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STRUCTURAL DEVELOPMENT OF BONE IN THE RAT UNDER EARTH GRAVITY, SIMULATED WEIGHTLESSNESS, HYPERGRAVITY AND MECHANICAL VIBRATION

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16. Abstract

Biophysical properties of bone in the femur and tibia of the rat were measured during eight months of exposure to earth gravity, hypergravities of 1.5, 2.0 and 2.5g, produced by continuous centrifugation, and during four months of exposure to mechanical vibration. Plaster cast immobilization of one hind leg was applied to simulate the hypodynamic state of weightlessness. Animals were successively sacrificed to determine the physical, mechanical and physiological parameters of bone. Mineralization was traced by periodic administration of tetracycline. Radiographic densitometry was employed in vivo and in vitro to follow up bone development.

At earth gravity normal aging takes place while physical dimensions, density, rigidity, microhardness, sound conductivity, and ash content of bone increase. Bone porosity and calcium content remain constant.

Rats under hypergravity have smaller rates of growth than rats at 1g. The differences are due entirely to differences in fatty tissue. Bone development as a function of age was found to be unaffected. Chronic vibration increases stiffness and microhardness of bone. In vibrated bone the active fronts of mineralization disappear and deposition becomes dispersed across the diaphysis. Immobilization significantly decreases bone density, ash and calcium content; immobilized bone becomes less porous and more brittle than normal.

In the hypergravity range investigated, no significant and systematic changes were found; however, simulation of weightlessness was found to produce pronounced atrophy of bone. Thus it does not seem possible to make simple extrapolations from the above 1g range to the below 1g range, since it is likely that there exists a threshold above which bone development is essentially normal while atrophy occurs below the threshold.

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ABSTRACT

Structural Development of Bone in the Rat Under Earth Gravity, Simulated Weightlessness, Hypergravity and Mechanical Vibration

Biophysical properties of bone in the femur and tibia of the rat were measured during the life span of from two to ten months under earth gravity, hypergravities of 1.5, 2.0 and 2.5g, produced by continuous centrifugation, and during four months of exposure to mechanical vibration. Plaster cast immobilization of one hind leg was applied in order to simulate the hypodynamic state of weightlessness.

Animals were successively sacrificed to determine the physical, mechanical and physiological parameters of bone. Mineralization was traced by periodic administration of tetracycline. Radiographic densitometry was employed in vivo and in vitro to follow up bone development.

At earth gravity normal aging takes place while physical dimensions, density, rigidity, microhardness, sound conductivity, and ash content of bone increase. Body weight growth is a logarithmic function of age. Bone porosity and calcium content remain constant.

Rats under hypergravity have definitely smaller rates of growth than rats at 1g. The respective differences are due entirely to differences in fatty tissue. Bone development as a function of age was found to be unaffected by the gravitational environments of the range administered in this study, except that, longitudinal bone growth is slower and the active zones of mineralization are wider at 2.5g than at normal gravity.

Chronic vibration increases stiffness and microhardness of bone. The active fronts of mineralization disappear and deposition becomes dispersed across the diaphysis.

Immobilization significantly decreases bone density, ash and calcium content; immobilized bone becomes less porous and more brittle than bone of normal subjects.

Subsequent to immobilization, density, compressive elasticity, porosity and calcium content return to normal level, observed in bone of corresponding age, irrespective of exposure to either earth gravity, hypergravity or vibration.

In the hypergravity range investigated, no significant and systematic changes were found; however, simulation of weightlessness was found to produce pronounced atrophy of bone. Thus it does not seem possible to make simple extrapolations from the above 1g range to the below 1g range, since it is likely that

there exists a threshold above which bone development is essentially normal while atrophy occurs below the threshold. This point could be clarified by space experiments.

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INTRODUCTION

Hypodynamia characterized by prolonged inactivity, such as immobilization, bed rest, or weightlessness, adversely affects the normal functioning of the physiological systems in man primarily through the homeostatic adaptation of the human body to its environment. The cardiovascular and the skeletal systems are profoundly affected and various methods have been established to counteract cardiovascular deconditioning. The atrophy of the skeletal system, however, has been less extensively studied and no specific methods have been recommended to alleviate bone deconditioning.

Present information on skeletal deconditioning has arisen from experimentation using immobilization, bed rest, and water immersion, and from space flight observations which have demonstrated the debilitating effects of inactivity ascribed primarily to the absence of mechanical stresses of weight bearing and countergravitational muscular activity. Clinical observations and experimental studies on various forms of immobilization reported negative calcium balance (Deitrick et al., 1948; Benson et al, 1962; Brannon et al., 1963; Vogt et al., 1965; Semb, 1966a), decreased mineral kinetics (Semb,

1966b; Sevastic et al., 1968), increased bone resorption (Landry and Fleisch, 1964; Frost, 1966), decreased weight and volume (Gillespie, 1954; Sevastic et al., 1968), decrease in bone density (Stevenson, 1952; Gillespie, 1954; Geiser and Treuta, 1958; Mack et al., 1968), and deterioration of the mechanical properties (Haike et al., 1966; Kazarian and von Gierke, 1970).

Pertinent information on the effects of weightlessness has been obtained in the relatively short space flights: namely, during the eight day Gemini V mission, a 10-20 % decrease in x-ray bone density was observed (Mack et al., 1967; Mack and LaChance, 1967). During the 22 days of weightlessness in the Soviet Kosmos-110 flight radiographic evidence of bone demineralization was reported together with high postflight blood and urine calcium concentrations in two dogs (David, 1963; Parin et al., 1966). A negative calcium balance was found during the fourteen day Gemini VII voyage although the astronauts consumed a calcium rich diet (Birge and Whedon, 1968).

It has been reported that physical activity tends to foster bone development: increases in bone weight, volume, density and breaking stress, as a result of physical exercise, have been found (Donaldson and Meeser, 1933; Saville and Smith, 1966; Smith and Felts, 1968; Saville and Whyte, 1969). Biochemical reactions and bone growth were intensified under mechanical loads and pressure (Whedon et al., 1949; Tvias, 1961; Solomons et al., 1965).

I. OBJECTIVES OF THE INVESTIGATION

The following study attempts to determine the role of mechanical stress in the structural development of bone and to discern the applicability of two particular stresses in prevention and alleviation of disuse bone atrophy.

Specifically, the effects of exposure to normal earth gravity, to simulated weightlessness, to the hypergravity of continuous centrifugation and to whole body mechanical vibration were to be investigated.

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 and vibration were then to be explored. From the musculoskeletal standpoint, immobilization partially simulates the state of complete inactivity and weightlessness. Consequently, exposure to earth gravity, hypergravity and vibration following immobilization should indicate the probable direction of bone development when similar mechanical stresses are applied to bone in space and in other forms of prolonged inactivity.

II. METHODOLOGY

A. MECHANICAL STRESS ENVIRONMENTS

1. Hypergravity:

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, but non-gravitational effects due to rotation are also present.

Animals can survive and grow under a wide range of hypergravity produced by chronic centrifugation. The influence of centrifugally produced hypergravity upon growth and body composition has been studied extensively and one of the most consistently reported effects is supression of growth. It is dependent on the magnitude of the artificial gravity and is influenced by the age, size and species of the experimental animals (Matthews, 1953; Steel, 1962; Bird et al., 1964; Wunder et al., 1963; Oyama and Pratt, 1965; Casey et al., 1967). Rats and mice were found to reproduce under conditions of chronic centrifugation, and animals thus born and reared appear normal in all respects except for a reduction in body weight (Oyama and Pratt, 1967).

The gravitational and rotational effects of centrifugation were separated by exposing labyrinthectomized hamsters to chronic centrifugation. No difference was found in the cumulative food consumption and body development between labyrinthectomized and normal subjects (Wunder et al., 1966). Centrifugation reduced the threshold of rotary perception in chronically centrifuged animals (Winget et al., 1962) indicating that adaptation takes place. Human subjects were also found to acclimate to rotation up to 10 RPM (Colehour and Graybiel, 1966).

Increased femur growth and circularization of cross section was found in mice under 4g (Wunder et al., 1960). Chronic centrifugation at 1.5, 2.0 and 3.0g increased the bone to muscle ratio in fowl. This was attributed to the smaller than normal weight of the centrifuged animals (Smith and Kelly, 1963).

The hypergravity experiments of this investigation are summarized in Table 1. Rats were exposed to 1.5 and 2.0g for two months and to 2.5g for up to eight months. In order to trace bone development, sample groups, consisting of two animals at earth gravity and four animals at hypergravity, were sacrificed successively in 30 day periods during the first two months of the investigation and in 60 day periods afterwards. Exposure to 2.0g hypergravity, after five weeks of immobilization, lasted for another five weeks.

Table 1. Design of Hypergravity Experiments

Gravity Level	Daily Exposure	Normal Animals		Immob. Animals	
		Duration of Test	No. of Animals	Duration of Test	No. of Animals
1.0g	24 hrs	8 months	14	5 weeks	7
1.5g	24 hrs	2 months	4	-	-
2.0g	24 hrs	2 months	4	5 weeks	4
2.5g	24 hrs	8 months	16	-	-

Apparatus. The hypergravity produced by chronic centrifugation is the vectorial sum of the earth surface gravitational acceleration and the imposed centrifugal acceleration, which latter is proportional to radius and square of angular velocity. 1.5 and 2.0g gravity levels were achieved in a 39 RPM centrifuge at radii of 0.65 and 1.07 m. Figure 1 shows the centrifuge with the animal capsules located at the different radii. 2.5 gravity was produced on another centrifuge with 2.28 m radius and 30 RPM shown in Figure 2.

The animal compartments, with twelve 8 mm dia. ventilation holes on the sides, were pivoted on the centrifuge arms in order to align the floor of the cage perpendicular to the resultant acceleration vector. At 1.5 and 2.0g the animals were housed individually in cages providing 20 x 25 = 500 cm^2 floor area. At 2.5g, initially six animals were placed in a cage of similar construction providing 23 x 51 = 1173 cm^2 floor area. The number of animals became, of course, successively less in the course of the experiment as the sampling groups were removed. Food was placed on the Sanicel bedding in the cages and distilled water, to keep the system free of contamination, was provided from a central tank through plastic tubes leading to a drinker valve (Upjohn Co.) in each compartment. The control animals under earth gravity were kept

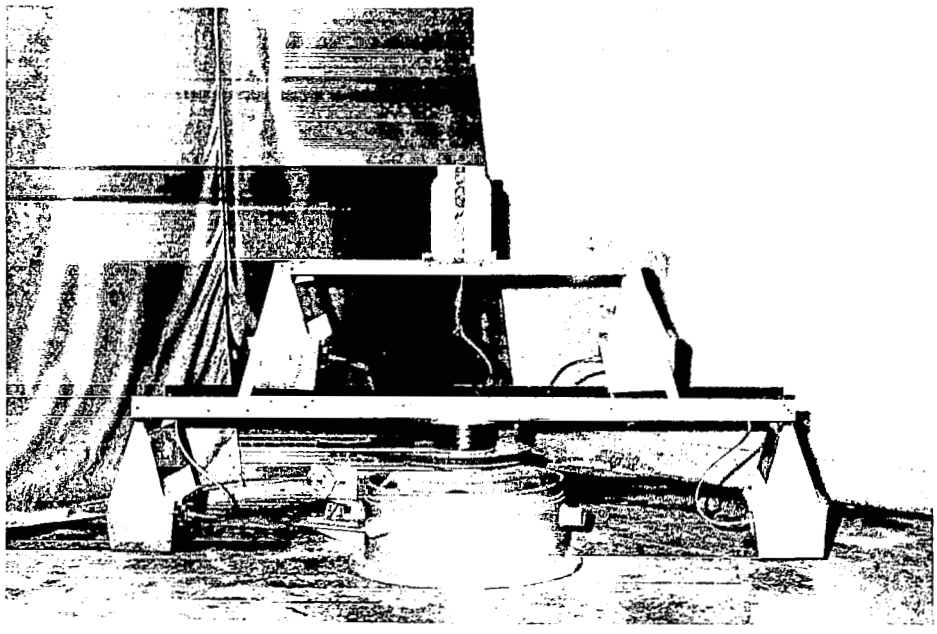

Figure 1. Centrifuge on which 1.5g and 2.0g hypergravity levels were produced

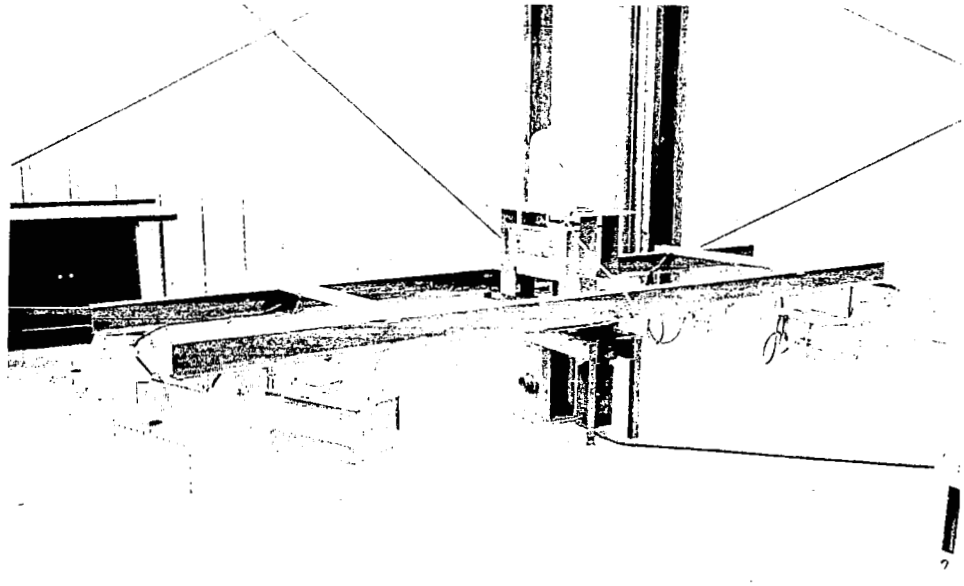

Figure 2. Centrifuge on which 2.5g hypergravity level was produced

in similar cages in the vicinity of the centrifuges.

The centrifuges were stopped twice weekly for about 30 min. for cage cleaning and resupplying food and water. Periods of acceleration for stops and restarts were 30 sec.

2. Vibration

Whole body vibration within the comfort limit of the subject could be another method of providing mechanical stresses to the skeleton in hypodynamia. Vibration constitutes a continuous, but alternating load on the bone. When placed on a vibrating platform, animals tend to damp the imposed vibration by physical activity of the legs. The feet follow the motion of the shake table, while the main body keeps relatively motionless. This results in continuous muscular exercise, which might be beneficial in counteracting lack of other physical activity.

Physiological, cyto-pathological and psychological effects of vibration have been extensively studied under a wide range of conditions (horizontal, transverse and vertical vibration), but relatively few investigations have studied the development of the skeletal system under vibration. The use of passive exercise by means of an oscillating bed significantly reduced the metabolic abnormalities of mineral secretion during inactivity in plaster immobilized patients (Whedon et al., 1949). No significant

alteration was found in the physical and biological character of the dental pulp and periosteum of rats under 43 Hz, 9.6g vibration (Restarski, 1945). The application of 50 Hz, 10.0g vibration for two to five hours daily increased the cross section of muscle fibers and decreased the fat content of muscle tissue (Hettinger, 1956). The endocrine system in both humans and rats adapted to repeated vibrational stress by an extinction of the stress response (Modignani et al., 1964; Sackler and Weltman, 1966).

Skeletal response to vibration of high frequency and high acceleration, produced by pneumatic tools, have been thoroughly investigated in occupational medicine. The observations reveal bone cysts (Beyer, 1943), stiff joints and x-ray evidence of osteoporosis (Gurdjian, 1945; Popova et al., 1966), decalcification (Beyer, 1943; Hunter, 1945), alterations of articulation (Beyer, 1943; Agate, 1947; Popova et al., 1966; Smith and Allen, 1969; Stewart, 1970).

The objectives and experimental conditions of such previous studies vary so much that the results cannot be used to forecast the type, frequency and duration of a vibratory environment which could be beneficial in hypodynamia. In this study the parameters of vibration were chosen in an attempt to avoid damaging effects on the animal during long term exposure. The maximum peak acceleration

is limited: one g, when superimposed on earth gravity, results in an alternating acceleration input from 2.0g in the lower half of the cycle to zero g in the upper half cycle. A pure mass leaves the shake table above this acceleration. In unrestrained animals, comprised of a system of masses, springs and dampers, the sinusoidal input motion might become amplified to an injurious degree even below 1.0g peak acceleration, depending on the instantaneous tension conditions of the musculature. In this investigation the peak table acceleration was one g.

The applicable frequency range is also limited. Low frequency vibration is harmful to many physiological systems, and the resonant frequency of the subjects must be avoided. High frequencies with the accompanying small amplitudes are likely to be damped out by the soft tissues. In this study 20 and 25 Hz were chosen with 1.25 and 0.80 mm double amplitudes. These two frequencies are above the resonant frequency of the rat and the motion of the shake table is damped by vigorous exercise of the leg. These two vibrations were selected on the basis of a series of experimental observations of rats exposed to vibration of constant 1.0g peak acceleration level at various frequencies in the range from 5 to 50 Hz. In 5-10 Hz, large amplitude vibration, the entire body of the animal moves in response to the shake table without any discrete physical

activity of the limbs. The dominant natural frequency of the rat is around 13 - 15 Hz. In this range the vibratory motion of the animal is greatly amplified over that of the shake table. In the range of 20 -30 Hz, rats damp the imposed motion with the legs. Above 30 Hz no apparent physical motion of the limbs can be observed.

The design of the vibration exposures is summarized in Table 2. In one series 20 and 25 Hz was applied for 2.5 hours twice daily with six hours of rest in between. In another series, the animals received 25 Hz for 12 hours each day without interruption.

Apparatus. Vertical sinusoidal vibration was generated with an electromagnetic vibrator (Textron Electronics, Model EA 1250MB) driven by a power amplifier (Textron Electronics, Model 2120MB). The frequency was regulated by an audio-oscillator (Hewlett Packard, Model 2021). The experimental animals were placed on the shake table into individual compartments, providing 200 cm^2 floor area (Figure 3.). The non-vibrated control subjects were kept in similar cages for the duration of each vibration session. Otherwise the animals were housed individually in regular laboratory cages with a floor space of 570 cm^2 available. The smooth, Plexiglas floor of the vibration compartments was covered with a thin layer of sawdust to absorb moisture. This bedding did not act as a damper because the paws of the animals were in direct contact with the

Table 2. Design of Vibration Experiments

Frequency	Daily Exposure	Duration of Test	No. of Animals	
			Normal	Immobilized
without vibration	24 hrs	4 months	22	-
	24 hrs	5 weeks	-	7
20 Hz	2x2.5 hrs	5 weeks	4	-
25 Hz	2x2.5 hrs	5 weeks	8	10
25 Hz	12 hrs	4 months	8	-

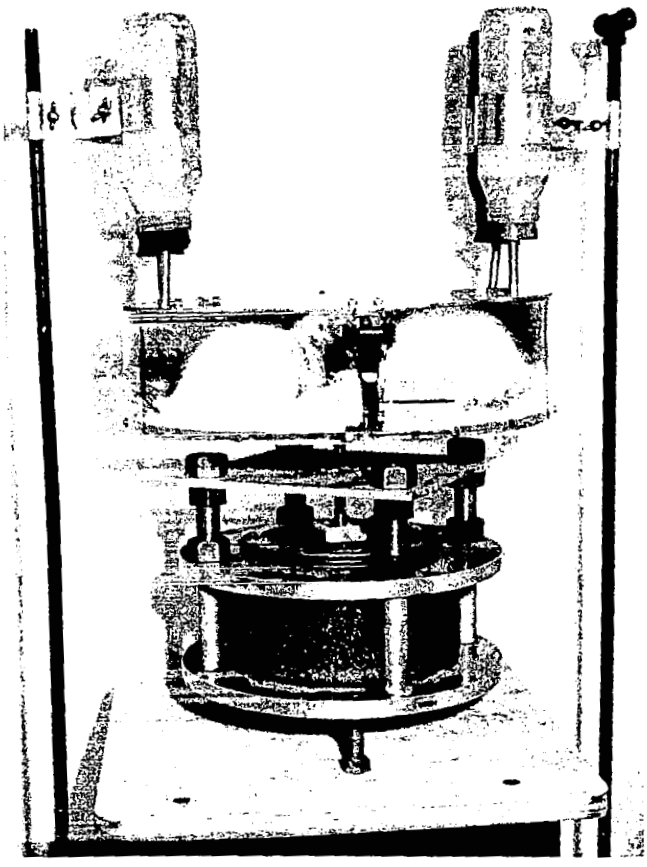

Figure 3. Electromagnetic vibrator with the animal compartments

floor. Both the vibrated and the control animals were provided with water from regular laboratory drinker bottles during the experimental session. The temperature in the vibration compartment was 2-3° C above ambient temperature of 24° C.

B. EXPERIMENTAL ANIMALS

Male Sprague-Dawley rats were used in the study. The rat was considered suitable because its relatively short life span 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. Consideration of the cost of acquisition and maintenance favored selection of rats.

All experiments were started with 60 day old animals weighing 250 $\pm$ 10 gr.* The animals, obtained from Laboratory Supply Company, Indianapolis, Indiana, were fed Purina Laboratory Chow and water ad lib. A food supplement was prepared from ground oyster shell mixed with melted sugar and was given free choice in order to be certain that the diet contained ample amounts of calcium and

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*In this investigation "gr" is used to abbreviate "gram" in order to distinguish it from "g", which is the gravitational acceleration at the earth surface.

phosphorous for bone development. Room temperature and humidity were kept essentially constant: 24° C and 60 % respectively. The daily lighting cycle was 12 hours on, 12 hours off.

For immobilization, a plaster cast (prepared from 2" fast setting plaster bandage) was put on the right hind leg of 60 day old animals under general Nembutal anesthesia. The cast surrounded the lower and upper part of the extremety completely and was extended in a funnel shape to partially cover the pelvis. See Figure 4. The cast weighed 30 to 40 grs. after drying. The animals were able to move around the cage with ease. The leg was left immobilized for five weeks and the cast was then removed under Nembutal anesthesia. The animals started using their legs 2-3 days after liberation. Exposure to centrifugation and vibration started on the third day after removal of the cast.

Only one extremity was immobilized, in order to make three comparisons possible between the free and immobilized legs for investigating the effects of mechanical stress environments on atrophic bone development:

1. Immobilized bone (at earth gravity) vs. free bone (at earth gravity): bone development in the immobilized limb can be compared to the free leg under natural environmental conditions;

Figure 4. Rat with immobilized hind leg in plaster of paris cast

2. Immobilized bone (under hypergravity or vibration) vs. free bone (under hypergravity or vibration);

3. Immobilized bone (at earth gravity) vs. immobilized bone (under hypergravity or vibration): in order to compare the effects of the applied environmental conditions directly on the development of atrophied bone.

C. ANALYTICAL PROCEDURES

The factors which influence the response of bone to stress and strain may be classified as (a) physical, (b) mechanical and (c) histological and physiological. The parameters, measured and analyzed in this study, are summarized below:

a. Physical properties of bone:

1. Weight
2. Geometry (volume, length, cross-sectional area)
3. Density

b. Mechanical properties of bone:

1. Compressive elasticity
2. Torsional elasticity
3. Microhardness
4. Sound conductivity

c. Histological and physiological properties of bone:

1. Histological solidity

2. Ash content

3. Calcium content

4. Mode of mineralization

Preparation of the specimen:

The subjects were killed with an overdose of Nembutal, then both hind legs were excised, disarticulated at the hip and ankle and cleaned of soft tissue. Care was taken to avoid injury to the surface of the bones. In order to remove all soft tissue, the bones were soaked for three hours in water containing 15 % coconut oil soap at 75° C. After this treatment all remnants of soft tissue were wiped off.

The bones were then dried in a vacuum oven for 36 hours at 65° C. Density of the bone as a whole was measured after drying, keeping the specimens in a desicator between the measurements to prevent any water absorption from the air.

In order to provide samples for the determination of all material properties and to be able to test bones of different size comparably in the various age and experimental groups, the femurs were cut into five sections proportionally to the total length, as

shown in Figure 5. For sectioning a diamond impregnated brass cutting disc was used on a lathe with water as lubricant, producing smooth parallel surfaces. The micrometer bype feed of the lathe allowed precise positioning and control of the lengths.

The sectioned bone samples were defatted in three separate 24 hour ether baths. The bones were then dried again for 24 hours in a vacuum oven at 65° C and all measurements were executed on dry specimens.

The preparation of the samples was kept strictly uniform, because the treatment procedure and the water content significantly influence the mechanical properties of bone (Ewans and Lebow, 1952; Ampiro, 1961; McElhaney et al., 1964). Since this investigation is concerned primarily with relative differences between the various groups rather than with absolute values, all measurements were made comparable with one another by conducting them on dry defatted specimens and under identical conditions at room temperature.

Measurement of weight, volume and density:

Density of the whole intact femur and tibia was calculated from the weight of the bones measured on an analytical scale to an accuracy of $\pm$ 0.1 mgr and from the volume determined according to Archimedes' principle, namely, from the weight loss upon

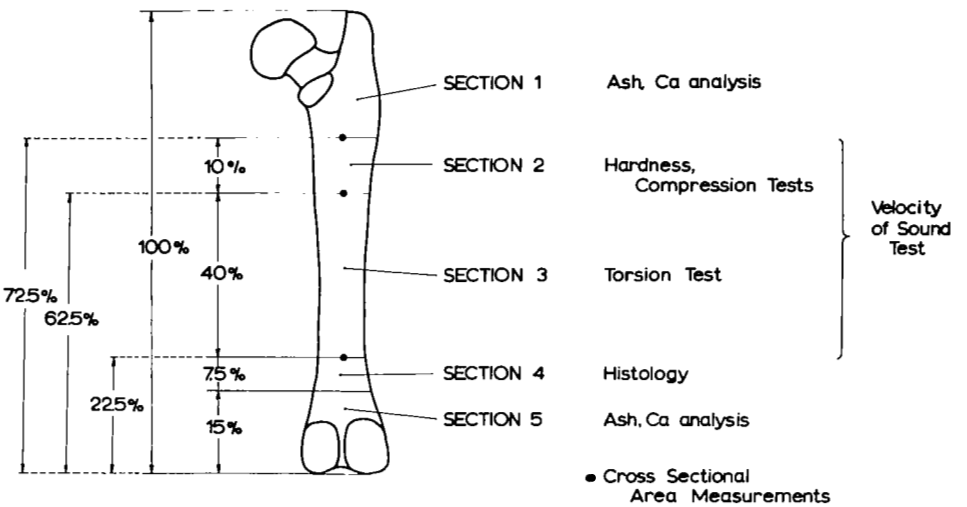

Figure 5. Test sections of the femur

immersion of the bones in distilled water. Before immersion, the water was degassed in a vacuum chamber for 30 min. and corrections for water temperature were applied.

Length and area measurements:

The length of femur and tibia was measured with a micrometer to $\pm$ 0.05 mm accuracy.

The cross-sectional area of the bone was measured at three sites along the diaphysis of the femur, at 22.5 %, 62.5 % and 72.5 % distance from the distal end, as shown in Figure 5.

The cross sections of the shaft were photographed on 35 mm film and printed on 8 x 10 in. paper with a final lineal enlargement of 7.55 corresponding to an area magnification of 57.00. The cross-sectional areas were measured by planimetry of the photographs along the periosteum and endosteum of the bone. An example of such a photograph is shown in Figure 6. For photography, the specimens were positioned uniformly in a mounting device in which the cross sections were pressed with springs against a microscope glass slide. A reference scale was placed in the plane of the cross sections for calibration.

The area enclosed by the periosteum is denoted as the total

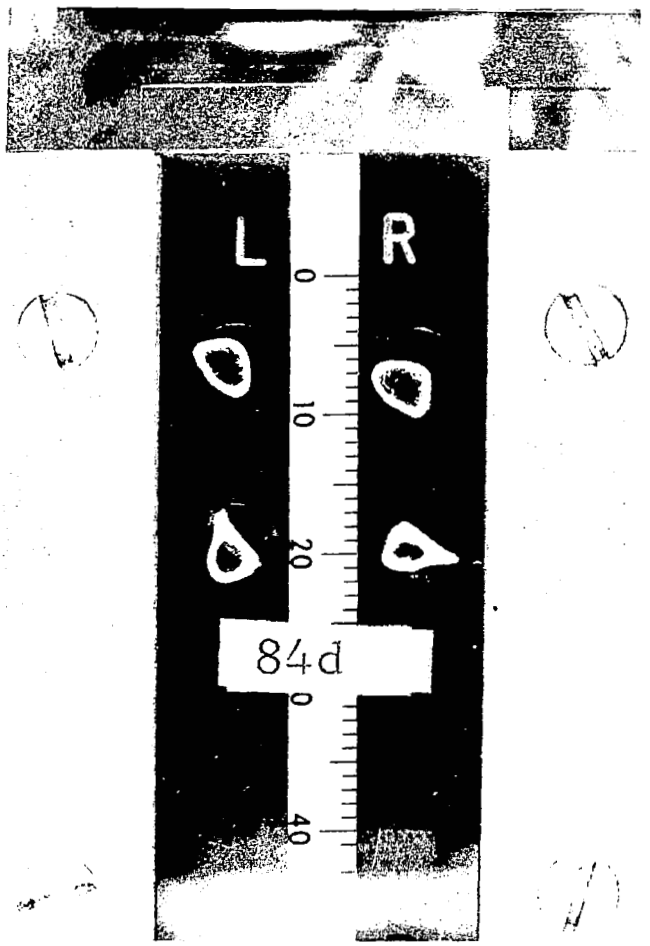

Figure 6. Photograph of femur cross sections as used for area measurements. The areas shown are located at a distance of 0.225L and 0.625L from the distal end

area. The cortical area represents the total area without the marrow space (encompassed by the endosteum).

Measurement of compressive elasticity:

The compressive elasticity of Section 2 (see Figure 5) of the femur was measured, applying the load in the direction of the longitudinal axis of the shaft. The compressive force was recorded as a function of change in length of the specimen until fracture occurred. The measurements were taken on a Model TT, Instron Universal Test Instrument with Type CD compression cell. The rate of loading was 0.05 inches/min., resulting in fracture of the specimen in 2-3 sec. A constant strain rate was used to avoid variations due to the viscoelastic and time-dependent response of bone, which have been observed, under different loading conditions (McElheaney, 1966; Burnstein and Frankel, 1968; Frankel and Burnstein, 1970).

The compressive spring constant of the material was computed from the force-compression relationship:

$$k = \frac{F_{max}}{\Delta L} \qquad (1)$$

where k - compressive spring constant (kp/mm)

F_{max} - ultimate compressive force (kp)*

ΔL - decrease in length due to compression (mm)

The ultimate compressive force was used, as by other investigators (Podrushniak and Suslov, 1967), in the computation because the breaking point was reached linearly ending in an abrupt fracture of the bone.

The modulus of elasticity was also determined from the compression tests. The modulus of elasticity is defined as the ratio of compressive stress to strain in the specimen:

$$E = \frac{s}{\epsilon} \tag{2}$$

$$s = \frac{F_{max}}{A} \tag{3}$$

$$\epsilon = \frac{\Delta L}{L} \tag{4}$$

where E - modulus of elasticity (kp/mm^2)

s - stress (kp/mm^2)

ϵ - strain (mm/mm)

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*In the metric engineering system, the unit kilogram is commonly used as kilogram mass or kilogram force. In conformity with standard use, the abbreviation kp for kilogram force has been used throughout the text of this study. It did not seem necessary to carry out this differentiation on the various figures. There kg was used universally.

A - average of the cross-sectional areas at the two ends of the shaft (mm^2)

L - length of the shaft before testing (mm)

The samples had columnar shape and were under compressive load, therefore, for elastic stability, the slenderness of the samples was calculated. In the computation, the bone shaft was assumed to be a hollow elliptical cylinder; both ends were considered as ellipses for which the long and short diameters were measured on the 8 x 10 in. photographs used in the cross-sectional area measurements. The slenderness of the samples was 3.4 to 3.6. Such a low value represents an extremely short column in which failure takes place due to compression and not to buckling.

Measurement of torsional elasticity:

Section 3 (see Figure 5) of the femur was tested in torsion by measuring the torsional moment as a function of the angular deformation. The measurements were taken on a Model TT, Instron Universal Test Instrument with a special attachment to transform the linear pull of the instrument into rotation. In the torsion device, developed for this purpose and shown in Figure 7, the distal end of the specimen is fixed with respect to the structure while the proximal end is twisted. Twisting was achieved by mounting the bone on a common shaft with a freely rotating disc and applying

linear pull tangential to the circumference of the disc. Ro reduce frictional losses, ball bearings and a 0.001" thick flexible steel ribbon were used to transmit the motion.

The longitudinal axis of the bone specimen was aligned with the axis of rotation by positioning the marrow cavity concentrically on conical guiding pins at both ends. The ends of the shaft were then embedded in a fast setting plaster of paris (Whipmix, setting time: 1 min., crushing strength: 8000 psi). The free length of the shaft between the embeddings constituted 30 % of the total femur length. The embedded specimens were dried in a vacuum oven for 24 hours at 65° C before testing.

A constant rate of loading was applied. The velocity of the linear pull was 2 inch/min., resulting in an angular velocity of 1.22°/sec. The torque vs. angular displacement relation was found to be linear ending in an abrupt spiral fracture of the bone.

The torsional spring constant of the bone material was calculated as:

$$\varkappa = \frac{T_{max}}{\theta_{max}} \qquad (5)$$

where $\varkappa$ - torsional spring constant (cmkp/rad)

T_{max} - ultimate torque (cmkp)

θ_{max} - ultimate angular displacement (rad)

The torsional spring constant expresses the elasticity of the bone in torsion, i.e. the torque required to produce unit angular deformation along the longitudinal axis of the femur. This term is used in this study to describe the torsional behavior of bone. It is a direct function of the shear modulus of the material, the polar moment of inertia of the cross section at the site of fracture, and is inversely related to the free length of the specimen:

$$\varkappa = \frac{GI_P}{L} \qquad (6)$$

where G - shear modulus of elasticity (kp/mm^2)

I_p - polar moment of inertia (mm^4)

L - free length of the test specimen (mm)

It was not practical to determine the shear modulus of bone from the torsional tests. Namely, the fractures were of the tensile type torsional failure, where cracks develop at a 45° angle to the axis of the shaft, as shown in Figure 8. The tensile modulus of elasticity is the determinant parameter of the material in such fractures. The site of the failure varied in the experiments. The cracks were extensive, frequently extending to the vicinity of the embeddings at the ends of the shaft. The cross-sectional shape and thus the polar moment of inertia differed considerably

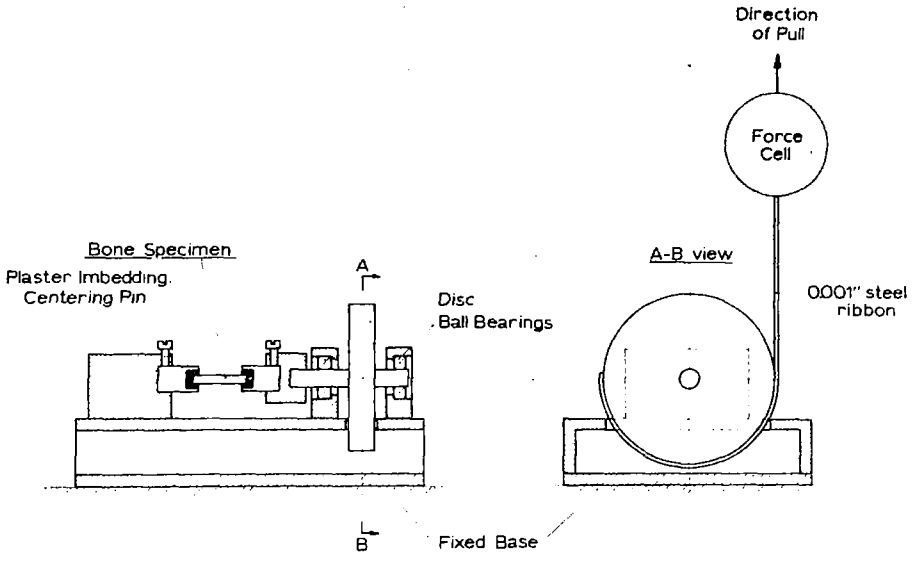


Figure 7. Outline of the bone mounting device used on tensile tester for measuring the torsional elasticity of the femur

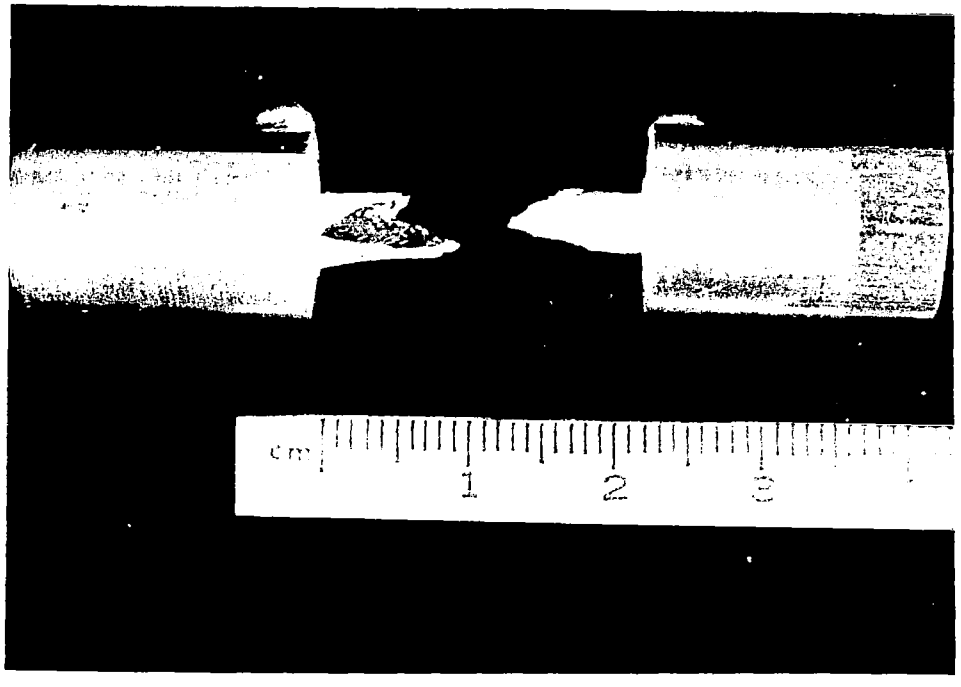


Figure 8. Spiral fracture of the femur under torsional load

from one end of the specimen to the other, also making it inadvisable to carry the torsional analysis further than determination of the torsional spring constant. Figure 9 shows an example of a torsional bone specimen illustrating the large variation in the shape and polar moment of inertia of the cross sections along the femur.

In order to illustrate this discrepancy, the polar moment of inertia, $I_p = \int_0^A r^2 dA$, was determined by numerical integration for Figure 9. The total cross sectional area, A, was divided into sections, equidistant from the assumed center of rotation by concentric circles, and the area of the bone inside the individual rings, called ΔA_i, was measured with a planimeter. The polar moment of inertia for each concentric ring, ΔI_{pi}, was calculated by multiplying the measured area and the square of the mean radius of the section, r_i:

$$\Delta I_{pi} = r_i^2 \Delta A_i \qquad (7)$$

and teh results for the individual sections were summarized:

$$I_p = \sum_{i=1}^{n} \Delta I_{pi} \qquad (8)$$

where n - total number of concentric rings. Computed by this method, the difference in polar moment of inertia at the ends of the specimen, as illustrated in Figure 9, is very large (18.17 mm^4 vs. 26.43 mm^4).

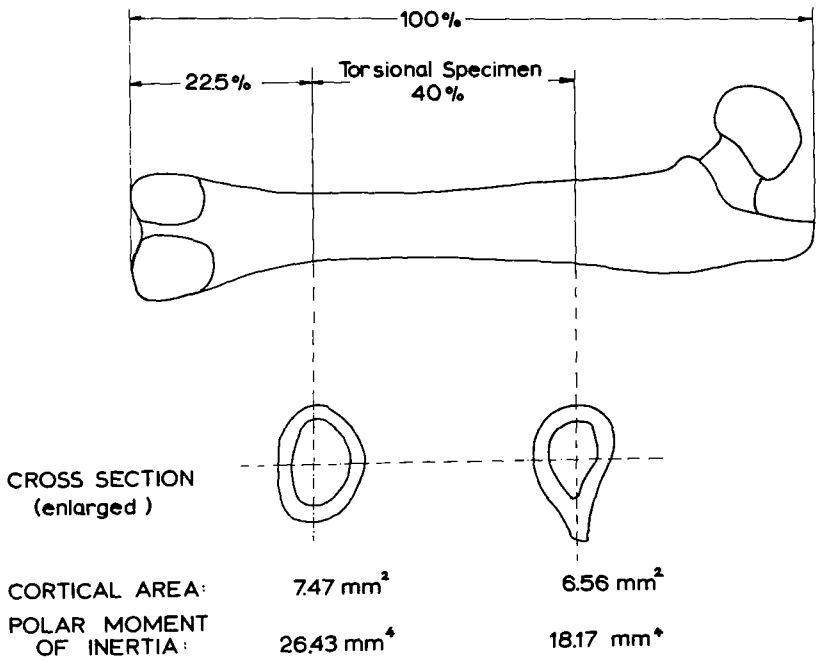


Figure 9. Anatomical location and geometry of the torsional specimen

Microhardness measurements:

Microhardness measurements were made with a Type MO Wilson-Tukon Microhardness Tester using the Vickers indenter. With this method, the microhardness of the material is determined by forcing a square base diamond pyramid having an apex angle of 136° into the specimen under constant load and measuring the diagonals of the recovered indentation. The unit Diamond Pyramid Hardness is defined as the load per area of surface contact in kp/mm^2. It was calculated from the average of the two diagonals in the pyramid indentation:

$$DPH = \frac{2F\sin(\frac{\alpha}{2})}{d^2} \qquad (9)$$

where DPH - Diamond Pyramid Hardness (kp/mm^2)

d - average length of the two diagonals (mm)

α - apex angle (136°)

F - loading force (kp)

The Vickers method was selected as most suitable for measurements on bone material, because the indentation of the pyramid is less influenced by the flatness and parallelism of the top and bottom surfaces of the specimen and is less sensitive to unevenness of the surface finish than other techniques. Because of the

relatively small area of indentation for a given load, it is also more suitable for microscopic testing. The penetration of the indenter is shallow with respect to other methods, being about 1/7 of the diagonal.

The microhardness tests were carried out on Section 2 of the femur (see Figure 5), on the surface between Sections 2 and 3 at 62.5 % distance from the distal end. The specimens were mounted on the microhardness tester in a holder formed of Kerr dental impression compound. The samples were pressed into the compound, softened previously in warm water, with a guided press mechanism to position the top surface of the specimen parallel to the bottom surface of the holder. Thus, when the holder was fastened to the table of the instrument, bone surface in the holder was set parallel to the table.

The site of the measurements was kept uniform because bone hardness at different locations varies within the cortical cross section. Figure 10 shows the test site. The axis of symmetry defined by the divider of the angle between the two flat sides of the bone sample was selected as a guideline. The first indentation was made along the symmetry axis, equidistant from the outer crest and the marrow cavity. Four more indentations were placed around the first one in a prearranged pattern, as indicated in the figure.

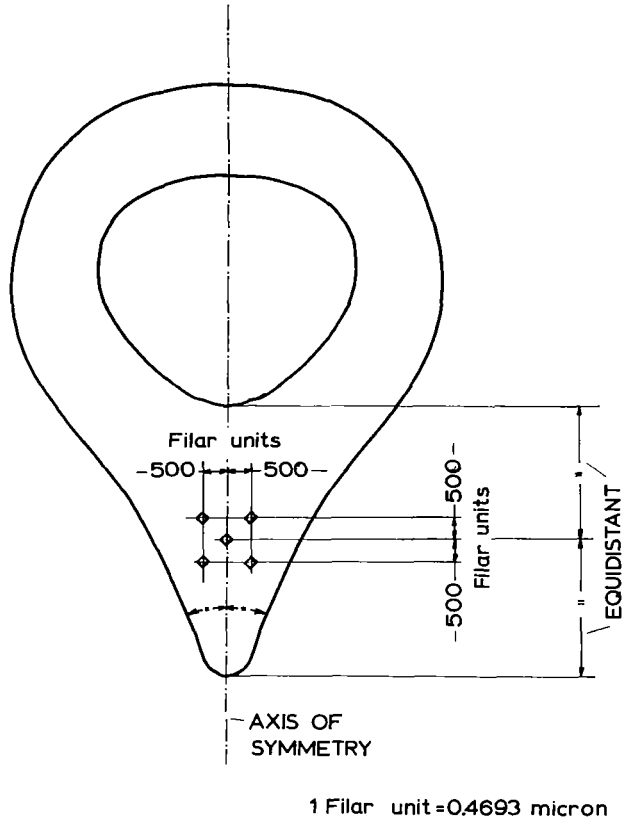


Figure 10. Location of the microhardness measurements on the bone cross section

In a few instances a major blood vessel (about the size of the indentation) was found at the preselected sites. In such cases, the test site was moved to a distance of twice the size of the blood vessel from the original location.

The indentations were made with 0.30 kp load. A bifilar measuring microscope was used with 20x lineal magnification to measure the diagonals of the indentations. Both diagonals were read in filar units (1 filar unit = 0.4693×10^{-3} mm) and the average of the two readings was considered to constitute one datum. For the various age and experimental groups, each consisting of several animals, the average and the standard deviation of the readings were calculated by pooling all five values obtained from the individual samples of the group. Thus the variation is based on the observed data, which is more meaningful than the variation of microhardness numbers would be, determined after converting the direct reading into hardness (by an inverse square function).

No additional surface finishing was needed because the high speed sawing of the bones provided the necessary smoothness for the microhardness tests. Duration of the indentation (while the pyramid was in contact with the bone) was also kept uniform because the bone material shows variations in microhardness as a function of the indentation period (Ampiro, 1961). The indenter made

contact with the specimen in 15 sec., and remained in contact for 18 sec. with constant load.

Measurement of conductivity of sound:

The conductivity of sound is another indicator of the mechanical properties of bone (Lange, 1970). Sound conductivity may be expressed numerically as the velocity of sound in the bone. The measurement is based on the principle that in solids the velocity of longitudinal sound waves is constant. It is a function of the modulus of elasticity and the density of the material and is independent of the sound frequency (Baumeister and Marks, 1967):

$$v = \sqrt{\frac{1 - \mu}{1 - \mu - 2\mu^2} \cdot \frac{E}{d}} \qquad (10)$$

where v - velocity of sound (m/sec)

μ - Poisson ratio (dimensionless constant)

E - modulus of elasticity (kp/m^2)

d - density (kg/m^3)

In this investigation the velocity of sound was measured on Sections 2 and 3 of the femur (Figure 5) using the ultrasonic velocity meter of the Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. The transit time of the

ultrasonic pulses through the specimen along the longitudinal axis was measured by placing it between two piezoelectric transducers, one serving as a generator and the other as a pickup of ultrasound pulses. Between the bone and transducers, Aquasonic 100 (Burdick Corp.) ultrasound transmission gel was used to provide acoustic coupling.

Pulses of three μsec (167 kHz) were passed through the bone, amplified and the signals displayed on an oscilloscope. The velocity was calculated from the transit time and the length of the specimen.

Determination of histological solidity of bone:

The histological study was aimed at establishing a quantitative description of the solidity of the femur on the microscopic level. Solidity is defined as the ratio of volume of solid matter to total volume of bone. Porosity is the complementary value of solidity, expressing the volumetric percentage of pores and cavities in the bone. The Zeiss point counting method was used to quantify solidity. The technique is based on Delesse's principle, as used in geology. According to this principle, the areal proportions of the components in a section of a composite material are equivalent to the volume proportions. The problem thus resolves itself into determination of the areas of the various components on a cut

surface. This was accomplished by using the grid developed for the Zeiss Integrating Eyepiece I (Hennig, 1958). The grid consists of a network of 25 points arranged at the vertices of equilateral triangles, as shown in Figure 11. The grid is placed on the histological sample and for each point, it is determined which constituent (space or solid matter) lies under it. The ratio of the components is computed from the counted points.

The theory of the point counting method was mathematically proved by Chayes (1956); the analysis of accuracy and the theory of statistical errors were worked out by Hennig (1958). The technique is used in histological examinations of both hard and soft tissue for quantitative description of histological changes (Chackley, 1943; Dunill, 1962, 1964; Dunill and Anderson, 1967).

For the histological study, a 30-35 μ thick slice was cut from the surface of Section 4 of the femur which is 22.5 % from the distal end. The bone slice was permanently mounted on a glass microscope slide with HSR mounting agent. Three 35 mm photographs were taken of different sites along the cross section with a Zeiss Universal Photomicroscope with 32.2x magnification. The three photographs covered 20-25 % of the total cortical area of the bone. The pictures were printed on 8 x 10 in. paper, which resulted in a total lineal magnification of 272.2. Figure 12 shows the

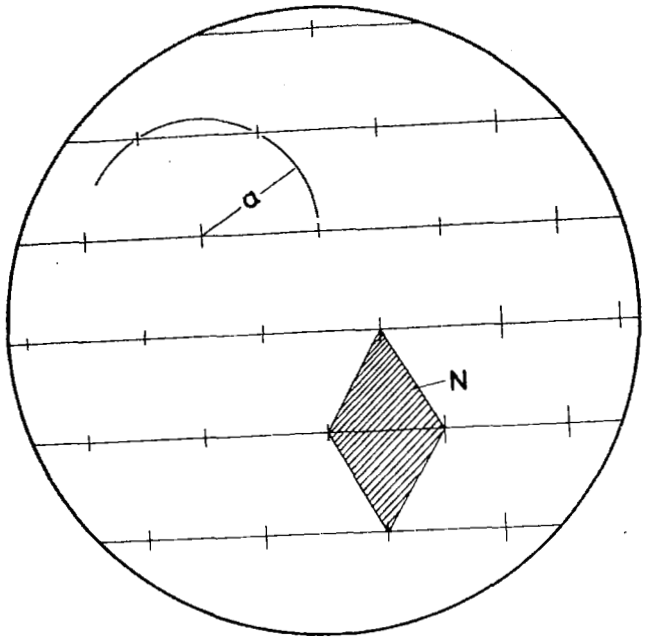


Figure 11. Network of 25 points, constituting the point counting grid of the Zeiss Integrating Eyepiece I. The interval between the points is "a", the area assigned to each point is "N", the area encompassed by the circle is 25N

Figure 12. Microscopic bone section for measuring histological solidity. 110x natural size

photograph of a histological microscopic bone section.

The grid of the Zeiss integrating Eyepiece I was also enlarged proportionally to the size of the bone sections on the 8 x 10 in. print by making a=23 mm ("a" is defined in Figure 11).

Three point counts were made on each of the three photographs, resulting in a total of nine readings for each bone specimen. In the nine readings, 225 points were counted. Based on statistical analysis, the absolute error in counting 225 points on a surface, which covers 20 % of the total area, is 1.79 % and the relative error is 8.11 % (as computed according to Hennig, 1958).

Determination of ash and calcium content:

The ash and calcium content of Sections 1 and 5 (Figure 5) of the femur, i.e. the hip and the knee joints, with the adjacent part of the diaphysis were determined.

The ash content expresses the amount of organic components in bone. It was computed as the percentage ratio of ash weight with respect to dry fat free weight (ash weight and weight of organic material).

The bones were ashed by placing the samples in Coors crucibles into a muffle oven for 16 hours at 650° C. The weight of the remnant was considered the ash weight of the specimens.

The calcium content was measured with atomic absorption spectophotometry. For the measurement a solution was prepared from the bone ash by dissolving it in 1 ml of 3N HCl and then diluting this solution with 1 ml 3x distilled H_2O. 50 µl of the solution was pipetted directly into 6 ml of 3x distilled H_2O and mixed. 100 µl of the mixture was further diluted in 2 ml of 0.1 % $LaCl_3$, resulting in a final dilution of 5040:1. The amount of calcium in the final solution was measured in an Instrumentation Laboratory Model 153 atomic absorption spectrophotometer of the Aging Research Laboratory, Veterans Administration Hospital, Lexington, Ky. The instrument was calibrated prior to each test with a standard calcium solution containing 5 πgr Ca/ml. Duplicate solutions were prepared from each bone sample and the average of the two readings was used to calculate the calcium content.

The calcium content of the fat free dry bone was calculated as the percentage ratio of the weight of the total amount of calcium in the sample to the fat free dry weight of the bone. The calcium content of the ash is the ratio of the calcium weight to the ash weight in the specimen.

Tetracycline labeling of bone mineralization:

Tetracycline hydrocloride was used to label the pattern and rate of bone growth in the experimental animals. Tetracycline and its compounds are deposited in vivo at the sites of active bone formation and can be visualized by fluorescence microscopy of the undecalcified bone sections (Frost, 1966).

Tetracycline belongs to a group of antibiotics which deposit with calcium at the calification fronts of the body. It is a temporally stable tissue marker which is deposited under natural circumstances.

New bone mineralization takes place in two functionally distinct phases (Frost, 1968): in the initial phase about 20 % of all the mineral that the organic component of the bone can accept is deposited in one day. The second phase lasts for several weeks during which the remainder of the mineral is deposited. Marking bone growth by tetracycline identifies the locations of new bone formation by labeling permanently the site of the first phase of mineralization of the organic matrix. The marker records where mineralization took place in the tissue regardless of composition, form, and intercellular morphological, energic or chemical mechanisms.

In this investigation, tetracycline was administered in the drinking water of the experimental animals (2 gram/liter) for 36 hours once in every 30 days. The microscopic bone sections, previously prepared for measuring the histological solidity of bone, were photographed at 10x lineal magnification on 35 mm Kodak Pan-X film through a 150 mμ ultraviolet interception filter (Zeiss No. 53). A high pressure mercury lamp served as the light source. The photographs covered 30-35 % of the lateral-posterior cortical area in the femur at a distance of 22.5 % of the total length from the distal end. Comparable location for the sampling site was necessary in the analysis to avoid the variations in bone turnover rates at different regions along the longitudinal axis and the circumference of the bone.

III. RESULTS

A. RESULTS FROM NORMAL SUBJECTS UNDER EARTH GRAVITY, HYPERGRAVITY AND VIBRATION

Body weight:

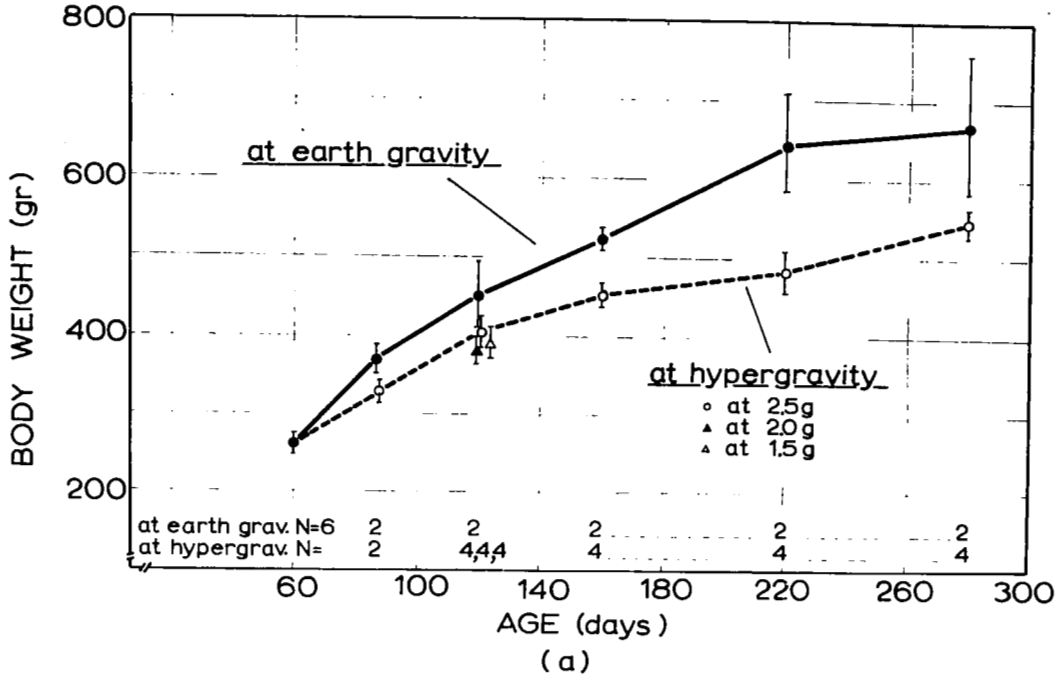
Interpretation of the observations in this study can only be made with due consideration of the temporal development of body weight of the experimental animals. The rats survived and physiologically adapted to hypergravity up to 2.5g with the one obvious effect of supression of body weight.

The growth pattern of the animals exposed to hypergravity on the centrifuge was divided into two periods. The first few days resulted in a decrease in body weight attrubutable to the combined effects of rotation and hypergravity accompanied by symptoms of motion-sickness. The second phase was marked by adaptation to the environment. The animals continued to grow after the temporary setback, but the rate of body weight increase was slower at hypergravity than at 1.0g. Significant differences between subjects at 2.5g and subjects at 1.0g persisted throughout the experiment and tended to increase with time.

Figure 13a shows the weights of the experimental animals at earth gravity and at hypergravity versus time. Each point on the

graph represents the average body weight of the group sacrificed at that age. The standard deviation of the data, indicated by vertical bars, shows that the difference between the groups is highly significant.

The average body weight of the vibrated animals did not significantly differ from that of the controls, as shown in Figure 13b. The points of this graph again represent the average weight of the groups at the time of sacrifice. The individual daily body weights, however, revealed unusual differences between two animal groups, caused by the feeding schedules. Namely, in the experiment with 12 hours of 25 Hz vibration, one group was exposed to vibration without food at daytime from 9.00 A.M. to 9:00 P.M. and the other group was on the shake table at night from 9:00 P.M. to 9:00 A.M. The control animals were similarly divided into two groups and respectively exposed, without food, to confinement in a restricted space, resembling the compartments on the shake table. Between the experimental sessions all rats were kept in regular laboratory cages and had free access to food. The body weight of the groups which fed in the daytime increased at a markedly lower rate than that of the groups which were able to feed at night, as shown in Figure 14. The differences between the corresponding vibrated groups and control groups are smaller than



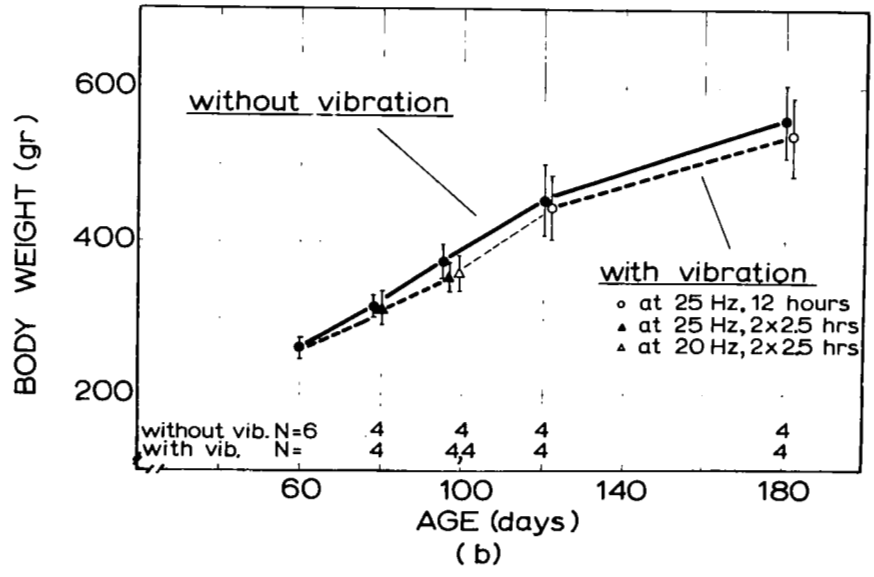


Figure 13. Body weight as a function of age
a) at earth gravity and at hypergravity,
b) without vibration and with vibration.
The points forming the curves constitute the group average with N representing the number of animals in each experimental and age group. The vertical bars through the points represent ±one standard deviation

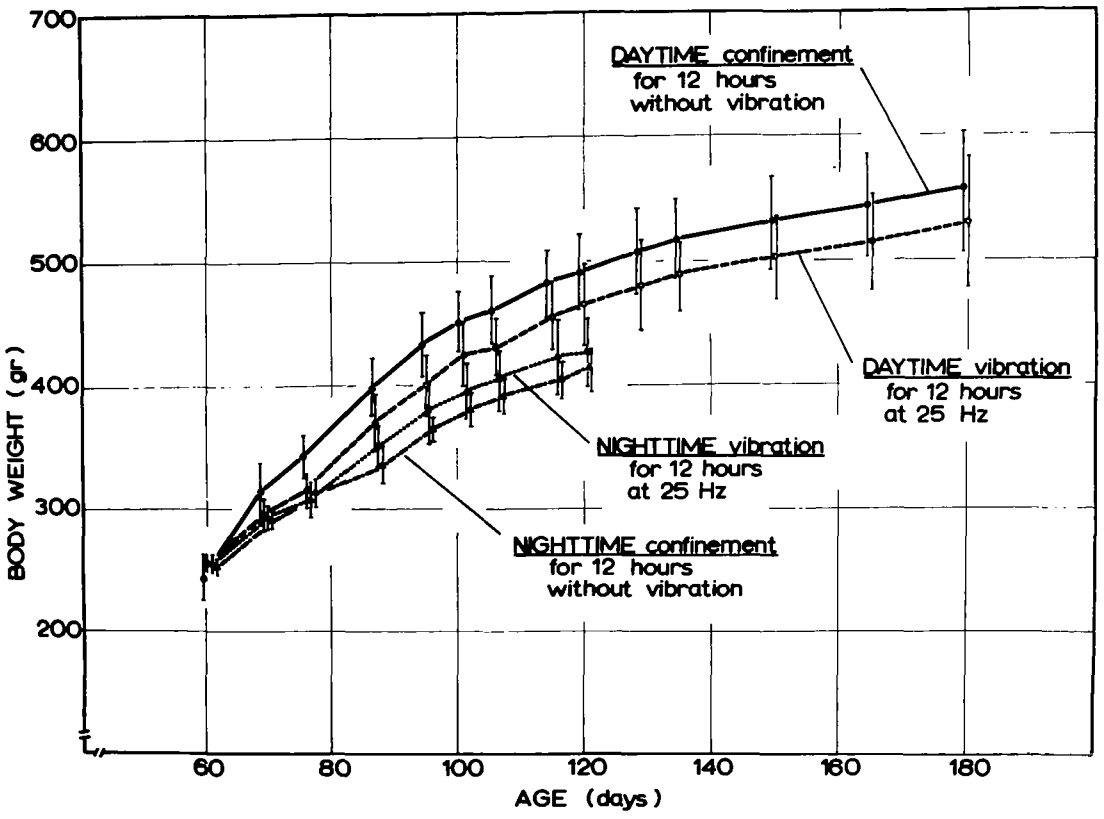


Figure 14. Effect of daytime and night-time feeding on body weight of rats. Rats fed at night in the Daytime Confinement and Daytime Vibration groups, and fed during daytime in the Nighttime Confinement and Nighttime Vibration groups. (Each point represents the average weight of four animals.)

the differences resulting from the two feeding schedules. Those differences must undoubtedly be attributed to the disruption of the normal circadian habits of the rodents. The rat is a nocturnal animal which feeds regularly at night. Food deprivation at night forced the animals into a reverse schedule resulting in a relatively slower rate of body weight gain.

Bone weight, volume and density:

Weight, volume and density of the intact femur and tibia are shown in Figures 15, 16 and 17 as a function of age. Each point on the graphs represents the average value for the bones in the right and left extremities of the animals sacrificed at that age. The process of normal aging is shown by the animals at earth gravity. At hypergravity the development of bone weight, volume and density followes the normal temporal pattern found at 1.0g. This fact, however, must be viewed with consideration of the large difference in body weight between the two groups. At any one age, the chronically centrifuged rats weighed significantly less than the controls of the same age at 1.0g, but this difference is not reflected in the respective weights, volumes and densities of the bones.

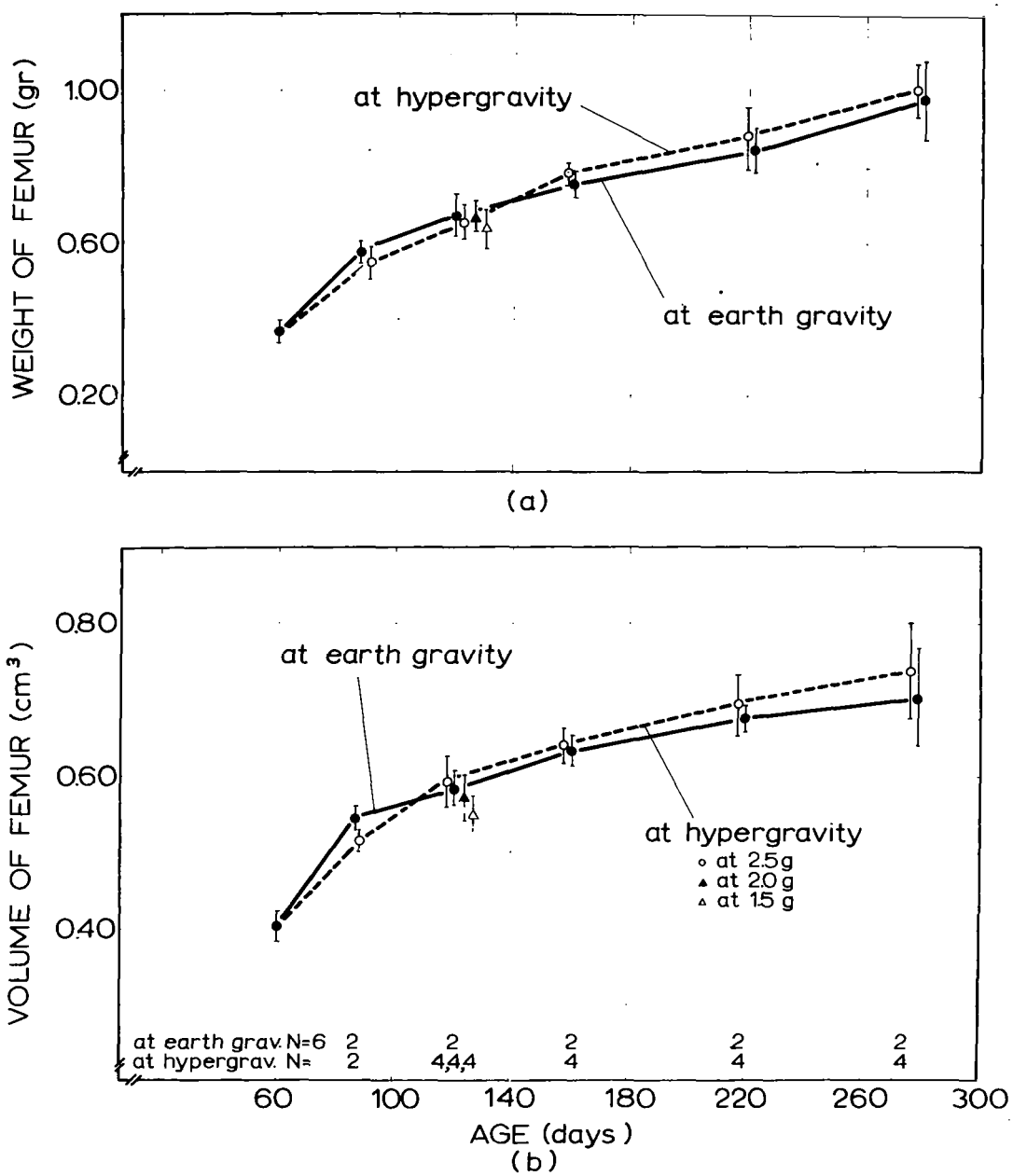


Figure 15. a) Weight and b) volume of the femur at earth gravity and at hypergravity

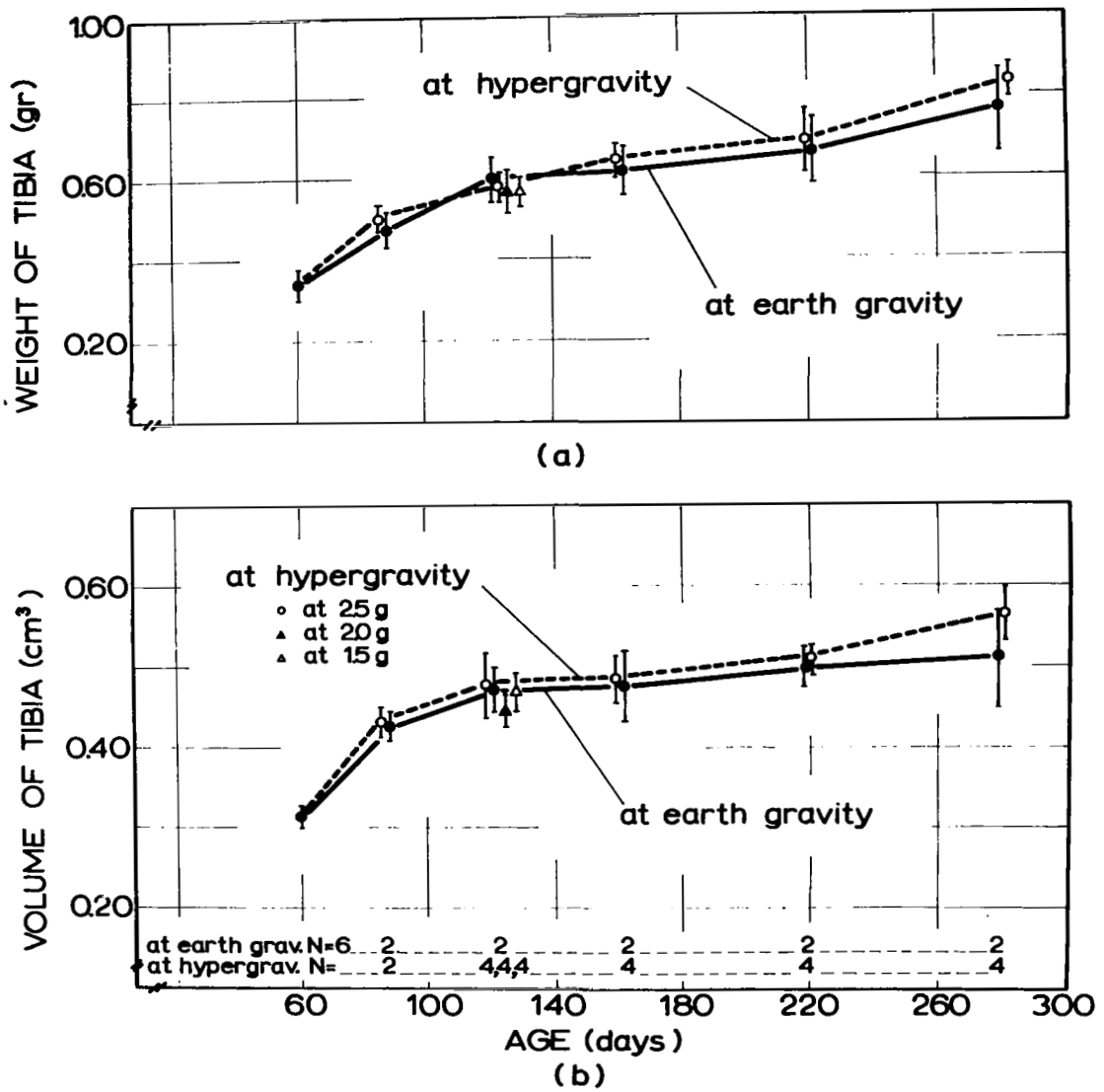


Figure 16. a) Weight and b) volume of the tibia at earth gravity and at hypergravity

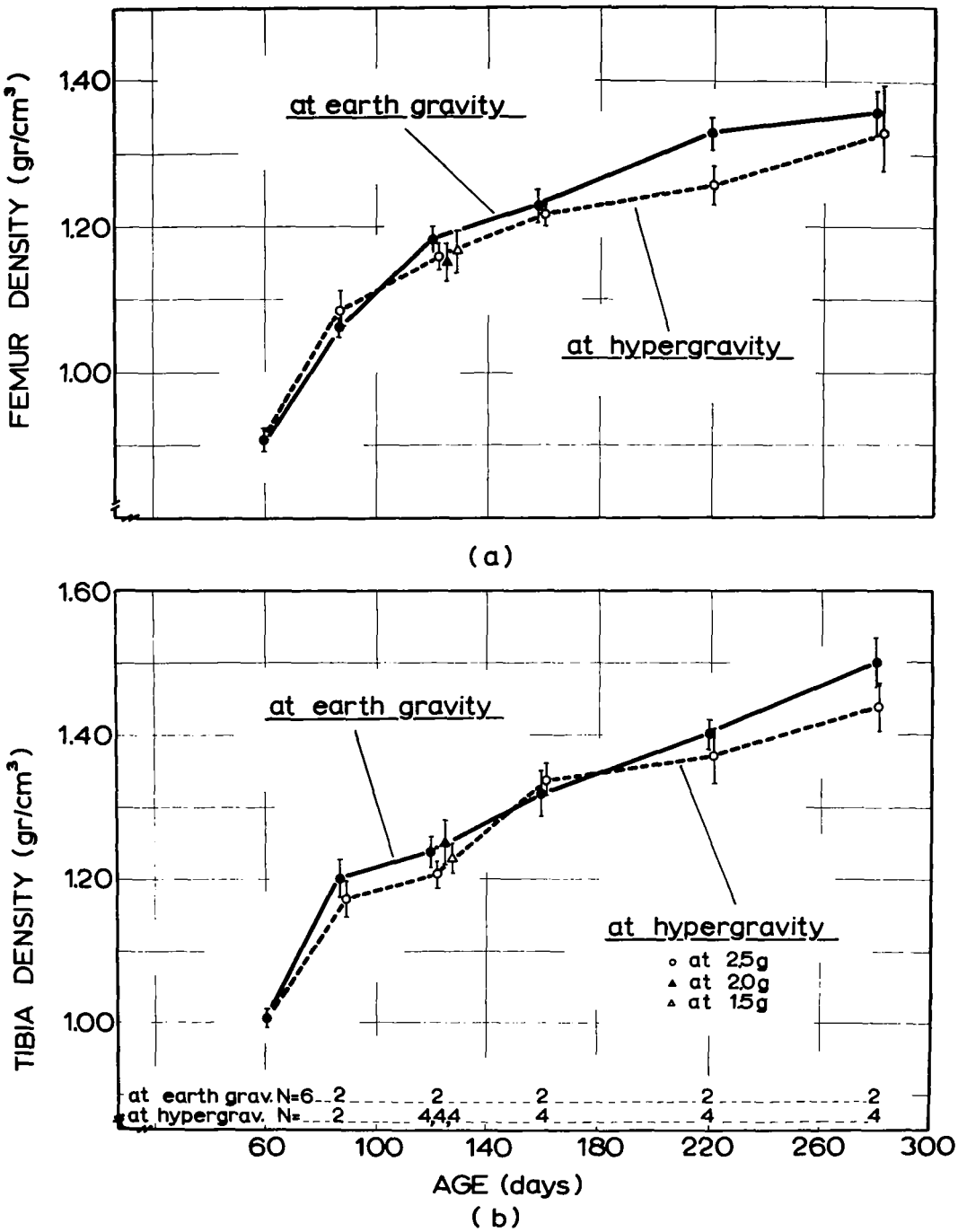


Figure 17. a) Density of the femur and b) density of the tibia at earth gravity and at hypergravity

Figures 18 and 19 show the weight and volume of the femur and tibia in rats exposed to chronic vibration against the weight and volume of controls. The density of the bones is shown in Figure 20 as a function of age. Vibration does not affect the normal development of the weight, volume and density of rat bone.

The weight, volume and density curves, as a function of age, resemble the growth curve, which tempts one to display the bone parameters not as a function of age (as above) but as a function of body weight. This has been customary in previous investigations (Wunder and Lutherer, 1964; Smith and Saville, 1966; Saville and Smith, 1966; Smith and Felts, 1968; Saville and Whyte, 1969). Bone weight, volume and density as functions of body weight were similarly plotted for rats used in this study, at earth gravity and at 2.5g hypergravity in Figures 21, 22 and 23. The regression lines of the data were calculated for the two groups with the method of least squares. The correlation coefficient of linearity and the F-ratio, expressing the variation of the data between and within the experimental groups, were also computed (according to Snedecor and Cochran, 1967).

Table 3 summarizes the results of the statistical analysis, carried out on the Laboratory's IBM 1800 computer. The correlation coefficients indicate that bone weight, volume and density of the

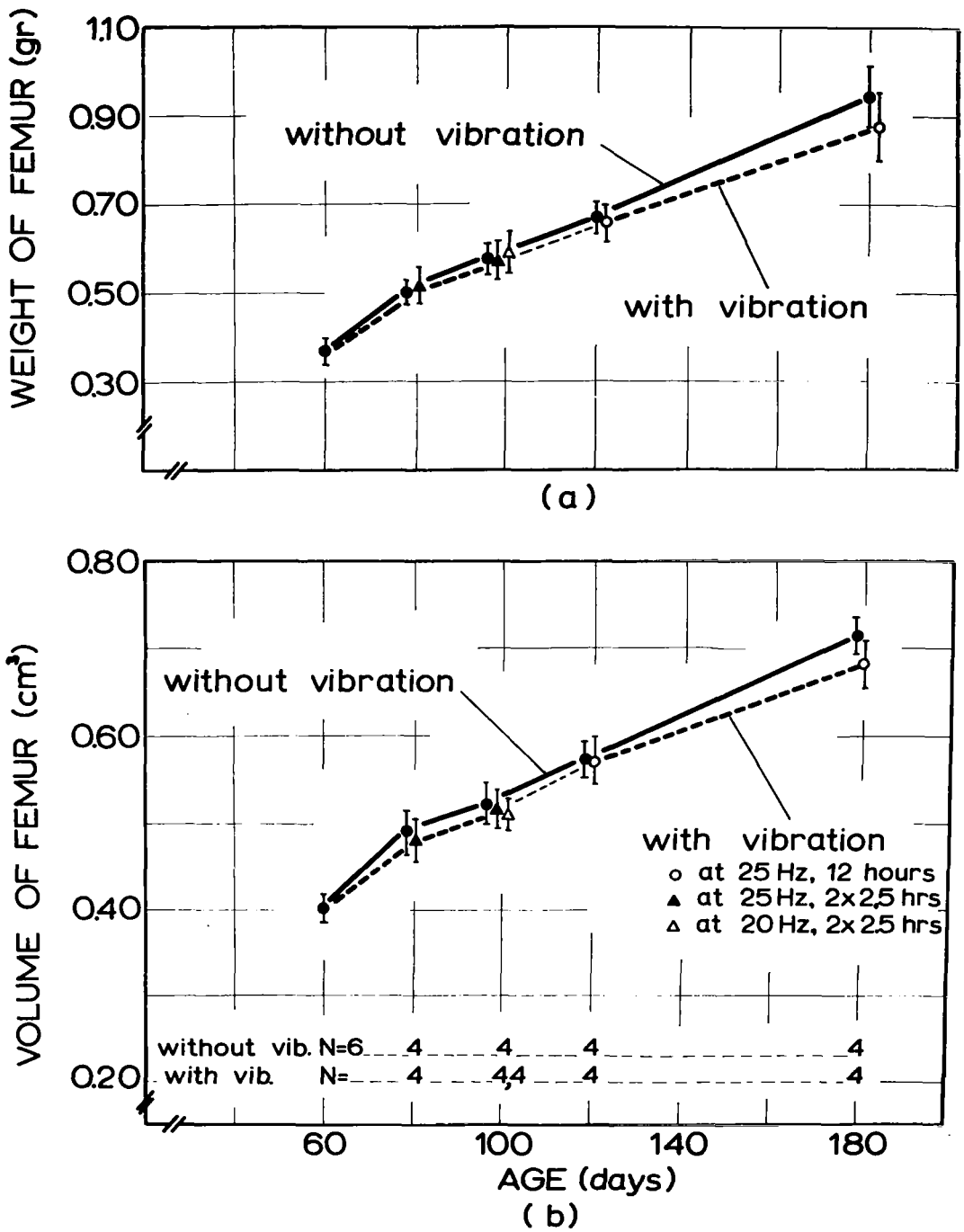


Figure 18. a) Weight and b) volume of the femur with and without vibration

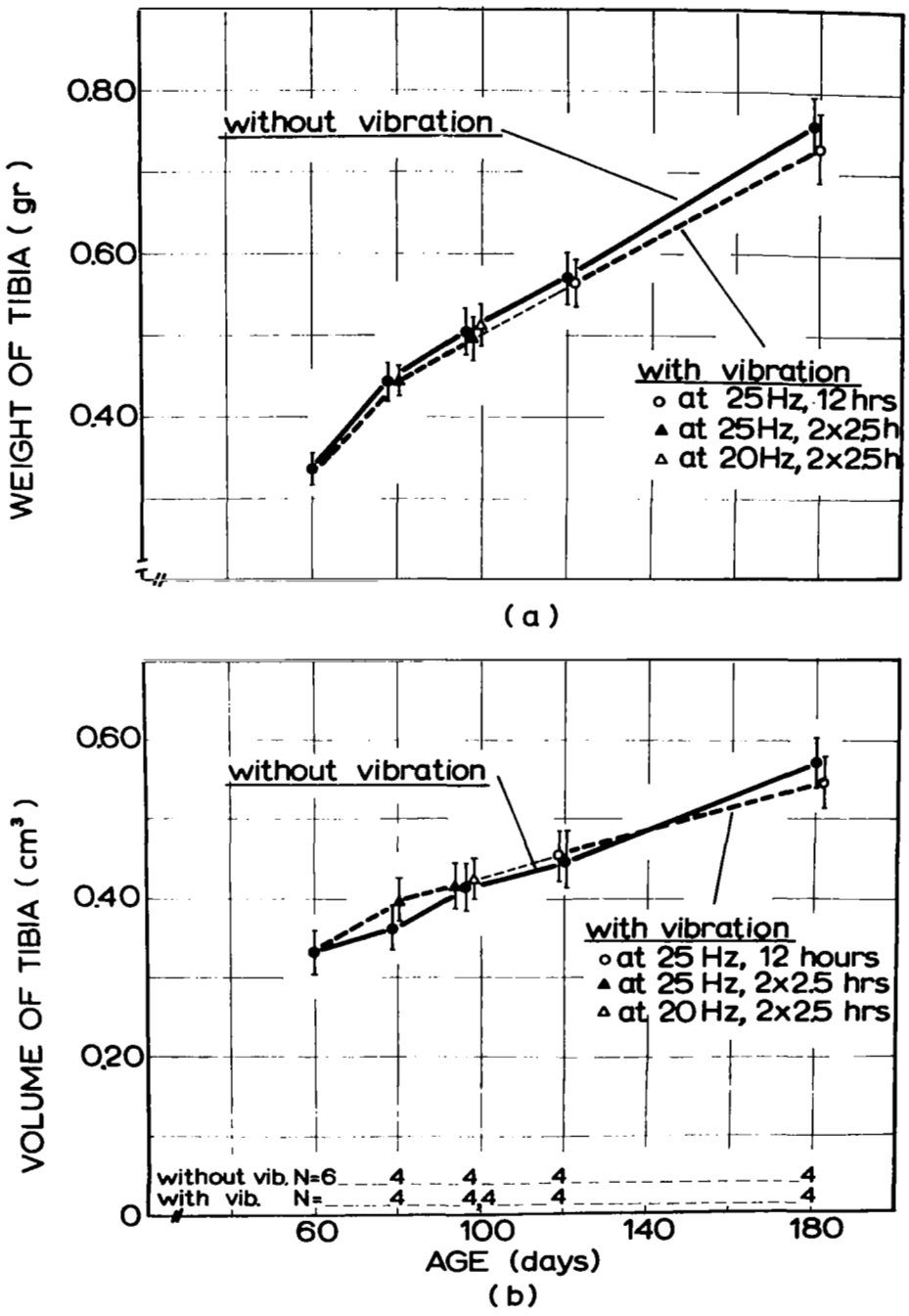


Figure 19. a) Weight and b) volume of the tibia with and without vibration

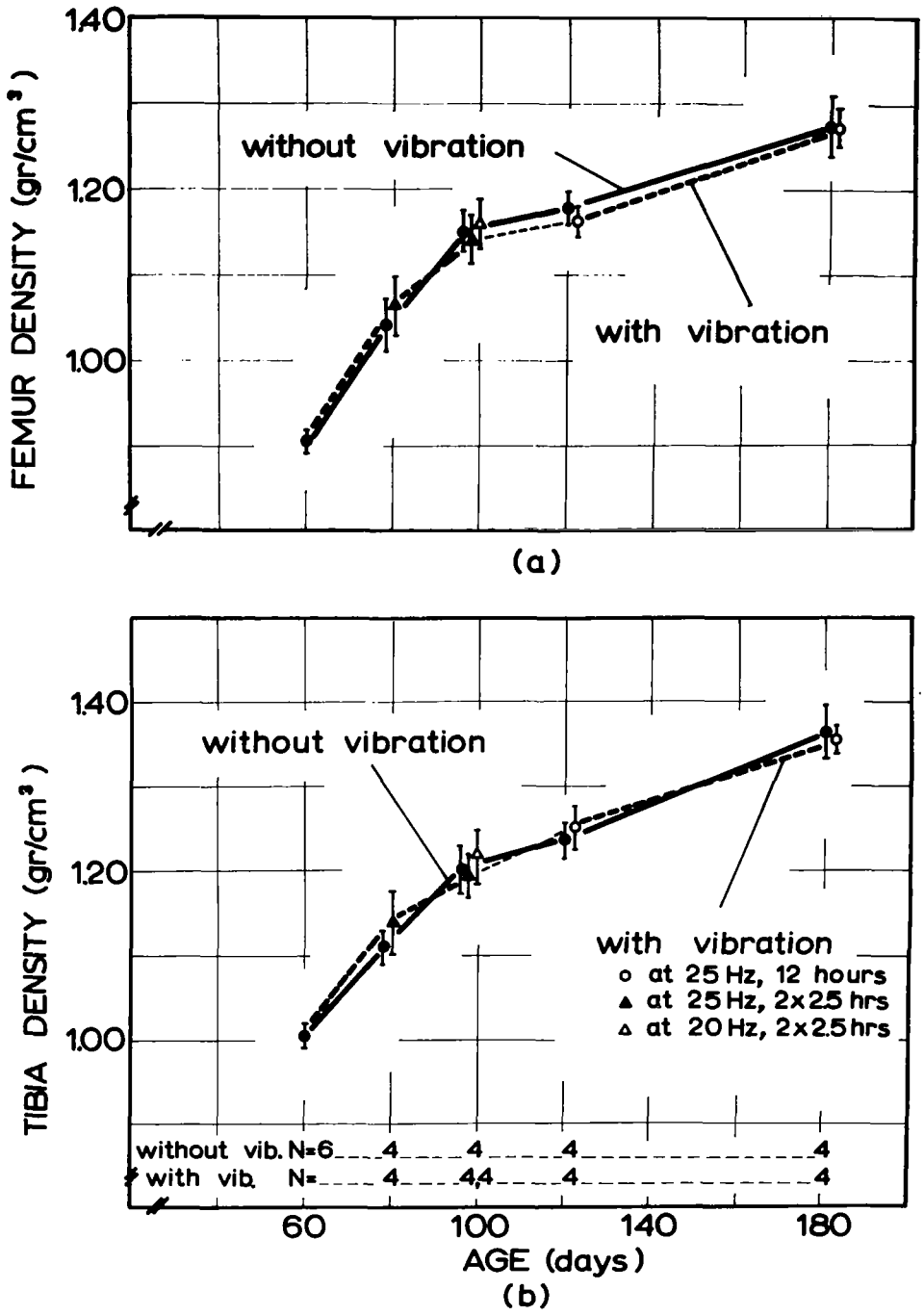
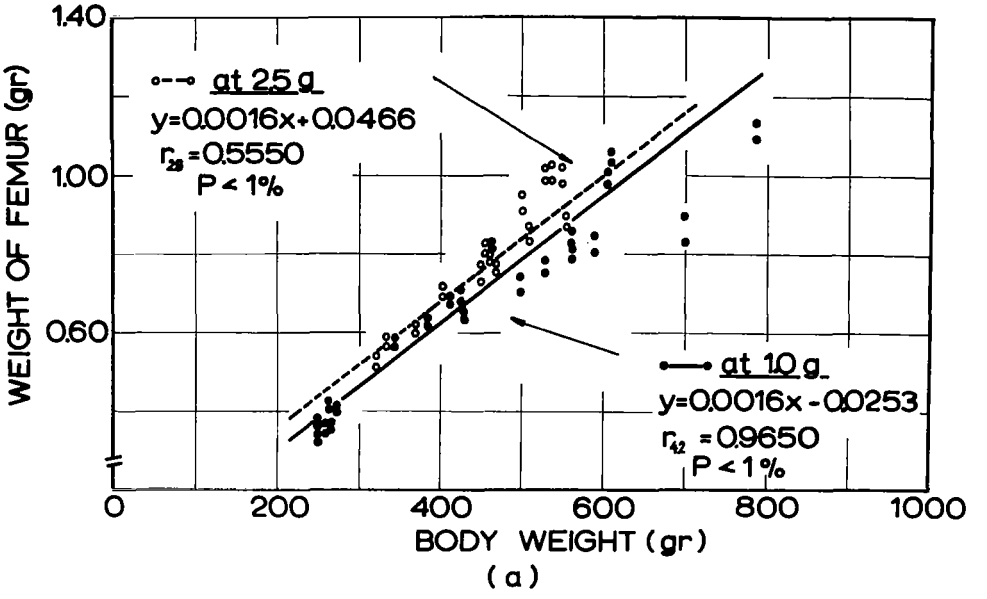


Figure 20. a) Density of the femur and b) density of the tibia with and without vibration



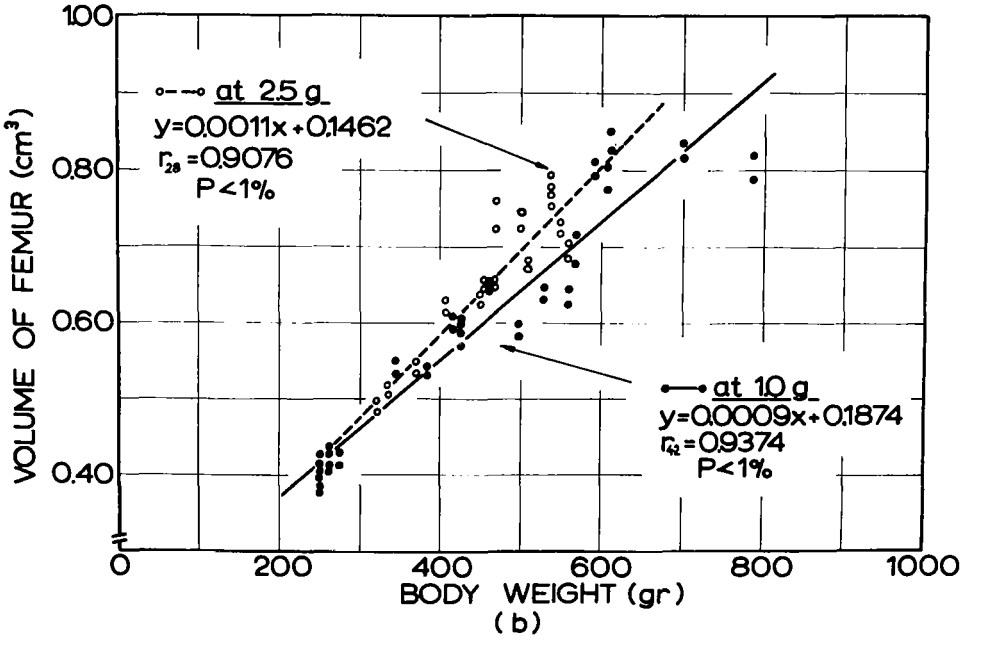


Figure 21. a) Weight of the femur and b) volume of the femur as functions of body weight, at earth gravity and at 2.5g hypergravity

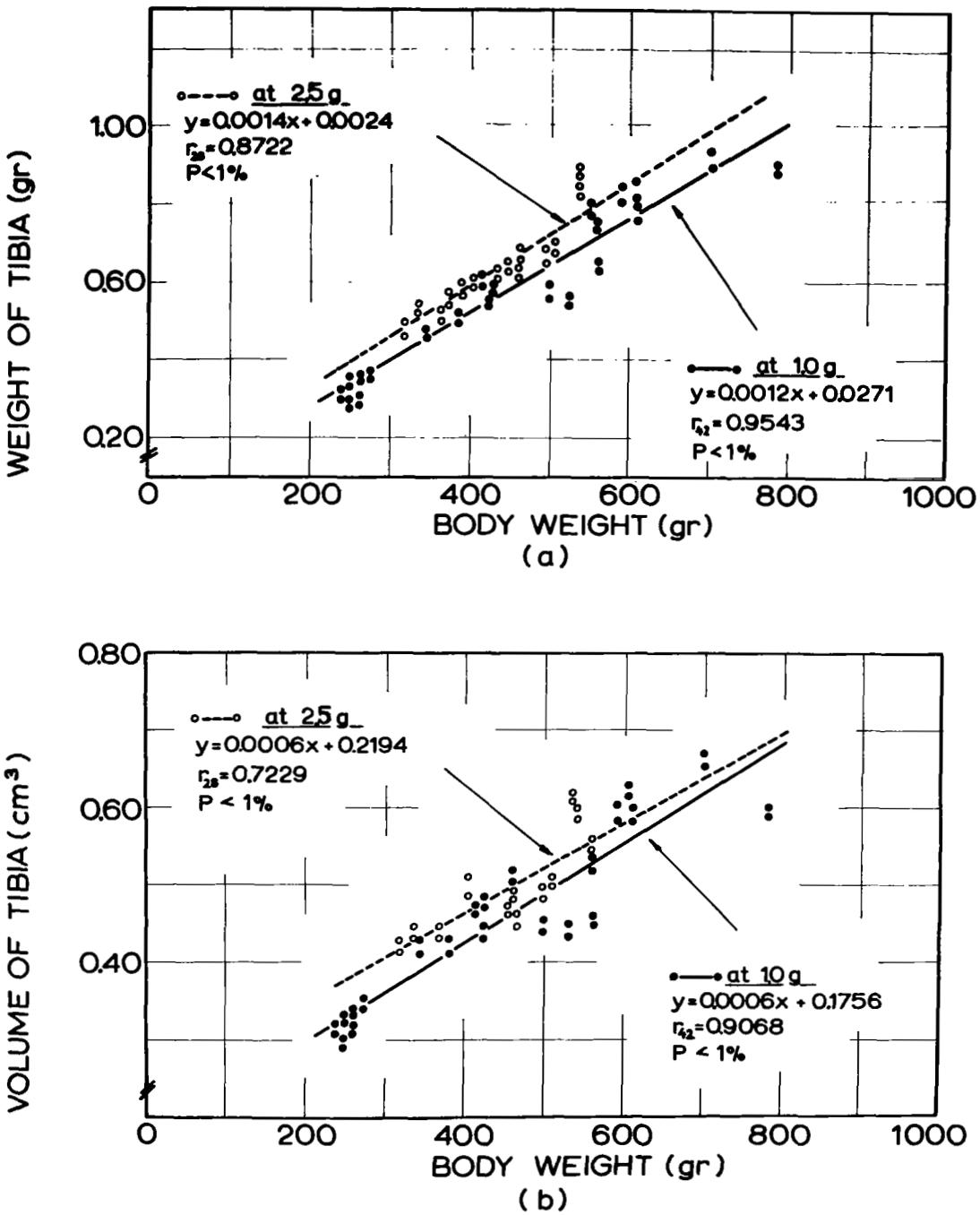


Figure 22. a) Weight of the tibia and b) volume of the tibia as functions of body weight, at earth gravity and at 2.5g hypergravity

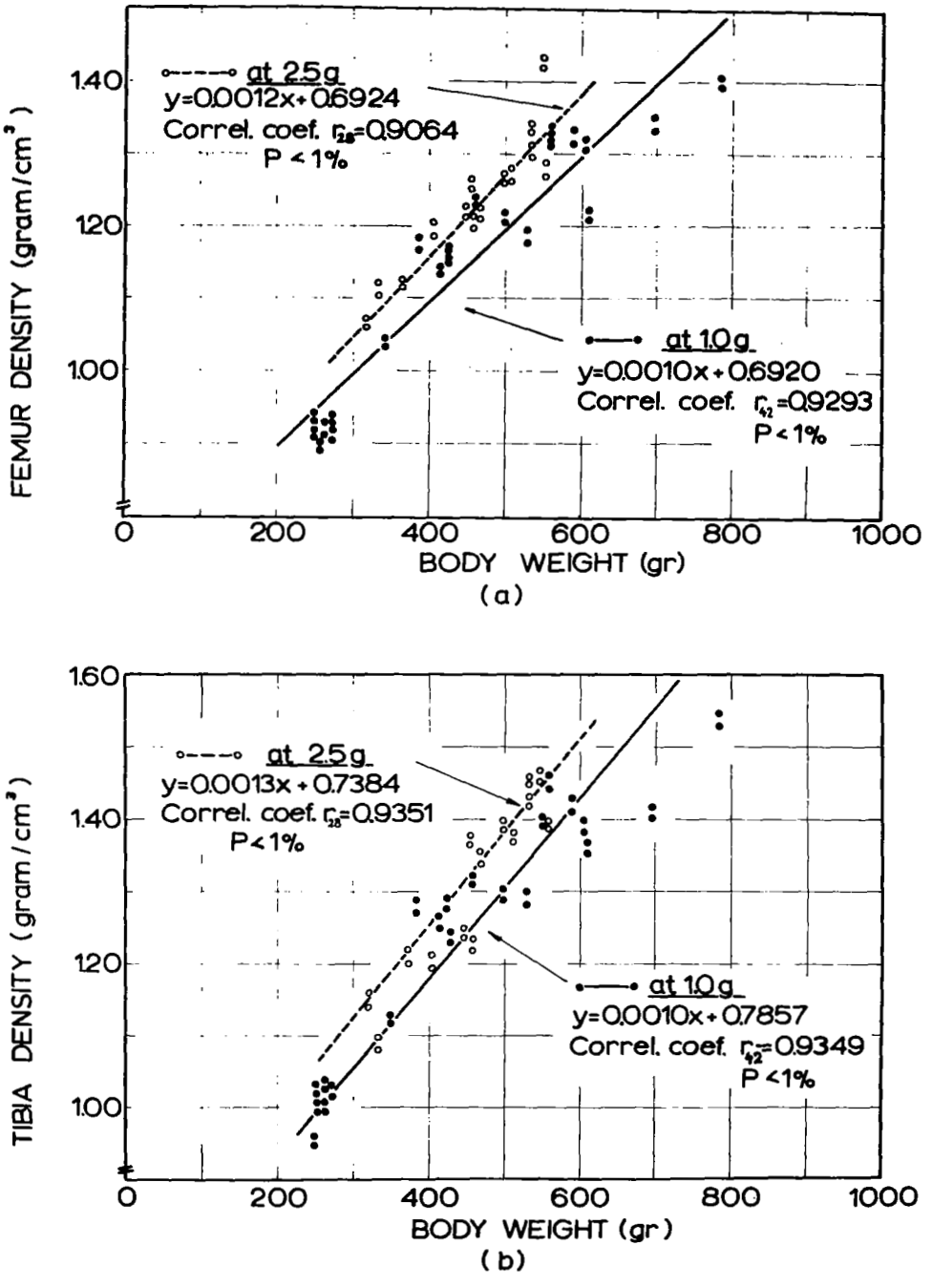


Figure 23. a) Density of the femur and b) density of the tibia as functions of body weight, at earth gravity and at 2.5g hypergravity

Table 3. Statistical Analysis of Weight, Volume and Density of Bone as Functions of Body Weight Between Experimental Groups at Earth Gravity and at 2.5 g Hypergravity

		Gravity Level	N	Regression Equation	Correlation Coeff.		F Ratio	
					r	P	F	P
Femur	Weight	1.0g	42	y = 0.001631x - 0.0253	0.9650	<1.0 %	1.2377	>10.0 %
		2.5g	28	y = 0.001680x + 0.0466	0.5550	<1.0 %		
	Volume	1.0g	42	y = 0.000912x + 0.1872	0.9374	<1.0 %	3.1257	> 5.0 %
		2.5g	28	y = 0.001096x + 0.1462	0.9076	<1.0 %		
	Density	1.0g	42	y = 0.001012x + 0.6920	0.9293	<1.0 %	6.4339	< 2.5 %
		2.5g	28	y = 0.001274x + 0.6925	0.9064	<1.0 %		
Tibia	Weight	1.0g	42	y = 0.001248x + 0.0271	0.9543	<1.0 %	3.4106	>10.0 %
		2.5g	28	y = 0.001443x + 0.0242	0.8722	<1.0 %		
	Volume	1.0g	42	y = 0.000633x + 0.1756	0.9068	<1.0 %	3.5402	>10.0 %
		2.5g	28	y = 0.000606x + 0.2194	0.7279	<1.0 %		
	Density	1.0g	42	y = 0.001021x + 0.7857	0.9349	<1.0 %	5.7567	< 2.5 %
		2.5g	28	y = 0.001285x + 0.7384	0.9351	<1.0 %		

femur and tibia in the rat are linear functions of body weight with the significance level of $P < 1\ \%$ both at hypergravity and at earth gravity.

The analysis of variance between and within the groups reveals that the density of both the femur and the tibia in the animals at hypergravity is higher than in animals of comparable weight at earth gravity. The difference is significant with $p < 2.5\ \%$ in the F-test. The difference in weight and volume of the femur of the animals at the two gravity levels is less significant statistically ($P > 10\ \%$ and $P > 5\ \%$ in the F-test). For the tibia, the F-test indicates that the differences are significant at $P > 5\ \%$.

Body weight, specifically fatty tissue weight, is affected by the environmental stress of gravity (see Figure 13). Therefore, displaying bone parameters as a function of body weight does not make explicit the fact that bone development at hypergravity follows the normal pattern of growth, i.e. the process of aging at high g progresses at the same rate as that which is observed at earth gravity. When body weight is used as the independent variable, the discrepancy in the weight of the different groups enters as a difference in either bone or soft tissue quality. However, when age is used as the independent variable, the bone

development of the various groups can be directly compared on a temporal basis. Based upon these considerations, the findings of this investigation are primarily displayed as a function of age.

Bone length and cross sectional areas:

Figure 24a shows the length of the femur and the tibia versus age. The small standard deviation of the data indicates that longitudinal bone growth is very uniform in the individual animals of the same age, and is not influenced by the relatively larger variations in body weight. At hypergravity (Figure 24a) the bones tend to be growing slower than at 1.0g. The lower hypergravity levels of 1.5 and 2.0g cause less deviation from the corresponding controls than 2.5g.

Chronic vibration does not change the normal pattern of longitudinal bone growth in the femur and the tibia, as shown in Figure 24b.

The cross-sectional growth of the femur is shown in Figure 25. The total area of each of the three locations, indicated in Figure 5, increases with age. Since both bone formation and resorption take place at the periosteal surface, an increase in total area means that bone forms faster than it resorbs. However, this increase is not the same at different locations along the diaphysis of the

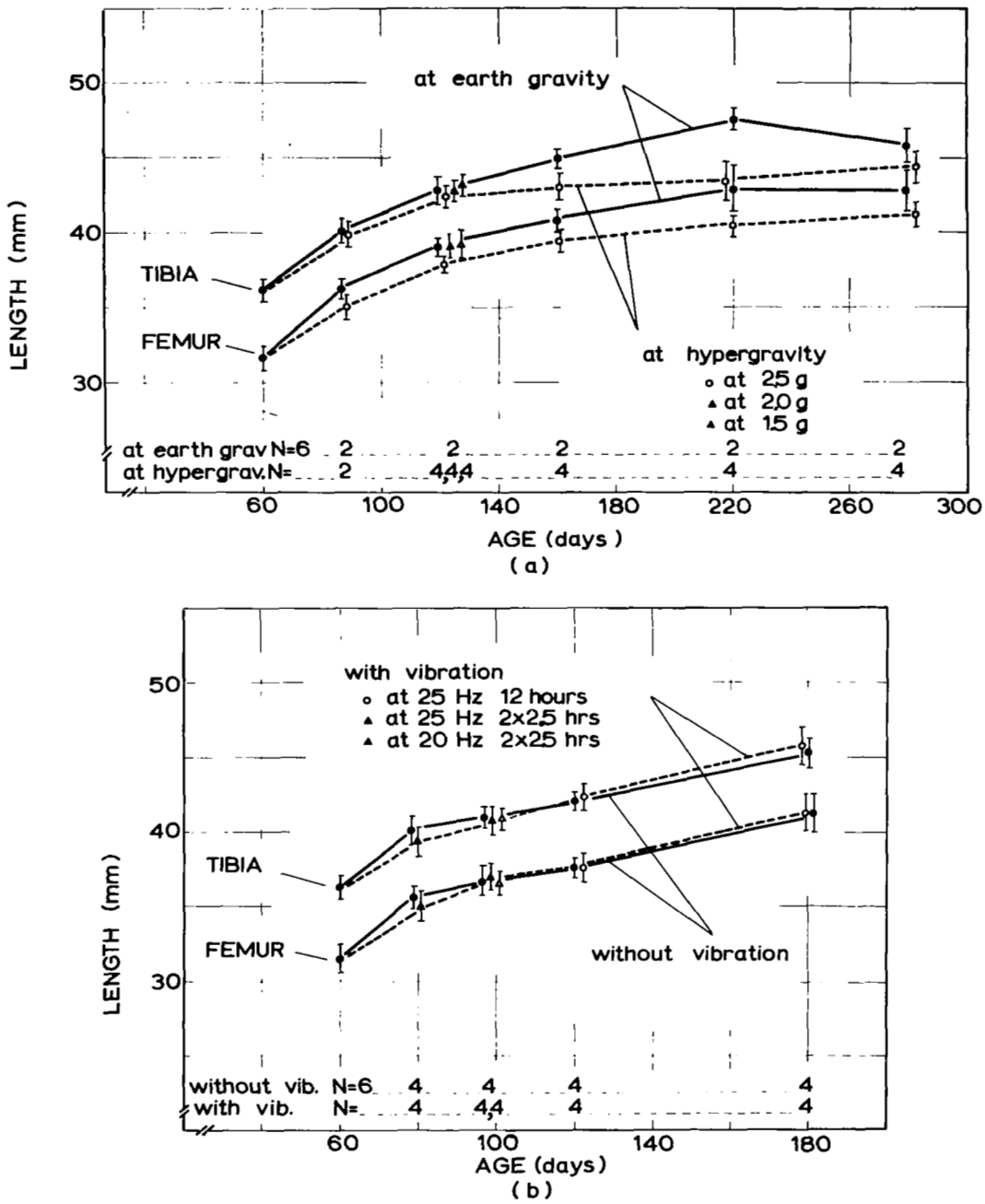


Figure 24. Longitudinal growth of the femur and the tibia
a) at earth gravity and at hypergravity
b) with and without vibration

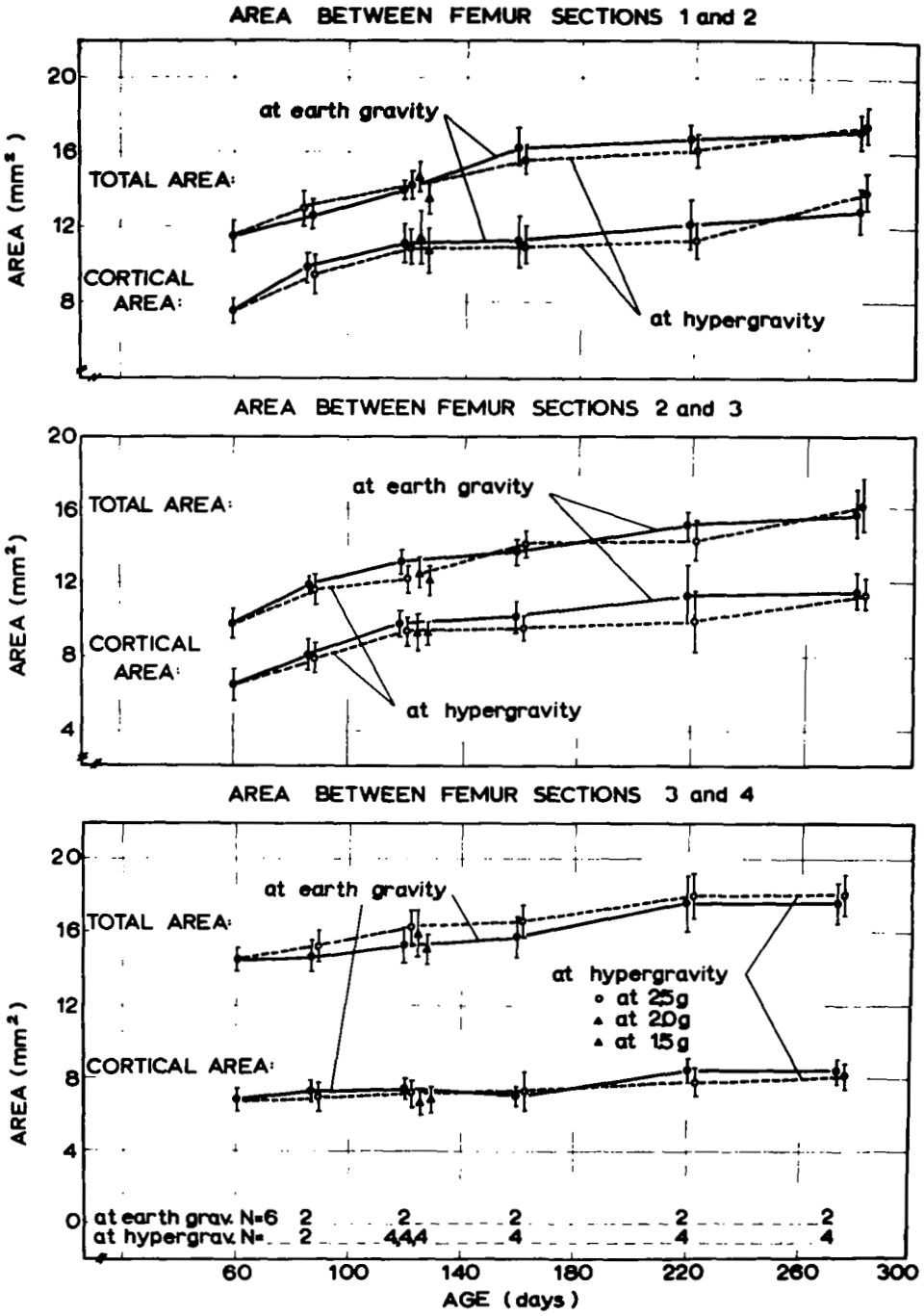


Figure 25. Cross-sectional growth of the femur at earth gravity and at hypergravity

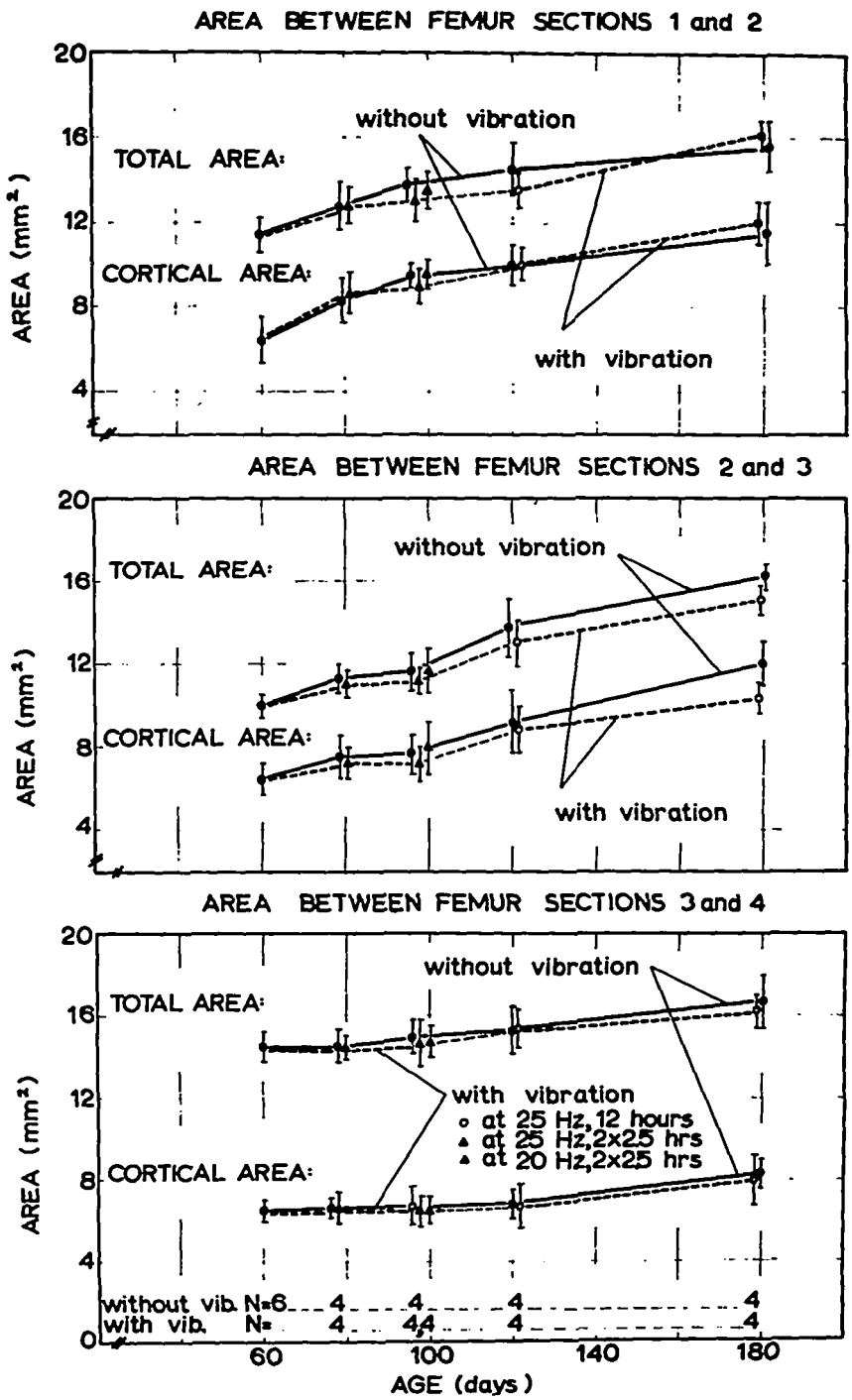


Figure 26. Cross-sectional growth of the femur with and without vibration

femur. At a distance of 0.725 length from the distal end and at 0.625L the cross section has a triangular shape and the marrow cavity is small with respect to the total area, as shown in the lower part of Figure 6. The rate of cross-sectional growth there is higher than at 0.225L where the shape of the cross section is elliptical with a relatively large marrow cavity as shown in the upper part of Figure 6.

The cortical area itself increases with age at 0.725L and 0.625L, but remains constant at 0.225L. Thus at this latter location the skeletal balance between the periosteal and endosteal surfaces is equal.

The pattern of cross-sectional bone growth at hypergravity was found to be similar to the cross-sectional development at 1.0g (Figure 25). Figure 26 shows that chronic exposure to vibration does not affect the cross-sectional growth either.

Compressive elasticity:

The steady increase of the compressive spring constant and of the modulus of elasticity during aging, found in this study and shown in Figures 27a and 28a, quantifies the well known fact that bone loses its elasticity with age. The standard deviation of the data in the compression test is relatively high because brittle

materials, such as dry bone, exhibit considerable scatter in breaking points under increasing compressive load (Shames, 1964). Chronic exposure to hypergravity does not seem to have a significant effect on the normal aging process of stiffening expressed by the spring constant as well as by the modulus of elasticity of bone (Figures 27a and 28a).

When only 2.5 hours of vibration was applied twice daily at 20 Hz and 25 Hz for 35 days, no other than normal stiffening occurred in the bone material: no change was found between the compressive elasticity of bone under vibration as compared to that of the non-vibrated controls. However, chronic vibration of 12 hours daily at 25 Hz did increase significantly both the compressive spring constant and the modulus of elasticity in the femur as Figure 29a and 30a show. During 60 and 120 days of vibration (at ages of 120 and 180 days respectively bone became significantly more rigid than bone in normal aging.

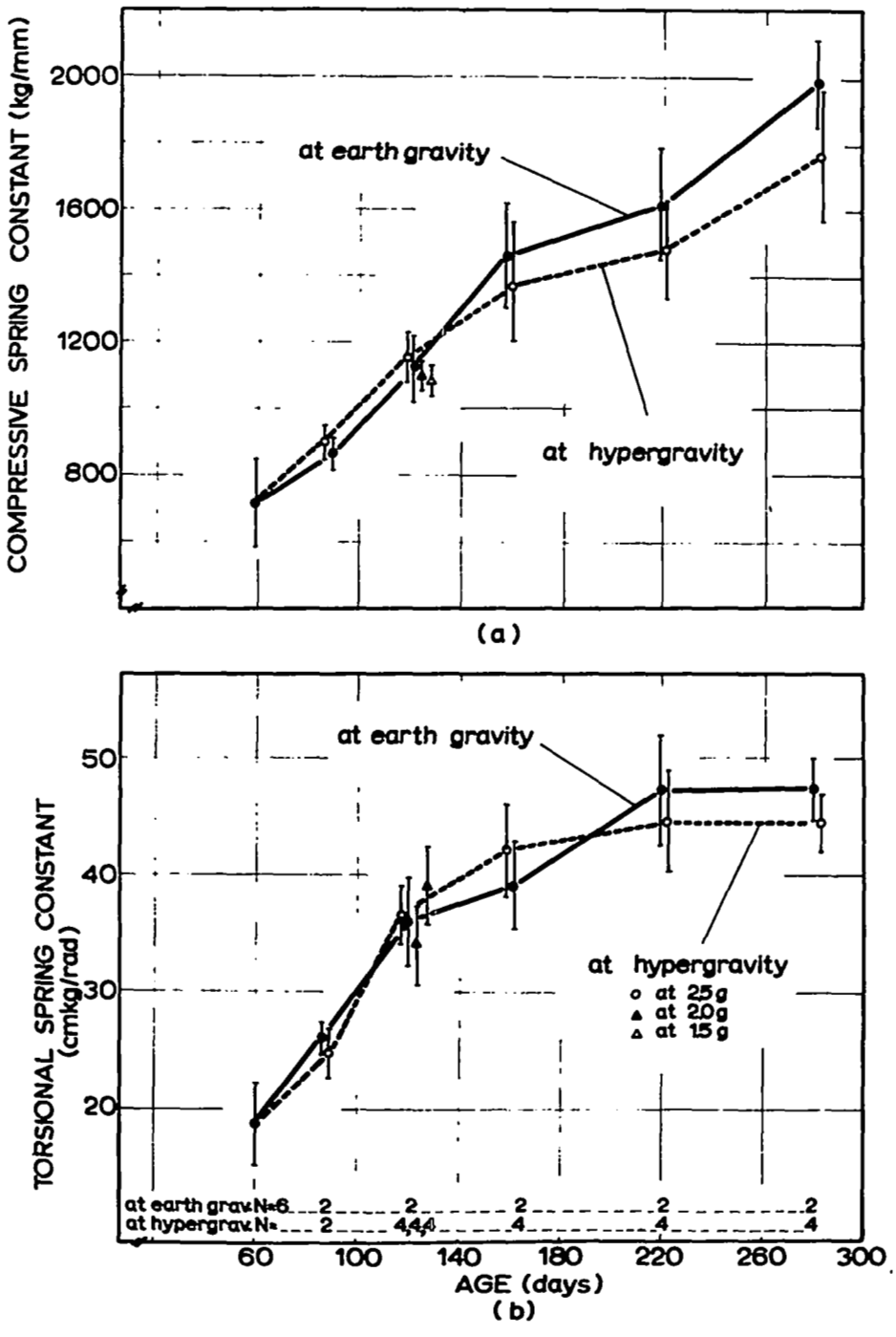


Figure 27. a) Compressive spring constant and b) torsional spring constant of the femur at earth gravity and at hypergravity

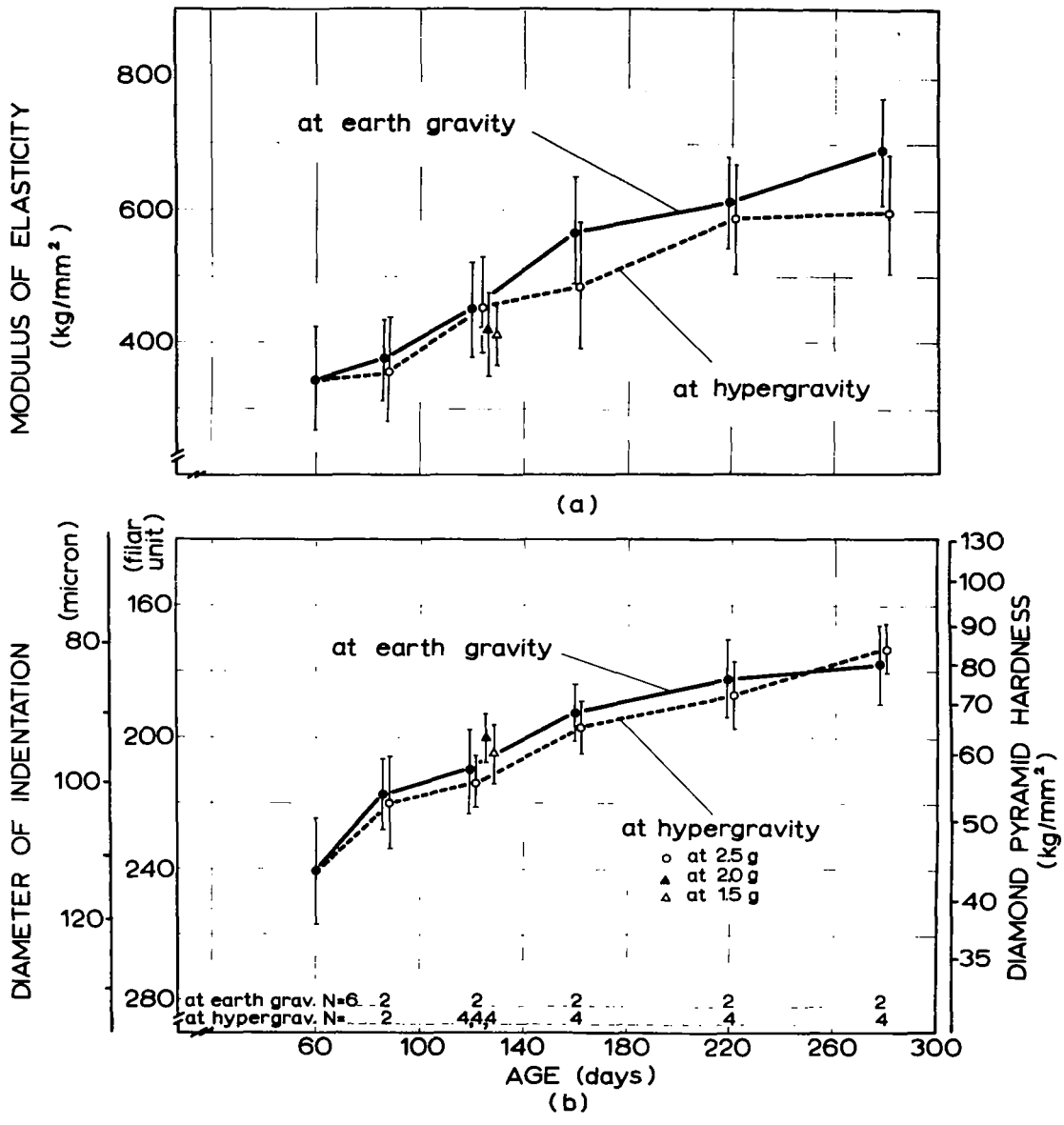


Figure 28. a) Modulus of elasticity and b) microhardness of the femur at earth gravity and at hypergravity

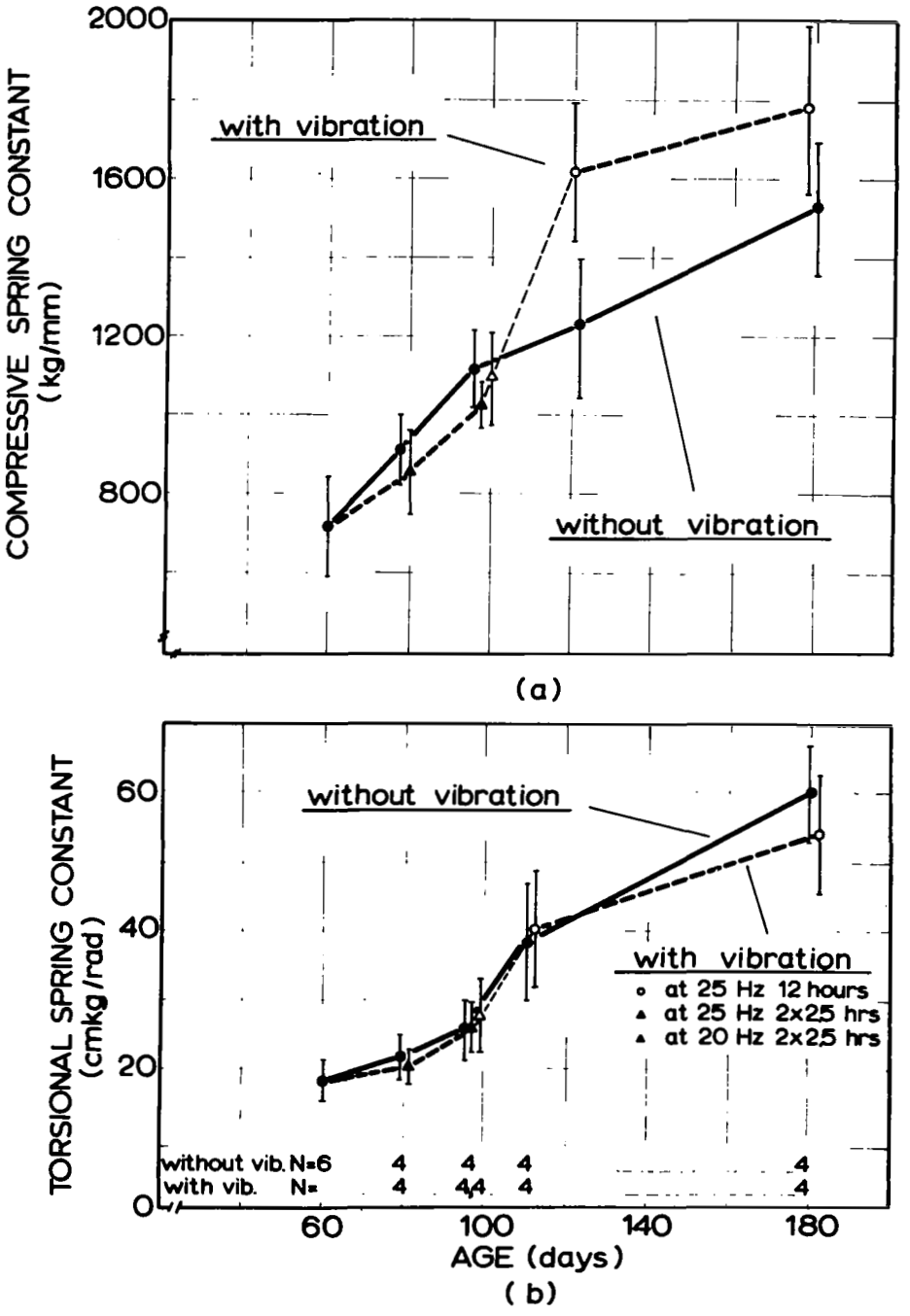


Figure 29. a) Compressive spring constant and
b) torsional spring constant of the
femur with and without vibration

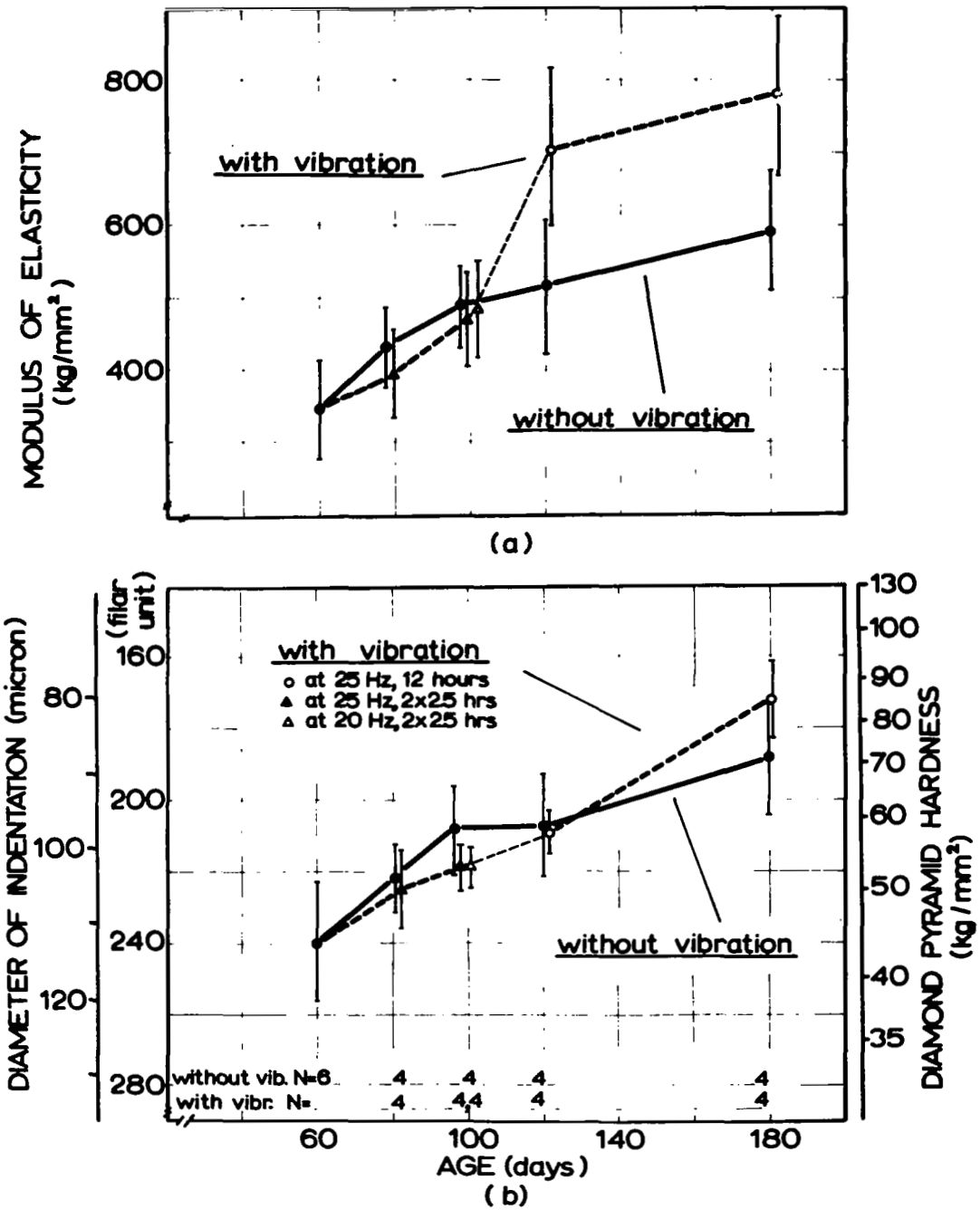


Figure 30. a) Modulus of elasticity and b) microhardness of the femur with and without vibration

Torsional elasticity:

As was to be expected, torsional stiffness of bone, as expressed by the torsional spring constant, was also found to increase with age reaching a maximum at around 220 days of age as shown in Figure 27b. No further increase was observed after that age.

Chronic exposure to hypergravity and vibration at the intensity levels investigated here did not change the torsional elasticity of the femur. This is shown in Figures 27b and 29b.

Microhardness:

Figure 28b shows that microhardness of bone increases with age. Chronic exposure to hypergravity does not influence the normal hardening process in the bone.

Long term chronic vibration, however, tends to increase hardness as shown in Figure 30b: after 120 days of vibration with 12 hours of daily exposure at 25 Hz, microhardness of vibrated bone has become higher than that of the non-vibrated controls.

Conductivity of sound:

Figure 31 depicts velocity of sound through Sections 2 and 3 of the femur versus age, at earth gravity and hypergravity. Sound conductivity through bone is found to increase rapidly in early age and more gradually later, especially in Section 3, which comprises 40 % of the total femur length and constitutes the major part of the diaphysis. This rise in velocity of sound and the corresponding rise in ash and calcium content, observed during the same period, make it apparent that conductivity of sound and state of mineralization in bone must be related.

Velocity of sound in homogeneous material is, as stated earlier, proportional to the square root of the ratio of modulus of elasticity and density. The function $\sqrt{\frac{E}{d}}$ versus age for femural bone was calculated from the measured values of "E" and "d", and entered in Figure 31a. It has essentially the same shape as the experimentally obtained velocity of sound curve, the only difference being that the calculated points are about $\Delta v = 150$ m/sec above those of the measured points. This must be due to the fact that bone is a porous and inhomogeneous material.

Chronic exposure to hypergravity and vibration does not affect the normal temporal increase in sound conductivity of bone to any significant degree (Figures 31 and 32).

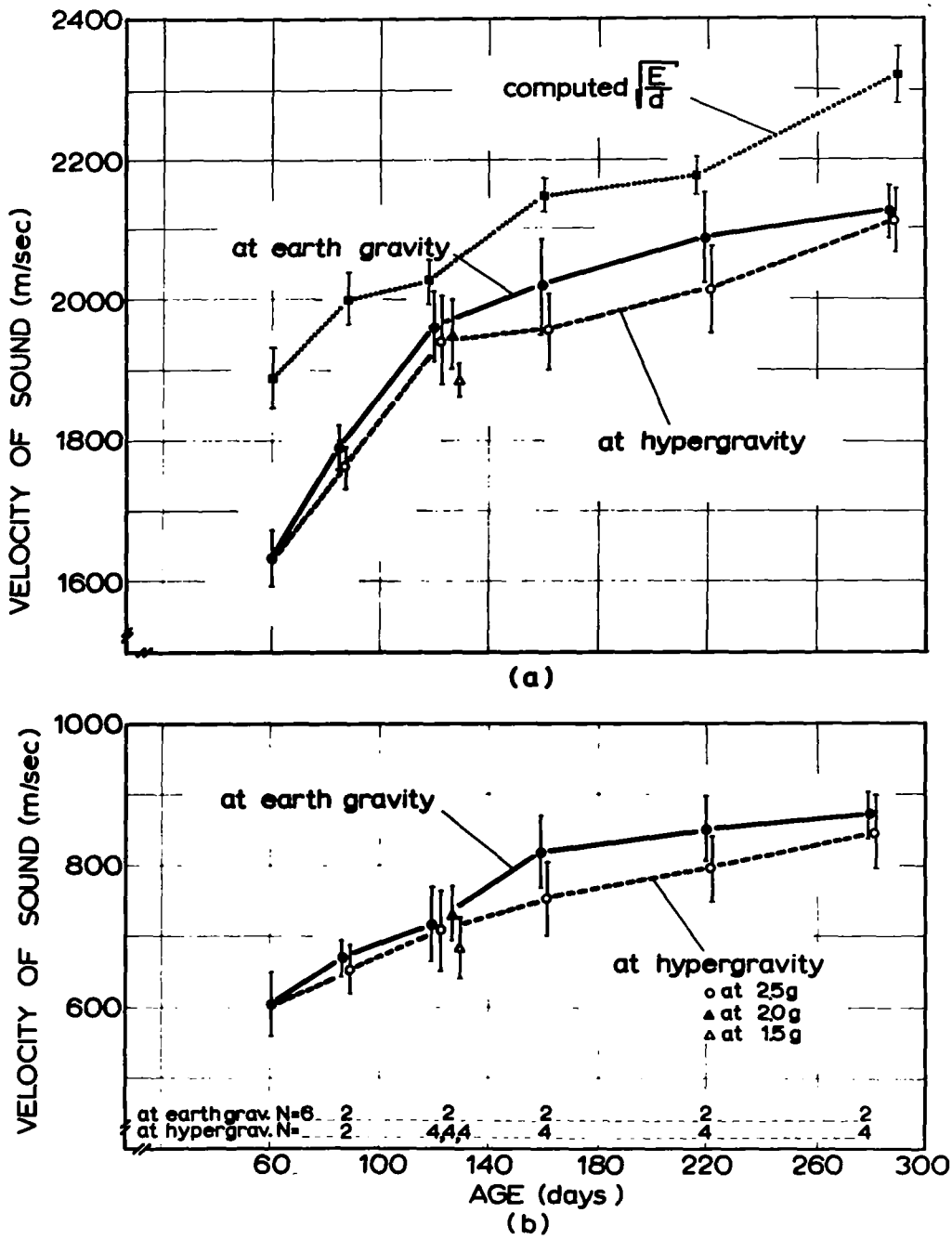


Figure 31. Velocity of sound in a) Section 3 and b) Section 2 of the femur at earth gravity and at hypergravity

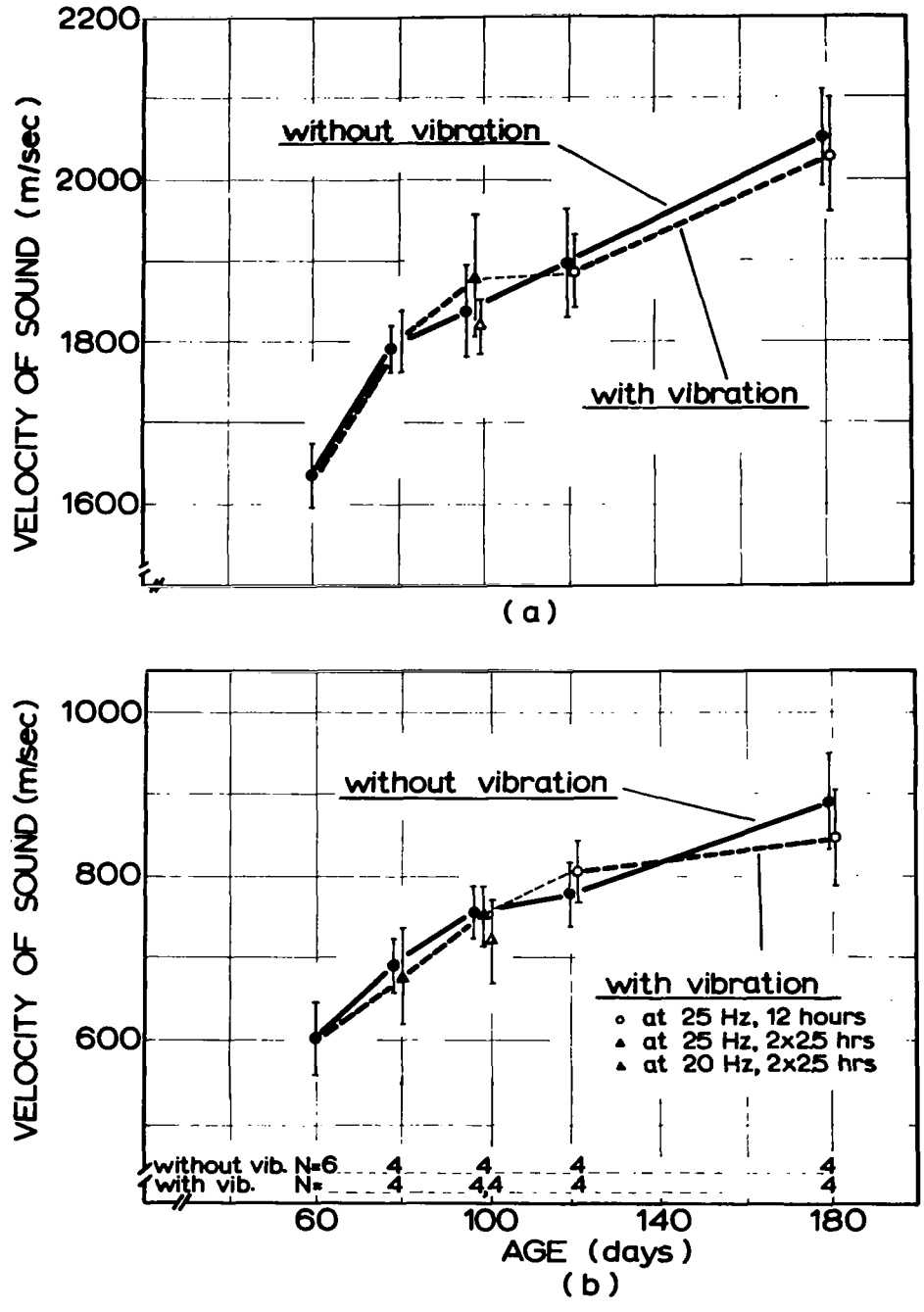


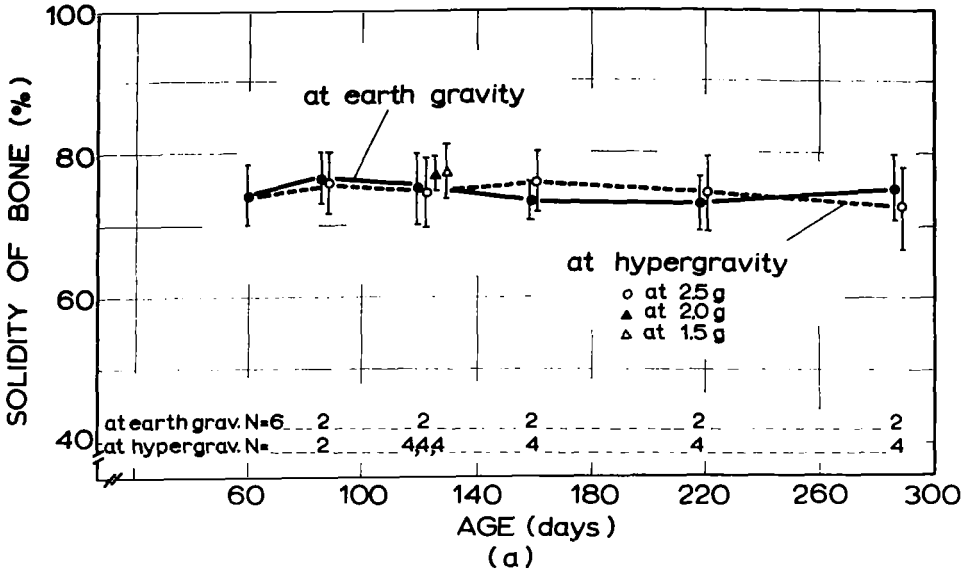
Figure 32. Velocity of sound in a) Section 3 and b) Section 2 of the femur with and without vibration

Histological solidity of bone:

The amount of solid matter in bone, expressed as percent of total volume, is shown in Figure 33. Solidity of bone remains almost constant over the time period of this investigation. The slight increase or decrease is of the same magnitude as the variability of the data and osteoporosis due to aging does not appear to occur in rats during the span of life which was studied in the experiment. This sorresponds with a slightly decreasing, but statistically insignificant change of bone solid matter found in humans with advancing age (Dunill and Anderson, 1967). Chronic exposure to hypergravity and vibration was found not to alter the normal porosity level of the femur.

Ash and calcium content:

The ash content found in the hip joint (Section 1, Figure 5) is plotted as a function of age in Figures 34a and 36a and that of the knee joint in Figures 35a and 37a. Ath the relaively young age of 60 days, the ash content of the femur is only 50 % of the dry weight in the knee joint and 54 % in the hip, showing that there is a high proportion of unmineralized organic matter present at this age. The ash content then increases up to the age of 160 - 180 days where a plateau of 64 % and 67 % is reached.



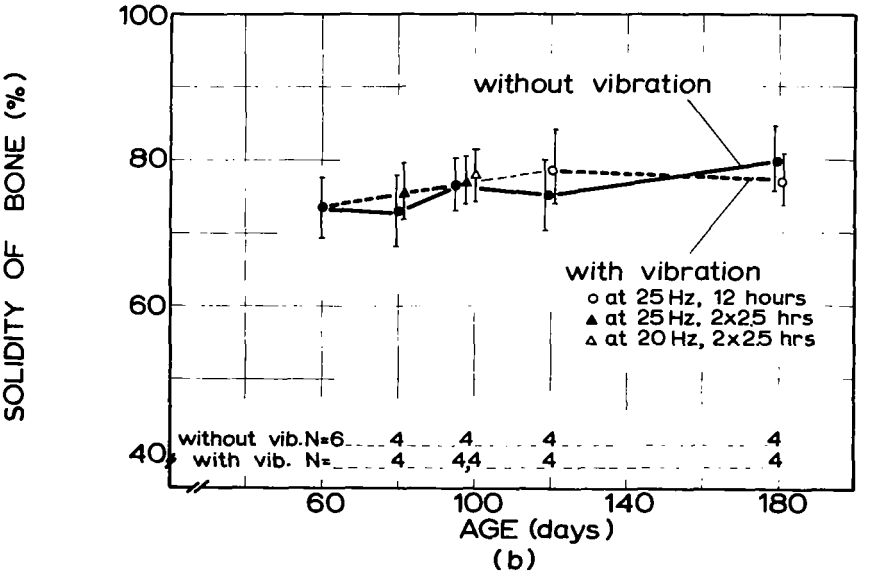


Figure 33. Solidity of bone in the femur
a) at earth gravity and at hypergravity
b) with and without vibration

The calcium content of the dry fat free bone, however, does not change with age as shown in Figures 34b, 35b, 36b and 37b. The level of calcium in the hip joint as well as in the knee joint remains almost constant around 20-21 % of the dry fat free weight.

However, the calcium content of the ash diminishes with age from 40 % to 30 - 32 % during the same period (see Figures 34c, 35c, 36c and 37c).

The findings reveal that unit weight of young bone contains smaller amounts of inorganic components than unit weight of older bone and that the calcium content of the whole bone (organic and inorganic) is constant while the proportion of calcium in the inorganic component (ash) decreases. Thus the increase in inorganic components must be due not to calcium deposition but to the deposition of other inorganic constituents (mainly carbonates, according to Fourman, 1960).

Figures 34, 35, 36 and 37 show the bone composition in the hip and knee joints of animals exposed chronically to hypergravity and vibration. Chronic exposure to hypergravity and vibration does not cause compositional changes in the femur.

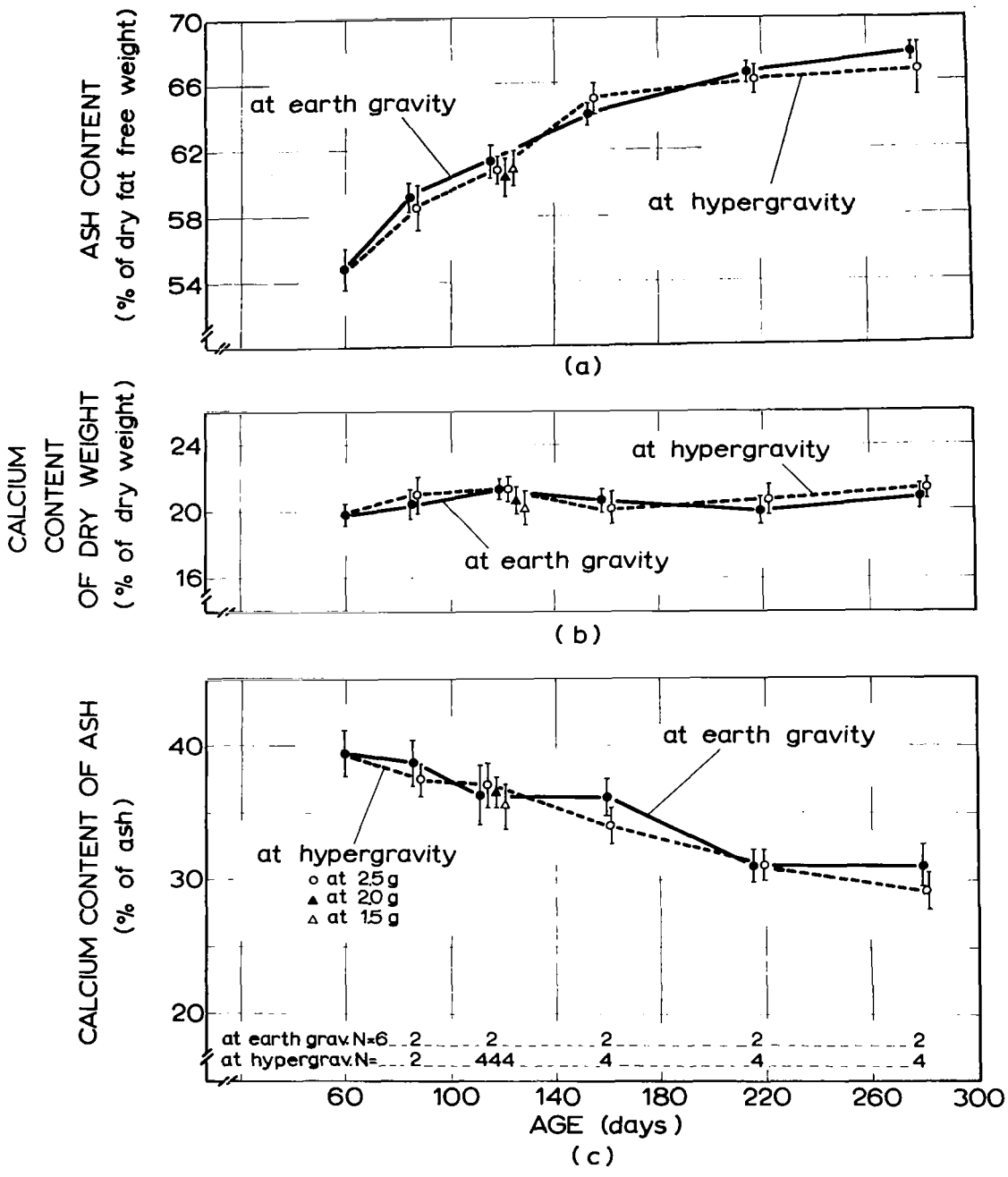


Figure 34. Compositional analysis of Section 1 (hip joint) of the femur at earth gravity and at hypergravity:
a) ash content of the bone
b) calcium content of the dry fat free bone
c) calcium content of the bone ash

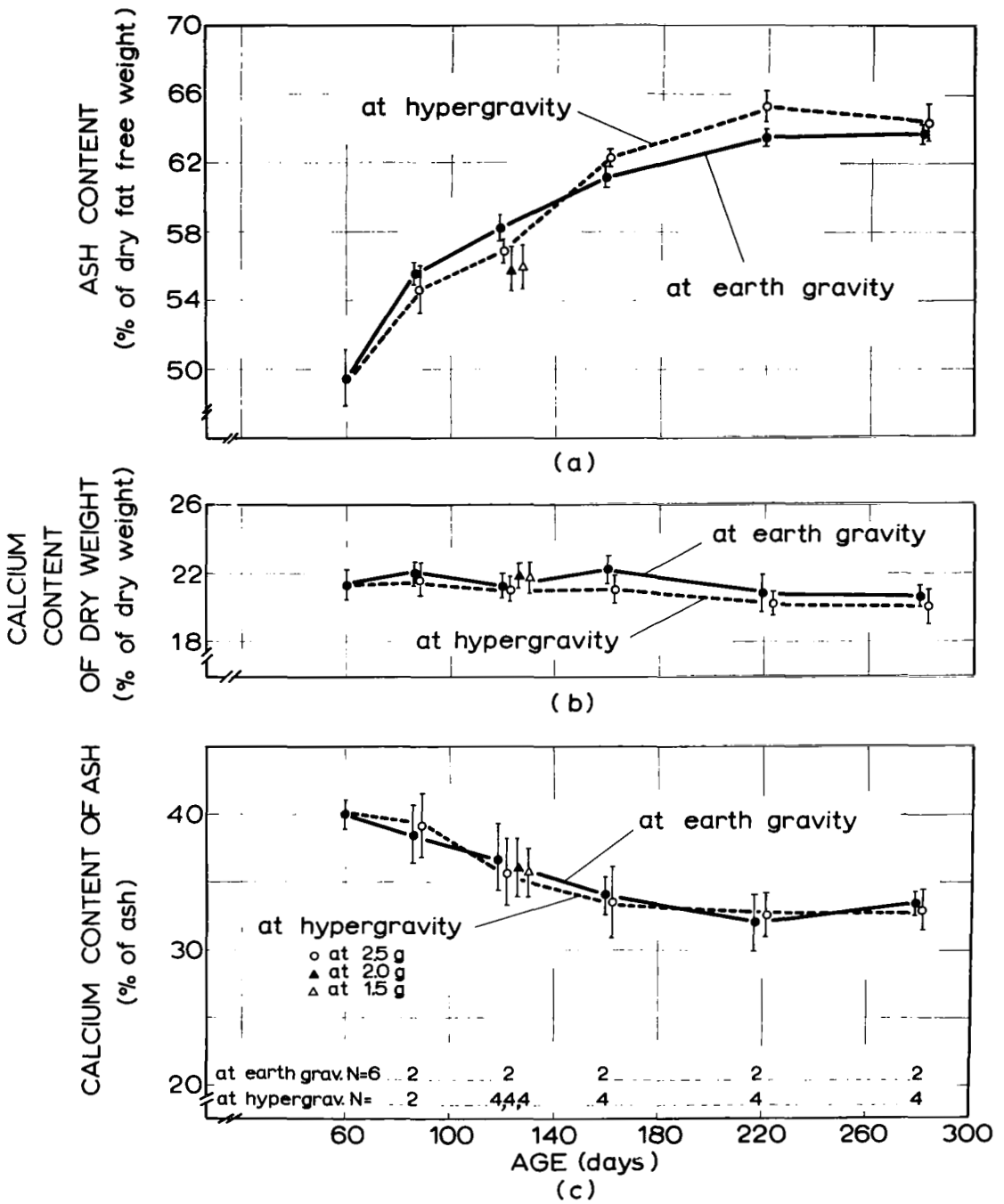


Figure 35. Compositional analysis of Section 5 (knee joint) of the femur at earth gravity and at hypergravity:
a) ash content of the bone
b) calcium content of the dry fat free bone
c) calcium content of bone ash

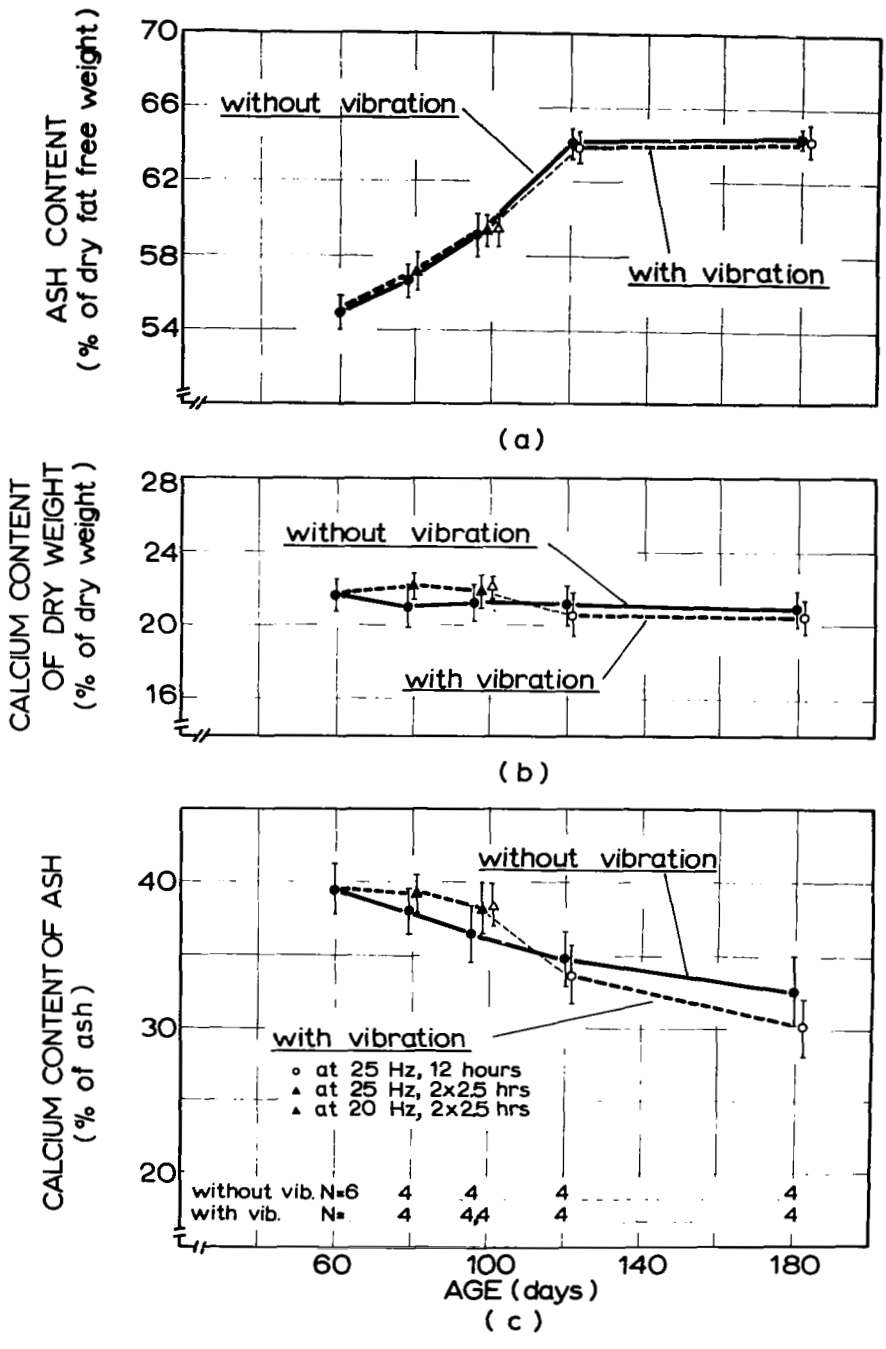


Figure 36. Compositional analysis of Section 1 (hip joint) of the femur with and without vibration
a) ash content of the bone
b) calcium content of the dry fat free bone
c) calcium content of bone ash

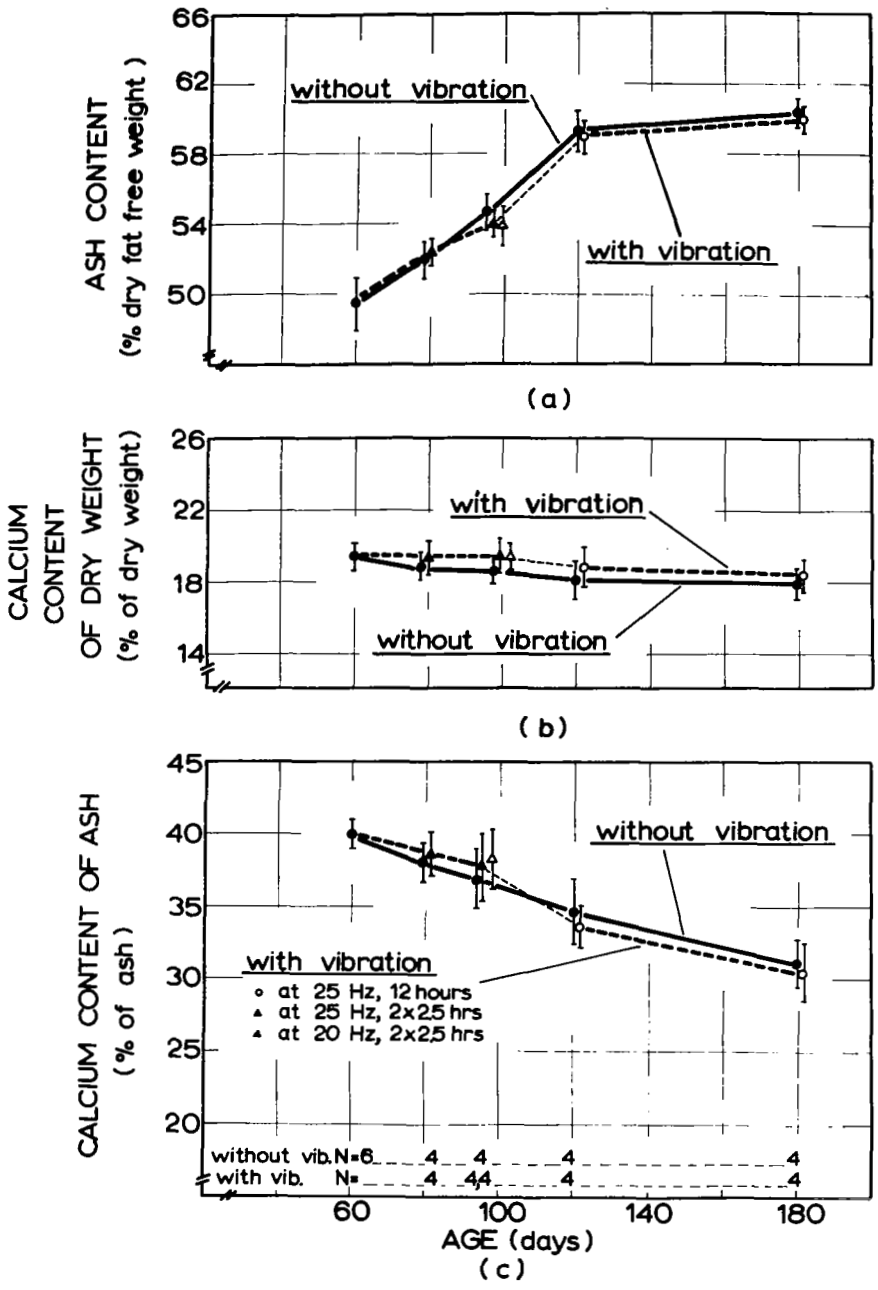


Figure 37. Compositional analysis of Section 5 (knee joint) of the femur with and without vibration:
a) ash content of the bone
b) calcium content of the dry fat free bone
c) calcium content of bone ash

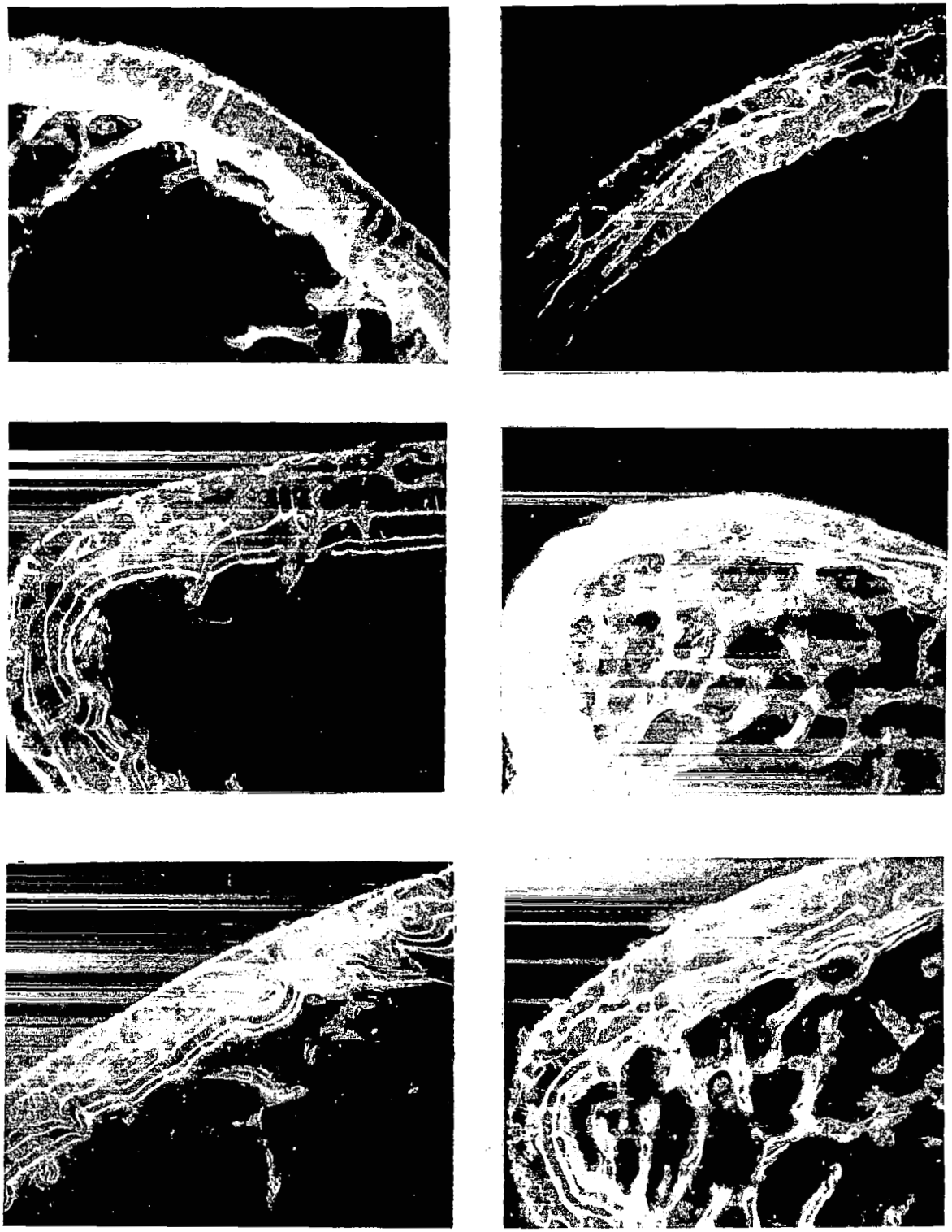
Mode of bone mineralization:

Table 4 contains the schedule of tetracycline bone labeling. The ages at which tetracycline was administered and the ages at which the rats were later sacrificed are listed.

Figure 38 shows the depositions of tetracycline in a diaphyseal cross section of the femur of animals at 2.5g gravity and correspondingly at earth gravity. The three samples shown were taken in two month periods after the administration of two, four and six doses of tetracycline. The bands represent the active front of bone growth where mineralization of the organic bone matrix took place during the 36 hours of tetracycline intake. Comparison of the respective patterns of mineral deposition reveals that the active zones tend to be wider at hypergravity than at 1.0g. This apparent variation could have two reasons: (1) The active layer of mineral deposition might actually be wider under hypergravity because osteoblastic activity of the tissue is more dispersed at high gravity than at 1.0g. (2) The planes in which the microscopic sections were cut might have been slightly different from one another. Femurs are shorter at hypergravity than at 1.0g, and thus the angle between the microscopic slice and the longitudinal zone of active cells along the shaft of the bone could be different between

Table 4. Schedule of Tetracycline Bone Labeling

Experiment	Successive No. of Doses	Age (days)	
		At administration of Tetracycline	At sacrifice
Centrifugation at 2.5g hypergravity	1	100	120
	2	153	160
	3	190	
	4	213	220
	5	250	
	6	273	280
Vibration with 25 Hz 12 hours daily	1	59	60
	2	90	
	3	115	120
	4	145	
	5	173	180

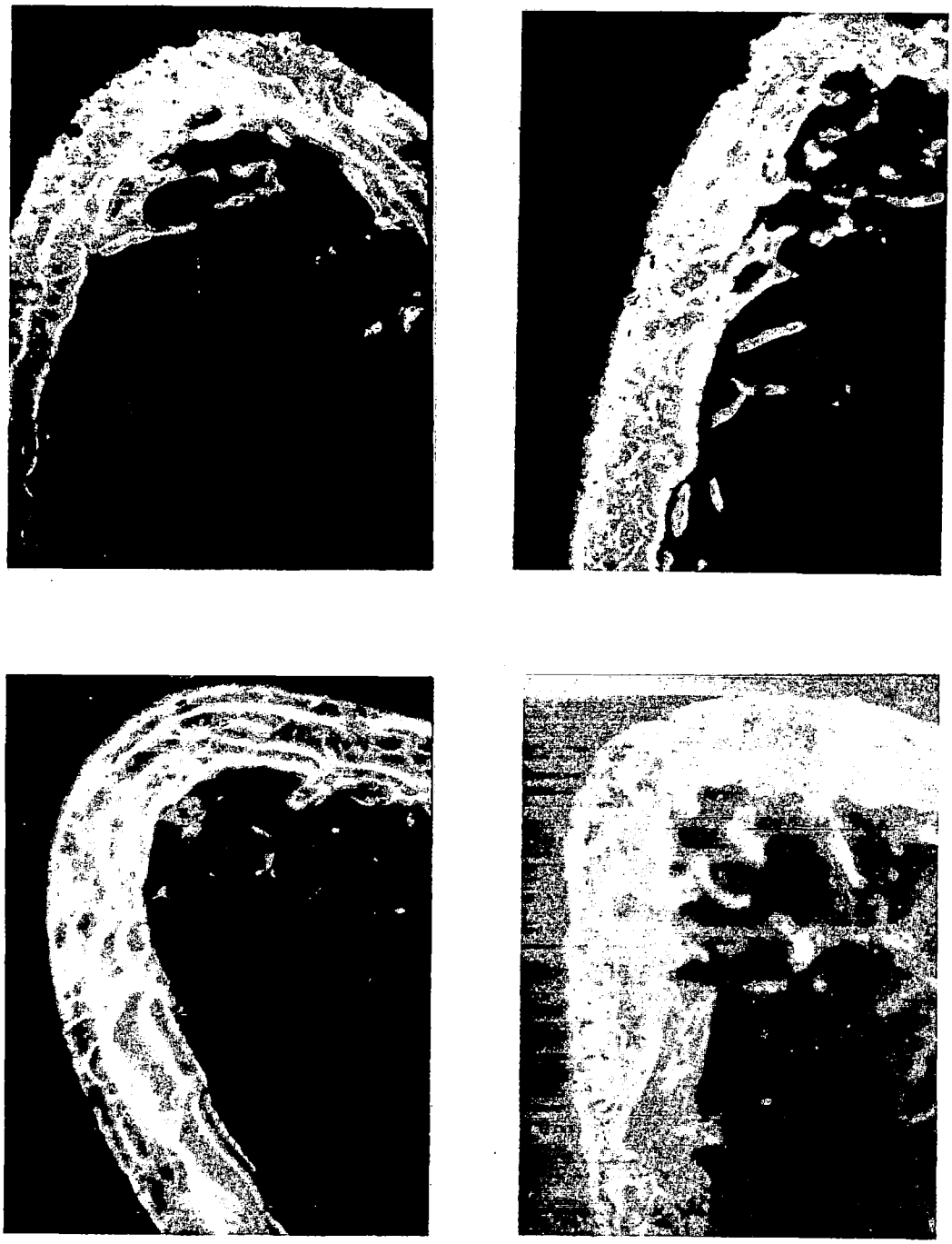


at earth gravity at hypergravity

Figure 38. Zones of active bone formation in a diaphyseal cross section of the femur, labelled by deposition of tetracycline, as visible under ultraviolet light after four, six and eight months of exposure to hypergravity. 22x natural size

the groups. Cutting the active layers at different angles would appear on the photographs as a variation in thickness even when the zones have the same width.

Figure 39 shows the effect of chronic vibration on the mode of bone deposition. The samples were taken from animals vibrated 12 hours per day for two and four months. Three and five doses of tetracycline had been administered to these rats. In the control animals, not exposed to vibration, the pattern of the active mineralization zones indicated by the tetracycline bands is regular and concentric. In the vibrated animals, however, no continuous lines are discernable, the sites of mineralization are dispersed across the diaphysis without well defined fronts of tetracycline deposition. Vibration profoundly alters the normal osteonal remodelling of bone. The mineralization of the organic matrix becomes discontinuous and dispersed in the femur diaphysis.



without vibration with vibration

Figure 39. Zones of active bone formation in a diaphyseal cross section of the femur, labelled by deposition of tetracycline, as visible under ultraviolet light, after two and four months of vibration.
22x natural size

B. RESULTS FROM IMMOBILIZED SUBJECTS UNDER EARTH GRAVITY, HYPERGRAVITY AND VIBRATION

Body weight:

After removal of the cast from the one hind leg, the weight of the "immobilized" animals was 342 ± 10 gram which is significantly less than the 375 ± 12 gram weight of normal rats of the same age (Figure 40 vs. Figure 13), i.e. immobilization decreases body weight development. Thirty-eight days later, at the end of the experiment, the previously immobilized animals still weighed less than normal rats of corresponding age, namely 432 ± 25 grams vs. 475 ± 32 grams.

Figure 40a shows that shortly after the start of exposure of these subjects to hypergravity (on day 98), there occurred the initial weight loss characteristic of first exposure to centrifugation. Body weight then started to increase in the second week of centrifugation and continued on an upward trend. However, the body weight of the "immobilized" animals at hypergravity stayed always below the weight of corresponding controls at earth gravity and also under the weight of centrifuged animals growing without prior immobilization.

Vibration after immobilization produces a similar effect on

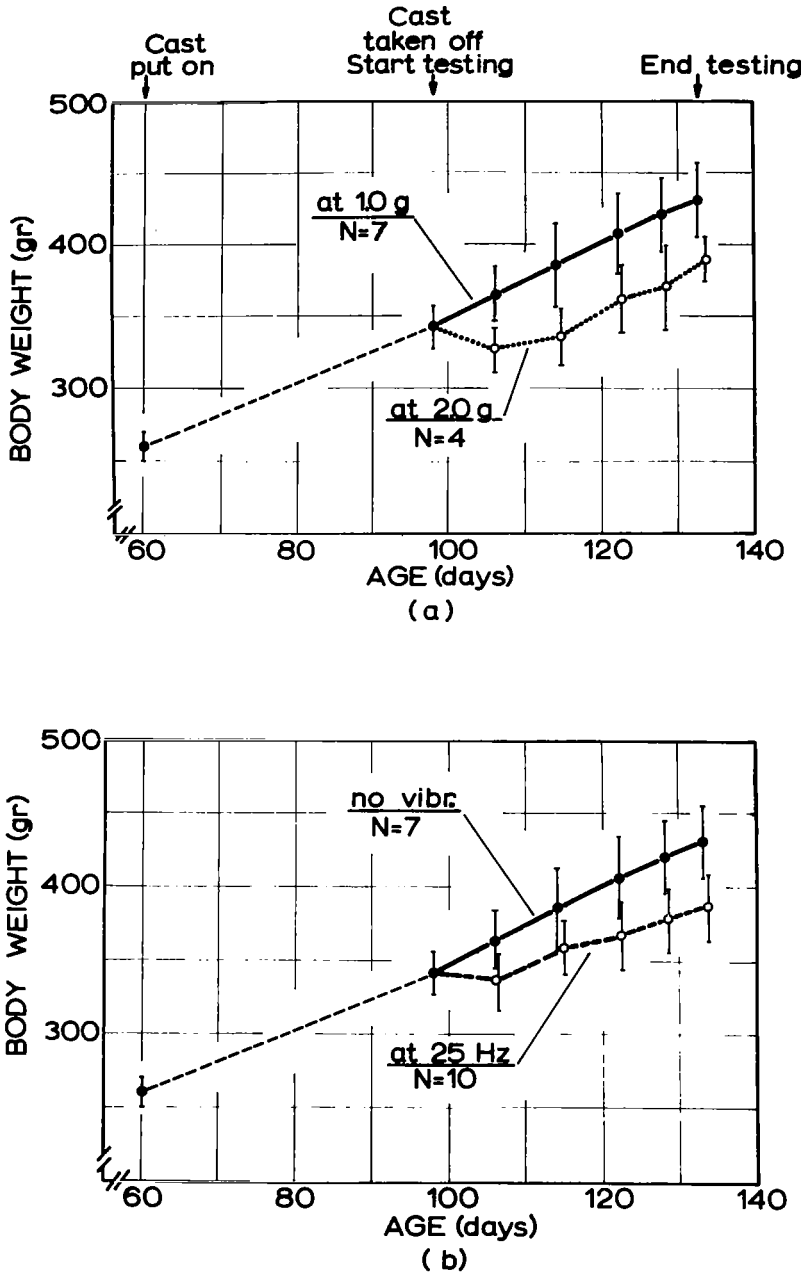


Figure 40. Body weight as a function of age during immobilization and subsequent exposure to
a) hypergravity and
b) vibration

the rate of body weight gain. After an initial decrease, the body weight starts increasing again, but it always remains less than that of other experimental groups (Figure 40b vs. Figure 13b).

Bone weight, volume and density:

As Figure 41 shows, at the end of the immobilization period, both weight and volume of the femur in the immobilized extremety were significantly lower than those in the corresponding free leg. After removal of the cast, weight and volume tended to regain the normal developmental levels of the immobilized leg but this is still not accomplished at the end of five more weeks of observation.

Figure 42 shows that there was no difference in weight and volume of the free and immobilized tibias at the time the cast was removed, and that the development of bone was normal.

Exposure to hypergravity and chronic vibration did not systematically influence the recovery pattern of previously immobilized bone (Figures 41, 42, 44 and 45).

Immobilization effects on bone density in both femur and tibia are shown in Figure 43. The pattern of normal bone development is represented by the density of the free leg, shown on the same figure. During five weeks of immobilization the density of

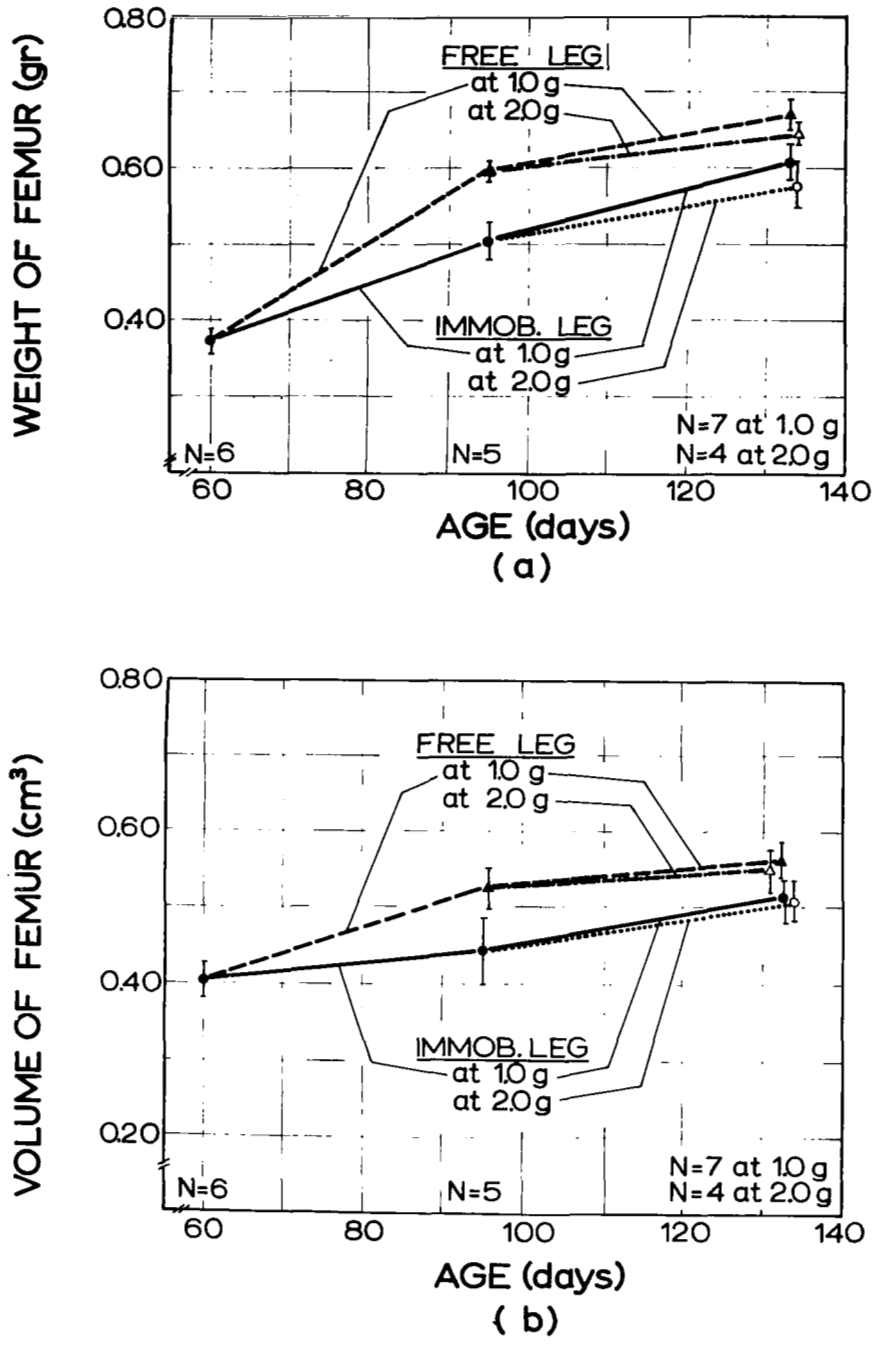
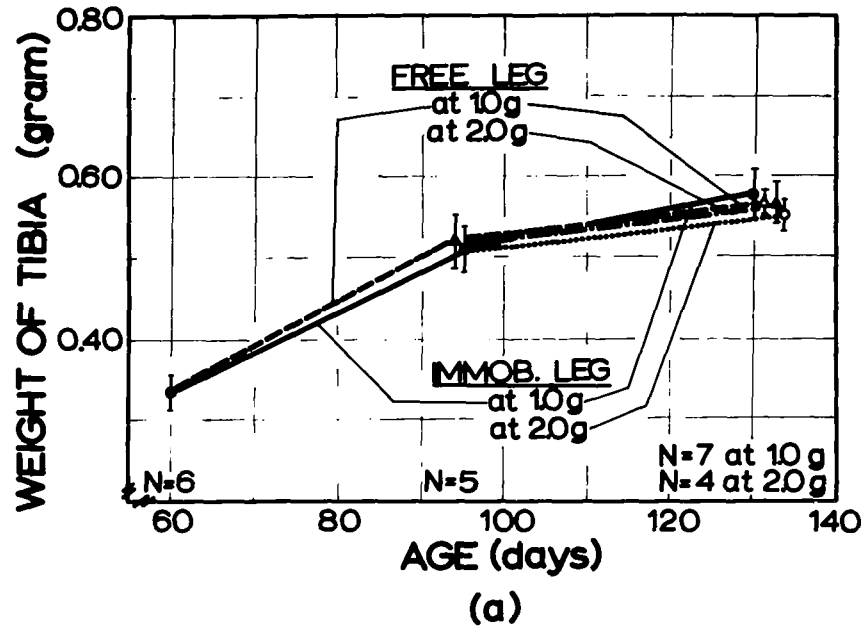


Figure 41. a) Weight and b) volume of the femur during immobilization and subsequent exposure to earth gravity and hypergravity



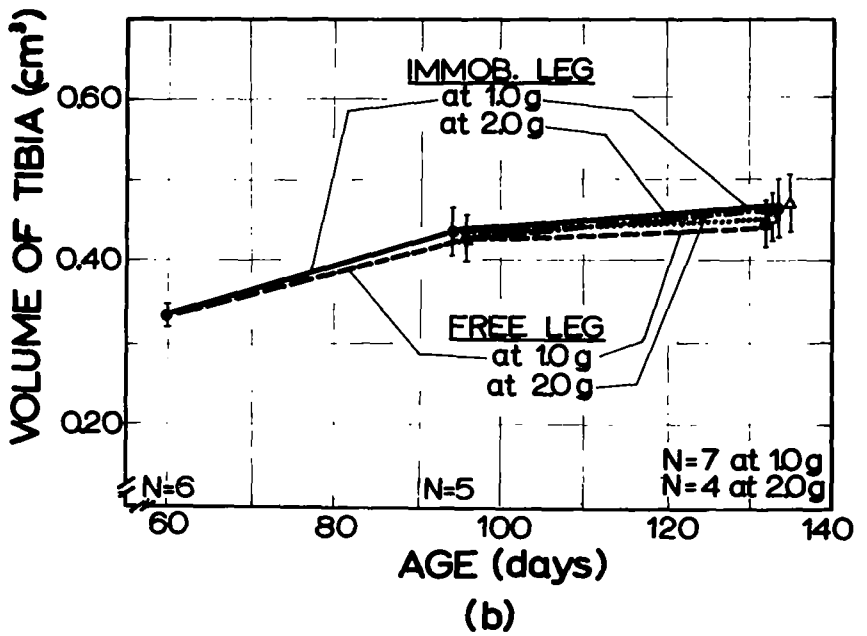


Figure 42. a) Weight and b) volume of the tibia during immobilization and subsequent exposure to earth gravity and hypergravity

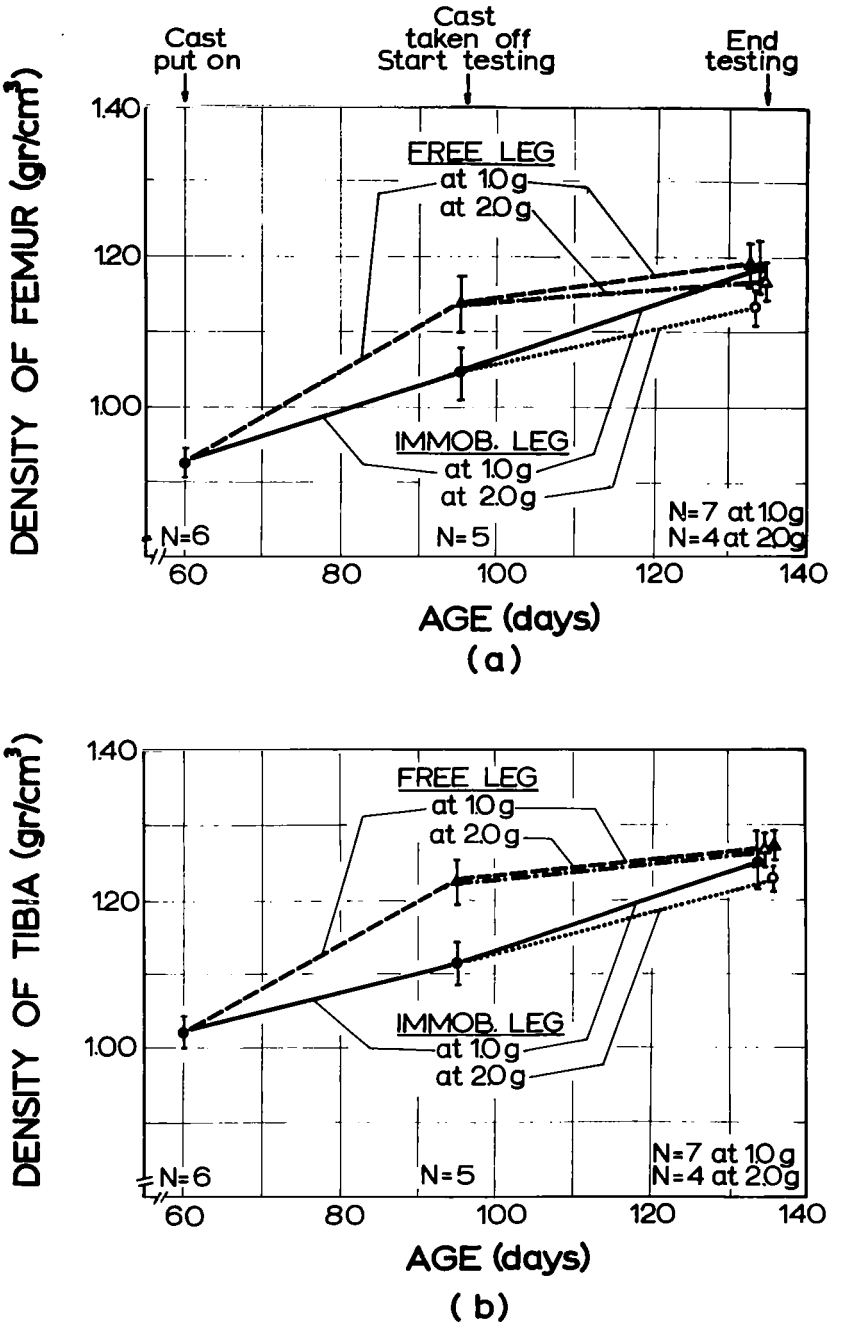
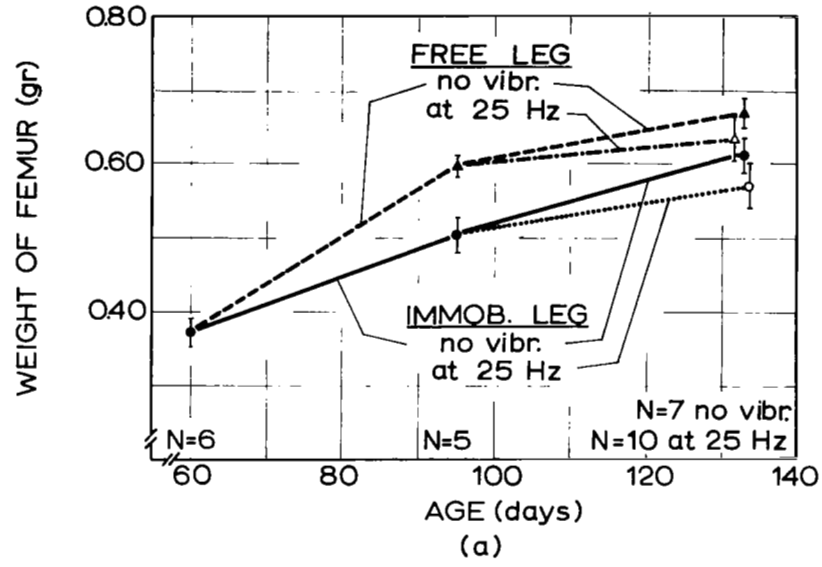


Figure 43. a) Density of the femur and b) density of the tibia during immobilization and subsequent exposure to earth gravity and hypergravity



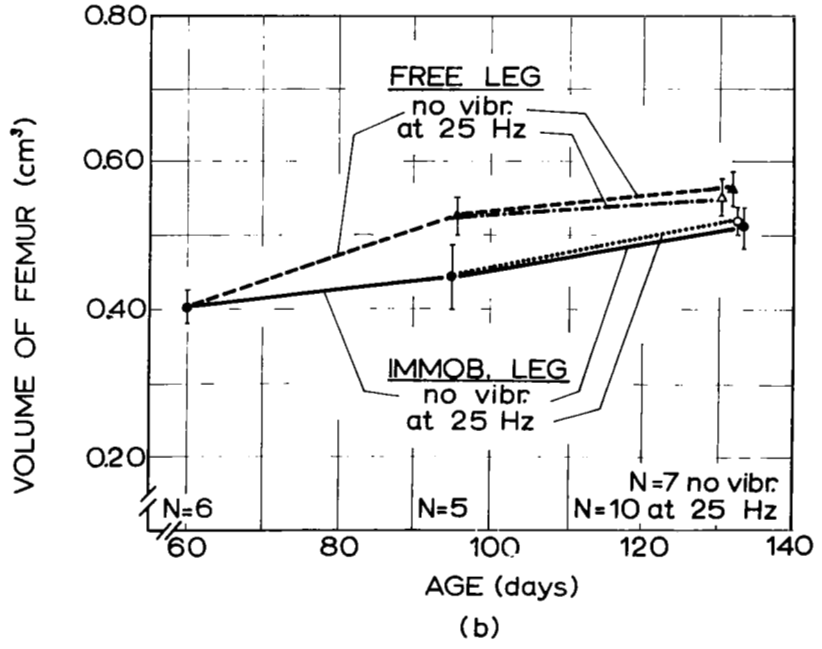


Figure 44. a) Weight and b) volume of the femur during immobilization and subsequent exposure to vibration

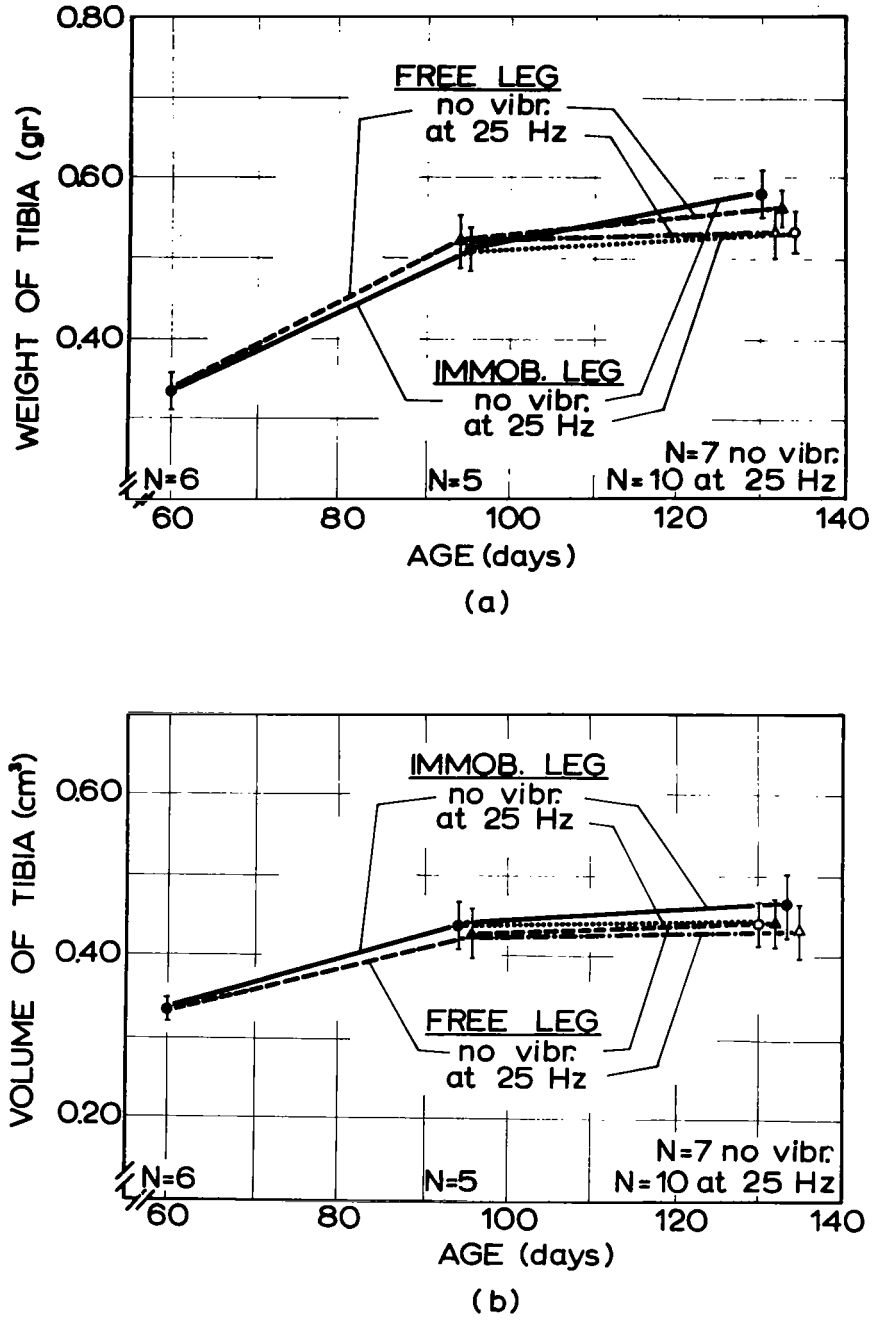


Figure 45. a) Weight and b) volume of the tibia during immobilization and subsequent exposure to vibration

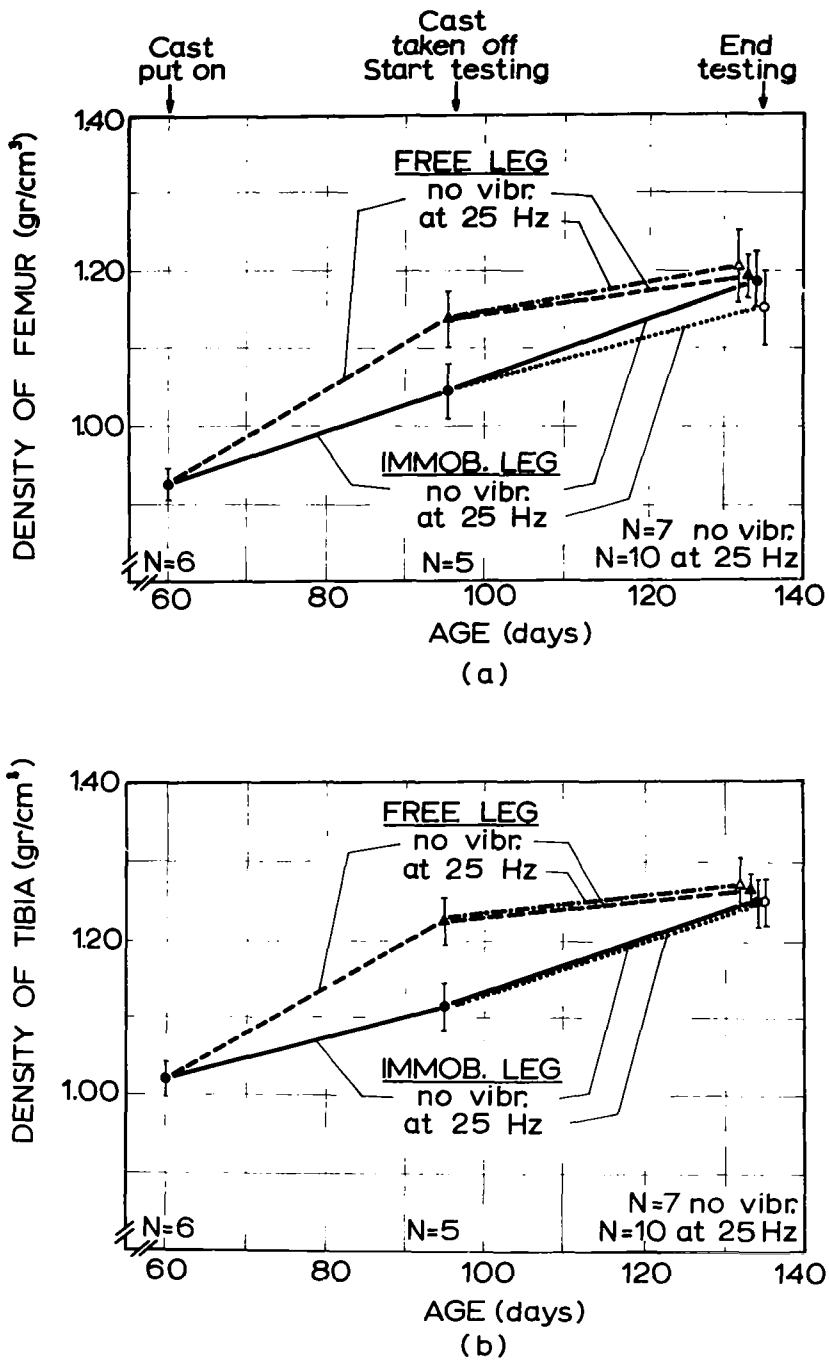


Figure 46. a) Density of the femur and b) density of the tibia during immobilization and subsequent exposure to vibration

bone in the cast increased, but at a greatly reduced rate with respect to that in the free leg and at the end of the five weeks the femur as well as the tibia were significantly less dense than the free leg. After the cast was removed, a faster than normal rate of bone density development was observed which in five weeks resulted in reaching the normal state of development. Exposure to hypergravity and chronic vibration did not change these trends to any significant extent (Figures 43 and 46).

Bone length and cross-sectional areas:

Figure 47 shows the longitudinal growth of the femur and tibia during immobilization. The longitudinal growth of bone is not affected by immobilization. Hypergravity and vibration during the following five weeks do not influence normal bone growth in either the formerly immobilized or the free extremity.

Plaster cast immobilization, however, does affect the cross-sectional growth of the femur in the vicinity of the epiphyses, as shown in Figure 48. Upon removal of the cast the total cross-sectional area of the immobilized leg is smaller than that of the free leg at 22.5 % and 72.5 % from the distal end of the femur. No such difference was observed at 62.5 % from the distal end.

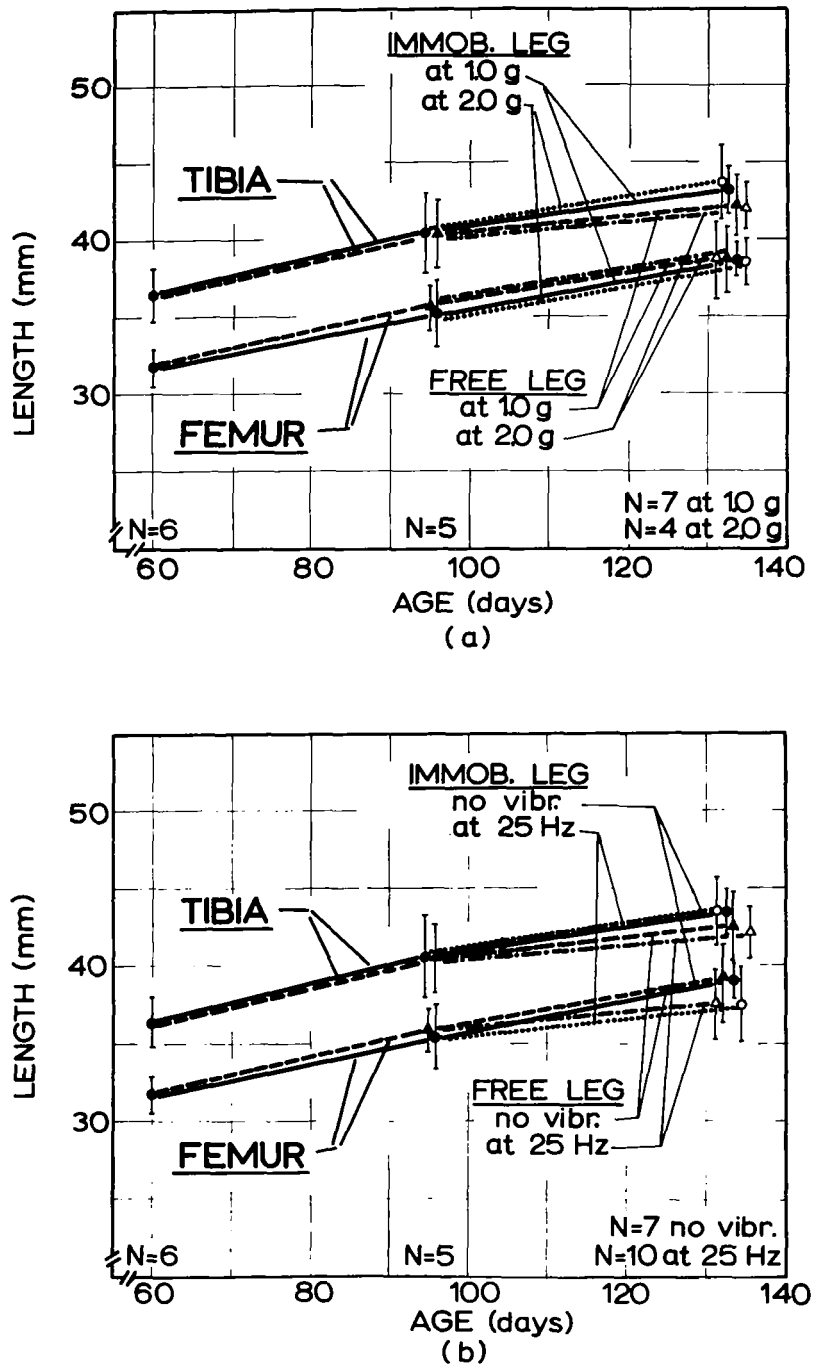


Figure 47. Longitudinal growth of the femur and the tibia during immobilization and subsequent exposure to
a) earth gravity and hypergravity
b) vibration

Immobilization tends to decrease the cortical area at 22.5 % of the total length, where the femur has an elliptical cross-sectional shape with relatively thin walls.

The differences in cross-sectional development of either extremity due to immobilization disappear in five weeks, irrespective of exposures to either earth gravity, hypergravity, or chronic vibration (Figures 48 and 49).

Compressive elasticity:

Bone becomes stiffer during immobilization than it would become during the same period of normal aging: Figure 50a shows that the compressive spring constant is somewhat higher in the femur of the immobilized leg than in the free extremity and Figure 51a shows that there is a pronounced difference between the moduli of free and immobilized bone.

After removal of the cast, spring constant (Figure 50a) and elastic modulus (Figure 51a) in the previously immobilized limb remain almost constant, while the rigidity of bone in the free leg increases steadily. After about five weeks bone elasticity becomes the same again in both extremities.

Exposure to hypergravity does not alter the recovery of the immobilized bone compared to recovery under earth gravity

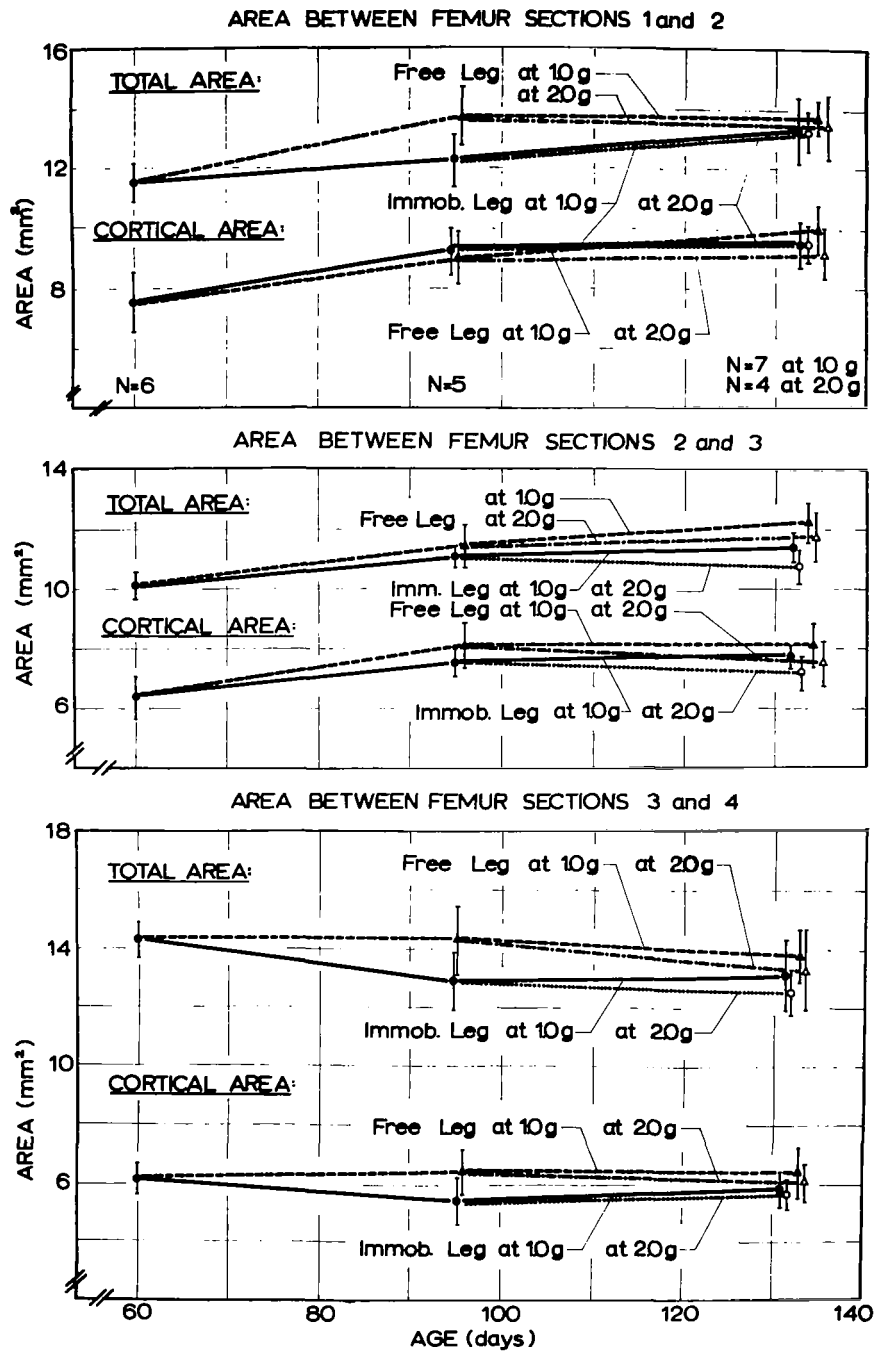


Figure 48. Cross-sectional growth of the femur during immobilization and subsequent exposure to earth gravity and hypergravity

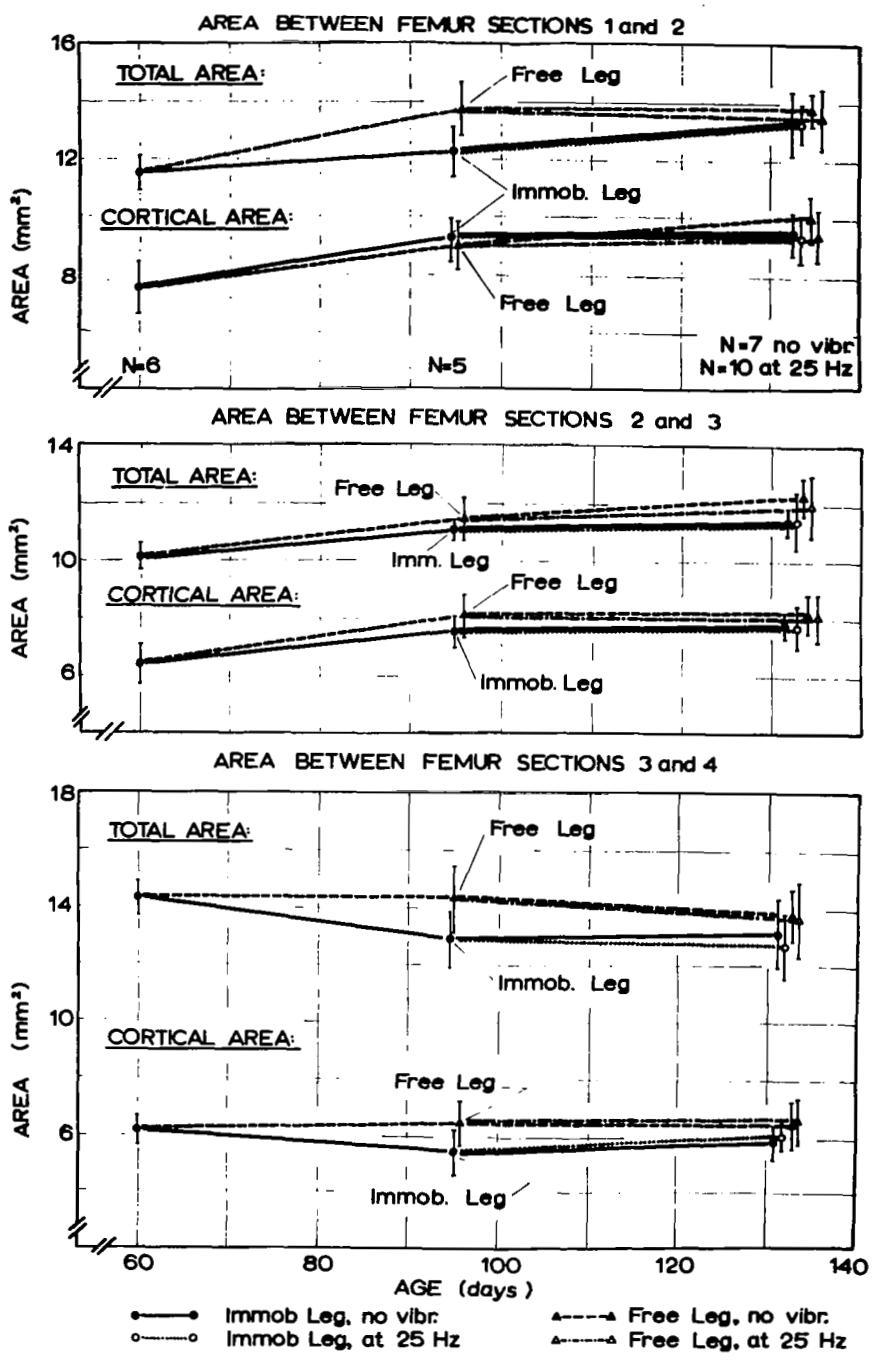


Figure 49. Cross-sectional growth of the femur during immobilization and subsequent exposure to vibration

(Figures 50a and 51a). Under chronic vibration, however, the compressive spring constant and the modulus of elasticity become higher than the corresponding properties of the non-vibrated controls, and this stiffening effect occurs in both the free and previously immobilized legs (Figures 52a and 53a).

Torsional elasticity:

Torsional elasticity, expressed by the torsional spring constant of the femur (Figure 50b), decreases at a higher rate during immobilization than in normal aging, but after removal of the cast, it remains approximately constant in the immobilized femur, while the free leg exhibits the normal aging process of becoming more brittle. After five more weeks there still remains an appreciable difference in elasticity in favor of the undisturbed leg.

Exposure to hypergravity and chronic vibration does not alter the development patterns observed in the 1.0g subjects (Figures 50b and 52b).

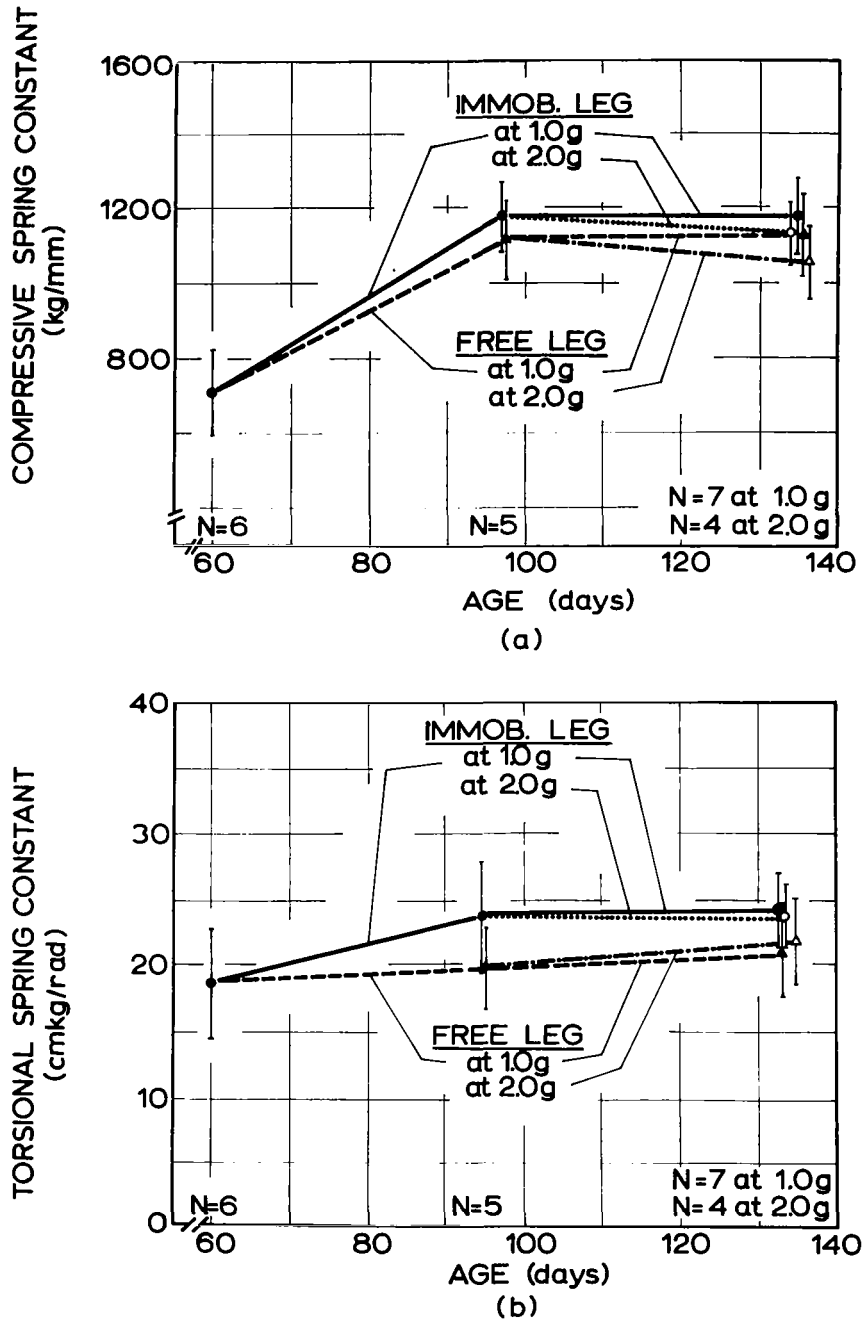
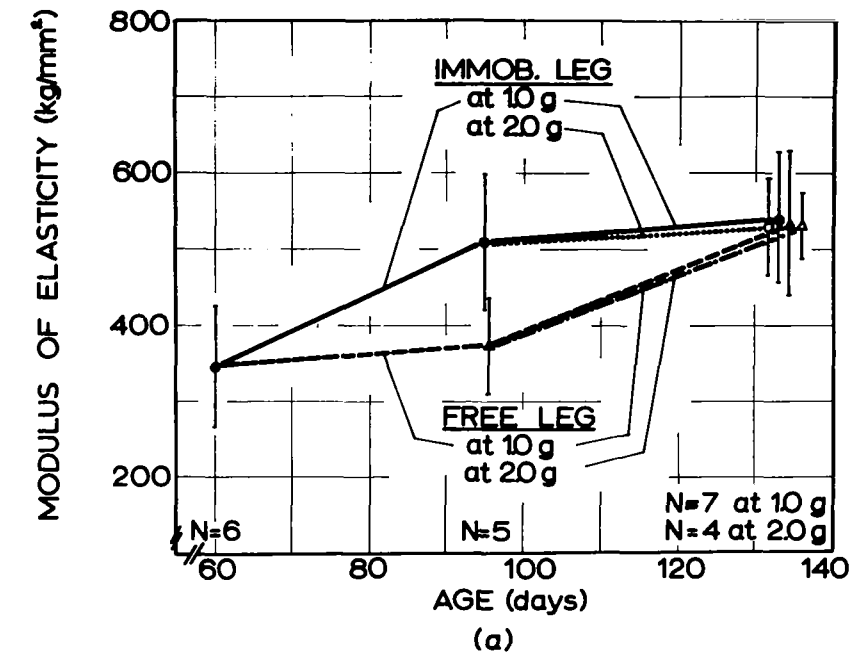


Figure 50. a) Compressive spring constant and b) torsional spring constant of the femur during immobilization and subsequent exposure to earth gravity and hypergravity



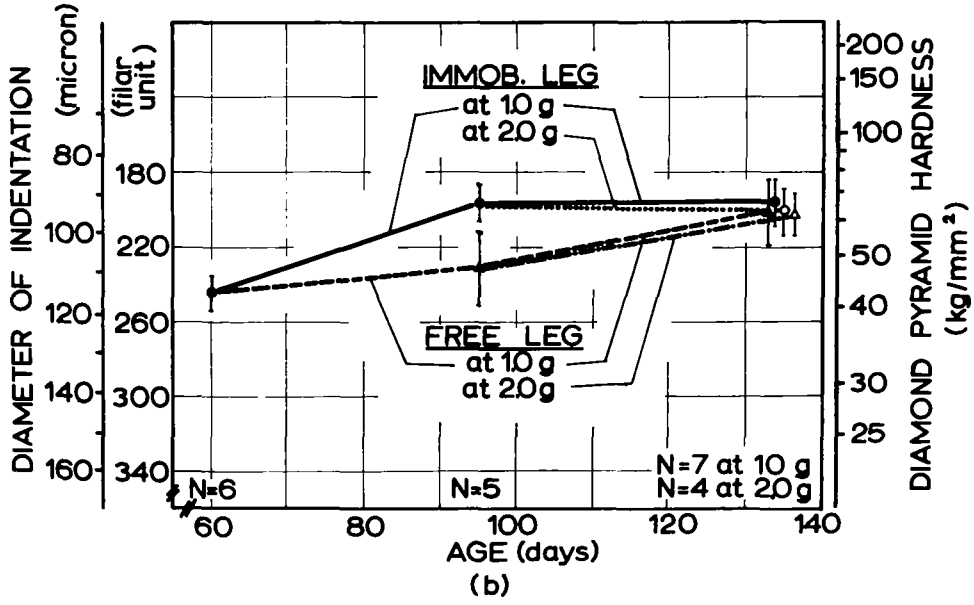


Figure 51. a) Modulus of elasticity and b) microhardness of the femur during immobilization and subsequent exposure to earth gravity and hypergravity

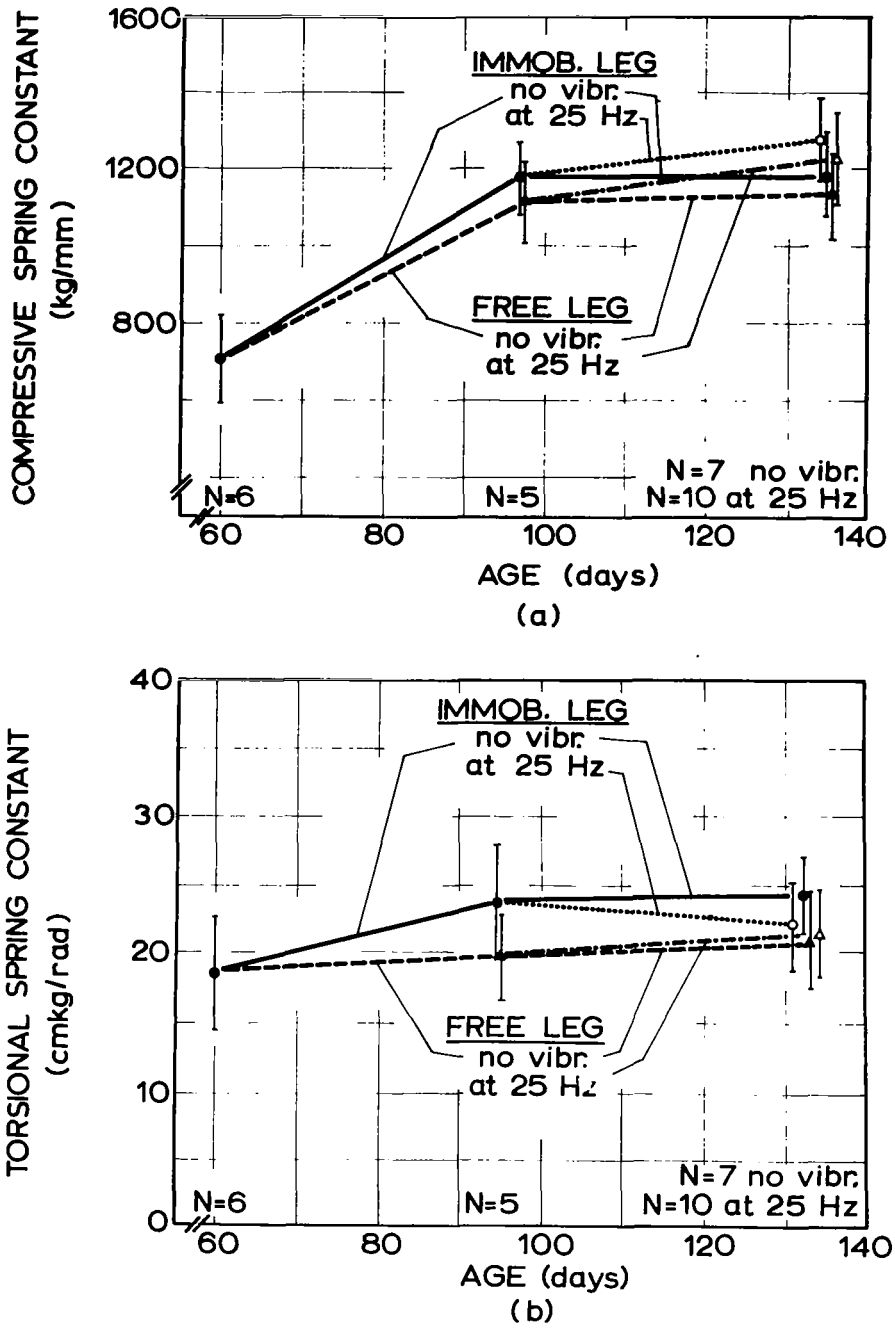
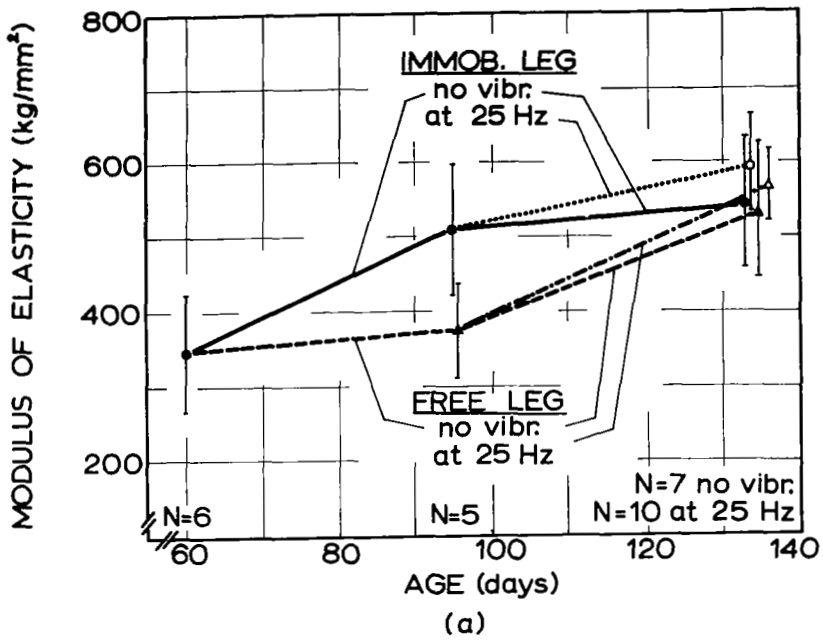


Figure 52. a) Compressive spring constant and b) torsional spring constant of the femur during immobilization and subsequent exposure to vibration



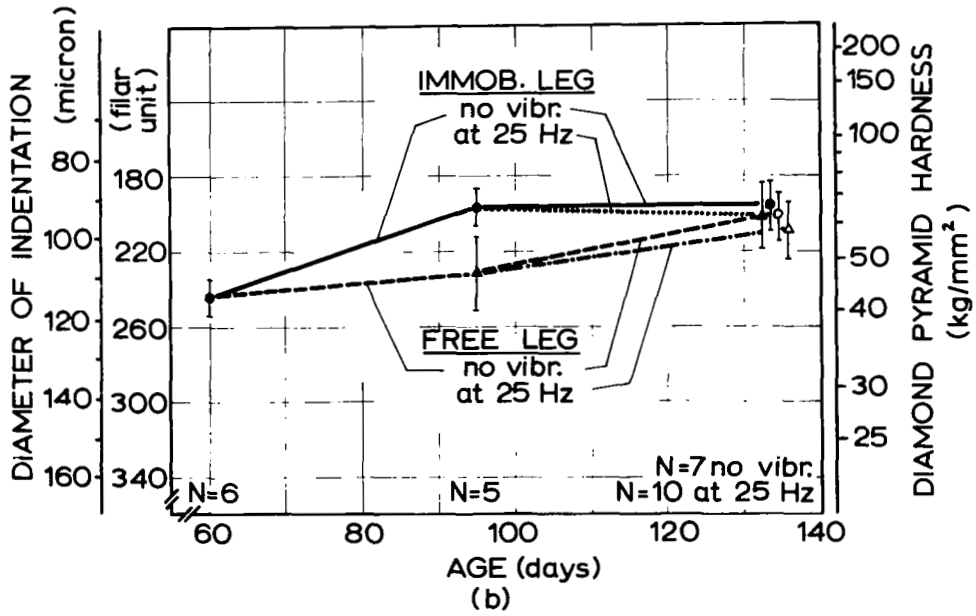


Figure 53. a) Modulus of elasticity and b) microhardness of the femur during immobilization and subsequent exposure to vibration

Microhardness:

Figure 51b shows that at the time of removal of the cast the immobilized femur had become considerably harder than the corresponding free femur. No further hardening with age took place in this leg after it was freed, but hardness in the free limb increased continually until it reached the same hardness level as the previously immobilized leg in five weeks. Again, the developmental patterns were unaffected by hypergravity or chronic vibration (Figures 51b and 53b).

Conductivity of sound:

Figures 54 and 55 show that the velocity of sound in bone increases with age, but is neither affected by immobilization nor subsequent exposure to hypergravity and chronic vibration.

Histological solidity of bone:

During immobilization, the immobilized bone apparently becomes more solid than the bone of the free leg, as shown in Figure 56. The number of pores in the bone immediately after removal of the cast is about 5.5 % less in the femur of the immobilized leg than in the corresponding free limb. The

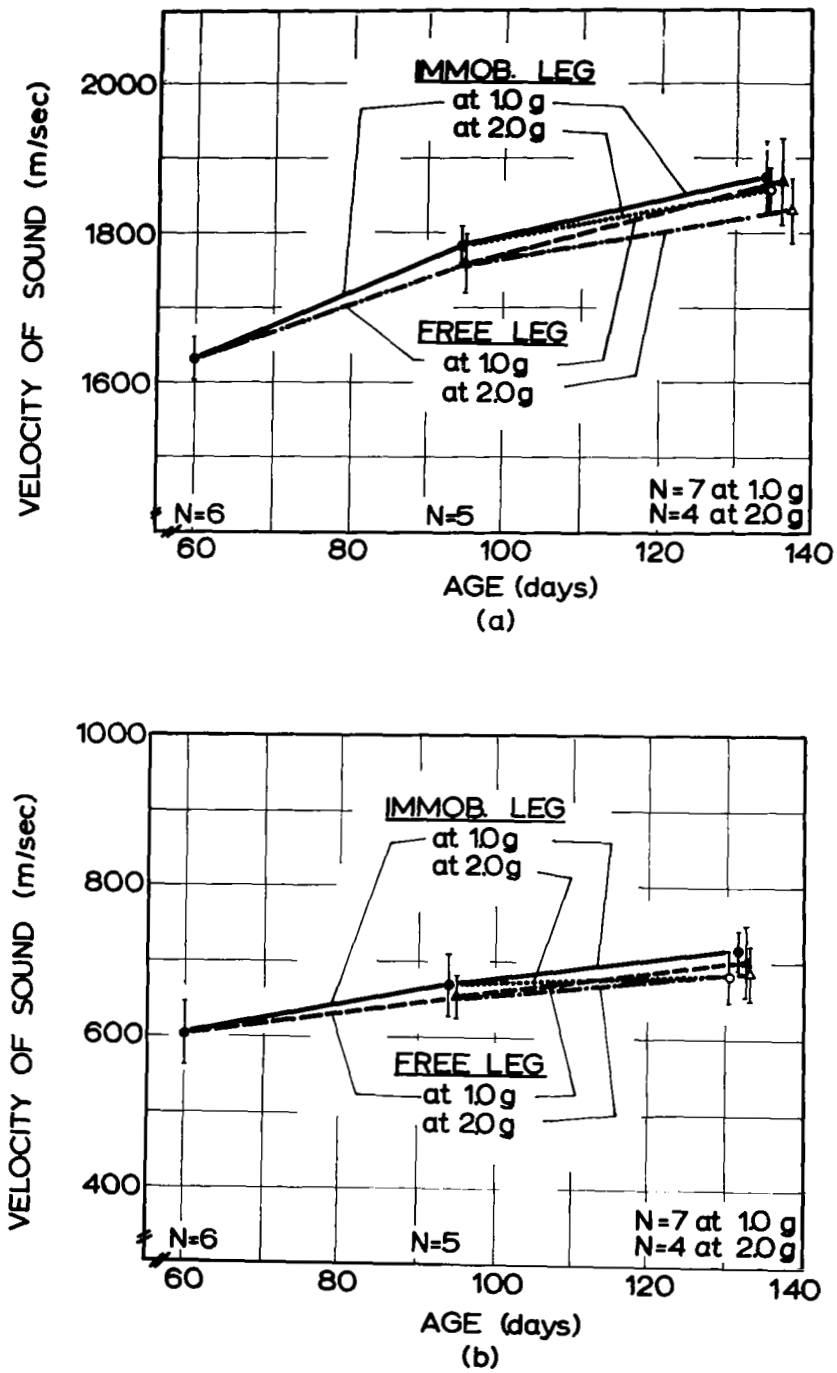


Figure 54. Velocity of sound in a) Section 3 and b) Section 2 of the femur during immobilization and subsequent exposure to earth gravity and hypergravity

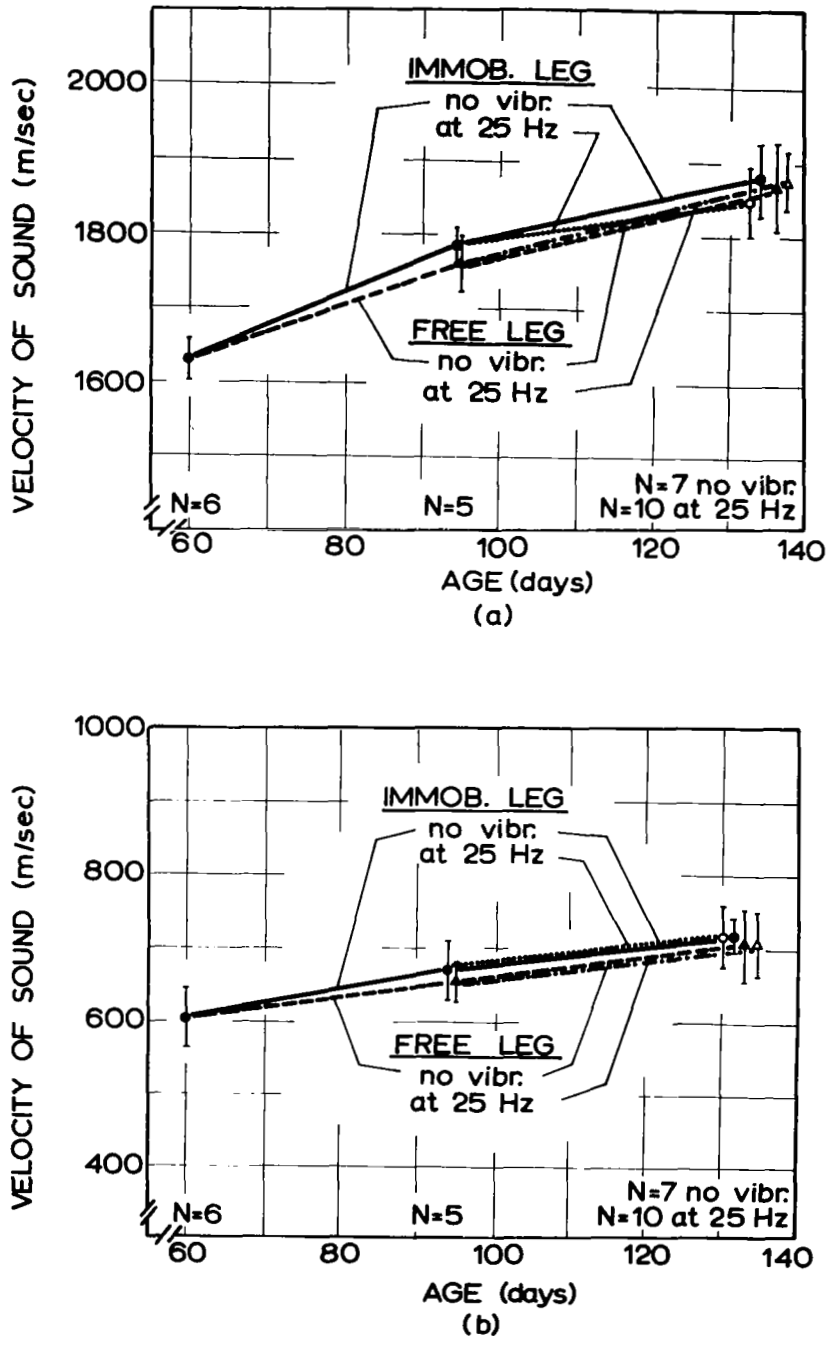


Figure 55. Velocity of sound in a) Section 3 and b) Section 2 of the femur during immobilization and subsequent exposure to vibration

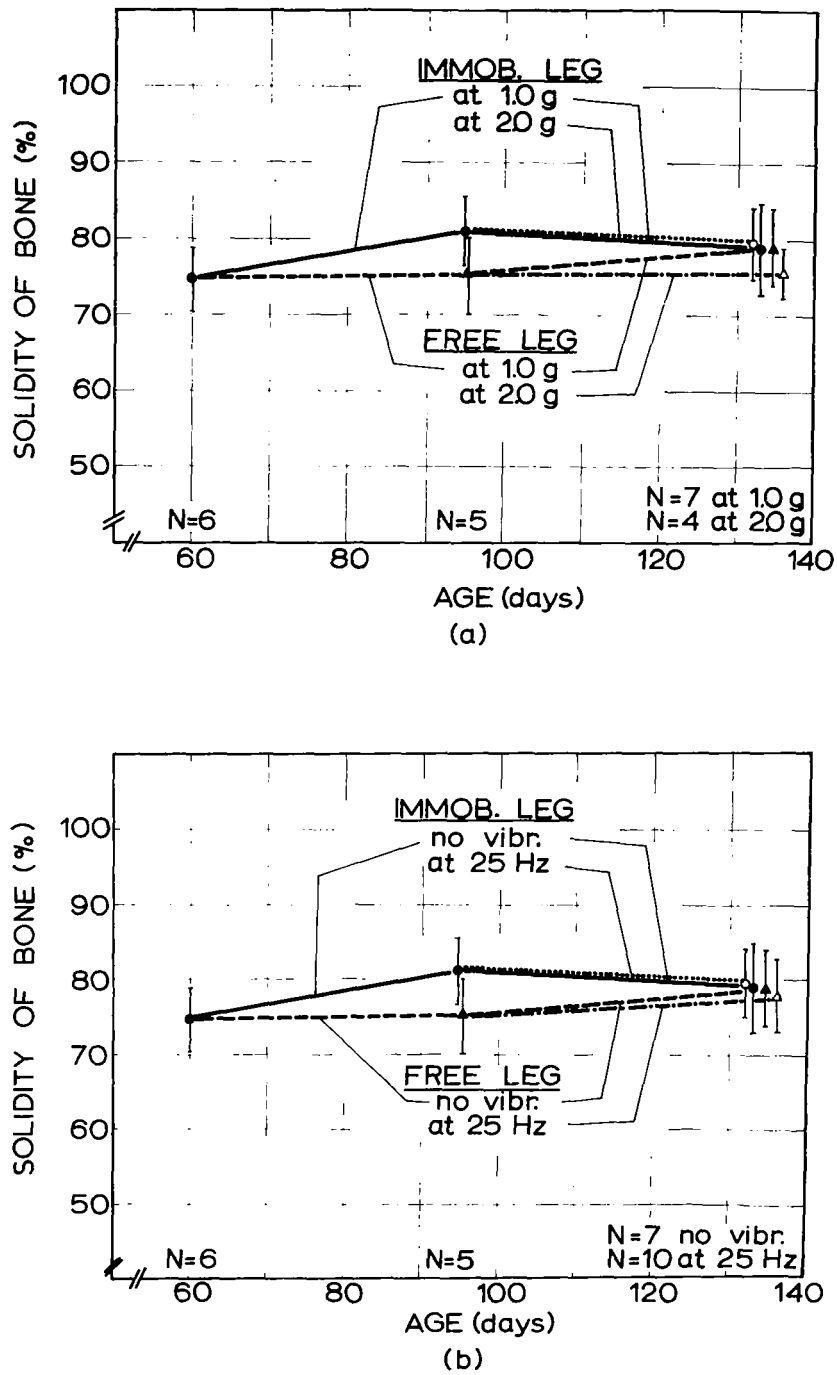


Figure 56. Solidity of bone during immobilization and subsequent exposure to a) earth gravity and hypergravity and b) vibration

variability of the data is of the same magnitude as the change itself, which therefore indicates only a trend. In five weeks after immobilization the difference in bone porosity disappears. Chronic exposure to hypergravity and vibration does not influence the final state of porosity in the femur.

Ash and calcium content:

In the hip joint of the femur, immobilization does not affect the normal temporal changes of the ash and calcium content of bone (Figures 57a, 57b, 57c). In the knee joint, immobilization decreases the ash content with respect to that of the free leg (Figure 58a). After immobilization is terminated the ash content increases faster in the immobilized knee than as in the free knee, but does not reach the free knee ash level in five weeks. The calcium content of the dry defatted bone is constant in the knee joint during and after immobilization (Figure 58b). The calcium content of the ash, however, becomes slightly lower during immobilization than corresponds to normal aging (Figure 58c), and the difference disappears in five weeks after removal of the cast.

Chronic exposure to hypergravity and vibration following immobilization does not alter the temporal pattern of bone ash and calcium content observed in animals exposed to 1.0g, as shown in Figure 57, 58, 59 and 60).

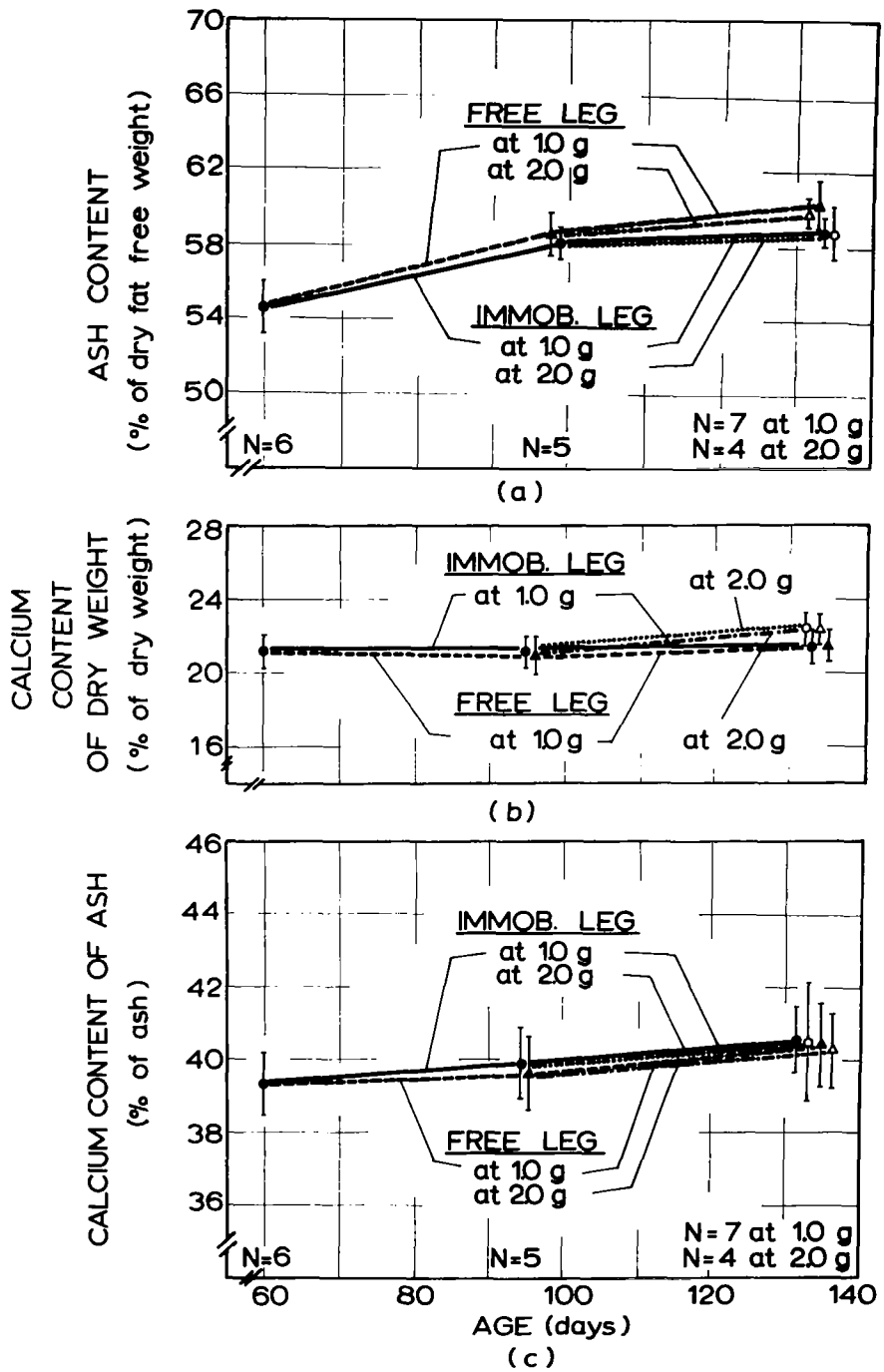


Figure 57. *Compositional analysis of Section 1 (hip joint) of the femur during immobilization and subsequent exposure to earth gravity and hypergravity:*
a) ash content of the bone
b) calcium content of the dry fat free bone
c) calcium content of bone ash

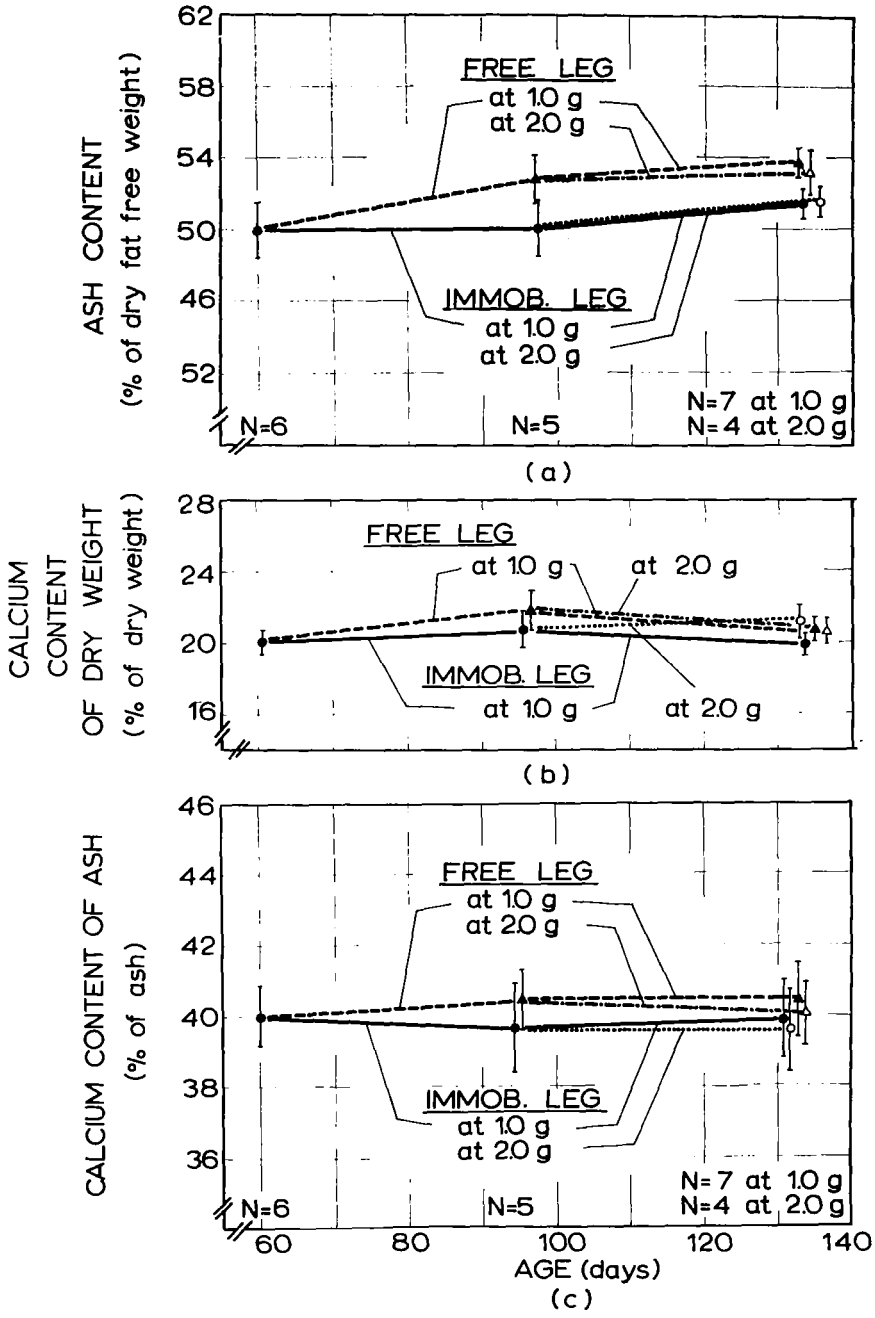


Figure 58. Compositional analysis of Section 5 (knee joint) of the femur during immobilization and subsequent exposure to earth gravity and hypergravity:
a) ash content of the bone
b) calcium content of the dry fat free bone
c) calcium content of bone ash

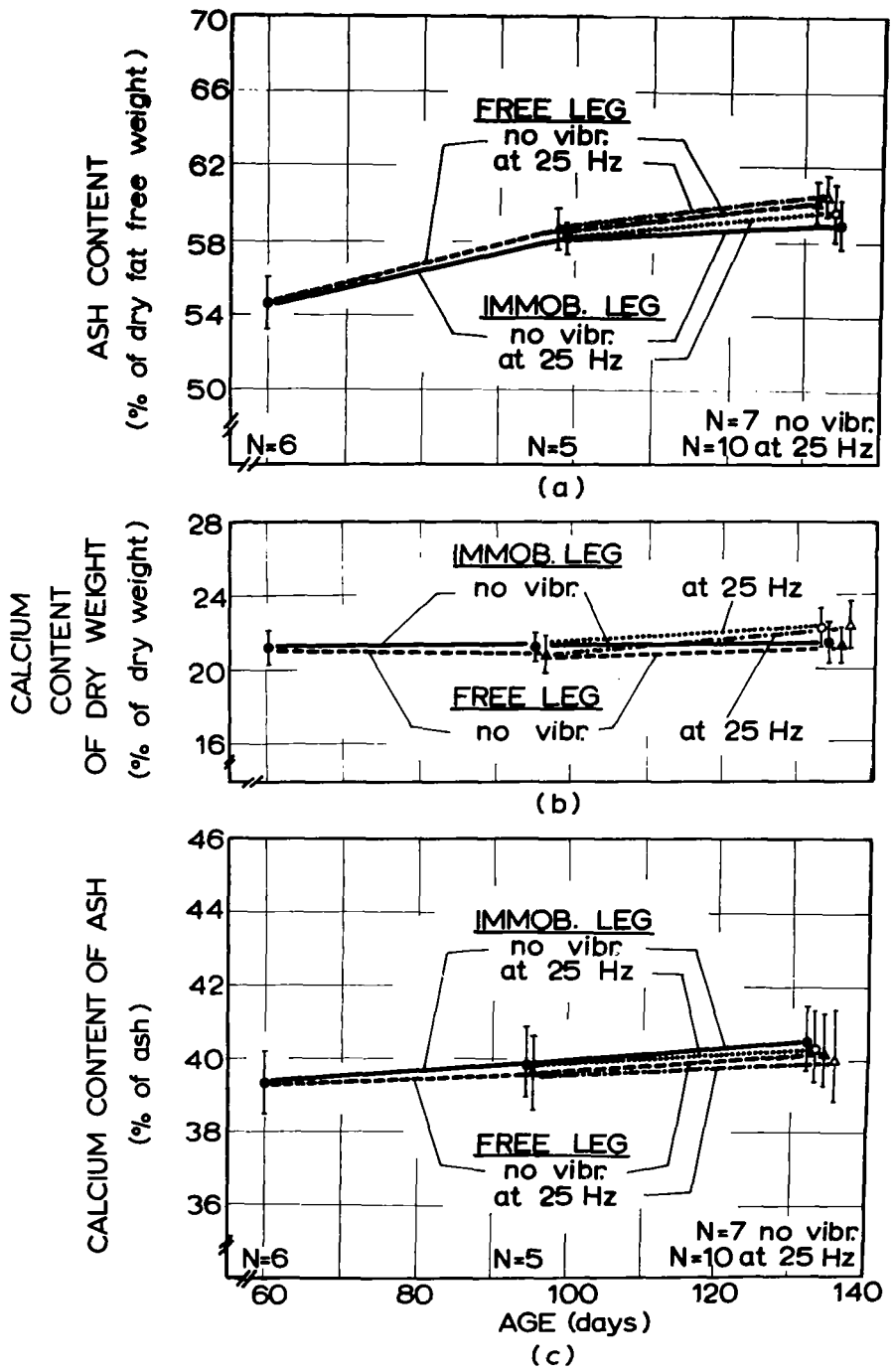


Figure 59. Compositonal analysis of Section 1 (hip joint) of the femur during immobilization and subsequent exposure to vibration:
a) ash content of the bone
b) calcium content of the dry fat free bone
c) calcium content of bone ash

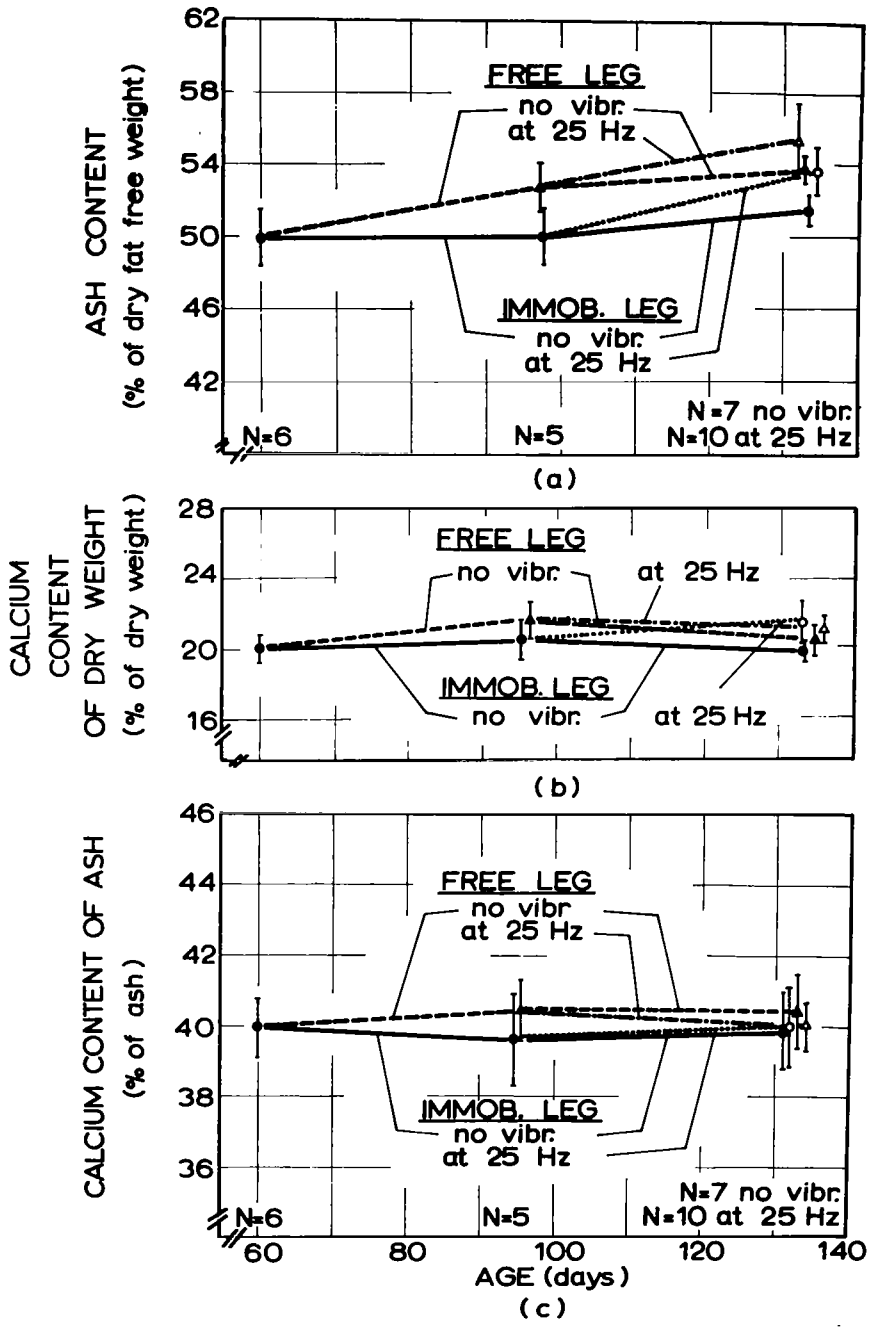


Figure 60. Compositional analysis of Section 5 (knee joint) of the femur during immobilization and subsequent exposure to vibration:
a) ash content of the bone
b) calcium content of the dry fat free bone
c) calcium content of bone ash

IV. RADIOGRAPHIC BONE DENSITOMETRY

Bone mass, mineral content and density can be quantitatively assessed from roentgenograms of bone. Such analyses usually consist of the photodensitometric comparisons of selected sites on the bone with a calibrated aluminum wedge on the same film. X-rays passing through bone are attenuated by specimen thickness and mineral concentration. Thus optical density of the roentgenographic bone image is related to the mass as well as to mineral content of the bone material. Marked departures (25-30 %) from normal bone mineralization and bone mass can be seen readily by visual inspection of the roentgenographs. Microdensitometers are used to detect smaller changes.

In the usual radiographic technique the light transmittance through the aluminum wedge is scanned with a microdensitometer to determine the film background optical density due to scattered x-rays, chemical fog, film base and emulsion layers. Next the bone image is scanned and the resulting trace is corrected for the previously determined optical density. Several systems have been built which automatize these procedures (Schaer et al., 1959; Cameron, 1965; Whedon et al., 1966; Colbert et al., 1967; Colbert and Garrett, 1969; Vogt et al., 1969; Progress in Methods of Bone

Mineral Measurement, 1970). The application of photodensitometric analysis of radiographs to bone has been reported in nutrition and growth (Williams et al., 1964; Whedon et al., 1966; Mack et al., 1968), in association with bed rest and space flight studies (Mack et al., 1967; Vogt et al., 1965; Vose 1969) and in calcification of dental tissues (Colbert et al., 1966).

In this study in vivo x-ray pictures of the right femur were taken in order to monitor successive states of bone development of the experimental groups exposed to 1.5 and 2.0g hypergravity, and to 2 x 2.5 hours of 20 and 25 Hz vibration. X-ray pictures of the stressed and control animals were taken in 18 day intervals. The rats were anesthetized with Nembutal, strapped into a Plexiglas frame with the right hind leg in a standardized position, as shown in Figure 61. In all x-ray photography a GE-100 DA-0064 type dental x-ray unit was used with aluminum filter and Plexiglas cone. The subjects were placed at a distance of 80 cm from the lens. Eastman Kodak Industrial Type AA, non-screen film was used in cardboard casettes with 70 kV, 15 mA and 3 sec exposure. The films were developed by the Oral Radiology Department, College of Dentistry, University of Kentucky, in a nitrogen burst automated processor.

The x-ray images were then analyzed in the microdensitometer

Figure 61. Positive radiograph of a rat and the calibrating aluminum wedge in the positioning device with the right hind leg in standard position for *in vivo* densitometry

of Fels Research Institute, Antioch College, Yellow Springs, Ohio. Their system, consisting of a Joyce-Loebl Mark II microdensitometer and an on-line Linc-8 computer, automatically scans the film and computes an index for the specimen which is proportional to the bone mass. Bone volume and density indices are also computed. The details of the method are described by Colbert and Garrett (1969).

The regression of the radiographic bone indices and the corresponding analytically determined bone parameters was calculated with the method of least squares, and the correlation coefficient of the data was also computed.

The radiographic bone mass index of the entire femur determined this way from in vivo x-ray pictures is plotted against the dry fat free weight of the bones in Figure 62a. Dry bone weight was determined by weighing after sacrificing the animals immediately after the x-ray pictures had been taken. The correlation coefficient, $r_9 = 0.7721$ is significant on $P < 5$ % level. On the same x-ray pictures a 10 mm long section of the bone was scanned along the midshaft of the femur in another series of measurements. The radiographic mass index of the midshaft section is plotted against the fat free dry weight of the femur in Figure 62b. The correlation coefficient of the relationship is $r_9 = 0.3293$ with the significance level of $P > 5$ %. The Spearman's rho of the measurements

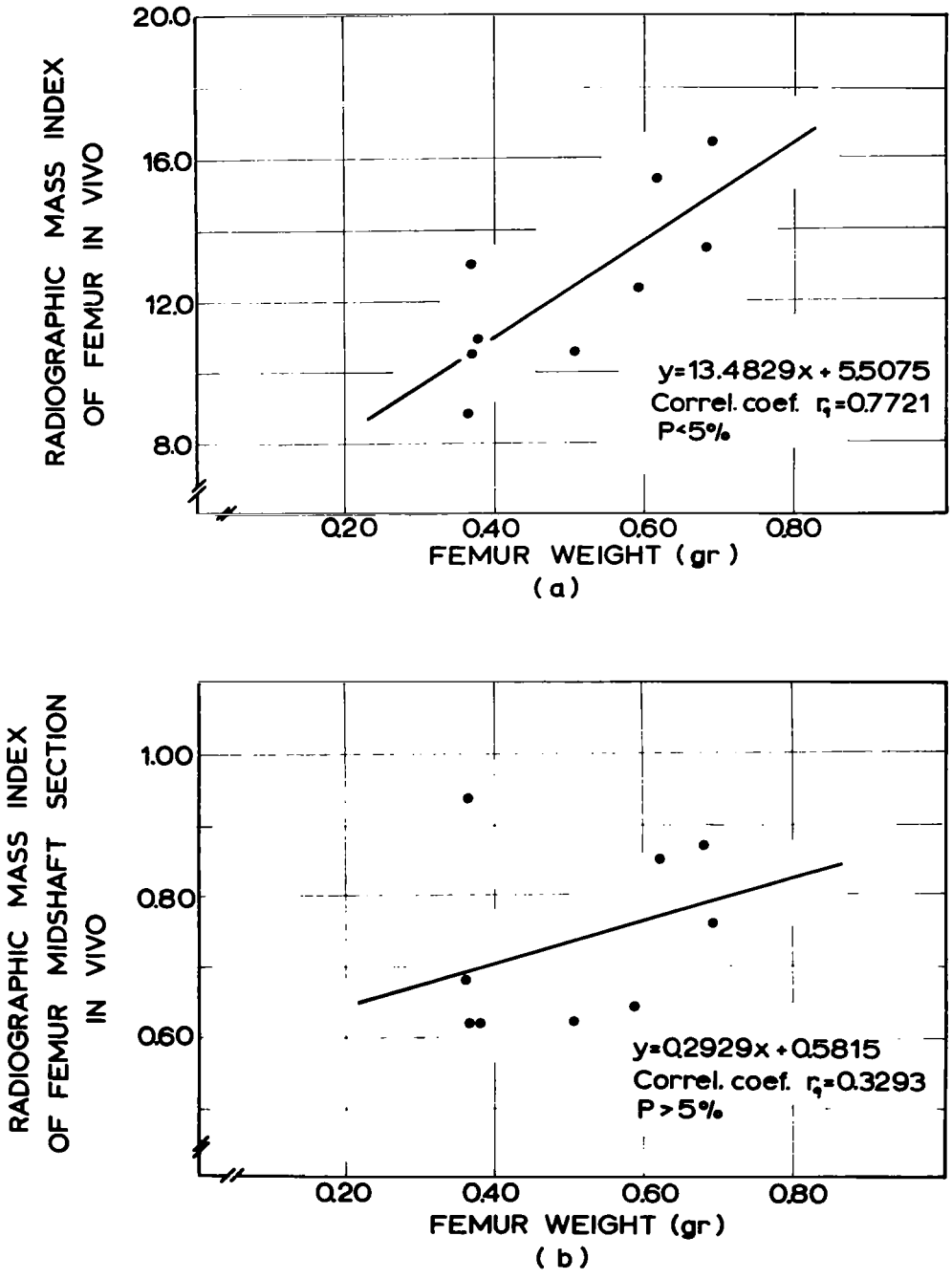


Figure 62. In vivo radiographic bone mass index of the whole femur and a 10 mm long midshaft section of the femur plotted as functions of the analytically determined bone weight

is $\rho = 0.63$ ($P > 5$ %). For the in vivo measurements, the significance levels of statistical analysis indicate a limited correlation between the radiographic bone index and the analytically determined weight of the femur.

However, in vitro radiographic bone indices of femur mass and volume show a much closer correlation with the analytical measurements. For this comparison the indices of bone mass, volume and density were determined from the x-ray picture of excised defatted dry femurs positioned on one film, as shown in Figure 63. The radiographic indices so obtained are plotted against their gravimetric counterparts in Figure 64. The correlation of the data is highly significant for mass and volume with $P < 5$ %.

Comparison of the radiographic in vivo and in vitro measurements of bone parameters leads to the conclusion that in the rat the photoabsorbency of the soft tissues overlying the femur influences the in vivo radiographic determination of bone indices so much that the in vivo technique was not extended over the complete range of conditions of this study.

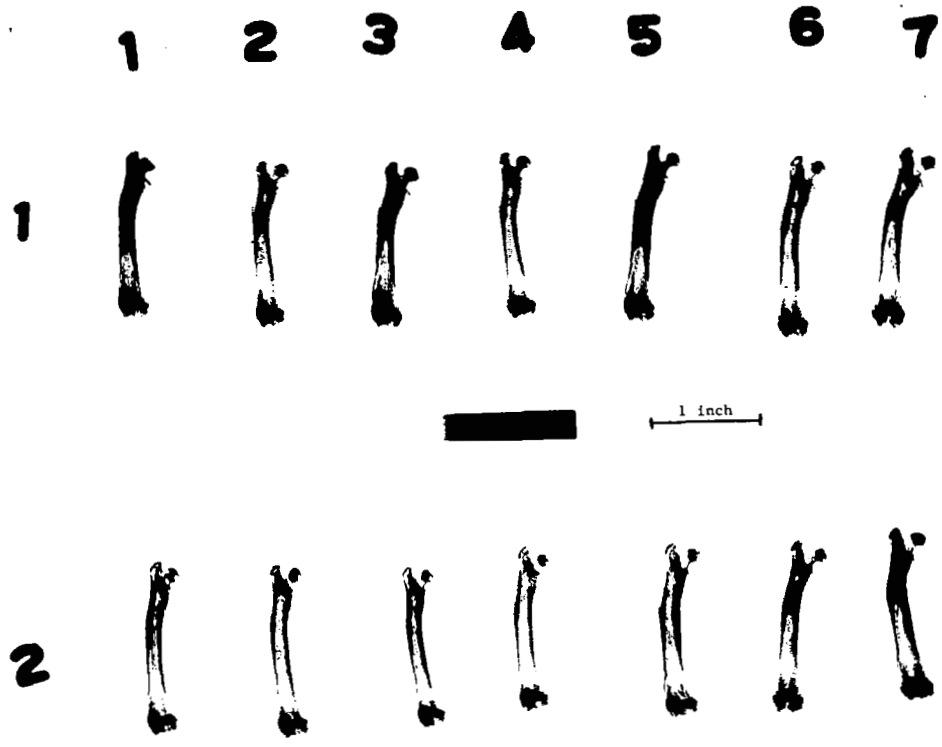


Figure 63. Positive radiograph of rat femurs positioned around the calibrating aluminum wedge for <u>in vitro</u> densitometry

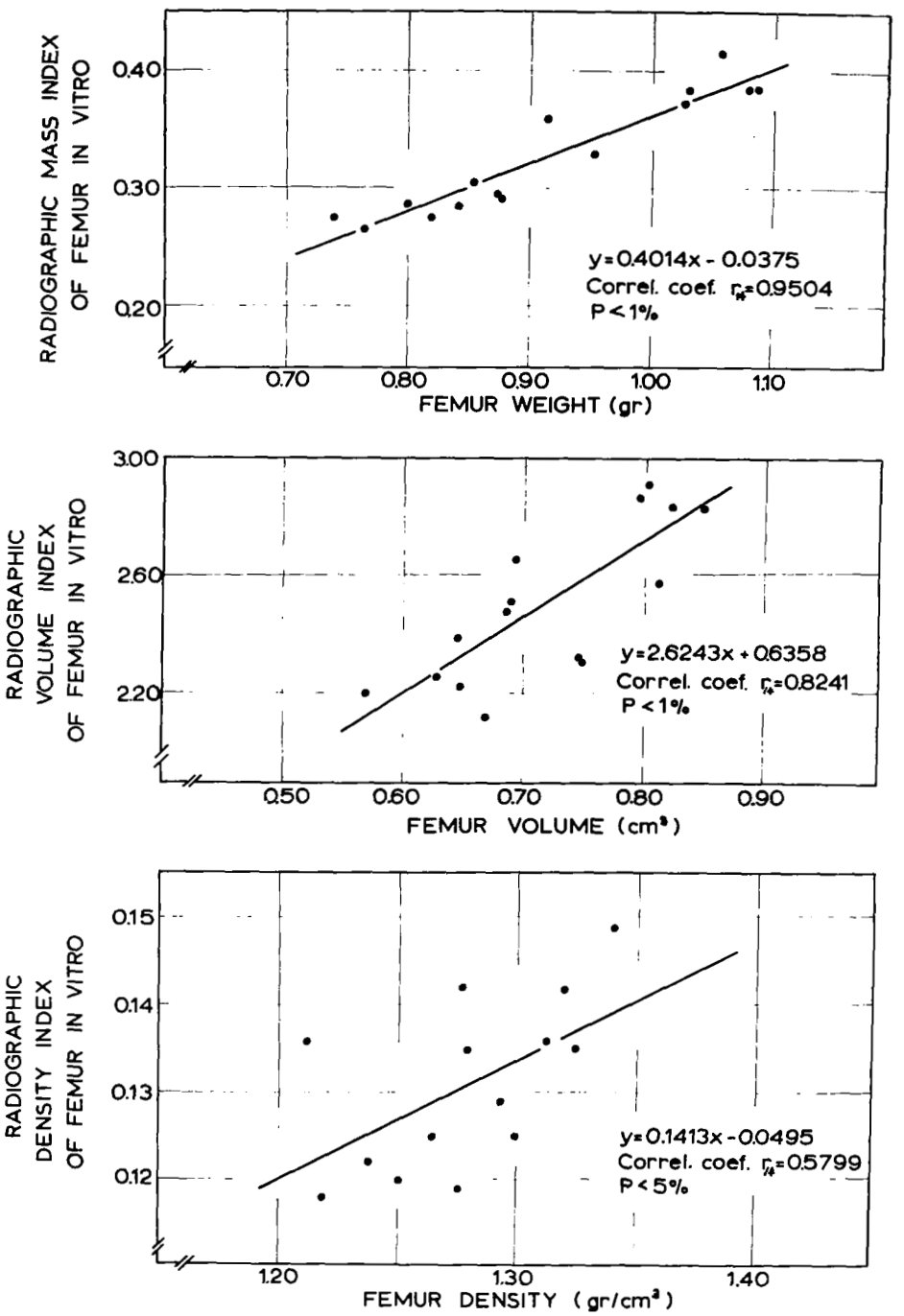


Figure 64. In vitro radiographic indices of bone mass, volume and density, plotted against the gravimetric weight, volume and density of bone

CONCLUSIONS

Temporal development of the structural properties of bone in the appendicular skeleton of the rat was investigated under earth gravity, hypergravity, mechanical vibration and hypodynamia of immobilization.

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 two to ten months. Body weight growth was found to be a logarithmic function of age [(Body Weight) $\propto$ 259 ln (Age), where Body Weight is in grams, Age in days], 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, microhardness and sound conductivity. The rate at which rigidity increases, unlike the growth rate, does not diminish during the eight months of observation

and is attributed to the progressive increase of ash content of the bone. The ratio of inorganic contituents of 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 the hip joint contains more inorganic components other than calcium than the knee.

The data obtained in this investigation are believed to be free of dietary influences. The chemical composition of bone is independent of diet (Weir, 1949; Frost, 1966). Dietary calcium does not

affect the mechanical properties of bone if the feed contains a minimum of 0.36 % calcium (Bell et al., 1941). Different calcium levels of the diet are not reflected in mineral secretion during either normal activity or chronic restraint (Pyke et al., 1968). 0.80 % calcium and 0.70 % phosphorous are required in the diet of rats for optimum growth, reproduction and calcification (Universities Federation of Animal Welfare Handbook, 1967, pp. 361-365). In this investigation regular laboratory feed containing a minimum of 1.30 % calcium and 0.94 % phosphorous (Purina Laboratory Manual, SP464A) was used which together with the oyster shell supplement more than met the requirements for normal bone development.

Chronic exposure to centrifugally generated hypergravity up to 2.5g does not affect normal development of bone in the rat: 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 control rats [(Body Weight) $\propto$ 200 ln (Age) at 2.0g and (Body Weight) $\propto$ 184 ln (Age) at 2.5g]. 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 (Bird et al., 1963; Oyama and Platt, 1965, 1967; Casey et al., 1967;

Atherton and Ramm, 1969). 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 wieder, 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 inhinbitory effect of increased gravitational load.

The apparent variation in the width of the tetracycline bands, which mark 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.

Chronic vibration does not affect body weight with respect to that of the controls of the same age. The aging process under vibration takes place at the normal rate in the gross physical properties of weight, geometry and density.

However, bone becomes more rigid under vibration than in normal aging. Modulus of elasticity and microhardness of vibrated bone are higher than those of non-vibrated bone. This is correlated to disorganization of mineral deposition: Labeling by tetracycline

shows that the regular zones of mineralization disappear under vibration and that mineral deposition becomes dispersed across the diaphysis. Under vibration the organic bone matrix is not mineralizing along the normal trajectories. Thus the crystalline bone structure becomes incapable of the same elastic deformation which uniformly deposited bone exhibits.

Bone composition, however, is not affected by vibration. The ash and calcium contents of the femur show normal temporal changes. Bone porosity is also normal.

Plaster cast immobilization of one leg, which served as simulation of the state of weightlessness, significantly influences body weight and bone development. The body weight of immobilized animals is always less than normal. The rate of body weight growth is significantly slower than normal during immobilization [(Body Weight) $\propto$ 164 ln (Age)], and somewaht faster than normal after immobilization [(Body Weight) $\propto$ 294 ln (Age)]. The development of some bone parameters is also retarded: weight, volume and density consitently 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 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. This has been observed to take place in humans during normal aging as well as in diseased bone (Jowsey, 1960, 1964).

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 sitffness 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 five weeks after removal of the cast. Exposure to hypergravity and vibration, following immobilization, retards the growth of body weight [(Body Weight) $\propto$ 220 ln (Age) under centrifugation and (Body Weight) $\propto$ 202 ln (Age) under vibration following immobilization], 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 2.5g 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 mechanical stress of 12 hour daily vibration at 25 Hz increases rigidity and microhardness of bone, which corresponds to an acceleration of the aging process, and results in mineral deposition which is quite disorganized compared to that of normal bone development. No other alterations in bone properties due to 12 hour vibration were observed and bone of animals exposed to only short daily periods of vibration develops normally. Further study is required to determine of there is a vibratory environment which is adequate for normal skeletal development, but does not simultaneously result in undesirable manifestations of aging and abnormal modes of mineral deposition.

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.

It is also known that psycho-physiological systemic stress

conditions (social-emotional disturbance, electric shocks, forced restraint, forced exercise, exposure to extreme temperatures, nutritional deficiencies, high oxygen pressure, anoxia) tend to inhibit bone growth and calcification and to develop osteoporosis in both humans and experimental animals (Selye 1950, 1951, 1956, 1962). Therefore the skeletal effects of psychological stresses must also be studied in considering the structural development of bone in an unconventional environment.

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 1g and an imperfect simulation of weightlessness by plaster cast immobilization had to suffice. No significant and systematic changes were found above 1g, 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 1g range to the below 1g 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.

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