experiments simulating microgravity (such as horizontal clinostat experiments) and by hypergravity experiments using low-g centrifugation. Data from these studies might explain the confusing nature of so many previous flight experiments.

Three strains of D. melanogaster should be studied for organismic responses: free flying (normal, wild); very poor flyers (miniatures); and a flying strain with no apparent memory (dunce). These tests should be maintained at 28°C, constant relative humidity, and fixed daylight (artificial) and darkness. The following will be examined in accordance with the suggested hypotheses: virgin females and males and third instar, fully fed larvae will be exposed to microgravity (both in the Spacelab, as well as on the ground in a horizontal clinostat) for 8 days (permitting the development of a second generation of F1 offspring in the case of the adult experiment). Counts will be made of the number of pupae produced and the percent of adult emergence, and longevity of the F1 offspring studied. In the case of the Earth-produced third instar larvae, comparable observations will be made for length of larvae period, percent of pupal development from larvae, and percent of adult emergence. At the cellular level, cytological observations are needed in space and biochemical studies of key brain and flight muscle after return from space of successfully emerging adults exposed to space and clinostat-simulated microgravity.

The use of the miniature strain is rather interesting since its reduced wings vibrate very rapidly, but its flight is very poor. Thus, if after F1 adult emergence this strain is returned to space, will its longevity be increased or decreased in accordance with Miquel's earlier findings that spaceflight-exposed adults flown on Earth prior to spaceflight show a reduced life span, whereas spaceflight-emerged adults show no reduction in life span over normal, Earth-emerged and only Earth-flown adults? The dunce strain, with a poor memory, is similarly interesting to test to determine whether its exposure to space microgravity might then have no effect on its flight behavior and related longevity after return to Earth's gravity.

Identification of gravity receptors concerned with spatial orientation, position, and geotropic responses can best be done at ground level by ablation (extirpation) and by neuroelectrophysiological confirmation, including the halteres, especially in the wild and miniature (adult) strains. Comparable studies of the effects of microgravity or hypergravity could then be made both at ground level and in space, of ground-eclosed and Spacelab-eclosed adults, of intact and haltereless

adults of all three strains. The panel would also like to see a low-g centrifuge developed for in-flight use both as an on-board 1 x g control condition and to explore gravity threshold responses (variable-g centrifuge). Similarly, a study of the effects of exposure (of both virgin parents allowed to mate within the clinostat as well as of fully fed third instar larvae) in a clinostat aboard a Spacelab might be an interesting alternative study, apparently never contemplated in previous spaceflight experiments.

By reducing the number of species to the four species (D. melanogaster, nematode, yeast, and Neurospora), one can narrow the focus to an in-depth concentration on species for which so much is known about normal reproduction and development. The short life span, the economy of space requirements and care, and the limiting of the number of new space modules that would be required for follow-up experiments would be minimized.

#### EQUIPMENT

As to equipment and other spaceflight facilities needed, aside from locker-type, temperature/humidity/light-controlled "cabinets" shielded from likely radiation influence, the equipment list should include on-board low-g centrifuges, clinostats (for comparison to ground-based clinostat studies), infrared video to observe (record) behavior, and time-lapse video microscopy to study early postfertilization egg development.





## AQUATIC INVERTEBRATE DEVELOPMENT WORKING GROUP

D. Meyers, Chairman

### INTRODUCTION

Little definitive evidence exists to show that gravity plays a major role in embryogenesis of aquatic invertebrates. Two reasons for this may be: 1) few studies have been done that emphasize the role of gravity (e.g., Lobster (Homarus)) statocyst development by C. W. Prentiss (Prentiss, C. W.: Bull. Mus. Comp. Zool. Harvard, vol. 36, no. 67, 1900), and 2) there simply may not be any gravity effect. The buoyant nature of the aquatic environment could have obscured any evolutionary effect of gravity. The small size of most eggs and their apparent lack of orientation suggests reduced gravitational influence. Therefore, we recommend that the term development, as applied to aquatic invertebrates, be loosely defined to encompass behavioral and morphological parameters for which baseline data already exist.

### PROPOSED EXPERIMENTS

In the defined context we have isolated two areas of promising research. The first deals with behavioral changes that occur as a necessary requirement for normal larval settlement and metamorphosis. Once larvae become competent to metamorphose, many species undergo a behavioral change. These changes include different tropisms or taxes in response to gravity, light, pressure, and currents. For example, many bryozoans, ascidians, and cnidarians demonstrate photo and/or geotropisms that eventually result in settlement onto a desirable surface. This stage is followed by metamorphosis that is triggered by a physical or chemical factor on the substrate. Such settlement systems are well known to Dr. Bonar, one of the panel members.

Chorymorpha, a marine cnidarian, presents another opportunity for promising investigation. After dropping onto the bottom as an egg, the developing animal puts down holdfast filaments into the substrate. At the tip of these stolon-like outgrowths are statocysts, each with a single statolith that lacks both cilia and neurological connections. This cnidarian appears to be a unique animal system analogous to many plant-growth orientation systems. As the cnidarian grows, more filaments are directed downward, regardless of body orientation. Dr. Campbell, another panel member, has conducted these initial observations.

### PROBABLE AREAS OF SUCCESSFUL RESEARCH

Investigation of cytoplasmic localization in relation to egg polarity and future embryonic organization of aquatic invertebrates offers an opportunity for comparisons to the well-established amphibian system. Egg rearrangement in some species is similar to that of amphibians, but with the advantage of undergoing more elaborate localizations. Suitable animals for study would be tunicates and annelids that produce larger eggs, thought to be more susceptible to gravitational effects.

Gravity receptor development could be of fundamental importance in interpreting the initial influences of gravity. Although no definitive evidence exists to directly support this approach, we believe it is an important topic to explore. As with larval settlement, ethology should be an essential component in analysis with the aim of correlating receptor development to behavioral responses. Two systems appear to offer potential based on previous studies. The first, Aurelia, a medusoid cnidarian, metusoid cnidarian, metamorphoses from polyp to ephyra stage and then to medusa. Each stage has its own orientation and associated behavioral patterns, and implies a modification of the statocyst system. Dr. Spagenberg specializes in Aurelia biology. Other animals that may be appropriate are gastropods and tunicates.

A second potential system is the rheoceptive system of Daphnia, a freshwater microcrustacean. Similar to the juvenile moths birthed in space, juvenile Daphnia born into simulated microgravity or neutral buoyancy appear to maintain directional movement not demonstrated by their parents born in 1 x g. This observation implies a developmental response to microgravity that could be studied histologically, morphologically, and behaviorally. Associated neuromuscular coordination would provide an excellent companion study. Panel Chairman Dr. Meyers discovered and continues to work on the gravity-dependent rheoceptive system of Daphnia (ref. 1).

Life history studies would be appropriate for all the systems described. Although little information exists, it is just that fact that should support further study. The ossification process is known to be affected in mammals exposed to microgravity. In contrast, the response of invertebrates (i.e., spicule formation) to microgravity is unknown and is an area which warrants investigation.

#### EQUIPMENT

All of our proposed experiments would require similar equipment. Environmental control vessels of aquatic containment (ECVAC) would be required to provide temperature, light, and gas composition controls. A high-magnification, high-resolution, infrared, video-monitoring system with real-time and time-lapse capabilities is essential. A method of fixing and preserving animals on the Shuttle is also required. A 1-g centrifuge for in-flight experiments would be an important experimental component. Beyond this, each experiment would have its own specific equipment needs.

#### CONCLUSION

We believe that little is known about the effects of gravity, or the lack thereof, on aquatic invertebrates because of limited opportunities in the past to isolate gravity as a variable. Encouragement of NASA's Gravitational Biology Program through this meeting and subsequent workshops will contribute to an increasing awareness of the potential of space research. But the lack of baseline data will be best addressed by conducting promising, exploratory experiments in the middeck locker section of the Space Shuttle. Even though such experiments may be viewed as inefficient, we believe NASA should commit itself to a small percentage of these studies on each flight. However, we do not wish to be misinterpreted. Most experiments carried on the Shuttle should be well supported by ground-based

studies. Some systems, though, defy efficient ground-based study and would require exploratory examination.

We would also like to emphasize our awareness of alternate systems that may prove to be as promising as those systems listed. The cell lineage of certain nematodes is well known and would provide easily observable evidence of potential gravitational effects. Sea urchins and their calcification system could be susceptible to gravity. Biochemical genesis as well as morphogenesis could also be considered in terms of enzyme production and muscle usage. Certain ascidians have tissue types with specific molecular markers that would allow gastrulation movements to be followed in connection with any gravitational effect. Therefore, we believe that areas of productive space experimentation with aquatic invertebrates will be dependent on a combination of well designed ground and flight experiments, as well as exploratory flight experiments based on promising systems.

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## REPORT OF THE MICROBIAL DEVELOPMENT WORKING GROUP

G. Nelson, Chairman

### INTRODUCTION

In formulating our ideas on the relationship of gravity to the development, growth, and reproduction of microorganisms, we have used a rather liberal definition of microorganisms which includes bacteria, yeasts, protists, filamentous fungi, and single cells in culture.

### EXPERIMENTAL ADVANTAGES OF MICROORGANISMS

A principal advantage of microorganisms as experimental subjects is the rigor with which they can be defined and controlled. As single cells, each cell may be regarded as identical to the others in the population. This property applies to the morphology, physiology, and genetic parameters of the cells. The growth and development of the population is subject to precise manipulation as the nutritional requirements are known and minimal media formulations have been developed. Growth and differentiation can be manipulated in a variety of ways, such as alteration of the culture temperature and food supply, or by use of mutants. Finally, the short generation times of microorganisms provide the opportunity to conduct multigenerational studies within practical time limits and, in a similar vein, cellular responses to various stimuli or stresses are conveniently monitored because of the rapid response times of single cells.

### EXAMPLES OF GRAVITY RESPONSES IN MICROORGANISMS

Several examples of gravity responses were identified during our discussions, but this list is not to be taken as exhaustive:

1. In Phycomyces, the fungi exhibit a profound negative geotropism as well as phototropism and an avoidance response. A large spherical sporocarp is lifted as much as 20 cm above the substrate on a stalk which has a diameter of about 100  $\mu\text{m}$ . A large vacuole near the growing tip of the fungus may be displaced opposite the gravity vector and thereby displace other organelles involved in growth and cell wall formation. The geotropic response has both a fast component and a slow component. The response to gravity is not required for normal development.

2. In Schizophyllum, the basidiomycete fungus, a normal response to gravity is required for spore dispersal. Schizophyllum produces a sporocarp which grows upward or outward from the mycelial substrate. As the structure matures, the gills comprising the spore-bearing surface of the sporocarp must orient precisely vertically. Spores are released from the innermost surface between the gills and are trapped by any significantly tilted gill surface. Only spores discharged between nearly vertically oriented gills are able to exit and to eventually germinate on the ground below.

3. In Paramecium, animals preferentially accumulate at the upper surface of the liquid. This response is probably an indirect effect of gravity. Paramecia swim forward in a homogeneous liquid, but are more dense posteriorly than anteriorly. As a consequence, they will swim upward in a liquid where hydrostatic pressure gradients are formed under gravity. Upward swimming is not caused by differences in gas partial pressures at the interface.

4. Cyanobacteria and sulfur-utilizing photosynthetic bacteria are final examples of a microbial response to gravity in their adjustment of position in a water column. These organisms possess gas vacuoles whose dimensions are regulated; this result in turn regulates cell buoyant density. This response may be required to maintain cells in an appropriate aerobic or anaerobic environment.

#### CONCLUSIONS FROM PREVIOUS EXPERIMENTS AND THEORETICAL TREATMENTS OF CELL BEHAVIOR IN MICROGRAVITY

A variety of experiments have been performed on microorganisms in space. Most of these have been designed to assess the space radiation environment and/or to assess the potential synergistic effects of radiation and other flight variables such as gravity, acceleration, and vibration. We feel that these studies have provided some provocative observations, especially regarding cell proliferation, but are not completely reliable for several reasons. First, poor controls were used in growth experiments. For example, the multiplication of amoebae was measured after growth on paramecia, which in turn were dependent on hay infusions for their nourishment. Such monoxenic cultures show considerable variation. Second, growth studies used end-point data rather than kinetic data. Third, cell volume changes were measured after fixation with glutaraldehyde, which is known to cause shrinkage of cellular structures. Better experiments are required to substantiate the notion that cells in microgravity grow faster and require less energy than those in 1-g controls.

Theoretical treatments of gravity-mediated movements of intracellular particles were found to be largely unsatisfactory. The principal defect was in modeling cells as homogeneous solutions of viscosity equal to that of water. This is completely erroneous. Cells are filled with inhomogeneous thixotropic gels criss crossed by cytoskeletal filaments and tubules, which are themselves interconnected and capable of considerable force generation. Cytoskeletal components are in association with actively changing surface membranes. Furthermore, the integrated structure of cells is under the control of multiple regulatory circuits and amplifying mechanisms. It is suggested that less attention be given to the identification of statolith-type gravity receptors and that more consideration be given to the possibility that the entire cell is a gravity-perceiving entity which has considerable potential for amplifying small perturbations (e.g., gravity-induced membrane deformations) into cell-wide responses (e.g., membrane depolarization followed by calcium influx and concomitant cytoskeletal rearrangement or changes in gene expression).

#### IDENTIFICATION OF IMPORTANT QUESTIONS AND RECOMMENDATIONS

The Microbial Working Group's consensus is that the most important question to be answered regarding the influence of gravity on unicellular systems, "Can a

generalized eukaryotic cell detect and use gravity?" Four aspects of cell development should be addressed in this context: growth, response to stress, movement, and the establishment of anatomical asymmetry. A generalized eukaryotic cell (e.g., a fibroblast) should be the object of choice to address the generality of gravity responses in cellular development. Lower priority should be given to systems that are highly specialized or have amplified their responses to gravity.

The strongest recommendation with regard to future experiments is the requirement for well-designed controls. Specifically, 1-g controls should be performed in flight as well as in parallel on the ground. The use of mutants should be encouraged whenever possible as the use of mutant strains provides specific phenotypic properties and removes some of the variation induced by the need for manipulation of the environment. Elevated background radiation levels should be matched in all controls. Assays for gravity effects should be rigorously tested in ground-based studies using clinostats, centrifuges, or natural gravity before experiments are flown. A programmatic corollary is that ground-based experiments be funded without the requirement for in-flight follow-on studies.

#### EXAMPLES OF EXPERIMENTS

The following several experiments are suggested for further evaluation and design to probe the relationship of gravity to cellular development.

##### Stress Proteins

It is well documented that prokaryotic and eukaryotic cells respond to a variety of stresses by synthesizing a group of 20 or so proteins immediately after a significant stress is applied and then terminating synthesis after homeostasis is restored. These proteins are highly conserved in species as phylogenetically diverse as *E. coli* and rodents. Originally they were identified as "heat-shock proteins" in bacteria, but a long list of other stresses such as anoxia or rapid metabolic shifts also produce the response. We suggest that if cells perceive rapid changes in acceleration or gravity as a stress, then they should produce the stress proteins. These experiments could be done easily on the ground by centrifuge upshifts or downshifts followed by protein assay using two-dimensional gel electrophoresis or nucleic acid hybridization to detect specific message synthesis. Similar experiments could be performed on metazoans and might later be extended to microgravity.

##### Phagokinetic Tracks

A remarkable property of cultured mammalian cells and some protists is the symmetry of movement and cytoarchitecture of sister cells following mitosis. This property is most dramatically demonstrated using the phagokinetic track assay of Gunther Albrecht-Buhler. In this assay, cells are plated onto cover slips previously coated with a suspension of colloidal gold particles and allowed to proliferate, develop, and migrate. As the cells move, particles are removed from the substrate by filopodia or other leading lamellar analogs, leaving a cleared zone behind that serves as a record of the cells' movements. It is observed that sister cells exhibit mirror symmetry of their tracks despite the fact that they were

derived from a cell that rounded up prior to dividing. If gravity is required for establishing cell polarity from which such symmetry is derived, then centrifugation or reorientation of seeded cover slips with respect to the gravity vector should perturb this track symmetry. This is an appropriate system for a good ground-based study. A corollary is that staining of cells using fluorescent antibodies to different cytoskeletal proteins should reveal differences in cytoarchitecture directly.

#### Growth of Cells

Growth of cells in suspension and on substrates should be measured in real time using time-lapse video techniques and modern optical techniques based on light scattering. Isotope labeling of cells grown axenically is desirable and mutants should be employed to vary growth requirements or behaviors of the cells. Accurate measurements of oxygen consumption and ATP levels should also be made. The hypothesis to be tested is suggested by previous experiments in microgravity, namely, that the growth rate and energy consumption of cells is inversely proportional to the strength of the gravity field.

#### Growth Rate

A simple growth-rate experiment would be to use a so-called racetrack tube for measuring fungal growth. In this experiment fungi are inoculated into one end of a glass tube filled halfway (radially) with nutrient agar and allowed to elongate along the long axis of the tube. Growth is measured by the linear rate of extension of the fungal hyphae. The tube itself could be oriented with respect to gravity fields of various strengths, and mutants could be used to control nutritional and circadian responses.

#### Radiation-Hazard Test

A final recommendation for in-flight experiments is to measure the radiation hazard aboard the Shuttle or Space Station by the "Ames Test" or suitable analog. This test measures induced-reversion mutation rates in Salmonella and has been successfully used to identify environmental mutagens/carcinogens. These methods are new and were not available on early flights. A similar experiment with a eukaryotic organism is desirable.

#### EQUIPMENT REQUIRED FOR IN-FLIGHT EXPERIMENTS

The following instruments are necessary for in-flight experiments: a centrifuge with a gravity range of 0 to  $1.5 \times g$ , a compound microscope with phase and interference optics fitted with a videotape system, and temperature- and humidity-controlled incubators.

The question was raised whether the validity of clinostat simulation of microgravity should be addressed by testing a small clinostat with a sensitive biological subject (e.g., plant coleoptile) using a flight centrifuge aboard the Shuttle at 1-g equivalent acceleration. This test would determine whether the motions of the clinostat accurately average the effects of gravity or whether new motions of



biological components are induced by the device's activity. We recommend that this issue be debated by experienced flight hardware designers.

It is also recommended that investigators be provided with access to experienced flight hardware-design engineers in an interactive setting as early as possible in designing flight experiments.

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1. Report No. NASA TM-86756	2. Government Accession No.	3. Recipient's Catalog No.	
4. Title and Subtitle NASA DEVELOPMENTAL BIOLOGY WORKSHOP: A SUMMARY		5. Report Date September 1985	6. Performing Organization Code
		8. Performing Organization Report No. 85264	10. Work Unit No.
7. Author(s) Kenneth A. Souza and Thora W. Halstead,* editors		11. Contract or Grant No.	
9. Performing Organization Name and Address Ames Research Center Moffett Field, CA 94035		13. Type of Report and Period Covered Technical Memorandum	
		14. Sponsoring Agency Code 199-40-22	
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration Washington, DC 20546		15. Supplementary Notes *National Aeronautics and Space Administration, Headquarters, Washington, DC 20645. Point of Contact: Kenneth A. Souza, MS 239-17, Ames Research Center, Moffett Field, CA 94035, (415)694-5251 or FTS 464-5251	
16. Abstract  The Life Sciences Division of NASA as part of its continuing assessment of its research program, convened a workshop on Developmental Biology to determine whether there are important scientific studies in this area which warrant continued or expanded NASA support. The workshop, convened on May 2-4, 1984, in Arlington, Virginia, consisted of six panels, each of which focused on a single major phylogenetic group. The objectives of each panel were (1) to determine whether gravity plays a role in the ontogeny of their subject group; (2) to determine whether the microgravity of spaceflight can be used to help understand fundamental problems in developmental biology; (3) to develop the rationale and hypotheses for conducting NASA-relevant research in developmental biology both on the ground and in space; and (4) to identify any unique equipment and facilities that would be required to support both ground-based and spaceflight experiments.			
17. Key Words (Suggested by Author(s)) Embryology Microgravity Reproduction Fertilization Development		18. Distribution Statement Unlimited  Subject Category - 51	
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 92	22. Price* A05

**End of Document**