Structural Development of Bone in the Rat under Earth Gravity, Hypergravity, and Simulated Weightlessness

JOHN P. JANKOVICH*
Naval Ammunition Depot, Crane, Ind.
AND
KARL O. LANGE†
University of Kentucky, Lexington, Ky.

Theme

HYPODYNAMIA characterized by prolonged inactivity, such as immobilization, bed rest, or weightlessness, adversely affects the normal functioning of the skeletal system in man primarily through the homeostatic adaptation of the human body to the absence of mechanical stresses of weight bearing and counter-gravitational muscular activity. Immobilization, bed rest studies, and spaceflight observations evidence such debilitating effects; whereas physical activity tends to foster bone development: increases bone weight, density, breaking stress, and growth. This study attempts to determine the role of mechanical stress in the structural development of bone and to discern the applicability of artificial gravity in prevention and alleviation of disuse bone atrophy.

Content

In order to be able to study bone development under stress, the mechanical properties of bone in normal aging under Earth gravity had to be determined as a first step. The effects of exposure to hypergravity were then explored. From the musculoskeletal standpoint, immobilization partially simulates the state of complete inactivity and weightlessness. Consequently, exposure to Earth gravity and hypergravity following immobilization indicates the probable direction of bone development when similar mechanical stresses are applied to bone in space and in other forms of prolonged inactivity.

Methodology

In a state of reduced activity, such as encountered in weightlessness, the skeleton can be exposed to mechanical loads by providing an artificial gravity with continuous centrifugation. This is a steady load, very similar to the gravity of Earth and animals can survive and grow under a wide range of hypergravity produced by chronic centrifugation. In this investigation rats were exposed to 1.5 and 2.0 g (at 39 rpm) for two months and to 2.5 g (at 30 rpm) for up to eight months, 24 hr/day. In order to trace bone development, sample groups were sacrificed successively in 30 and later in 60 day periods. Exposure to 2.0 g hypergravity, after five weeks of immobilization, lasted for another five weeks.

Male Sprague-Dawley rats were used in the study because the relatively short life span of the rat makes it possible to embrace a considerable part of the life cycle within a reasonable period of time. Moreover, successive sacrifice of groups of several animals, necessary to obtain statistically significant results, required a large population of subjects. For immobilization, a plaster cast was put on the right hind leg under general Nembutal anesthesia. The cast surrounded the lower and upper part of the extremity completely and was extended in a funnel shape to partially cover the pelvis. The leg was left immobilized for five weeks. The animals started using their legs 2–3 days after liberation. Exposure to centrifugation started on the third day after removal of the cast.

The following parameters, which influence the response of bone to stress and strain, were measured in the femur: a) physical properties—weight, geometry (volume, length, cross-sectional area), density; b) mechanical properties—compressive elasticity, torsional elasticity, microhardness; and c) histological and physiological properties— porosity, ash content, calcium content, mode of mineralization (labelled by deposition of tetracycline).

Results

At Earth gravity the normal process of aging takes place and the properties of bone change significantly during this process. These changes were measured over the rat's life span of from 2 to 10 months. Body weight growth was found to be a logarithmic function of age, i.e., body weight gain is substantial in early age and becomes smaller with increasing age. Certain structural properties of bone display a similar trend of gain at a high rate during early age and lower rate later. The development of weight, volume, density, longitudinal, and cross-sectional growth of bone follows this pattern. During aging at Earth gravity bone steadily loses elasticity, i.e., it becomes more brittle, as reflected in increases of the modulus of elasticity, torsional spring constant, and microhardness. The rate at which rigidity increases, unlike the growth rate, does not diminish during the 8 months of observation and is attributed to the progressive increase of ash content of the bone. The ratio of inorganic constituents to total bone material becomes larger with age as a larger portion of the bone matrix mineralizes, resulting in a stiffer, more brittle material. Porosity of bone does not change with age during the life span investigated here. It should be noted, however, that porosity was measured in the vicinity of the distal end of the femur. At this location the cross-sectional area was found to have the least rate of growth and the area of the marrow cavity was found not to change appreciably with time. These facts indicate that the rate of bone remodelling is less intensive at this testing site than elsewhere along the femur. The ash content of the bone is not uniform along the femur. The hip joint contains 4% more ash than the knee joint. This difference appears to be significant since it persisted throughout the investigation without any tendency to disappear. There is no difference, however, in the calcium content of the two joints, indicating that

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* Research Engineer.

† Professor of Mechanical Engineering. Associate Fellow AIAA.

the hip joint contains more inorganic components other than calcium than the knee.

Chronic exposure to centrifugally generated hypergravity up to 2.5 g does not affect normal development of bone: the structural properties are essentially identical to those observed under Earth gravity in animals of comparable age although the function of body weight gain of centrifuged animals is significantly lower than that of the controls. Other investigators have found that soft tissue organ development and function are not altered by chronic centrifugation either and attributed the characteristic weight decrement to a reduction of fat tissue. The findings of this study complement these investigations by establishing that hard tissue development is essentially normal under hypergravity conditions. Only two effects of hypergravity are observed: the active zones of mineralization tend to be wider, and the longitudinal growth of femur and tibia is slower. The decrement in bone length can simply be attributed to the smaller stature of the centrifuged animals, or, possibly, to the inhibitory effect of increased gravitational load. The apparent variation in the width of the tetracycline bands, which marked mineralization, might not be real because in the geometrically shorter bones the zones of fresh bone deposition at a specified distance from the distal end could appear differently than in bones of larger size.

Plaster cast immobilization of one leg, which served as simulation of the state of weightlessness, significantly influences body weight and bone development. The rate of body weight growth is slower than normal during immobilization and somewhat faster than normal after immobilization. The development of some bone parameters is also retarded: weight, volume and density consistently have lower values during immobilization than during corresponding normal aging. Lower bone density is largely due to the reduction of ash content found in the immobilized knee joint. Calcium content of the immobilized knee joint is also lower than that of the free knee; the calcium content of the hip, however, was found to be identical in both the immobilized and the free leg. Growth of the cross-sectional area is retarded in the vicinity of the knee joint only. Porosity in this region decreases which may mean that in disuse some of the vascular channels become inactive and occluded with mineralized connective tissue. Compressional and torsional rigidity and microhardness become higher than normal during inactivity, i.e., acceleration of the aging process takes place. However, after the bone has been freed, the aging process is suspended: the stiffness level remains constant until the normal process of aging reaches the state of bone development produced prematurely by the accelerated aging of immobilization. Most effects of immobilization disappear within 5 weeks after removal of the cast. Exposure to hypergravity, following immobilization, retards the growth of body weight but does not interfere with subsequent return of all bone parameters to normal level.

The measurements made in this study clearly show that chronic exposure to hypergravity up to 2.5 g 1) has no adverse effects on bone development in normal rats and 2) permits regain of normal state of development in previously immobilized bone comparably to Earth gravity. Thus it may be assumed that, in the absence of Earth gravity and in hypodynamia, centrifugation could safely provide the condition for normal bone development. The information obtained in this study applies to bone in the rat. It may well represent general trends, but before extending it to apply to other species, and especially to man, due study and consideration should be given to the respective differences in construction, organization, development and mechanical behavior of bone.

The stress environments which are of specific practical interest in connection with manned space flight lie, of course, in the range between weightlessness and Earth gravity. This laboratory study, of necessity, had to restrict itself to artificial gravities greater than 1 g and an imperfect simulation of weightlessness by plaster cast immobilization had to suffice. No significant and systematic changes were found above 1 g; however, simulation of weightlessness was found to produce pronounced atrophy in bone. Thus it does not seem possible to make simple extrapolations from the above 1 g range to the below 1 g range, since it is likely that there exists a threshold stress above which bone development is essentially normal, while atrophy occurs below the threshold. Space experiments will be needed to clarify this point.