

Space Research with Intact Organisms: The Role of Space Station Freedom

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ABSTRACT

The study of intact organisms has provided biologists with a good working knowledge of most of the common organisms that have evolved in the 1 g environment of Earth. Reasonably accurate predictions can be made about organismal responses to most stimuli on Earth. To extend this knowledge to life without gravity, we must have access to the space environment for prolonged periods. Space Station Freedom will provide a facility with which to begin this type of research. Spaceflight research to date has been limited to relatively short-term exposures that have been informative but incomplete. This paper provides a brief background of known changes that have occurred in intact organisms in the space environment and proposes the kinds of experiments that are needed to expand our knowledge of life on Earth and in space.

INTRODUCTION

The greatest challenges and the greatest opportunities for space and gravitational life science research will come with the study of intact organisms. Such research will utilize many species, from simple prokaryotes through humans. They, with their multiple systems, have evolved over countless generations under the constant influence of the Earth's gravitational field into the familiar plants and animals that we recognize today. These organisms have been studied, analyzed, and dissected functionally, morphologically, and chemically by today's scientists and their predecessors. Most biologists would agree that we have a good working knowledge of most of the common organisms in our environment, at least at the organ and system levels. In recent decades increasingly sophisticated research tools have allowed scientists to probe more deeply into biological function. These efforts have begun to provide an understanding of basic mechanisms at the cellular and subcellular levels of organization. Reasonably accurate predictions can be made about animal responses to most stimuli on Earth. No such storehouse of knowledge exists concerning organismic response to the stimuli

found in space. Only within the past few years has there been the opportunity to study organisms exposed to the space environment, removed from an absolute environmental constant, "the force of gravity." The evolution of all life has occurred in the 1 g environment that our bodies recognize as the norm.

Viewed from another perspective, the law of gravity is the one law that cannot be broken, modified, or ignored as long as we continue to live on the face of the Earth. An excellent analogy to the problem of trying to study the effects of gravity while restricted to ground-based facilities was suggested by A.H. Brown. Imagine a student of the effects of light being unable to utilize darkness as a test paradigm. The student might modify the position of the light, or make it brighter (hypergravity), but could only turn it off for brief instants (free fall). Without the ability to investigate the role of darkness for prolonged periods, could the real roles of light with all their subtleties ever be established? For that reason the opportunity to examine the behavior and function of organisms removed from their hereditary gravitational environment is unique.

To date that opportunity has been more promise than fulfillment. There have been a number of preliminary descriptive reports of the immediate, short-term responses during and following exposure to the weightless and the combined weightless and high radiation environments of space. These studies have been informative and in many cases intriguing. Unfortunately, they leave many questions. In almost no instance have adaptive responses been carried to new stable endpoints. Developmental biology and multiple generational studies are still dreams awaiting the availability of long-term laboratories in space.

Space Station Freedom, even with its diminished capacity following restructuring, will provide a facility in which such studies can begin to be made. What could and should be studied? How can biologists most effectively utilize the life science research facilities on Freedom? The intent of this paper is to provide a brief background of the changes that have been noted in intact organisms exposed to space and suggest some examples of the kind of experiments

that might provide new and exciting information on the role of gravity in the evolution of life as we know it and how gravity has shaped function and morphology in every intact organism — terrestrial and aquatic.

THE EFFECTS OF SPACEFLIGHT

Humans

The intact organism that has been studied most often in both the American and Soviet, now Russian, programs is the human being. By and large these studies have dealt with problems of immediate concern to operational medicine. Certainly we can now recognize and even anticipate a number of acute and semi-chronic adaptive responses to microgravity. The most prominent changes can be directly related to the weightlessness that is a characteristic of spaceflight. Other changes may be due to anxiety, changes in activity, or generalized stress. It should be emphasized that many of the changes that occur are truly adaptations to a novel environment and are appropriate as long as one remains in space. There are no obvious major "in-flight" detrimental manifestations once the very acute alterations of the first few days, such as space motion sickness, subside. Real and potential problems become evident following return to Earth's gravity field.

Spaceflight produces many changes in the human body. Some are minor and both develop and subside in the first few days, such as motion sickness, which is present in about 60% of space travelers. Facial edema, decreased red cell mass, and a transient neutrophilia are also components of the early response to microgravity. The body's immediate responses also include shifts in fluid from the dependent portions of the body, as well as decreases in the size of the various water pools, including blood volume. The fluid shift to the upper body begins to occur in humans as they lie in a leg-elevated position prior to launch. The shift results in a condition that has been colloquially called "bird legs" because the shift greatly reduces leg girth.

There are changes in the cardiovascular system that are often described as deconditioning. In actuality, the appearance of deconditioning becomes apparent primarily following return to Earth, and is characterized by decreases in stroke volume, blood pressure, and an increase in heart rate.

Some of the changes that occur during spaceflight are more serious and occur more slowly, such as skeletal muscle atrophy, bone demineralization, and psychosocial problems. An increased potential for radiation damage is superimposed when the flight is into deep space instead of low-Earth orbit. Skeletal muscle and bone atrophy represent major long-term

adaptive responses that most investigators feel are analogous to disuse atrophy on Earth. It has been determined that the most prominent muscle changes are in the slow-twitch antigravity muscles, which are tonically active on Earth but not required for maintenance of posture while in space. Morphologically, individual muscle fibers are diminished in size. Functionally, based on enzyme concentrations, there is a switch from slow-twitch to fast-twitch fiber types. Similarly, the lack of weight-bearing stresses on the skeletal system in space decreases the need for large, dense bones. Massive remodeling of the skeleton is initiated with calcium mobilization dominant over calcium deposition. The result is an osteoporosis-like decrease in bone mass, which may be continued well beyond the one year that Soviet cosmonauts have spent in space. Overall, the rate of calcium loss from the body in humans is of the order of 1% per month. However, the loss is not uniform in all parts of the skeleton, and the complex changes may affect structure more than mass.

There are also alterations in the neurovestibular system. The most notable alteration occurs in the first few days of flight as a malaise that is variously called space adaptation syndrome, or more explicitly, space motion sickness. It is a transient response seen in over half of all space travelers. Although the specific etiology is still open to debate, there is a reasonable consensus that it is related to sensory conflicts between the visual and vestibular systems with additional central nervous system modification of the activity of the autonomic nervous system. Of greater consequence are more chronic central nervous system changes that do not appear to be manifested while in flight but become prominent following return to Earth. These include both sensory and motor effects such as altered balance and hand-eye coordination. A good review of the effects of spaceflight on physiological systems has been presented in the recent book by Nicogossian et al. (1989). Additional information is available in the Proceedings of the Space Life Sciences Symposium (1987).

Given the good hindsight present in most of us, many of these responses now seem eminently predictable. However, prior to spaceflight most of these changes were not particularly anticipated. With that as a background, how well are we able to foretell the responses that are likely to be seen in humans and other mammals maintained for prolonged periods in space or in reduced gravitational fields?

Other Animals

Although there are more data available on humans than on other organisms, there have been some studies conducted with plant and other animal spe-

cies. By and large data collected from mammalian vertebrates, such as non-human primates and rodents, indicate that their changes are similar to the responses seen in humans. Certainly there are differences in magnitude, but the basic adaptations are analogous. Bone loss, muscle atrophy, and cardiovascular and sensory-motor changes are evident following return to Earth. To date, inflight measurements, other than observational, have not been made on animals.

Much of the flight data on other organisms, although tantalizing, is fragmentary. Certain simple studies must be repeated or extended for longer times. It is not the intent of this paper to present a broad review of past and current space research on intact organisms, but rather to cite some examples as a prelude to defining our thoughts on where organismic space research is needed as the opportunity develops to utilize the facilities of the space station.

A study of the effects of five days of spaceflight on avian embryogenesis demonstrated that two-day-old embryos did not survive, although they continued to grow for the first day or two following launch. Conversely, nine-day-old chick embryos were capable of continuing their development and were ultimately hatched following return to Earth (Vellinger and Deuser, 1990). Calcium mobilization from the shell was not impaired in the older embryos and their growth following hatching appeared normal (Hester et al., 1990). However, they had a decreased vestibular response to gravitational stimuli (Jones et al., 1990).

In a preliminary experiment, it was found that encysted brine shrimp (*Artemia*) embryos, when activated in space, grew and developed normally for the rest of the flight. Hatching and survival rates were comparable to ground-based controls (DeBell et al., 1991). Other invertebrates also appear able to develop in space. Jellyfish (*Aurelia*) polyps, when activated during spaceflight with iodine or thyroxine, undergo metamorphosis to produce free-swimming ephyrae that appear normal (Spangenberg, personal communication). Further, it has been reported that paramecia multiply more rapidly in space than do ground controls (Richoille et al., 1986). Based on the responses of these very diverse invertebrate species, it would appear that aquatic invertebrate development during a single generation is not adversely affected by microgravity. Conversely, invertebrate aging and longevity were detrimentally affected by spaceflight in a terrestrial organism, the common housefly (*Musca domestica*). The flight animals had a greater rate of mortality and an increase of brain lipofuscin (Marshall et al., 1990). Increased brain lipofuscin concentration is associated with aging in humans.

Plants

Plant growth is also affected by the microgravity of space. Most reports have indicated that development halts at or just before flowering. In general, both root and shoot growth has been less than seen in ground controls (Halstead and Dutcher, 1987). In only one instance have plants (*Arabidopsis*) been carried throughout a complete reproductive cycle with flowering and seed development (Merkys and Laurinavichius, 1983). Root growth in the absence of a guiding gravity vector becomes random, and no longer orients toward ground water and nutrients. A unique, recent report states that root growth is markedly enhanced during spaceflight with little influence on shoot growth (Levine and Krikorian, 1991).

Chromosomal aberrations are also more common in plants grown in space. Basic biochemical changes have been recorded. A number of researchers have noted decreases in starch-containing amyloplasts as well as the cell wall constituents, cellulose and lignin (Halstead and Dutcher, 1987). Corn and mustard spinach seedlings exhibited a decrease in the amount of starch in amyloplasts, with an increase in the number of lipid vacuoles. Fatty acid metabolism was also modified with a decrease in the C-18 unsaturated fatty acids and an increase in the C-16 saturated fatty acid (palmitic), which is more typically a component of animal fat (Lewis and Moore, 1990).

THE FUTURE — LONG-TERM EXPOSURE TO SPACE

The exploration of space is, and should be, a transitional, stepwise process. We must walk before we run and we must float a little in low-Earth orbit before we cast ourselves on the ocean of interplanetary space. As noted above, our knowledge of the effects of space on biological function is not only rudimentary and fragmentary, it is also, with only a few exceptions, based on very short-term exposures. In these brief excursions there has been little to indicate that adaptations have reached stable new set points. In many cases the assumption has been made that acclimation is complete, but that is more conjecture than fact.

Several important questions must be addressed concerning the effects of the space environment, both the lack of a gravitational stimulus and the presence of increased quantities of a unique radiation, on intact organisms. The first question involves the gravitational stimulus in a single life cycle. Here there is a distinct difference between plant and animal kingdoms. It has been repeatedly shown that germination and early plant growth are not greatly affected by the space environment, whereas maturation, flowering,

and seed production are clearly inhibited. What is not known is to what extent that inhibition is due to environmental factors other than gravity. Habitats with poorly engineered provisions for optimal plant growth and inadequate monitoring equipment will fail to expose the real effects of gravity. NASA needs to work closely with the plant science community to develop sealed habitats that will effectively isolate the gravity variable. For instance, light intensity, spectra, and duration are all important variables that must be measured and controlled. Plant hormones and byproducts such as ethylene have not been measured, due in part to resource limitations, but their lack or excess may significantly modify plant growth characteristics and the completion of maturation with viable seed formation.

In the animal kingdom, not only is there scant data on fertilization, differentiation, and embryogenesis, but later events in the developmental life cycles are unknown. In part the discrepancy is due to the difference in generation time. Only a few invertebrate animals have sufficiently short life cycles to allow generational studies with our current spaceflight systems. To date there are no data to support or refute the hypothesis that a vertebrate animal can come to sexual maturity and reproduce in space. We do not know whether gametogenesis will occur, the estrus cycle will be initiated, fertilization will take place, and, in the case of mammals, that gestation, parturition, and lactation will be normal. The one data point that we have on early avian embryogenesis indicates complete failure; all of the two-day-old embryos died within the first 48 hours. Conversely, amphibian embryogenesis was successful. *Xenopus* eggs fertilized in space developed into tadpoles, which subsequently underwent metamorphosis following return to Earth (Souza, personal communication).

Assuming that mammalian reproduction is possible through parturition, there will be other logistic problems associated with non-primate postnatal development. Imagine a litter of mice, or rats, or pigs, or puppies born in space. What kind of nest must we devise to allow the female access to her young for nursing? A unit or facility must be small enough to retain the young, yet allow the dam to enter for nursing and social interaction, and then leave to acquire food and water and eliminate body wastes, and still prevent the neonates from floating off. Such a unit will be a challenge to develop. Will the young be able to seek, find, and attach to the mammary gland to gain nutrition and the psychosocial interactions necessary for later life? Will the lack of the communal relationships of a traditional nest and the modification of early neonatal imprinting impair them as adults in their interaction with others of their species, as well as with humans?

The phenotypic changes seen on exposure to space are similar in plants and terrestrial vertebrates. There is a decrease in those morphological elements that are required to sustain the organism in the Earth's gravitational field. Practically and philosophically there is no difference between a decrease in cell wall lignin and cellulose, and a decrease in bone mass and atrophy of the antigravity muscles. Certainly the mechanisms are unique, but the fundamental changes are the same. Without a gravitational stimulus there is a decreased requirement for the structures that organisms have developed to support themselves on Earth and stand against the Earth's gravitational pull. What about aqueous organisms? We do not refer to the benthic animals that must support themselves against a gravitational field on the ocean's floor, but rather to the neutrally buoyant organisms that are free swimming. Would their morphologic development in space be modified? Is there a basis for suggesting that trout or shrimp depend on other than the resistance of their environment for bone and muscle development?

Today we cannot even say what the phenotypic expression will be in a terrestrial vertebrate conceived and grown to maturity in space. There is no predictive basis for describing the morphological changes that will occur. Bone and muscle mass will be diminished, of that there is no question, but relative changes in different parts of the skeleton and alterations in total skeletal muscle are conjectural.

Of even greater interest and concern is the generational stability of phenotypic expression. How stable and invariant is the gene pool when intact organisms are continually exposed to a new environment? For how many generations will adaptive change continue to occur? While F_1 , F_2 , and F_3 generations will express different phenotypes as they mature, will the changes be apparent at birth? How rapidly will individual adaptation be translated into a new genetic stability? This is an important question for both the plant and animal kingdoms. Will there be a difference? Are either plants or animals inherently more adaptable to dramatic climatic changes such as the removal of an heretofore environmental constant?

An even more basic question is, how adaptable are we? Certainly intact organisms inhabit almost all regions of the Earth, including many that at one time seemed too inhospitable for survival. The human race, in its development, has spread over most of the Earth's surface, thriving from Arctic to tropics, from mountain to lowland, from desert to rain forest. Based on our ubiquitous presence we could be commended on our adaptability. These adaptations, however, have occurred over countless generations. The very basic question is, can we or

other gravity-developed organisms survive and adapt in a weightless environment? Have intact organisms in general become too specialized, too dependent on gravity, to exist and conform to a zero gravity life?

There are two major reasons why the study of humans on Space Station Freedom will not provide the answers to basic questions of adaptation. First, there is the question of time. Time as a factor in chronic adaptation of even an individual on the space station is extremely limiting, to say nothing of generational effects. The nominal crew stay on orbit following permanent manning of the facility will be 90 days, perhaps extending to 180 days — essentially one half a year. We would hold that the adaptation of a mature adult human over such a brief period of his/her life span will not answer basic questions of adaptation, nor of our ability to adapt to an essentially gravity-free life. A life span of 90 years is not at all uncommon in today's world, and a small six-month segment will not provide definitive answers to the question of long-term adaptation. It is reasonably clear from the few Soviet cosmonaut exposures of longer than six months that adaptive end-points were not present in some measured systems such as bone.

The second reason deals with the fact that many of the recognized rapid adaptations to space living are seen as detrimental upon return to Earth. Sufficiently detrimental that a major program is being instituted — Biomedical Monitoring and Countermeasures — to insure that on Space Station Freedom humans do not adapt in ways that might prove detrimental to their subsequent life following spaceflight. To the extent that this program is effective, space adaptation will be not only reduced, but prevented in the human population. To understand chronic multigenerational effects of spaceflight, it will be necessary to utilize smaller animals with rapid reproductive cycles as models of the likely responses in our species.

Questions such as those posed in the preceding paragraphs need to be addressed. We need to establish research goals that will provide fundamental information on how gravity has and does shape life on Earth. A first step in providing some answers will come from utilization of the life science research facilities on Space Station Freedom. That facility will provide a beginning in the quest for basic information on the role of gravity in the development of life. It is, however, the next logical step.

As the space station is currently configured, in the assembly phase, which includes man-tended capability crews that will be present for limited periods while the space shuttle is present, the major research emphasis will be on materials research rather than life sciences. As permanent manned capability

is developed, the Space Station Freedom program will have a gravitational biology facility, and the centrifuge facility will be added with plant and animal habitats. With these components in place it will be possible to conduct experiments leading to answers to some of the biological questions raised above. The centrifuge is designed to provide long-term exposure to 1 g fields as a control, to condition plants and animals to the force of gravity prior to initiating experiments, and also to have the capability of exposing plants and small animals to variable g forces as might be encountered on the moon or Mars.

In conclusion, we have a rare opportunity. Simultaneously we can begin to exploit the space frontier and enhance our basic knowledge of life here on Earth. The ability to conduct long-term experiments with intact plants and animals, and to have a centrifuge for providing 1 g controls and for studying gravitational thresholds, will provide important new insights. Results emanating from such work will be used in countless applications which cannot be predicted at this time. Such has always been the course of major new enterprises.

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