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Animals and spaceflight: From survival to understanding

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Abstract

Animals have been a critical component of the spaceflight program since its inception. The Russians orbited a dog one month after the Sputnik satellite was launched. The dog mission spurred U.S. interest in animal flights. The animal missions proved that individuals aboard a spacecraft not only could survive, but also could carry out tasks during launch, near-weightlessness, and re-entry; humans were launched into space only after the early animal flights demonstrated that spaceflight was safe and survivable. After these humble beginnings when animals preceded humans in space as pioneers, a dynamic research program was begun using animals as human surrogates aboard manned and unmanned space platforms to understand how the unique environment of space alters life. In this review article, the following questions have been addressed: How did animal research in space evolve? What happened to animal development when gravity decreased? How have animal experiments in space contributed to our understanding of musculoskeletal changes and fracture repair during exposure to reduced gravity?

Keywords: Vertebrates, Development, Musculoskeletal, Weight-bearing, Fracture Repair

Introduction

Spaceflight officially began on October 4, 1957, with the launch of the Russian Sputnik satellite. In July 1958, the United States created the National Aeronautics and Space Administration (NASA) from the National Advisory Committee for Aeronautics. This review explores the contributions of animal research from the beginning of spaceflight to a dynamic program investigating the reduction of human spaceflight risks, and selectively presents data that best illustrate the uniqueness of the space environment. The following questions are addressed: How did animal research in space evolve? What happened to animal development when gravity decreased? How have animal experiments in space contributed to our understanding of musculoskeletal changes and fracture repair during exposure to reduced gravity?

The authors have no conflict of interest.

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How did animal research in space evolve?

Animal research in space evolved over time; it has been divided into three phases for this review: the early years, the research phase, and future animal research.

The early years

On November 3, 1957, Sputnik 2 was sent into space carrying the first animal[1]. The Russian dog, Laika, was instrumented and multiple physiological parameters were down-linked for the first 4.5 hours. The down-linked data showed that her physiological parameters returned to normal after insertion into orbit, indicating that she adequately tolerated launch and the space environment. Six other dogs were orbited by Russia between 1960-1961. This series of animal flights proved that short duration spaceflight in low earth orbit would be safe from a biological and medical perspective; these flights paved the way for the launch of Yuri Gagarin on April 12, 1961.

The launch of Laika spurred U.S. interest in orbiting monkeys as these animals could be trained to carry out tasks during launch, weightlessness, and re-entry. The first US nonhuman primate in orbit was the chimpanzee, Enos, launched on

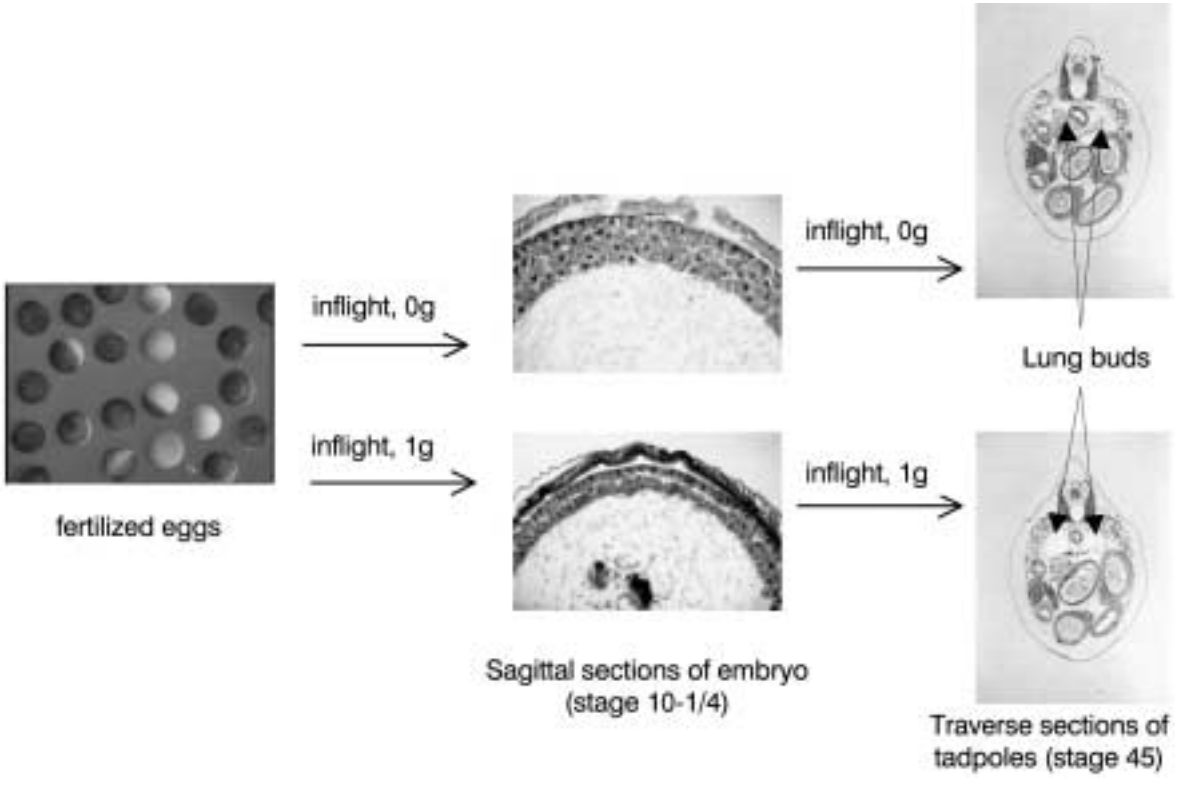

Figure 1. Fertilized frog eggs on Earth rotated so that the heavier vegetal pole was on the bottom and the lighter animal pole was on the top; this rotation was hypothesized to be required for normal development. In space, the eggs appeared to develop normally whether or not they rotated. Several interesting abnormalities were noted in histological sections taken from the embryos and tadpoles. In the embryos grown in microgravity, two extra cell layers were noted at the blastula stage and yet no gross abnormalities were found in the tadpoles. In the microgravity-grown tadpole, the lung buds did not appear to inflate. Pictures courtesy of Kenneth Souza, NASA Ames Research Center.

November 29, 1961[2]. Shortly after this successful mission, John Glenn was launched into space on February 20, 1962.

In summary, animals preceded humans at every stage of early spaceflight from suborbital to orbital flights. The early phase ended with the Apollo Lunar Missions (December 1968-December 1972).

The research phase

After the Apollo lunar series ended, the next 30 years (1973-2003) of animal research in space focused on categorizing flight physiological changes and their risks. During this period of time, spaceflight was limited to low Earth orbit, but innovative and often unexpected Life Sciences research results were emerging and were changing physiology textbooks. As the complexity of the scientific questions increased from the early survival/death questions to mechanisms of adaptation to space, a complementary increase in the complexity of the spacecraft and payloads was required. The pre- and post-flight animal data obtained from unmanned biosatellite missions were verified and elaborated upon by Shuttle Spacelab experiments that required in-flight animal dissection and tissue fixation. In-flight sampling avoided the complications of physiological readaptation during re-entry and the several hours that typically elapsed post-flight at 1-G before animals could be handled, although in-flight manipulations increased the complexity of the mission. Data obtained from Spacelab missions provided insights into physiological adaptation to space that were not possible with only pre- and post-flight sampling. Our present understanding of the physiological changes induced by spaceflight is derived largely from these three decades of animal space research.

Future animal research

U.S. animal experiments in space became limited after 2003 due to the retirement of Spacelabs (in 1998) and the Shuttle (by 2010) and the loss of research capabilities on the International Space Station (ISS) due to U.S. funding shortfalls. These changes necessitated an emphasis on payloads that could be automated to sample and to analyze specimens during the mission. While such automation is feasible with microbes, cells, and tissues, it presents very difficult problems

for vertebrates. A possible exception may be the limited use of the Mouse Drawer System (MDS) on ISS, a habitat under development by the Italian Space Agency. With limited animal habitats available for long duration missions, animal research on the ISS appears to be bound for a lengthy hiatus, creating a delay in obtaining answers to some of the major risks of long-term spaceflight, risks that ethically cannot be obtained with humans (e.g., radiation, wound healing). Delayed development of U.S. animal habitats promises to curtail the ability of scientists to resolve these important risks.

Summary

The early years of animals in space (1957-1972) focused on survival, safety, and performance; the driver for this phase was the race to the moon. Then (1973-2003), the program became more ambitious. Researchers investigated how animals adapted to the space environment to understand the risks to humans during long duration spaceflight; the driver for the research phase was to understand the physiological changes induced by spaceflight in low Earth orbit. Since 2003, with the retirement of Spacelabs and the postponed development of animal facilities for the ISS, animal research in space has been curtailed in the U.S. due to changes in NASA priorities; the driver for this phase is returning to outer space.

What happened to animal development when gravity decreased?

This question cannot be fully answered by spaceflight experiments conducted to date; however, studies with tadpoles, birds, and rats suggested that interesting developmental changes occurred in reduced gravity and indicated that gravity may play a crucial role in animal development.

Tadpoles

The most elegant and definitive developmental biology experiment in space[3] used the amphibian as a model (Figure 1). This experiment had an on-board 1-G centrifuge control. On Earth, scientists observed that fertilized frog eggs rotated upon sperm penetration so that the heavier vegetal pole was on the bottom and the lighter animal pole was on the top; this rotation was thought to be essential for normal development. Upon fertilization, the egg began to divide and formed the embryo that, after an appropriate time, emerged from the jelly-like envelope as a tadpole.

When female frogs were sent into space and induced to shed eggs that were artificially inseminated, the eggs placed in reduced gravity habitats did not rotate and yet, surprisingly, the tadpoles emerged and appeared normal. After return to Earth within 2-3 days of hatching, the tadpoles metamorphosed and matured into normal frogs.

Initial development appeared normal during spaceflight, yet some morphological changes in embryos and tadpoles did occur. The embryo had a thicker blastocoel roof (Figure 1) that would be expected to create abnormalities in the tadpole, but no deformities appeared, suggesting plasticity of the embryo. Also, the flight tadpoles did not inflate their lungs until they returned to Earth while the lungs of on-board 1-G controls were normally inflated (Figure 1). The lungs of flight animals appeared normal by the time the tadpoles were 10 days old, approximately a week after they returned from space. This finding raised multiple questions: If the lungs don't inflate and the animals remain in space, then would the gills remain as the tadpoles metamorphosed into frogs? If the gills resorbed without inflated lungs, would the defect be lethal? Why didn't the lungs inflate? We don't know the answers to these questions, but we do know that air bubbles were present in the tadpole aquatic habitat on orbit and that the 1-G on-board control lungs were normal (Figure 1). Possibly the flight tadpoles had difficulty finding and/or penetrating the air bubbles due to a lack of directional cues or other factors.

This developmental study produced multiple important findings[3]. It showed that vertebrates can be induced to ovulate in space and that rotation of fertilized eggs was not required for normal development in space. The flight-induced changes, including a thicker blastocoel roof with more cell layers and lungs that did not inflate, appeared correctable when the animals were returned to Earth at 2-3d of age in this experimental paradigm. In conclusion, the vertebrate embryo was very adaptive and the system was plastic, yet the long-term fate of the animal throughout its life in space remains unknown.

Quail

Adult quail on the Russian MIR space station adapted quickly to the space environment; they soared rather than flapped their wings for flight and they were able to firmly grasp the perch for normal feeding. On the other hand, fertilized quail eggs appeared to undergo normal embryogenesis in space, but serious problems occurred after hatching[4]. When a cosmonaut took a hatchling from its habitat, the chick appeared content as long as it was held. But once released, the bird first flapped its wings for orientation and began to spin like a ballerina, then kicked its legs causing it to tumble –it became a spinning ball. The cosmonaut noted that the chick would fix its eyes on the cosmonaut while trying to orient in space. When placed in their habitat, the chicks had difficulty flying to their perch to eat, and, unlike the adults, had difficulty grasping the perch for stability when eating. The hatchlings ate normally only when fed by the crew and would have died without crew intervention.

Rats

In rats, spaceflight influenced events underlying the postnatal development of motor function similar to those noted in hatchling quail[5]. Such events are probably dependent on the

age of the animal, the duration of altered gravitational loading, and the specific motor function. Neurolab (STS-90), a 16-day mission, and NIH-R3 (STS-72), a 9-day mission, included neonatal mammals that were launched into space on postnatal day 14 or 15, respectively. Data from the two spaceflights suggested that surface righting (i.e., the ability of the animal to flip over when placed on its back) did not develop normally in rats spending 16 days in space, but did develop normally in animals spending nine days in microgravity. Walton's data[5] suggested that there are critical development periods during which biomechanical loading of limbs was essential to give cues to nerves that innervate muscles. Without the cues, brain and limb innervations developed abnormally and animals developed abnormal locomotion. Dr. Danny Riley found delayed development of certain nerve connections to muscles in the Neurolab neonates. The connections returned to normal after landing on Earth, yet fibers in hindlimb muscles did not reach normal size even after a month post-flight. The Riley team found similar results in neonates that were not allowed to bear weight on their hindlimbs on Earth[6]. Their data suggested that biomechanical loading of limbs during early development might be essential for innervation of muscles and development of normal muscle fiber size.

Summary

These vertebrate studies suggested that embryonic development in frogs and birds proceeded normally in space, although unexplained changes occurred during embryogenesis and early development. In fact, developmental transition from tadpole to air-breathing frog could be lethal if the lungs did not inflate as the gills were resorbed. In birds and rats, biomechanical loading could be required for Earth-like development and innervation of certain structures. We learned that space habitats in which early development occurs might have to be designed very differently than conventional Earth cages. Without gravity, rat and bird neonates floated freely. Without a surface to crawl against, the animals thrashed about and their health degraded if the housing provided was too large and lacked appropriate surfaces for the animals to cling to. In space, animals are able to use all 3-dimensions of their habitat rather than the 2-dimensions available in Earth habitats. One might conclude that space habitats should be sized to the individuals, based on data suggesting that more confining habitats could protect neonates until they were able to grasp and walk. Only after development of appropriate motor function should cage size be enlarged to accommodate the mature animal. Cages that accommodate all stages in the life of vertebrates are critical if we are to understand the influence of gravity on development of vertebrate systems in a free-fall environment. If the habitat for a particular species was not compatible with survival throughout the life cycle, then evolution of that species would not occur in that environment. Experiments with frogs, quail, and rats need to be repeated and continued beyond the neonatal phase of life to determine if reproduction through multiple generations might be possible in the absence of gravity.

How have animal experiments in space contributed to our understanding of musculoskeletal changes and fracture repair during exposure to reduced gravity?

Male and female rats (primarily weanling, juvenile, or young adults) have flown on multiple spaceflight missions including the unmanned Russian Cosmos series (782, 936, 1129, 2044 missions lasting 14-19.5d), multiple Shuttle Spacelab missions including Spacelab 3 (STS-51B, 7d), Space Life Sciences 1 (STS-40, 9d), Space Life Sciences 2 (STS-58, 14d), Life and Microgravity Mission (STS-78, 17d), Neurolab (STS-90, 16d), and Shuttle mid-deck flights including adult male rats on STS-29 (5d) and female mice on STS-108 (12d). These missions form the basis of our current knowledge of the response of the musculoskeletal system to reduced gravity.

Skeleton

The musculoskeletal system on Earth has evolved to allow locomotion in a gravity field. Although changes in weight-bearing bones during spaceflight might be anticipated, there is no *a priori* reason to expect alterations in non-weight-bearing bones unless spaceflight induced a systemic response (Figure 2). Changes in mineralization rates and bone maturation in the lower jaw were reported following the 7d flight of juvenile rats aboard Spacelab 3[7]. In fact, the hinge of the lower jaw, which is loaded during eating but is non-weight-bearing, may be exquisitely sensitive to the altered loading environment in microgravity. Data from the periodontal ligament of the maxilla of juvenile rats flown on the 18.5d Cosmos 1129 flight suggested that precursor cells that become osteoblasts may differentiate at a slower rate in the jaw[8], and fewer osteoblasts would form less bone. These changes in the jaw suggested a systemic effect of spaceflight on the skeleton; however, they may also reflect a difference in muscle activity during spaceflight. On Earth, the jaw drops open with gravity, and muscle action is required to keep the jaw closed; during spaceflight, no muscle action is required to keep the jaw closed.

As anticipated, weight-bearing bones showed changes during flight. Bone formation at the periosteal surface of both the humerus and tibia was depressed about 40% during spaceflight[9,10]. Exposure to microgravity for one week suppressed bone formation[11]; within three weeks of spaceflight, growth in diameter in the long bones may have even ceased (Figure 3)[9,10]. Bone strength remained approximately the same as when the animals were launched into space rather than increasing as in Earth-based controls[12,13]. Spaceflight did not appear to affect bone elongation[14]. Interestingly, few changes in humeral cortical bone biochemical and mechanical properties were noted in young adult rats after 14d of spaceflight[15].

The amount of mineral added per gram of bone tissue was the same whether the rat was growing in space or on Earth[16].

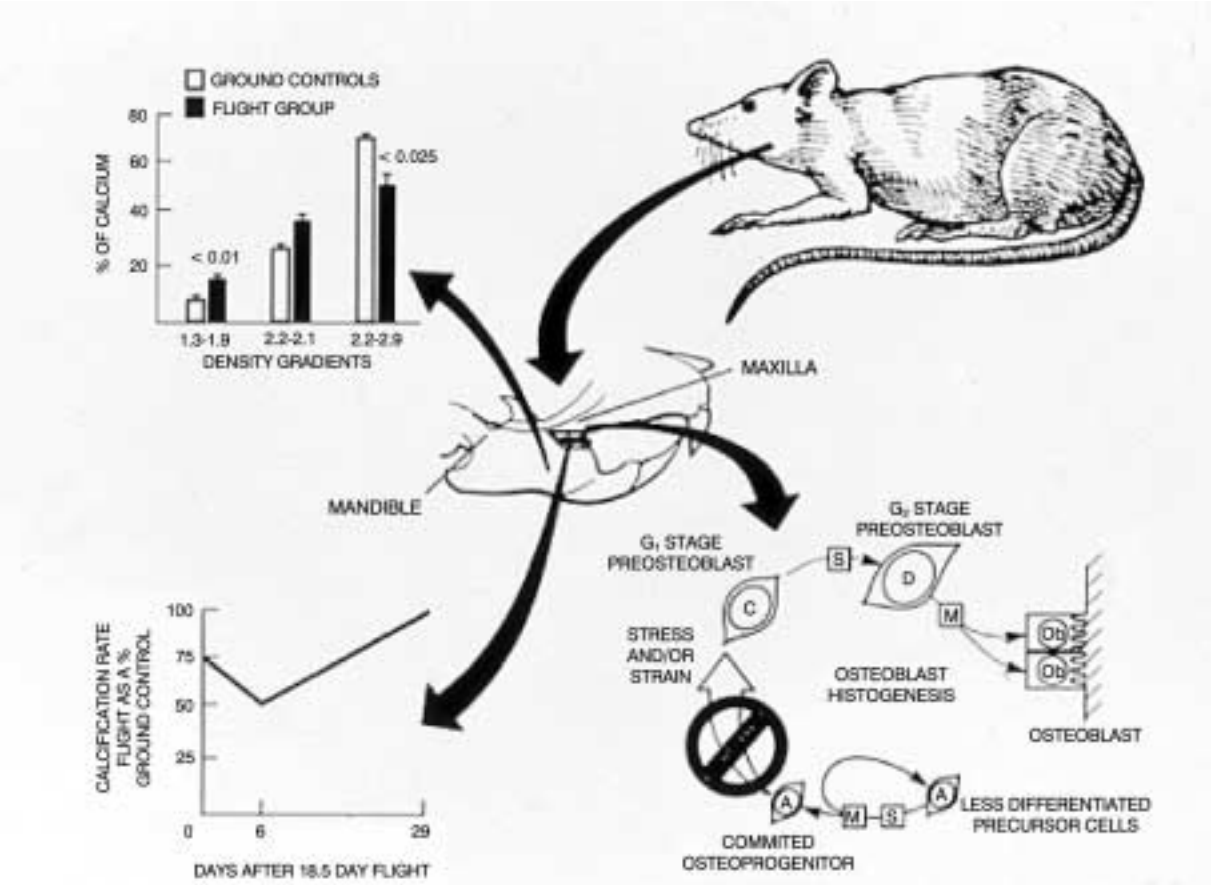

Figure 2. This figure depicts some of the flight changes reported in the jaws of growing male rats. The panel in the upper left shows data from Simmons et al.[7]. Calcium measured in three different density fractions shows a significant shift of calcium into the least-dense fraction and a significant decrease of calcium in the most-dense fraction indicating that the bones from flight rats are less mature than the bones of the ground controls. The lower left panel shows that the rate of mineralization or calcification is decreased about 25% in alveolar bone around the mandibular molars during spaceflight[7]. The lower right panel shows a scheme of production of osteoblasts[8]. The less-differentiated precursor cells (A) are stimulated to become bone cells. DNA is synthesized (S) and the cell divides (M=mitosis) to form a committed osteoprogenitor cell and, perhaps, another less-differentiated precursor cell. When a load is placed on the cell, the size of the nucleus increases (C to D) as the cell passes through various growth (G) and synthesis phases before dividing to become two osteoblasts. Continuous renewal of the osteoprogenitor pool is necessary to maintain normal bone formation. During flight, stimulation of committed osteoprogenitor cells may be negligible, causing an increase in this cell pool and a decrease in the number of C/D cells. Following flight, several days elapse before bone formation returns to normal which could reflect a delay in completing the cell cycle.

The total amount of the major bone protein, collagen, was normal, but collagen concentration may be slightly increased during flight[16]. Cytoskeletal elements in the osteoblasts, aligned to direct and orient collagen fibers as they leave the bone cell, may be disrupted in space; this malfunction could alter the ability of collagen to form a stable structure[17,18]. The organization of the organic matrix in flight rats may be partially responsible for the smaller size of the bone crystals[7,17]. Some of the blood vessels just beneath the outer surface of bone appeared to be blocked with debris in flight rats, probably decreasing the blood supply[18]. This change may have contributed to localized bone changes. The collagen organization, the smaller crystals, and the change in blood flow in growing rats during flight could have added mass and mineral to bones without increasing strength or stiffness.

When growing rats were spun on a short radius centrifuge at 1-G during flight, bone strength increased similarly to experimental control rats even though bone formation was still suppressed. Centrifuged rats ate less food and grew more slowly than either flight or experimental control rats; the decreased rate of bone mineralization may have reflected the slower growth rate rather than any change in bone structure[13]. Also, centrifuged animals formed bone immediately upon return to Earth while non-centrifuged flight rats showed no evidence of bone mineralization for several days post-flight.

Young animals grow rapidly; therefore, both bone formation and resorption rates are extremely high. In these young

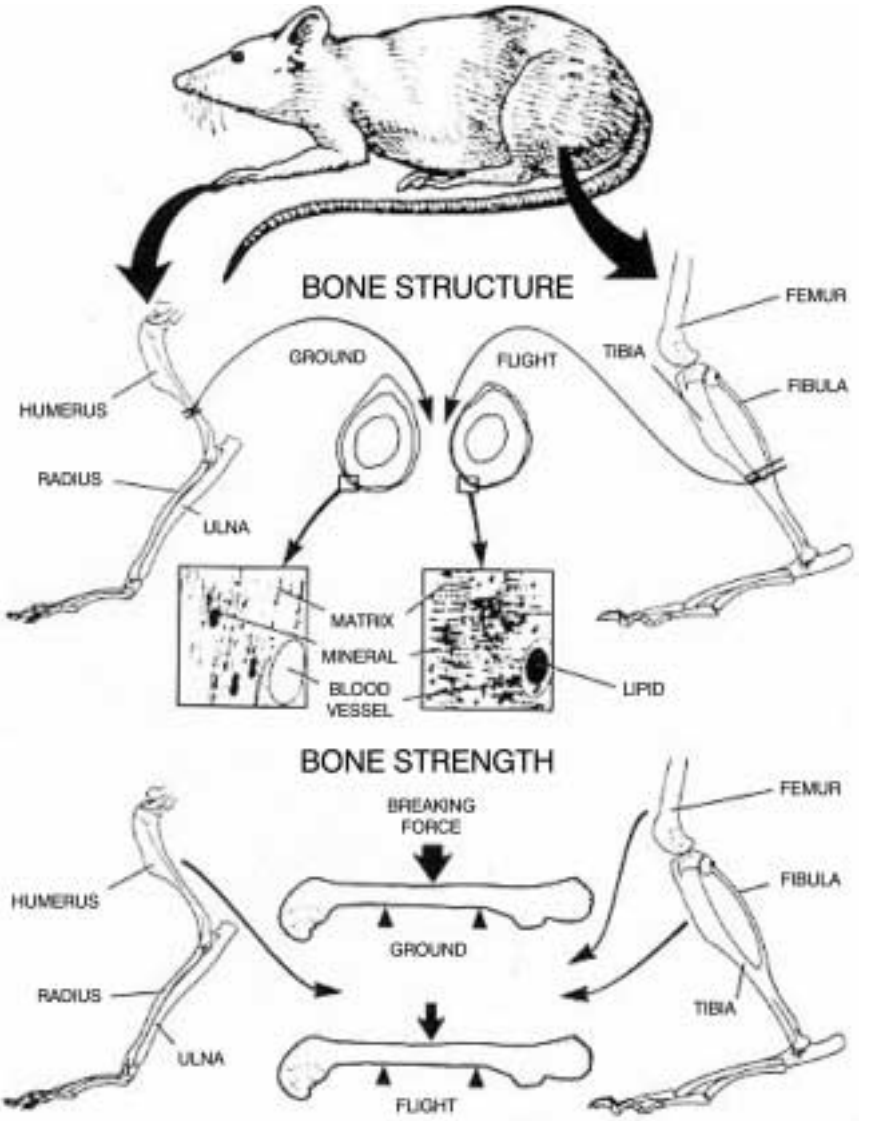

Figure 3. During spaceflight, changes have been noted in both the forelimbs and hindlimbs of growing male rats. Studies on the humerus and tibia have shown a decrease in the amount of bone formed during flight. During the same period of time, the ground controls form about 45% more bone at the surface of the bone shaft. If a similar microscopic area from each section is enlarged (see boxes), further differences between the flight and ground control rats can be seen. In the flight bone, surface blood vessels appear to be blocked with debris and lipid deposits, the mineral may aggregate in smaller crystals, and the collagen may be somewhat disorganized in a convoluted pattern. These changes may be responsible for the changes in bone strength. Data from the humerus, femur, and tibia suggest that the flight bone is about the same size as the ground control bone, yet bone strength is significantly less in the flight animals.

rats, the primary bone defect associated with decreased loading appeared to be a suppression of bone growth in diameter rather than bone loss. Interestingly, weanling male rats flown for 17d showed virtually no bone changes[19]. Cosmos 2044 carried young adult male rats (109d of age); fewer changes were reported during the 14d mission than occurred in younger rats[15,18,20-22], yet lumbar vertebral disks were smaller in the flight group[22]. Data from this flight were compared with ground controls and few skeletal differences were noted[23]. Several excellent reviews have been written recently on the bone response to flight[24,25]. Spaceflight studies with adult rats (at least 6-months-old) are needed to compliment the data available from these younger animals, if we are to understand the risks of long duration spaceflight to adult bone.

Skeletal muscle

Skeletal muscles exert forces that allow the skeleton to move the body or to maintain a position. During growth, muscles increase in size to support the growing skeleton.

When loading forces decrease in a growing animal, postural muscles initially atrophy while other muscles either are not affected or grow more slowly. The decrease in muscle mass during unloading in such animals can be attributed primarily to a change in the size and/or number of muscle fibers. During short duration spaceflight in growing rats, the proportion of muscle fiber types change. Fast-twitch fibers, used for fine skilled movements, become predominant so that the postural slow-twitch fibers appear to decrease. This change will cause about a 15% loss of muscle mass after a spaceflight of one week in rat muscle primarily used for postural support. For example, the soleus muscle in the lower leg of Spacelab 3 flight rats decreased about 15% from the basal muscle mass and 35% from the flight controls, indicating atrophy rather than merely decreased growth in this postural, primarily slow-twitch muscle[26]. In contrast, the gastrocnemius, a primarily fast-twitch muscle in the lower leg, decreased about 20% from ground controls, but increased about 10% from the basal muscle mass, indicating only growth suppression and not atrophy. True muscle atrophy (loss of muscle mass from the initial size), rather than a decrease in normal growth, occurred only in gravity-sensitive or postural muscles during spaceflight. Cosmos 2044 data from multiple investigators[23,27-34] defined multiple muscle changes that occurred during this 14d flight; the changes included preferential atrophy of extensors over flexors; transformation of slow fibers into fast or intermediate fibers; reduced protein synthesis and increased degradation; primarily contractile protein lost; decreased force, power, and locomotor co-ordination; disproportionate loss of actin thin filaments to myosin thick filaments; increased fiber velocity from increased expression of fast myosin isozymes and shortening of slow-twitch muscles, and post-flight eccentric contraction-like lesions, connective tissue tears, and microcirculation thrombi. Two-month-old female mice also exhibited muscle atrophy, but unlike rats, the mice showed only minimal changes in isoform expression and a significant decrease in muscle oxidative capacity after 12d in space[35]. Excellent reviews of skeletal muscle adaptation to spaceflight include those by Adams, Caiozzo, and Baldwin[36], Baldwin and Haddad[37], Desplanches[38] and Fitts, Riley, and Widrick[39].

Fracture repair

Only two spaceflight studies have investigated fracture healing in rats[23,32,40,41]. Adult male rats on STS29 had a fibular osteotomy induced 5d before launch. Following the 5d flight, the authors reported that periosteal osteogenesis and vascular channel development were similar, chondrogenesis was less advanced, and angiogenesis in osteotomy gap was less advanced in flight rats as compared to ground controls[40]. The authors concluded that bone healing may be impaired during space travel and recommended long-term fracture repair studies in space. On Cosmos 2044, 110d old male rats received a central break of the fibulae and crush of the soleus/gastrocnemius muscle with hemostats 3d before flight[23,32,41]. Following the 14d flight, the authors reported that fibula callus volume was smaller, consolidated bone fragment strength was decreased, and muscle repair was less organized with more macrophages and blood vessels without chronic inflammation in flight rats as compared to ground controls. The authors concluded that fracture healing in space is inhibited due to decreased callus size and strength and that muscle repair is delayed and could be suboptimal if scar tissue forms.

Summary

The major findings regarding changes in the musculoskeletal system of animals during spaceflight include:

1) Young rats exhibited a growth defect; adult animals are more likely to show true bone loss.
2) Bone strength decreased over time in space.
3) Bone loss could be associated with muscle loss; muscle mass was lost more rapidly than bone during flight and returned to normal more rapidly following flight.
4) Functional/phenotype changes in postural muscle fibers could cause post-flight damage and altered locomotion.
5) Fracture healing may be delayed during spaceflight; however, to understand the fracture repair process during long-duration spaceflight the fracture should be induced during, rather than before, flight.

Conclusions

Animal research in space evolved from survival missions to complex scientific investigations that probed molecular mechanisms of adaptation to spaceflight. Animals in space have contributed significantly to our understanding of the effects of gravity on living systems including humans. The exciting, rapid, progress that was made from 1973-2003 was slowed thereafter due to priority shifts within NASA. We have been left with snapshots of spaceflight changes with fascinating findings and few answers about long-term low-gravity effects on individuals.

Developmental studies in frogs, quail, and rats suggested that the space environment presents unique challenges for survival, and that certain structures (particularly the weight-bearing musculoskeletal system) may become vastly different after multiple generations in space.

Spaceflight changes in the skeletal system have been documented particularly in the jaw and long bones of growing rats. Changes included suppressed osteoblast production, decreased bone growth and maturation, altered vascularity, decreased crystal size, altered matrix configuration, and diminished strength in bones compared to ground controls. The flight-induced alterations affected bone strength more than bone size. Bone growth and strength in space may be adequate for microgravity, but inadequate for return to earth's gravity after extended spaceflight. In particular, the bone changes suggested that the skeletal system in growing rats was very sensitive to lack of gravity, and might be an

excellent model system for elucidating microgravity-related responses that could have relevance to other organ/tissue systems. Postural, antigravity muscles lost mass and transitioned from a slow-twitch to a fast-twitch fiber phenotype in space. Strength and the proportion of slow-twitch fibers in postural muscles decreased during spaceflight. Decreased fitness of muscle fibers may be responsible for the rips and tears found following spaceflight. Fracture repair may be delayed in space if the defect is induced before flight; to understand the fracture healing process during long duration spaceflight, the fracture should be induced during flight.

Our understanding of the response of growing muscle and bone to spaceflight has increased significantly in the past thirty years, but many questions remain unanswered. The extent, duration, and type of muscle fiber or blood vessel changes during flight are not fully understood. Whether alterations in bone during spaceflight resulted from, or caused, changes in calcium metabolism is unknown. If and when bone mineralization ceased during spaceflight has not been adequately documented. Whether total musculoskeletal turnover is altered during flight or whether the effect of spaceflight on the musculoskeletal system is limited to specific tissues or specific areas within tissues begs for further study. Whether muscle contributed to, or caused, any of the bone changes has not been studied. Whether the low-gravity induced changes can be fully reversed following return to Earth after long duration flights is not known.

Adapting to spaceflight is not the issue; returning to Earth is. Spaceflight seems to validate the Functional Use Hypothesis, which is: use it or lose it[42]. How much bone/muscle strength can be lost in space before return to Earth becomes impossible? We can venture into space, but could we come home after multiple generations?

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