THE EFFECT OF RESTRAINT ON CALCIUM AND PHOSPHORUS BALANCE AND Ca^{45} DEPOSITION IN BONES OF IMMATURE MACACA NEMESTRINA PRIMATES

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We hereby recommend that the thesis prepared under our supervision by Amelia Su-hui Chen entitled THE EFFECT OF RESTRAINT ON CALCIUM AND PHOSPHORUS BALANCE AND Ca^{45} DEPOSITION IN BONES OF IMMATURE MACACA NEMESTRINA PRIMATES

be accepted as fulfilling this part of the requirements for the Degree of Master of Science.

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INTRODUCTION

Although man has entered space and returned successfully to earth, the long term effects of weightlessness on the physiology of man is not yet fully understood. The creation of a prolonged gravity-free environment has not been possible on earth, since a number of problems exist in attempting to evaluate the influence of weightlessness within a ground-based laboratory.

The problem of prolonged weightlessness has been reviewed by McCally and Lawton (1). Of the methods available that simulate certain selected physiological effects of subgravity environments, the bed rest technique approaches the condition of weightlessness. The effect of gravity force acting down the long axis of the body is removed when the subject maintains a horizontal recumbent position. Muscular activity is reduced greatly although it is not completely restricted.

Bed rest involving human subjects and restraint experiments using primates have been conducted extensively at the Texas Woman's University Research Institute. Also, this Institute has been the first to study changes in bone density during bed rest as well as in space. A general finding in human subjects during prolonged bed rest was a loss of bone density and a negative calcium balance. Calcium found to be

lost in the urine unquestionably came from the skeletal system. Urinary loss of calcium indicated an imbalance between the rate of formation and the rate of breakdown of bone material.

Furthermore it has been shown that the most important factors that cause the increase in negative calcium balance and loss of bone density during prolonged bed rest has been due to the reduction of stress and strain of bone by interaction of muscle and gravity. The reduction of stress and strain secondary to gravity and muscle activity results in a net osteoclastic activity. Simulated weight bearing exercise also has been found in the TWU Laboratories to be effective in preventing calcium loss from bone.

The general finding in primate studies conducted at the Texas Woman's University has been the fact that bone density has decreased during restraint. Both calcium and phosphorus balance also have become less positive or even somewhat negative during a restraint period. Even if a slight negative calcium balance is found in some animals, however, it has not been sufficiently great to indicate the degree of bone density loss during the restraint period, although the direct measurement of bone density losses has correlated with bone mass closely in a study by Al-Shawi (2). In animals which have not completed growth of the skeletal system, calcium has been found to move from formed areas to

growing portions of the body, such as the epiphyseal cartilage, rather than being excreted in the urine and feces.

The main objectives of this study are to determine:

1. The effect of restraint on calcium and phosphorus balances in Macaca nemestrina primates.

2. The location of the deposition of intravenously injected radioactive Ca^{45} in selected areas of bones by autoradiography and by radio-assay.

REVIEW OF LITERATURE

Muscular Inactivity. When primates are placed in restraint on couches at about a 20^{o} attitude, the effect of longitudinal pressure on bone is greatly reduced and the muscular activity is almost eliminated. It is well known that prolonged muscular inactivity causes bone atrophy, immobilization, osteoporosis, and an increased negative calcium balance.

As early as 1921, Allison and Brooks (3) reported an experimental study of bone atrophy resulting from inactivity. Howard, Person, and Bingham (4) reported in 1945 that bone atrophy and a constant increase in urinary excretion of calcium and phosphorus were found in 17 subjects at bed rest with complete leg and partial body casts. Deitrick et al. (5) in 1948 conducted an extensive investigation. In this study, four healthy young men were placed in bivalved plaster casts extending from the umbilicus to the toes for six to seven weeks. Both urinary and fecal calcium excretion increased during immobilization. The maximum calcium excretion was reached by the fourth to the fifth week. Total calcium loss ranged from 5.0 to 23.9 gm. The calcium content in the urine was twice as much during immobilization as during ambulation. These indicate the probable loss of mineral from the skeletal system.

An investigation which involved six healthy young men was reported by Vogt, Mack, Beasley, and Spencer in 1964 (6). This showed losses in calcium through the urine and feces as well as reduction in bone mass by densitometry during bed rest in the first phase of the study. During the second phase, systematic isometric exercise was administered and bone density was not reduced to such an extent as was shown when the isometric exercise program was not followed.

In experimental animals, Kharmosh et al. (7) showed that the degree of osteoporosis in immobilized animals correlates with changes in muscle mass. In this study, a rabbit's hind limb was denerved by hemilaminectomy. The muscle and bone ash weight on the paralyzed side was expressed as a portion of that which was on the control side. Total radioactivity and specific activity ratios of bone ash also were determined. Muscle and bone atrophy were in evidence at once, continued for 10 weeks and then ceased. Bone deposition was not changed until some time between 10 days and four weeks, when muscle atrophy increased by 30 per cent. This finding suggests that muscle atrophy leads to bone atrophy. The mechanism of bone atrophy includes increased bone resorption followed by increased accretion, until equilibrium is reached and the atrophy is discontinued.

In primates, Gross et al. (8) studied calcium, nitrogen, and phosphorus mobilization resulting from conditions of inactivity in

Macaca irus monkeys. The monkeys were immobilized by surgical denervation, plaster casts, and tranquilization. The animals were placed in the plaster casts in a sitting or supine position. Calcium, phosphorus, and nitrogen balance during inactivity were studied. Calcium was measured by flame photometry, while nitrogen and phosphorus were measured colorimetrically. The animals inactivated by surgical denervation and plaster casts showed a negative nitrogen balance which could be due to disuse atrophy of the skeletal muscle. The urinary phosphorus excretion increased in the inactivated animals by several times, but there was no significant change in calcium balance observed in the inactivated animals.

Mack et al. (9, 10) studied the physiological and metabolical changes in Macaca nemestrina (pigtail monkeys) on two types of diet during restraint and non-restraint. Four groups of primates were fed two different diets. Diet A was high in protein and Diet B was high in fat, carbohydrate and major minerals. Animals on each diet were restrained on couches for 35 days, with exposure to the Biosatellite simulated reentry profile conditions on the 35th day of study. The bone density decreased during the restraint period, regardless of which diet was fed. A high calcium level in the diet tended to effect an increase in bone density. The urinary calcium excretion was not always in a negative correlation with the rate of the bone density.

Brooks (11) studied the effect of restraint and programmed exercise on bone mass and calcium balance of the Macaca nemestrina primates. Six primates were used. The restrained animals were found to decrease in bone mass, with negative calcium balance found on various days. The overall calcium balance was positive, however, during the 14 day restraint period. The exercised animals, on the contrary, were found to decrease in bone mass and to be in negative calcium balance during 14 days.

Van Zandt (12) studied the effect of a new type of restraint garment on Macaca nemestrina primates. Four primates were placed in restraint garments, which were designed for use in a Biosatellite flight, on couches for 13 days. Bone mass was lost in the skeletal sites where the restraint from the garment was most pronounced. The excretion of calcium and phosphorus decreased at the same time that loss of bone mass was taking place. This could be due to the fact that the food intake was not sufficient to maintain body metabolism adequately.

A comprehensive review of literature on primates has been given by Varner (13), Al-Shawi (2), and Lin (14), former graduate students at the Texas Woman's University.

Mack (15) studied the effect of programmed exercise on bone density and calcium balance of human beings during prolonged bed rest.

The data showed that those who exercised regularly excreted less calcium and phosphorus than those who did not exercise. Bone density values supported this finding.

Donaldson et al. (16) conducted an investigation on mineral balance during a prolonged simulated non-gravitational environment. Three healthy adult males were restricted to complete bed rest for 30 to 36 weeks. Urinary calcium excretion was elevated throughout the bed rest period. Mean calcium balance during bed rest ranged from -200 to -256 mg. per day for all subjects. This represents an estimated loss of 0.5 to 5.5 per cent of the skeleton. The recovery of calcaneus mineral to values above the initial level was observed 5 - 10 months following reambulation. Disuse osteopenia therefore may be reversible. Serum parathyroid hormone concentration increased during bed rest.

Trueta (17) stated: "The ability of the osteoblast to synthesize the required protein for its matrix depends also on the availability of amino acids and other constituents required for the synthesis. It has long been known that immobilization, bed rest and lack of muscle activity are all factors contributing to osteoporosis. Why this is so has not yet been fully explained, although the osteoblast-osteocyte syncytium appears to be adversely affected by the lack of activity seems unquestionable." In order to find some explanations of the

osteoporosis of inactivity, a study was made by Trueta and Geiser (18). They used 82 rabbits of both sexes from three to 12 months of age for this experiment. In the first experiment, 40 rabbits had the calcaneral tendon partially removed. The animals were killed at short intervals, from two to 89 days, in order to collect information on the successive changes taking place in the bones. The first changes of bone in radiographs were visible one week after tenotomy. One of the changes was an early fading of the fan of trabeculae in the calcaneum.

The second experiment was to investigate the part that simple immobilization may play in causing bone changes. Twenty-two rabbits had their right hind leg immobilized by means of a padded plaster cast extending from the toes to just above the knee. The immobilization in plaster caused changes like those obtained from tenotomy.

In experiment 3, four animals were immobilized in plaster of Paris and stimulated by faradic electricity. One leg of each of these animals was immobilized and a window was cut in the plaster cast over the calf muscle in order to insert a fine electrode. Through the window, faradic stimulation was applied twice daily in two animals and eight times daily in the other animals. In the rabbits stimulated twice daily, no significant difference was shown between the bone rarefaction of control animals that had been immobilized without faradic stimulation. After three weeks, the two animals with more extensive stimulation had

only slight bone rarefaction limited to the fan of trabeculae in the tuber calcani. Bone rarefaction had increased somewhat after a few weeks, but not as extensive as the severity of that observed in animals immobilized for the same period in the former experiment.

In these studies, the authors summarized that they succeeded in causing rarefaction of calcanuem of animals in all experiments soon after the bone was removed from muscular compressing forces. New bone was generated when the calcaneum was subjected to the stress and strain of muscle contraction. Therefore, the presence or absence of pressure forces was accountable for the balance between bone formation and bone removal in the calcaneum of rabbit.

In these experiments, bone rarefaction was found to be accompanied by a great increase in vascularity in size and number, near the osteoblasts. In order to find whether or not the muscle contraction contributed to the blood flow in the intra-ossesous circulation, another experiment was conducted by deValderrama, Fernandez, and Trueta (19) in 1965. In this study, normal adult dogs were used. The intra-osseous pressure in the tibia of the dog was determined during various conditions of muscle activity. The investigators concluded that muscular contraction seemed to influence the blood flow in bone in two ways: (a) by the occlusion of venous outflow, which leads to an increase in the blood

pressure in marrow, and (b) by rapidly emptying the intra-osseous veins during muscle relaxation.

In the review of Hattener and McMillan (20), there are three factors in the etiology of disuse osteopenia. These include: a). reduction of stress and strain secondary to weight bearing and muscle tension; b). neuronally mediated influences; and c). vascular and blood flow changes. It was concluded that, although specific vascular and neuronal influences can not be excluded in the causation of disuse osteoporosis, the more important determinant appears to be mechanical strain and compression of bone by the interaction of muscle and gravity.

In explanation of biologic effects of strain on bone, recent investigations have disclosed the finding that bone collagen and collagen apatite junction can function as piezoelectric transducers of mechanical stress and strain (i.e., functional pressure) into electrical signals (21). The electrical stimulus affects not only the alignment (and probably the aggregation pattern) of extracellular marcromolecules, but also the behavior of cells (their migration, nutrition, specialization, and proliferation rates). Osteoblastic activity occurs due to an increase in relative electronegativity; osteoclastic activity occurs under the influence of electropositivity. The areas under compression develop an increased electronegativity and develop electropositivity, according to Becker and

Bassett (22). Becker (23) and Jahn (24) have proposed a closed system to explain the process of osteogenesis in response to mechanical stress.

By modifying the Becker and Jahn's system, Stubbs (25) presented a quantitative feedback mechanism which explains both normal bone maintenance and bone demineralization in reduced gravitational conditions. Change of stress results in changed bone deformation (strain). A strain input causes the piezoelectric elements to produce a current outflow. Compression causes an increased electrical negativity and tension causes a decreased negativity or a relative positivity. At no time is osteoblastic or osteoclastic activity zero. An increased electronegativity results in net osteoblastic activity and a reduced negativity results in net osteoclastic activity. Under normal conditions, bone maintenance would occur. If the bone is subjected to reduced stress as in muscular inactivity or in an agravic condition, the loss of compressional strain causes decreased electronegativity which would result in net osteoclastic activity and lead to the bone demineralization.

Calcium^{45} Metabolism. Ca^{45}, a pure beta emitter with a half life 164.5 days, has been commonly used as a tracer to study calcium metabolism in the skeleton of experimental animals. The two common methods for measuring the radioactivity after introduction into an organism are: a). autoradiography, by which radioactivity is recorded on

a photographic emulsion; and b). radio-assay, by counting the number of disintegrations per unit of time.

In respect to skeletal deposition, Steven and Ray (26) in 1967 stated that the bone-seeking isotopes might be incorporated into the skeleton by three principal processes: rapid ion-exchange (27); long-term exchange (28); and incorporation into the mineral phase during de novo bone formation or "accretion" (29). The first two of these are physico-chemical processes, independent of living cells, while the last is associated with cellular activity. Also, Claus (30), in 1958 stated that, in the new born calf the Ca^{45} was deposited mainly along the epiphyseal plates with a smaller amount in the subperiosteal region, except at the ends where deposits were heavy. There was no evidence of haversian deposition in autoradiographic studies. In three-month-old pigs, Ca^{45} was present in the bone, primarily in the region of epiphyseal plates 2 1/2 minutes after injection. Sections of bone from pigs sacrificed after 60 days still retained Ca^{45}, but the radioisotopes had disappeared from the region of the epiphyseal plate and were located mainly in the shaft region of the bone.

Jowsey et al. (31) in 1965 stated that the rate of disappearance of Ca^{45} from the blood of three dogs showed that it is most rapid in the youngest and least rapid in the oldest dog. The autoradiographs of the femors showed, that in the nine-month-old dog, more than half of the

Ca^{45} is associated with bone formation, both at 24 hours and at three weeks after injection, while in the 2.5-year-old dog, this is true of only 1/5 of the Ca^{45}. In the ten-year-old dog, there is a return toward the pattern shown by the young dog, although still less than 50 per cent is associated with bone formation. Therefore, only in a young growing animal is bone formation quantitatively more important than these other factors (physico-chemical exchange) in the deposition of calcium. In an adult animal, only 20 per cent of Ca^{45} is retained as a result of bone formation.

In a study of uptake of radioactive Ca^{45} in rats, Ray (32) in 1962 reported that adult rat bone killed by repeated freezing and thawing and then reimplanted in the body took up about 70 per cent as much Ca^{45}, C^{14} ad did normal living bone. The difference between living and dead bone was greater in young rats, but the devitalized bone in the rat still accumulated appreciable amounts of the Ca^{45} in the slowly exchangeable fraction of the mineral.

Salomon and Ray (33) in 1966 studied autoradiographic distribution and localization of Ca^{45} in undecalcified fresh and devitalized rat bone in comparison with that in normal rats. Ca^{45} was distributed over the entire cortex, metaphysis and epiphysis, with concentration in the subendosteal zone in the normal rats and fresh transplants, and in the subperiosteal zone in the devitalized implant. The entire hypertrophic

cartilage zone was labeled. On a cellular level, concentration of Ca^{45} was observed in and around osteocytes and lacunae. Osteocytes in the devitalized bones of rats also took up and concentrated radiocalcium. In Ca^{45} uptake, the only difference between living and devitalized bone was in the subendosteal and subperiosteal accumulation of radioisotope.

The finding by Ray (32) that the subsequent fall in Ca^{45} of the dead bone was very slow and bore no relationship to the isotope level in the blood was difficult to correlate with the belief that physico-chemical exchange plays only a limited part in the deposition of bone-seeking isotopes in the adult skeleton and that it is rapidly reversible. In order to check this finding, Steven and Ray (26) in 1967 studied the distribution of isotopes following a single injection of Ca^{45} and C^{14}-proline in young rats in which one tibia had previously been removed, killed and reimplanted. The dead tibia took up 25 per cent as much Ca^{45} or C^{14} as did the living tibia. The determination of the "accretion rate" by kinetic analysis of Ca^{45} data showed that this was much too high unless the physico-chemical process of uptake of Ca^{45} by bone was taken into account.

Ellsasser et al. (34) in 1969 compared the kinetic and autoradiographic distributions of Ca^{45} and Ba^{133} in ten-year old dogs killed seven days after injection. The rate of long-term transfer from blood to the skeleton was measured at five days and found to be larger for Ba^{133}

than for Ca^{45}. This was due to an increased uptake of Ba^{133} on the inactive bone surface. Furthermore, the study of the shape of the curve of specific activity in the plasma suggests that some kind of active transport may be counteracting the diffusion of Ca^{45} from blood to bone, and might be related to the endosteal cells lining the bone surface. The ratio of diffuse specific activity in the mid-shafts of the long bone to the average specific activity of the skeleton was only 1:6. A larger fraction of skeletal activity, however, probably was associated with increased uptake of Ca^{45} in the relatively young bone which results from recent haversian remodeling. The diffuse specific activity of Ca^{45} throughout the skeleton increased with increased bone metabolism. This could be a regulation by the osteocyte at the lacunar-canalicular level.

Simmons and Marshall (35) in 1970 studied bone growth and calcium metabolism in marine acellular-boned toadfish by tetracycline and by Ca^{45} labeling over a 26-day period. The uptake of Ca^{45} in the vertebrae and jaw appeared to be related to the surface area of bone rather than the mineral content of the bone. In a study of appositional bone growth by the separation of two tetracycline labels, only a single subperiosteal fluorescent band was observed. This was probably the result of a very low appositional rate of bone growth. The fall in the plasma radioactivity was almost inversely proportional to time since injection. No diffuse labeling could be detected, due presumably to the absence of osteocytes, in lacunae and canuliculi which, in cellular bone, permits

diffusion of calcium ion from extracellular fluid to bone crystals deep within the skeleton. The diffuse component, if present, was less than one-fifth that expected in cellular bone.

Popove and Kuznetsov (36) in 1966 used radioactive tracers to show accumulation of Ca^{45} in the callus after fractures of the long bones in rats. This accumulation was most marked during maximal development of the callus.

Hibi (37) in 1969 carried out an experimental study on the healing process of fractures using Ca^{45} in rats in which complete subcutaneous fracture of the middle of the right tibia was manually induced. He found that, along with the healing of fractures, Ca^{45} deposition into the portion of fracture gradually becomes intense, becoming most intense three weeks after fracture. On the other hand, increase in the resorption of bone salt was noted in the bone other than the site of the fracture. Changes in the epiphysis was especially large, with a decrease in calcium deposition and an increase in resorption. Due to the reaction of the body to local changes, such as fracture, these changes probably take place. The supply of calcium salt necessary for the calcification of callus is mainly conducted systemically via a blood stream in addition to the lacal supply.

Bosch (38) in 1969 studied the plasma Ca^{45} clearance by the tibia in the immature dog. The data best fit the hypothesis that the

volume rate of plasma following through the skeleton is not uniformly distributed among capillaries able to exchange calcium with bone mineral and capillaries unable to do so, and that this non-uniformity is a determinant of the quantity of bone mineral calcium which can exchange with systemic circulation.

Movshev (39) in 1968 studied the Ca^{45} metabolism in intact and burned rats by kinetic analysis. The kinetic equation reflects a decrease in Ca^{45} in the blood, an increased incorporation into bone and an increased Ca^{45} excretion from blood, both three and 15 days after burn.

Wanner et al.(40) in 1956 have presented findings to show that the monkey is superior to the rat and the dog in metabolic studies related to calcium metabolism. They injected Ca^{45} intravenously and observed that the ratio of Ca^{45} excretion in urine and feces during the next five days was 1:22 in the rat, 1:10 in the dog, 2:1 in the monkey, and 2:1 in the human being. It has since been determined that the urine:feces excretion ratios of six young men subjects ranged from 0.50:1 to 3.2:1 and averaged 1.9:1.

Also, Harris et al. (41) in 1961 fed radioactive Ca^{45} to rhesus monkeys both by intravenous injection and by stomach tube and in the diet. The excretion of calcium five days after its receipt in the diet was 5.3 per cent in the urine and 28 per cent in the feces. The author stated

that the urine:feces ratio of Ca^{45} in their primate study was in close agreement with data previously obtained with children.

PLAN OF PROCEDURE

The data presented in this report were obtained from eight healthy male Macaca nemestrina primates (pigtail monkeys). The primates were housed in the primate laboratory at the Texas Woman's University.

Feeding Method

The diet for the primates was Purina Monkey Chow. The food consumed by each monkey was weighed daily. During the restraint period, the food was fed by hand. Water was taken ad libitum. All of the animals were offered approximately the same amount of food each day. During the ambulatory periods the animals usually ate all of the food given them. During the restraint periods, however, some of the animals refused some of their food.

Collection of Excreta

Excreta were collected from the individual metabolism cages, with the feces weighed and the urine measured. During the restraint period, the urine was collected by means of a urine collector fitted to a plastic tube which was connected to a bottle under the couch. The feces of the restrained animals was caught immediately below the anus in a plastic container placed under the couch.

Urine and fecal samples were collected each morning. Urine samples were measured and were acidified with hydrochloric acid. An aliquot sample from each animal was stored in a labeled plastic bottle. Fecal samples were collected in two-day lots and weighed, and then were blended with a measured amount of distilled water, with an aliquot placed in a crucible and weighed. The sample was ashed in an oven at 650° C overnight. The ash was dissolved in hydrochloric acid, diluted to 100 ml. and aliquots of the solution were used for analysis.

Analysis of Food and Excreta

Calcium in urine, feces, and food was determined by the method of Ferro and Ham (42, 43), using the pH adjustment suggested by Chiamori and Henry (44).

Phosphorus in urine, feces and food was analyzed by the method of Fiske and Subbarow (45) as modified by Dryer et al (46).

Details of these analyses are given in the Appendix.

The study was designed to determine the location of the deposits of intravenously injected Ca^{45}. Eight animals were placed in separate metabolism cages for 46 days, which was intended to serve as an ambulatory period. Then all animals were placed in couches in restraint garments that were designed for use in a Biosatellite flight. The animals were injected intravenously with 100 uc of Ca^{45} on the twelfth or

thirteenth day of restraint. The monkeys were sacrificed at various times from nine to 30 days later. One of the restraint animals (No. 545) was removed from the couch and became a control animal for the remainder of the group on the day that they received the injections of radioactive calcium. This was done because this primate began hemorrhaging from an ulcer produced by his catheter. Another animal (No. 775) was injected with Ca^{45} and then was sacrificed two days later so as to determine the immediate location of the deposition.

Following the injection of Ca^{45}, the other animals were sacrificed according to the following schedule:

Primate 745	9 days	Primate 547	28 days
Primate 695	13 days	Primate 708	29 days
Primate 758	14 days	Primate 545	30 days
Primate 787	24 days	Primate 757	30 days

The tibiae, fibulae, radii, and ulnae were removed and dried in an oven. These bones from the left limbs were cut into five segments so that the end pieces contained the epiphysis and endochondral bone adjacent to it. Then the diaphysis of the bone was cut into three approximately equal pieces. Figure 1 shows how each bone was cut. Each piece of bone was weighed and ashed overnight at 650° C. The ash was weighed and dissolved in 6N HCl and neutralized with NH_4OH to a pH such that the bone mineral just remained in solution and then

was made up to a 50 ml. volume. A one milliliter aliquot was placed on an aluminum planchet, dried, and the Ca^{45} activity was determined on a Beckman Wide Beta Counter.

The bones of the right limbs were cut into longitudinal and cross sections and polished on fine emory paper to produce a flat smooth surface for use in preparation of autoradiographs. These sections were placed on type AA X-ray film and placed in a cardboard holder for three days. A weight was placed on the holder to maintain close contact between the bone and the film. The films then were developed in an X-ray developer by the usual technics.

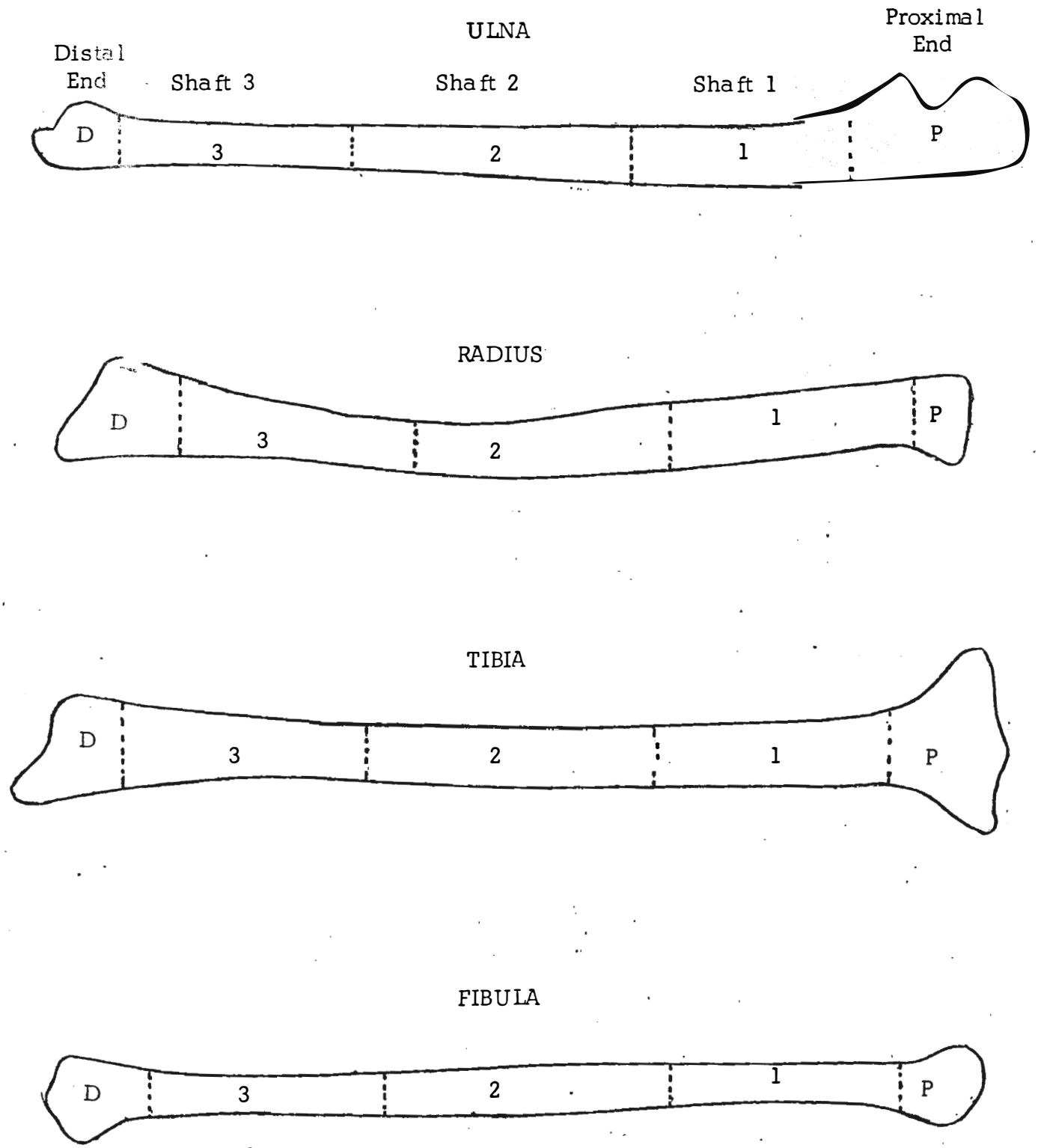

Figure 1. SEGMENTS OF BONE USED FOR Ca^{45} DETERMINATION

PRESENTATION OF FINDINGS WITH DISCUSSION

All of the animals used in this study had been selected from the animal colony on the basis of the fact that roentgenographs of the hands and feet had shown that they had some or all of their epiphyses in an immature state. There was a wide variation, however, in their skeletal maturation. This was determined to be especially true at the end of the study when the autoradiographs were made. Some animals were found to be very immature while others had some mature epiphyses and some nearly mature. Because in examining the data it appeared that there were differences based on the maturation status of the animals, it was determined that, if the animals were divided into two groups, many of the results which would have otherwise been difficult to explain were much more easily understood. Thus on the basis of the autoradiographs the animals were divided into two rather empirical groups. The first group of animals was found to be quite immature, and this included animals 695, 787, 708 and 757. The remaining animals (545, 547, 745, 758 and 775) were more mature and they were placed in the second group.

Calcium Balance Data

Table I, Part A through Part H gives the data on calcium intake, excretion and balance for all the primates throughout the study.

Summary A gives the comparison of the mean daily calcium balance during the ambulatory period and the restraint period.

Ambulatory Period. All the primates in the study were in positive daily calcium balance during this period. The most positive was primate 745 with a mean of +288 mg. per day and the least positive was primate 545 with a mean of +100 mg. per day.

Restraint Period. Primate 695 was in negative daily calcium balance for 11 of 25 days of restraint. Although the overall mean balance was slightly positive, it was significantly less so than during the ambulatory period, with a difference which was statistically significant ($P < 0.02$), by the "t" test.

Primate 758 was in negative calcium balance with a mean of -116 mg. per day for the overall period. Highly negative daily balances were found for most days of this period. When this period was compared with the ambulatory period, a highly significant statistical difference was noted ($P < 0.001$).

Primate 757 was in calcium equilibrium for the overall period, although negative balances were found for 21 of the 41 days of the restraint period. A significant difference was found when this period was compared with the ambulatory period ($P < 0.01$).

Primate 745 appeared in negative calcium balance with a mean of -77 mg. per day for the overall period. Also, the daily balances were found to be negative most of the days during this period. A highly significant difference between the overall and the ambulatory period was shown by statistical analysis ($P < 0.001$).

Primate 547 appeared to be in negative calcium balance for the overall period (mean of -42 mg. per day). Negative daily calcium balances were found for 19 of the 39 days of restraint, with most of these occurring during the first two weeks. The calcium balance was different during this period from that during the ambulatory period by a highly significant difference ($P < 0.001$).

Primate 708 was found to be in slightly positive calcium balance during the overall period, with a mean of +29 mg. per day, although it was much less positive during this period than during the ambulatory period by a highly significant difference ($P < 0.001$).

Primate 545 was in negative calcium balance with an average of -150 mg. per day for the overall period. Almost all of the daily calcium balances were negative except during the first three days. The difference between this period and the ambulatory period was distinctly significant ($P < 0.01$).

Primate 787 was in daily negative balance for one-half of the period. The average overall calcium loss was 60 mg. per day. There were no data for the ambulatory period to be used for comparative purposes.

In summary, most primates in the study were in negative calcium balance during the restraint period. Primates which were in positive calcium balance were much less positive during the restraint period than during the ambulatory phase of the study. Statistically, most primates had a highly significant difference between the restraint period and ambulatory period ($P < 0.001$). When the data for all seven primates were combined for the purpose of comparing the calcium balance during the restraint with that of the ambulatory period, it was seen that the balance in the restraint period (mean of -47 mg. per day) was appreciably less than that of the ambulatory period (mean of +167 mg. per day), with a highly significant difference between the periods shown by the "t" test ($P < 0.001$). The mean difference between these periods is 214 mg. per day.

When the animals were divided into the two groups on the basis of their skeletal maturation, it was found that the more mature animals had a significantly higher loss of calcium during the restraint period ($P < 0.05$). The immature group had a mean daily calcium balance of -6 mg. per day while the older group had a balance of -84 mg. per day.

During the ambulatory period there was no difference between the two groups (See Summary C).

Food intake remained fairly constant during the study. Most of the animals ate well both in the restraint and ambulatory periods. Animals number 745 and 787 rarely consumed all of the feed offered them while they were restrained. This was true for several others during the first two weeks, although after that they ate better. Most of the variation in calcium intake was due to the difference in calcium content of the monkey chow. Samples from each bag used were analyzed. The calcium content of the feed used during the ambulatory period ranged from 7.44 to 9.33 mg. per gram. During the restraint period, however, a new shipment of feed was used, which later was found to contain 5.80 mg. of calcium per gram.

Phosphorus Balance Data

Table II, Part A through Part H gives the data on phosphorus intake, excretion and balance throughout the study. Summary B gives the comparison of mean daily phosphorus balance during the ambulatory period and the restraint period.

Ambulatory Period. All of the primates in the study were in overall positive phosphorus balance during this period. The most positive was primate 745 with a mean of +162 mg. per day. The least positive was primate 545 with a mean of +59 mg. per day.

Restraint Period. Primate 695 was in daily positive phosphorus balance with a mean of +97 mg. per day. There were no changes which were statistically significant during this period.

Primate 758 was in negative phosphorus balance for the overall period, with a mean of -22 mg. per day, and with the difference between the two periods highly significant ($P < 0.001$).

Primate 757 was in positive daily phosphorus balance for all except 10 of the 41 days of restraint, with a mean of +71 mg. per day. There was no statistically significant difference between the ambulatory and restraint periods.

Primate 745 was in slightly negative phosphorus balance for the overall period with a mean of -5 mg. per day, with a highly significant change found during this period as shown by the "t" test ($P < 0.001$).

Primate 547 was in daily overall positive phosphorus balance, with a mean of +41 mg. per day. On the other hand, it was in negative balance for 13 of the 39 days of restraint. Statistically, no significant change was shown between the ambulatory and the restraint periods.

Primate 708 was in positive phosphorus balance during the overall period, with an average of +55 mg. per day. A negative daily balance was found only for eight of the 40 days of the period. The "t" test did not show a significant change during this period.

Primate 545 was in daily negative phosphorus balance for about one-half of the period and was in overall negative balance with a mean of -20 mg. per day. This negative balance was not significantly different ($P < 0.10$) than the positive balance observed during the ambulatory period.

Primate 787 was in negative phosphorus balance for the overall period, with an average of -25 mg. per day and in daily negative balance for 14 of 33 days of restraint.

Although some individual variations were observed in the responses of the primates in the study, the "t" test for all of the seven primates indicated the phosphorus balance to be less positive during the restraint period (mean of +42 mg. per day) than that during the ambulatory period (mean of +94 mg. per day), with a moderately significant difference ($P < 0.05$).

As had been found in the calcium balance data, the older primates also had a less positive phosphorus balance than did the more immature animals ($P < 0.05$). The young group had a mean daily balance of +54 mg. while the older animals had a balance of +7 mg. per day (See Summary C).

SUMMARY A

SUMMARY OF MEAN DAILY CALCIUM BALANCE

(milligrams)

Days	Primate Number	Intake	Excretion	Balance
Ambulatory Period				
46	695	1161	1001	+160
46	758	1166	1015	+151
46	757	1163	1044	+119
46	745	1162	873	+288
46	547	1160	1025	+136
46	708	1169	948	+221
46	545	1174	1074	+100
None	787	--	--	--
Restraint Period				
25	695	673	637	+37
26	758	711	827	-116
41	757	707	706	+ 1
21	745	508	585	-77
39	547	648	690	-42
40	708	682	653	+29
13	545	678	836	-159
33	787	529	589	-60

SUMMARY B

SUMMARY OF MEAN DAILY PHOSPHORUS BALANCE

(milligrams)

Days	Primate Number	Intake	Excretion	Balance
Ambulatory Period				
46	695	752	663	+88
46	758	756	660	+96
46	757	753	660	+93
46	745	754	592	+162
46	547	753	685	+68
46	708	757	664	+93
46	545	760	701	+59
None	787	---	---	--
Restraint Period				
25	695	527	429	+97
26	758	558	581	-22
41	757	575	504	+71
21	745	395	400	- 5
39	547	526	485	+41
40	708	564	508	+55
13	545	504	524	-20
33	787	434	459	-25

SUMMARY C

COMPARISON OF MEAN CALCIUM AND PHOSPHORUS BALANCES BETWEEN IMMATURE AND MORE MATURE PRIMATES

(milligrams per day)

Primate	Calcium Balance		Phosphorus Balance	
	Ambulatory Period	Restraint Period	Ambulatory Period	Restraint Period
Immature Group				
695	+160	+37	+88	+97
757	+119	+ 1	+93	+71
708	+221	+29	+93	+55
787	X	-60	X	-25
Weighted Mean	+166	- 6	+91	+54
More Mature Group				
547	+136	-42	+68	+41
745	+288	-77	+162	- 5
545	+100	-159	+59	-20
758	+151	-116	+96	-22
Weighted Mean	+169 (N.S.)	+84 ($P < 0.05$)	+96 (N.S.)	+ 7 ($P < 0.05$)

Autoradiography

As may be observed in the autoradiographs presented in the figures, the Ca^{45} which was administered to the primates was taken up in several distinct parts of the bones in addition to a general diffuse uptake over the entire bone. These locations differ only slightly with the age of the animals. The heaviest concentration of Ca^{45} was found in the area next to the epiphyseal cartilage in which growth of the long bones was taking place. As may be seen in the autoradiographs of the immature animals (Figure 2 - A), there was a very dark region of calcifying material, a gray area of hypertrophic cartilage which appears to contain a relatively high diffuse concentration of Ca^{45} and a narrow band of cartilage with little or no activity. In the more mature animals, the cartilage itself is not visible at all, although in most bones an area of high Ca^{45} concentration is found at the position where the cartilage had been located (Figure 2 - B).

In all animals, regardless of age, there was evidence of haversian deposition. This can be seen as small lines in the longitudinal sections of the bone shaft (Figure 3 - B) and as dots in the cross-sections (Figure 4).

All bones also showed evidence of Ca^{45} deposition in the subperiosteal and subendosteal regions. There appeared to be little or no difference between the immature and nearly mature animals. There

might possible be slightly more subendosteal deposition in the more immature animals as evidenced by the greater darkening of the film under the marrow cavity of the bones of these animals.

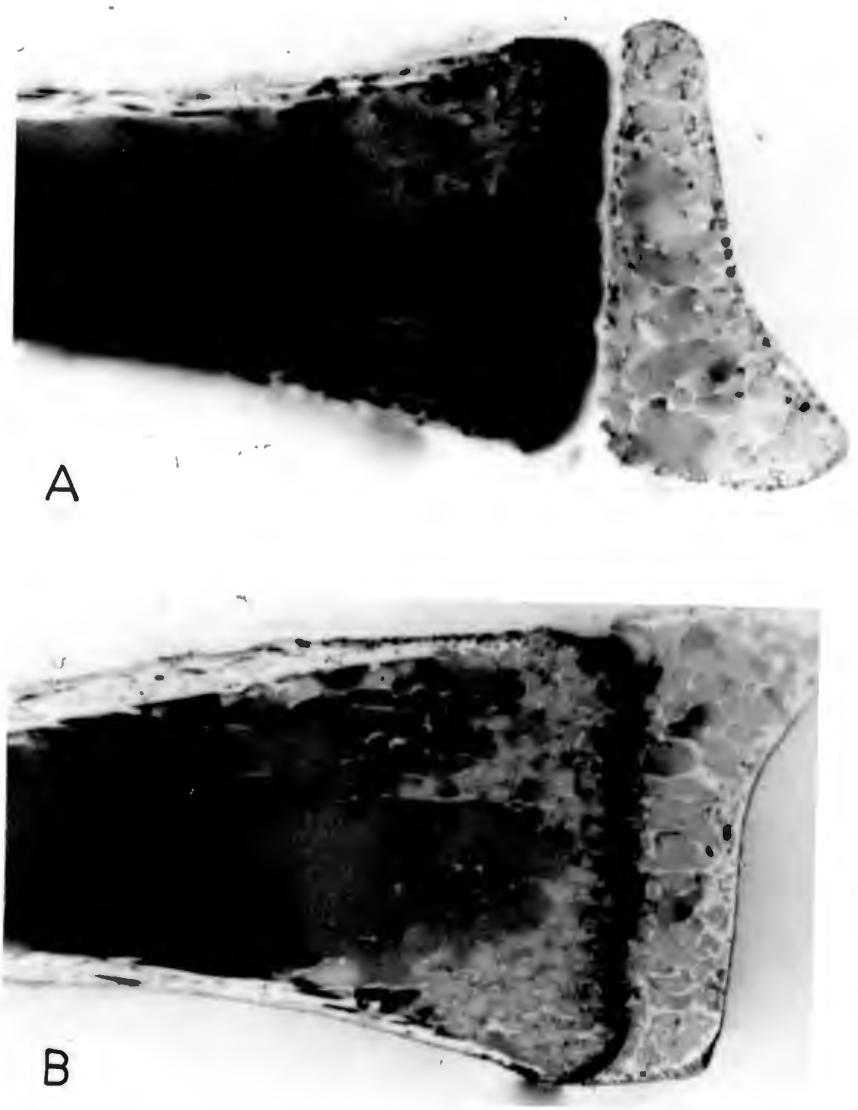

Figure 2. AUTORADIOGRAPHS OF THE DISTAL END OF THE RADIUS OF (A) AN IMMATURE PRIMATE AND (B) A MORE MATURE PRIMATE

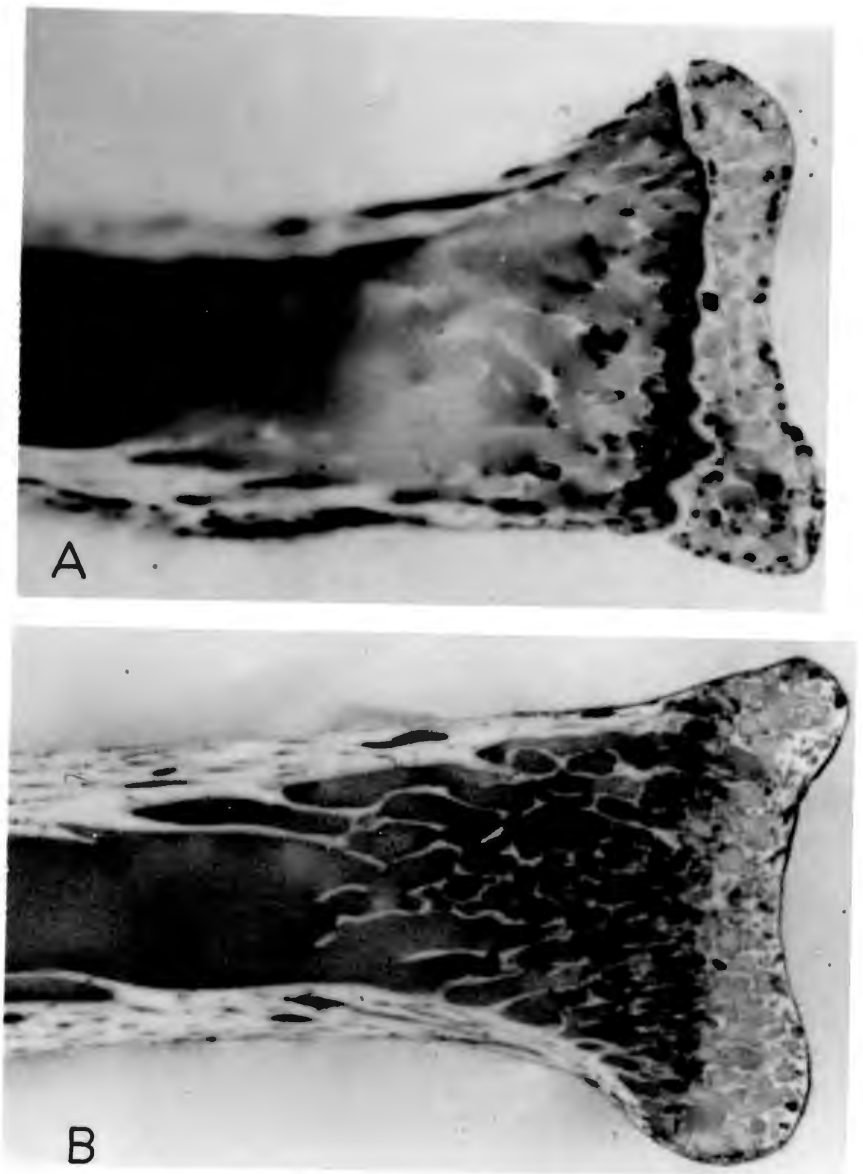

Figure 3. AUTORADIOGRAPHS OF THE PROXIMAL END OF THE RADIUS OF (A) AN IMMATURE PRIMATE AND (B) A MORE MATURE PRIMATE

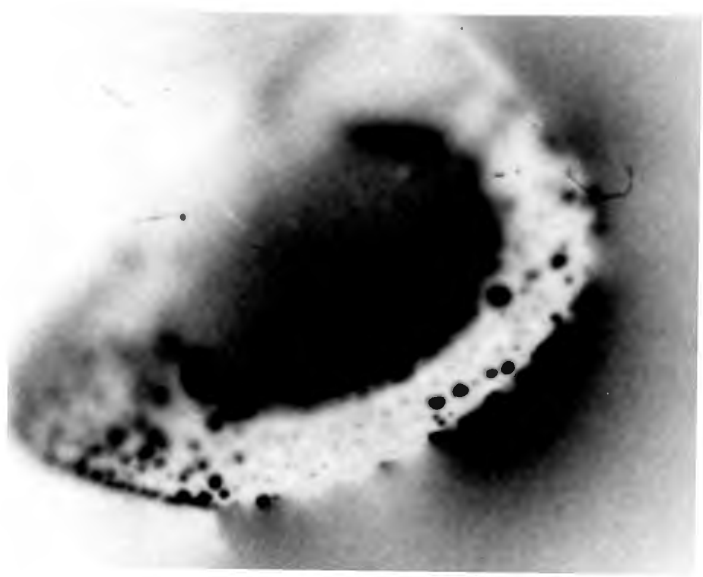

Figure 4. AUTORADIOGRAPH OF A CROSS-SECTION OF THE TIBIA OF A PRIMATE

Radioassay

The Ca^{45} specific activity of the ashed bones is presented in Tables III through VI. It was observed that most of the activity was located in both the proximal and distal ends of the long bones, with a smaller amount in the shaft. This is in agreement with the finding from the autoradiographs. In all four of the bones examined, both ends had higher specific activities than the shaft, but in three of the four bones one end consistently had a higher count than the other. Only in the radius was there an approximately equal deposition of Ca^{45} in both the proximal and distal ends. In the ulna, the distal end had a much higher specific activity than the proximal end, while in the tibia and fibula the proximal end was the most active. The actual total count in the distal end of the radius was much greater than that of the proximal end. Because of the difference in size of the two ends (the proximal end is much smaller), however, their specific activities were similar.

An effort was made to determine whether the Ca^{45} that was deposited immediately following the injection was transferred later to other portions of the bone. One animal was sacrificed two days after the injection and others were sacrificed at intervals until the termination of the study after 30 days was reached. No differences could be observed when the distribution of the Ca^{45} between the five segments of each bone were compared. The ratios of the specific activity of the

center section of the shaft were not significantly different for the animals sacrificed at different times during the study.

When the specific activities of the animals which had been classified as immature were compared with those of the more mature primates, it was found that there was much more Ca^{45} deposited in the bones of the younger animals. These data are presented in Figures 5, 6, 7, and 8 and in Summary D. When the ratio of the specific activity of the center section of the shaft was compared to that of the end segments, there were no significant differences between the two groups of animals, although in three of the four bones studied, the mean ratios of the immature animals were higher than those of the more mature animals (See Summary E). Thus there seems to be a trend which indicates that the immature animals were depositing more Ca^{45} in the epiphyseal area in relation to that deposited in the shaft than were the more mature animals.

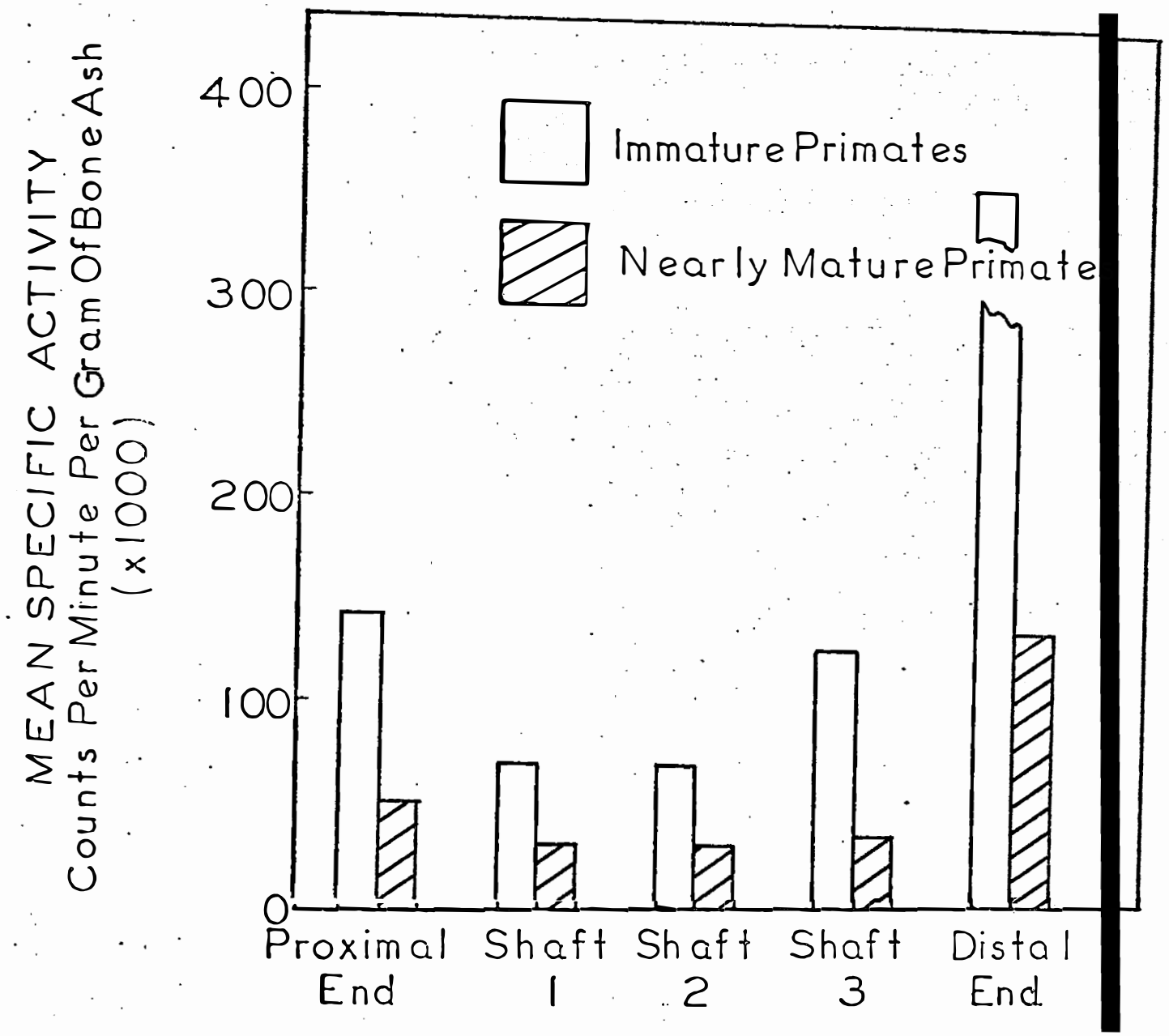

Figure 5. COMPARISON OF MEAN SPECIFIC ACTIVITY OF CA^{45} IN THE ULNA BETWEEN IMMATURE AND NEARLY MATURE PRIMATES

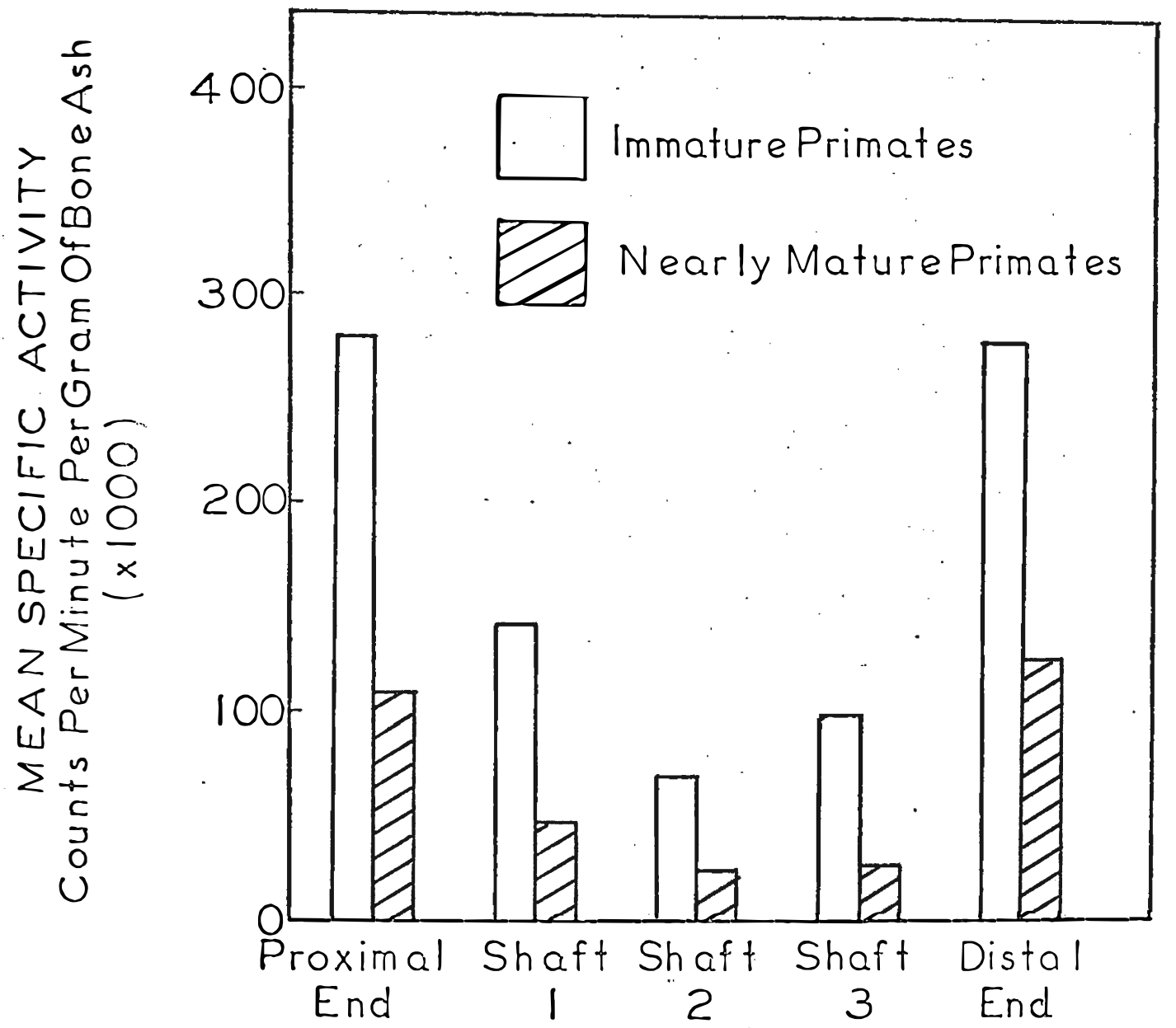

Figure 6. COMPARISON OF MEAN SPECIFIC ACTIVITY OF CA^{45} IN THE RADIUS BETWEEN IMMATURE AND NEARLY MATURE PRIMATES

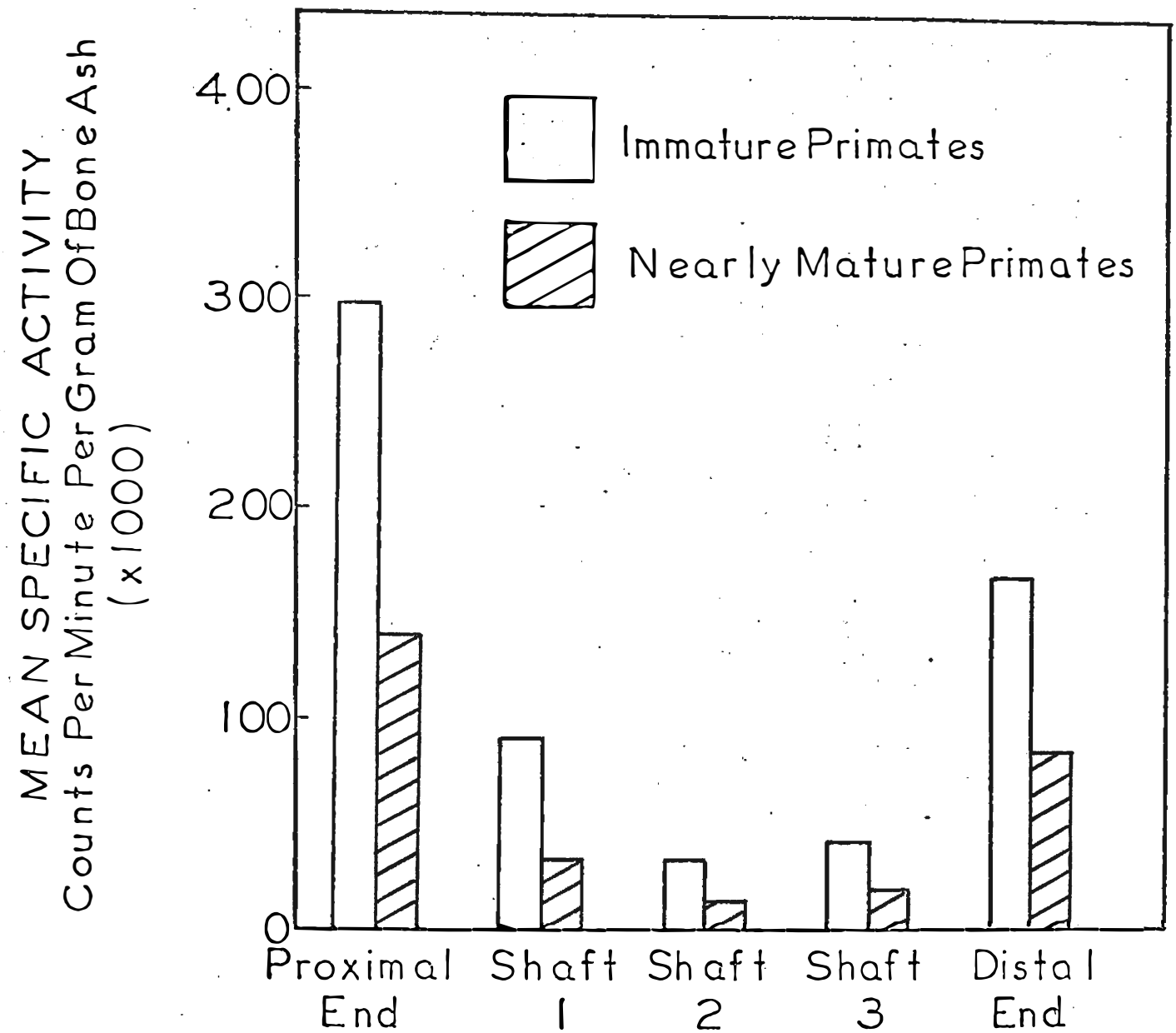

Figure 7. COMPARISON OF MEAN SPECIFIC ACTIVITY OF CA^{45} IN THE TIBIA BETWEEN IMMATURE AND NEARLY MATURE PRIMATES

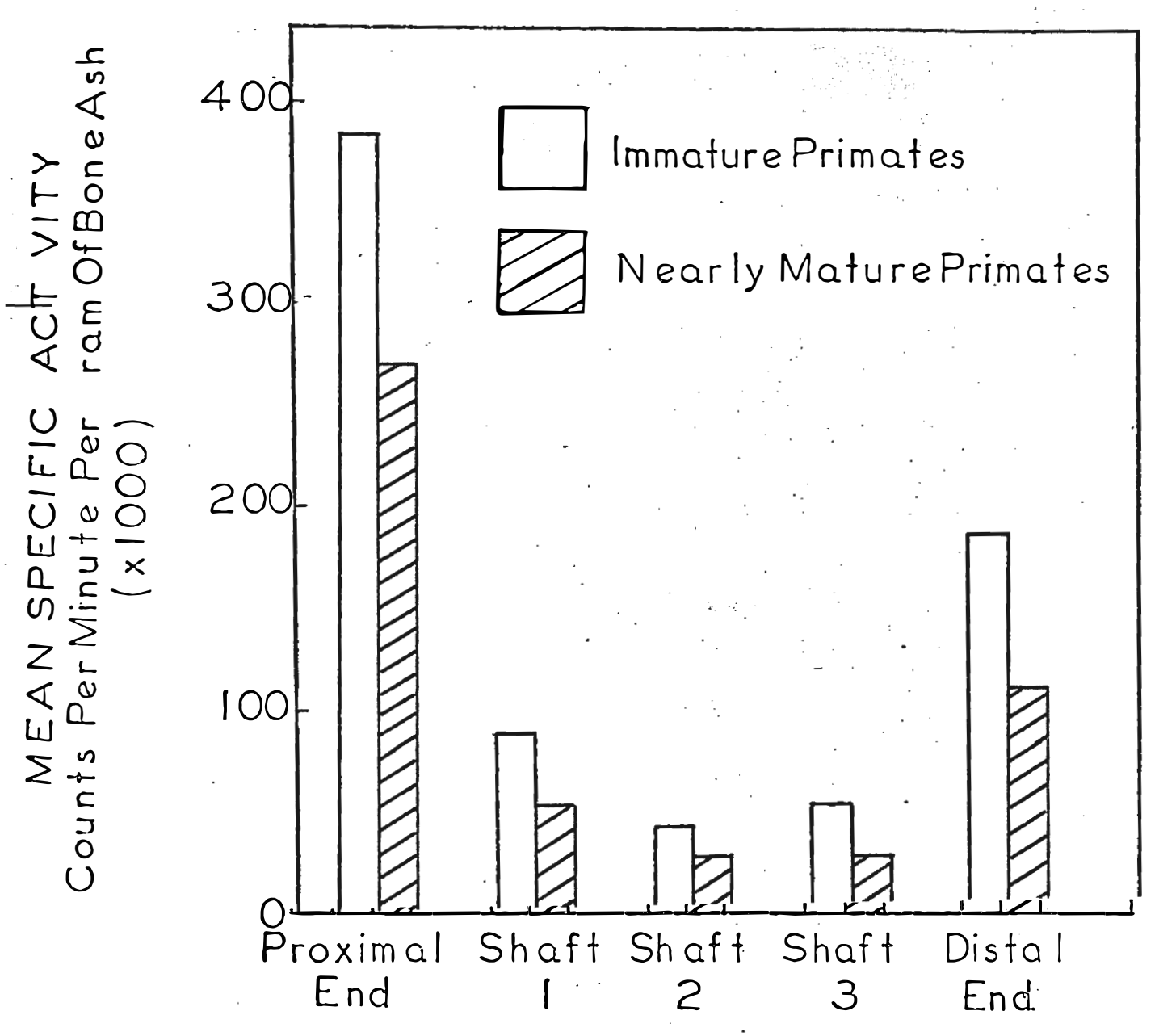

Figure 8. COMPARISON OF MEAN SPECIFIC ACTIVITY OF CA^{45} IN THE FIBULA BETWEEN IMMATURE AND NEARLY MATURE PRIMATES

SUMMARY D

COMPARISON OF MEAN SPECIFIC ACTIVITY OF CA^{45} BETWEEN IMMATURE AND NEARLY MATURE PRIMATES

(Counts per minute per gram of ashed bone)

	Proximal End	Shaft 1	Shaft 2	Shaft 3	Distal End
Ulna					
Immature group	144,799	71,969	74,801	129,509	361,395
Nearly mature group	49,096 ($P<0.05$)	30,813 ($P<0.10$)	30,739 ($P<0.10$)	37,429 ($P<0.05$)	139,991 ($P<0.02$)
Radius					
Immature group	283,891	144,441	72,870	100,416	284,808
Nearly mature group	114,889 ($P<0.10$)	47,104 ($P<0.10$)	26,926 ($P<0.10$)	31,784 ($P<0.01$)	127,266 ($P<0.10$)
Tibia					
Immature group	303,998	94,157	34,457	43,680	173,531
Nearly mature group	141,089 ($P<0.20$)	36,528 ($P<0.20$)	16,486 ($P<0.20$)	20,624 ($P<0.20$)	84,912 ($P<0.20$)
Fibula					
Immature group	303,188	88,689	41,491	54,701	191,175
Nearly mature group	274,346 (N.S.)	55,016 (N.S.)	28,403 (N.S.)	27,227 ($P<0.20$)	114,601 (N.S.)

SUMMARY E

COMPARISON OF MEAN RATIO OF THE SPECIFIC ACTIVITY OF THE PROXIMAL AND DISTAL ENDS TO THE SPECIFIC ACTIVITY OF THE CENTER SHAFT BETWEEN IMMATURE AND NEARLY MATURE PRIMATES

	Proximal End	Distal End
Ulna		
Immature Group	2.4	5.6
Nearly Mature Group	1.8	5.0
	(N.S.)	(N.S.)
Radius		
Immature Group	3.9	3.8
Nearly Mature Group	4.3	4.8
	(N.S.)	(N.S.)
Tibia		
Immature Group	8.8	5.4
Nearly Mature Group	8.0	5.0
	(N.S.)	(N.S.)
Fibula		
Immature Group	10.3	4.9
Nearly Mature Group	9.6	3.9
	(N.S.)	(N.S.)

SUMMARY AND CONCLUSION

The study was designed to determine the effects of restraint on calcium and phosphorus balance and the location of the deposition of injected Ca^{45} in the long bones during the restraint period. Eight young male primates (Macaca nemestrina) maintained on monkey chow diet, were kept in cages for 46 days as an ambulatory period. Then all were placed in restraint on couches. The animals were injected intravenously with 100 μc of Ca^{45} on the 12th or 13th day of restraint and sacrificed at various times after administration of Ca^{45}. The long bones were dried and cut into five sections. Left limbs were used for radioassay, while right limbs were used for autoradiographs.

During the ambulatory period, all of the animals were in positive calcium balance, while during the restraint period, they either were in negative or less positive calcium balance with a mean of -46 mg. per day. This indicated that there was a slight loss of calcium through the urine and feces during the restraint period.

The phosphorus balance was less positive during the restraint period than during the ambulatory period.

By comparing the results of radioassays and autoradiographs, it was shown that Ca^{45} was deposited over the entire bone with higher concentrations at both epiphyseal ends, with smaller deposition in the shaft.

In the autoradiographs, there also was evidence of Ca^{45} deposition in the subperiosteal and subendosteal regions and discreet haversian deposition in the dense cortical bone of the shaft.

On the basis of the bone maturation in the autoradiographs, the animals were divided into two groups, immature and nearly mature groups. The younger immature primates had a significantly less loss of calcium and phosphorus through the urine and feces, than that of the older nearly mature primates during the restraint period.

Radioassay data showed that the younger immature primates tended to have a higher uptake of Ca^{45} in each segment of the bone than the older nearly mature primates, with a statistical probability approaching significance. Also, the younger immature animals had a more distinctly high concentration of Ca^{45} deposition in the epiphyseal cartilage region than that of older nearly mature primates in the autoradiographs.

Therefore, it is possible that in immature primates, the calcium lost during restraint could partly be deposited at the epiphyseal cartilage

for growth instead of being excreted through urine and feces. Thus, while older mature animals would be in negative calcium balance during restraint, younger immature animals could go through a restraint period and still maintain a positive calcium balance if the calcium intake were adequate.

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A P P E N D I X

Methods Used in the Determination of Calcium and Phosphorus in Urine, Feces, and Food

Analytical Procedure for Calcium

Preparation of Samples. For urine samples, 10.0-milliliter aliquots were placed in test tubes and acidified to at least pH 1 with several drops of concentrated hydrochloric acid. The tubes were placed in a boiling water bath for about 30 minutes, and then were cooled to room temperature. The urine was transferred to 25-milliliter volumetric flasks, and the test tubes rinsed twice with distilled water, which also was transferred to the flask. The pH was adjusted to 4.5 with ammonium hydroxide and hydrochloric acid. Methyl red was the indicator used. The flasks then were made up to volume with distilled water.

Dried feces, or food was ashed at 600° C. The ash then was dissolved in 6N hydrochloric acid and made up to a 100-milliliter volume with distilled water.

For the ashed feces samples, aliquots of the solution were pipetted into volumetric flasks. The size of the aliquots and the flasks was dependent on the amount of calcium in the solution. If the approximate concentration was known, the aliquots were such that the final concentration in the flasks was approximately 10 milligrams of calcium per 100 milliliters (at least between 5 and 20). The flasks were filled

about three-fourths full, several drops of methyl red were added to each, and the pH was adjusted to the orange neutral point. The flasks then were filled to the mark.

Procedure. The standard solution was made to contain 10 milligrams per 100 milliliters of calcium. Two milliliters of the pH-adjusted samples, the standard, and a water blank were pipetted into 15-milliliter conical centrifuge tubes. One milliliter of chloranilic acid reagent was added to each tube, and the tubes were agitated by twirling in order to provide thorough mixing. The tubes then were permitted to stand for at least two hours, after which they were centrifuged at 1800 revolutions per minute for 15 minutes. The supernatant liquid was decanted, the tubes were inverted on absorbant paper, and were allowed to drain for two minutes. The mouths of the tubes were wiped with tissue paper. The precipitate was washed with about four milliliters of 50 per cent isopropyl alcohol using a fine stream from a polyethylene wash bottle. The precipitate was dispersed and resuspended in the alcohol by twirling. The tubes were centrifuged, decanted, and drained again.

Then two drops of water were added to the packed precipitates, which were broken up by tapping the ends of the tubes with the forefinger so that the precipitate was resuspended in the water. Six milliliters of the EDTA solution were added to each tube, and the tubes were twirled

until the precipitate was dissolved completely. The absorbance of each tube was determined at 520 millimicrons (Coleman).

Calculation.

$$\frac{\text{Concentration of standard}}{\text{Optical density of standard}} = K$$

$$K \times \frac{\text{Optical density}}{\text{of sample}} \times \text{Dilution} \times \frac{\text{Volume of Sample}}{100} = \text{milligrams Ca}$$

Analytical Procedure for Phosphorus

Preparation of Samples. The urine dilution for phosphorus depends on the daily volume and the diet. It ranged generally from 5 to 25 times.

Ashed feces, or food was dissolved in 6N hydrochloric acid, and the samples were diluted so that the diluted solution contained an amount of phosphorus which will fall within the range of standards. A 100 times dilution was found usually to be correct for ashed feces samples.

Procedure. Standard solutions were made to contain 0.25, 0.50, 1.00, and 1.50 milligrams per 100 milliliters of phosphorus, respectively.

Two milliliters of each working standard, each sample solution, and a water blank were placed in medium-sized test tubes. Two

milliliters of 5N sulfuric acid were added to each tube and mixed; two milliliters of ammonium molybdate solution were added to each tube and mixed. Then four milliliters of N-phenyl-p-phenylenediamine solution were added to each tube and mixed. These were allowed to stand for 15 minutes. The contents of the test tubes were poured into 19 millimeter Coleman cuvettes and read in the Coleman Spectrophotometer at 700 millimicrons versus a water blank which was carried through the complete procedure.

TABLE I

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART A. PRIMATE 545

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	1162	1585	-423	25	1221	1094	+127
2	1141	1636	-495	26	1203	1129	+ 74
3	1097	742	+355	27	1185	953	+232
4	1143	624	+519	28	1192	894	+298
5	1150	1216	- 66	29	1222	1096	+126
6	1116	1130	- 14	30	1172	977	+195
7	1216	1516	-300	31	1180	1064	+116
8	1189	1444	-255	32	1159	994	+165
9	1044	706	+338	33	1264	919	+345
10	1061	698	+363	34	1289	1012	+277
11	1061	1227	-166	35	1276	1145	+131
12	1038	1231	-193	36	1279	1045	+235
13	1033	936	+ 97	37	1269	1028	+241
14	1020	959	+ 61	38	1294	1040	+254
15	1032	919	+113	39	1302	984	+318
16	1049	946	+103	40	1313	1058	+255
17	1052	973	+ 79	41	1334	913	+421
18	1098	970	+128	42	1306	981	+325
19	1064	1209	-145	43	1349	1128	+221
20	1064	1012	+ 52	44	1288	1203	+ 85
21	1049	949	+100	45	1339	1486	-147
22	1061	1087	- 26	46	1356	1505	-149
23	1074	1002	+ 72	Mean	1174	1074	+100
24	1204	1043	+161				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART A. CONTINUED, PRIMATE 545

Restraint Period (13 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	1248	384	+864	66	750	551	+199
48	685	391	+294	67	742	588	+154
49	697	431	+266	68	762	812	- 50
50	163	409	-246	69	762	820	- 58
51	559	928	-369	70	758	620	+138
52	685	961	-276	71	748	615	+133
53	736	963	-227	72	748	509	+239
54	721	1383	-662	73	768	513	+255
55	732	1375	-643	74	727	612	+115
56	734	890	-156	75	733	586	+147
57	402	901	-499	76	739	526	+213
58	719	901	-182	77	749	578	+171
59	727	955	-228	78	752	728	+ 24
Mean	678	836	-159	79	750	772	- 22
				80	748	752	+496
Recovery Period (28 days)				81	750	239	+511
				82	761	374	+387
Day	Intake	Excretion	Balance	83	752	363	+389
				84	764	719	+ 45
60	721	1561	-840	85	749	698	+ 51
61	752	1594	-842	86	750	705	+ 45
62	748	1034	-286	87	798	706	+ 92
63	726	1017	-291	Mean	749	698	+ 51
64	736	739	- 3				
65	731	712	+ 19				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART B. PRIMATE 708

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	1138	818	+320	25	1184	931	+253
2	1160	871	+289	26	1174	942	+232
3	1113	942	+171	27	1201	1031	+170
4	1161	781	+380	28	1193	1006	+187
5	1161	1207	- 46	29	1171	1001	+170
6	1153	1212	- 59	30	1144	970	+174
7	1210	868	+342	31	1172	953	+219
8	1181	917	+264	32	1204	998	+206
9	1019	962	+ 57	33	1283	949	+334
10	1036	927	+109	34	1271	992	+279
11	1045	866	+179	35	1283	1067	+216
12	1031	896	+135	36	1238	1094	+144
13	1042	722	+320	37	1265	1193	+ 72
14	1041	772	+269	38	1289	1193	+ 96
15	1015	954	+ 61	39	1285	1047	+238
16	1050	981	+ 69	40	1305	1059	+246
17	1048	747	+301	41	1334	900	+434
18	1076	732	+344	42	1328	873	+455
19	1066	714	+352	43	1274	1136	+138
20	1038	664	+374	44	1314	1096	+218
21	1075	871	+204	45	1293	988	+305
22	1070	872	+198	46	1244	1065	+179
23	1195	898	+297	Mean	1169	948	+221
24	1190	923	+267				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART B. CONTINUED, PRIMATE 708

Restraint Period (40 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	1330	554	+776	71	561	649	- 88
48	679	570	+109	72	751	345	+406
49	406	562	-156	73	739	346	+393
50	202	545	-343	74	621	721	-100
51	374	509	-135	75	738	497	+241
52	674	541	+133	76	743	680	+ 63
53	744	522	+222	77	765	668	+ 97
54	729	946	-217	78	735	591	+144
55	731	931	-200	79	755	618	+137
56	745	826	- 81	80	751	676	+ 75
57	352	773	-421	81	755	656	+ 99
58	713	782	- 69	82	778	610	+168
59	728	806	- 78	83	771	699	+ 72
60	738	892	-154	84	779	661	+118
61	745	910	-165	85	331	629	-298
62	707	683	+ 24	86	318	649	-331
63	708	696	+ 12	X	X	X	X
64	731	659	+ 72	X	X	X	X
65	727	633	+ 94	X	X	X	X
66	719	605	+114	X	X	X	X
67	713	678	+ 35	X	X	X	X
68	729	575	+154	X	X	X	X
69	726	620	+106	Mean	682	653	+ 29
70	744	607	+137				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART C. PRIMATE 547

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	1149	1791	-642	25	1177	1110	+ 67
2	1157	1688	-531	26	1187	1036	+151
3	1114	751	+363	27	1159	696	+462
4	1127	1016	+111	28	1206	813	+393
5	1140	1299	-159	29	1205	929	+276
6	1105	1195	- 90	30	1135	950	+185
7	1185	1046	+139	31	1175	921	+254
8	1147	1050	+ 97	32	1178	924	+254
9	1016	691	+325	33	1254	922	+332
10	1030	731	+299	34	1261	884	+377
11	1054	876	+178	35	1273	912	+361
12	1053	940	+113	36	1216	926	+290
13	1027	974	+ 53	37	1252	1096	+156
14	1040	991	+ 49	38	1257	1109	+148
15	1042	926	+116	39	1259	1092	+167
16	1054	882	+172	40	1336	1063	+273
17	1068	1072	- 4	41	1288	1009	+279
18	1067	1019	+ 48	42	1307	885	+422
19	1027	1062	- 35	43	1288	1134	+154
20	1065	908	+157	44	1288	1163	+125
21	1050	1019	+ 31	45	1302	1173	+129
22	1081	988	+ 93	46	1336	1195	+141
23	1082	1040	+ 42	Mean	1160	1025	+136
24	1181	1258	- 77				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART C. CONTINUED, PRIMATE 547

Restraint Period (39 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	1278	475	+803	71	530	608	- 78
48	156	424	-268	72	732	688	+ 44
49	268	435	-167	73	717	591	+126
50	138	409	-271	74	759	562	+197
51	273	629	-356	75	735	612	+123
52	569	673	-104	76	741	699	+ 42
53	741	721	+ 20	77	739	639	+100
54	667	864	-197	78	410	500	- 90
55	714	752	- 38	79	758	978	-220
56	491	881	-390	80	751	760	- 9
57	388	971	-583	81	752	743	+ 9
58	516	946	-430	82	763	630	+133
59	714	942	-228	83	765	675	+ 90
60	736	549	+187	84	762	482	+280
61	730	553	+177	85	523	438	+ 85
62	708	976	-268	X	X	X	X
63	718	811	- 93	X	X	X	X
64	716	592	+124	X	X	X	X
65	718	611	+107	X	X	X	X
66	707	679	+ 28	X	X	X	X
67	722	582	+140	X	X	X	X
68	708	1287	-579	X	X	X	X
69	719	910	-191	Mean	648	690	- 42
70	743	615	+128				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART D. PRIMATE 745

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	1155	606	+549	25	1197	804	+393
2	1184	575	+609	26	1174	857	+317
3	1104	730	+374	27	1208	822	+386
4	1166	877	+289	28	1181	749	+432
5	900	1080	-180	29	1210	953	+257
6	1130	1029	+101	30	1144	929	+215
7	1188	789	+399	31	1194	947	+247
8	1186	807	+379	32	1205	936	+269
9	1041	994	+ 47	33	1256	755	+501
10	1030	963	+ 67	34	1270	799	+471
11	1058	814	+244	35	1177	963	+214
12	1049	845	+204	36	1270	920	+350
13	1010	961	+ 49	37	1293	775	+518
14	1016	968	+ 48	38	1288	831	+457
15	1028	747	+281	39	1264	809	+455
16	1033	765	+268	40	1304	876	+428
17	1048	761	+287	41	1312	954	+358
18	1059	784	+275	42	1328	883	+445
19	1074	942	+132	43	1289	1051	+238
20	1074	924	+150	44	1299	1043	+256
21	1070	859	+211	45	1321	1037	+284
22	1075	786	+289	46	1344	1056	+288
23	1083	889	+194	Mean	1162	873	+289
24	1156	935	+221				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART D. CONTINUED, PRIMATE 745

Restraint Period (21 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	1047	316	+731	X	X	X	X
48	609	389	+220	X	X	X	X
49	0	339	-339	X	X	X	X
50	172	344	-172	X	X	X	X
51	479	392	+ 87	X	X	X	X
52	661	251	+410	X	X	X	X
53	432	375	+ 57	X	X	X	X
54	711	689	+ 22	X	X	X	X
55	520	689	-169	X	X	X	X
56	280	798	-518	X	X	X	X
57	443	760	-317	X	X	X	X
58	705	749	- 44	X	X	X	X
59	582	1169	-587	X	X	X	X
60	518	764	-246	X	X	X	X
61	620	713	- 93	X	X	X	X
62	578	737	-159	X	X	X	X
63	358	736	-378	X	X	X	X
64	514	449	+ 65	X	X	X	X
65	718	607	+111	X	X	X	X
66	621	524	+ 97	X	X	X	X
67	0	389	-389	X	X	X	X
Mean	508	585	- 77	X	X	X	X

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART E. PRIMATE 757

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	1148	1324	-176	25	1190	971	+219
2	1154	1397	-243	26	1184	923	+261
3	1049	900	+149	27	1205	892	+313
4	1134	861	+273	28	1175	784	+391
5	1135	1221	- 86	29	1152	958	+194
6	1114	1167	- 53	30	1179	918	+261
7	1110	1124	- 14	31	1163	1028	+135
8	1176	1162	+ 14	32	1160	1049	+111
9	1018	961	+ 57	33	1288	929	+359
10	1042	1040	+ 2	34	1277	933	+344
11	1042	1108	- 66	35	1263	1201	+ 62
12	1050	1166	-116	36	1219	1223	- 4
13	1027	805	+222	37	1279	1077	+202
14	1027	836	+191	38	1282	1203	+ 79
15	1016	920	+ 96	39	1280	1155	+125
16	1053	914	+139	40	1312	1177	+135
17	1086	877	+209	41	1328	1192	+136
18	1076	892	+184	42	1303	1164	+139
19	1069	887	+182	43	1304	1229	+ 75
20	1061	846	+215	44	1342	1113	+229
21	1104	1060	+ 44	45	1323	1084	+239
22	1032	1059	- 27	46	1327	1083	+244
23	1070	1163	- 93	Mean	1163	1044	+119
24	1174	1039	+135				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART E. CONTINUED, PRIMATE 757

Restraint Period (41 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	1291	469	+822	71	533	496	+ 37
48	188	479	-291	72	774	489	+285
49	583	484	+ 99	73	754	497	+257
50	274	447	-173	74	770	603	+167
51	588	750	-162	75	744	432	+312
52	670	783	-113	76	750	855	-105
53	734	817	- 83	77	762	846	- 84
54	749	1006	-257	78	747	612	+135
55	740	933	-193	79	760	620	+140
56	748	586	+162	80	752	709	+ 43
57	307	473	-166	81	761	709	+ 52
58	742	462	+280	82	778	763	+ 15
59	751	488	+263	83	778	900	-112
60	795	1124	-329	84	770	865	- 95
61	752	1100	-348	85	669	850	-181
62	727	764	- 37	86	755	841	- 86
63	718	782	- 64	87	778	844	- 66
64	635	527	+108	X	X	X	X
65	722	1273	-551	X	X	X	X
66	723	651	+ 72	X	X	X	X
67	735	676	+ 59	X	X	X	X
68	695	745	- 50	X	X	X	X
69	741	684	+ 57	Mean	707	706	+ 1
70	745	510	+235				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART F. PRIMATE 758

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	1152	1067	+ 85	25	1213	832	+381
2	1164	1035	+129	26	1197	838	+359
3	1104	1128	- 24	27	1166	942	+224
4	1137	1172	- 35	28	1189	911	+278
5	1149	1143	+ 6	29	1163	1012	+151
6	1098	1088	+ 10	30	1162	1050	+112
7	1120	989	+131	31	1200	937	+263
8	1208	996	+212	32	1221	909	+312
9	1042	1004	+ 38	33	1263	1127	+136
10	1062	1012	+ 50	34	1269	1117	+152
11	1048	1018	+ 30	35	1270	880	+390
12	1078	1030	+ 48	36	1270	934	+336
13	1034	975	+ 59	37	1264	1060	+204
14	1055	941	+114	38	1286	1002	+284
15	1043	956	+ 87	39	1274	1241	+ 33
16	1027	1005	+ 22	40	1288	1210	+ 78
17	1079	880	+199	41	1311	1021	+290
18	1054	957	+ 97	42	1322	877	+445
19	1080	963	+117	43	1334	826	+508
20	1027	947	+ 80	44	1328	870	+458
21	1047	961	+ 86	45	1234	1303	- 69
22	1046	1214	-168	46	1328	1362	- 34
23	1061	956	+105	Mean	1166	1015	+151
24	1194	996	+198				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE ~~STUDY~~

(milligrams)

PART F. CONTINUED, PRIMATE 758

Restraint Period (26 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	1287	798	+489	71	574	677	-103
48	990	908	+ 82	72	769	611	+158
49	687	840	-153	X	X	X	X
50	294	805	-511	X	X	X	X
51	619	673	- 54	X	X	X	X
52	660	655	+ 5	X	X	X	X
53	715	661	+ 54	X	X	X	X
54	719	776	- 57	X	X	X	X
55	711	800	- 89	X	X	X	X
56	764	1148	-384	X	X	X	X
57	322	1146	-824	X	X	X	X
58	720	1094	-374	X	X	X	X
59	736	1136	-400	X	X	X	X
60	718	1130	-412	X	X	X	X
61	746	828	- 82	X	X	X	X
62	742	941	-199	X	X	X	X
63	700	970	-270	X	X	X	X
64	598	736	-138	X	X	X	X
65	738	756	- 18	X	X	X	X
66	730	570	+160	X	X	X	X
67	728	522	+206	X	X	X	X
68	742	760	- 18	X	X	X	X
69	742	827	- 85	Mean	711	827	-116
70	733	726	+ 7				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART G. PRIMATE 695

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	1144	1030	+114	25	1190	1050	+140
2	1180	1249	- 69	26	1188	1013	+175
3	647	1051	-404	27	1180	874	+306
4	1167	1305	-138	28	1188	1059	+129
5	1189	1520	-331	29	1167	518	+649
6	1101	1411	-310	30	1183	610	+573
7	1124	929	+195	31	1164	1020	+144
8	1157	894	+263	32	1205	989	+216
9	1033	789	+244	33	1266	933	+333
10	1056	924	+132	34	1288	949	+339
11	1050	846	+204	35	1277	1267	+ 10
12	1037	792	+245	36	1265	1240	+ 25
13	1041	936	+105	37	1290	1050	+240
14	1027	893	+134	38	1270	1019	+251
15	1021	944	+ 77	39	1278	942	+336
16	1054	974	+ 80	40	1299	999	+300
17	1060	820	+240	41	1280	1249	+ 31
18	1097	846	+251	42	1321	1136	+185
19	1092	1097	- 5	43	1293	827	+466
20	1061	1136	- 75	44	1345	767	+578
21	1061	919	+142	45	1363	1217	+146
22	1070	842	+228	46	1362	1233	+129
23	1077	948	+129	Mean	1161	1001	+160
24	1190	991	+199				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART G. CONTINUED, PRIMATE 695

Restraint Period (25 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	1278	256	+1022	71	568	501	+67
48	275	269	+ 6	X	X	X	X
49	679	400	+279	X	X	X	X
50	943	409	+534	X	X	X	X
51	704	835	-131	X	X	X	X
52	411	831	-420	X	X	X	X
53	734	878	-144	X	X	X	X
54	726	680	+ 46	X	X	X	X
55	722	586	+136	X	X	X	X
56	240	598	-358	X	X	X	X
57	366	580	-214	X	X	X	X
58	730	600	+130	X	X	X	X
59	725	672	+ 53	X	X	X	X
60	741	902	-161	X	X	X	X
61	729	928	-199	X	X	X	X
62	719	721	- 2	X	X	X	X
63	610	754	-144	X	X	X	X
64	601	597	+ 4	X	X	X	X
65	711	599	+112	X	X	X	X
66	719	568	+151	X	X	X	X
67	721	576	+145	X	X	X	X
68	713	965	-252	X	X	X	X
69	720	747	- 27	Mean	673	637	+37
70	752	468	+284				

TABLE I, CONTINUED

DAILY CALCIUM BALANCE THROUGHOUT THE STUDY

(milligrams)

PART H. PRIMATE 787

Restraint Period (33 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
50	205	561	-356	74	499	364	+135
51	571	510	+ 61	75	533	359	+174
52	502	533	- 31	76	576	325	+251
53	545	520	+ 25	77	456	322	+134
54	586	717	-131	78	191	347	-156
55	564	744	-180	79	517	363	+154
56	487	430	+ 57	80	603	731	-128
57	306	569	-263	81	457	753	-296
58	676	569	+107	82	579	964	-385
59	429	564	-135	X	X	X	X
60	724	663	+ 61	X	X	X	X
61	660	944	-284	X	X	X	X
62	696	915	-219	X	X	X	X
63	561	895	-334	X	X	X	X
64	490	664	-174	X	X	X	X
65	565	565	0	X	X	X	X
66	656	329	+327	X	X	X	X
67	494	326	+168	X	X	X	X
68	574	505	+ 69	X	X	X	X
69	504	480	+ 24	X	X	X	X
70	553	318	+235	X	X	X	X
71	425	313	+112	X	X	X	X
72	711	1129	-418	Mean	529	589	- 60
73	569	1137	-568				

TABLE II

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART A. PRIMATE 545

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	736	918	-182	25	764	651	+113
2	723	921	-198	26	753	653	+100
3	695	448	+247	27	741	725	+ 16
4	724	447	+277	28	746	727	+ 19
5	729	787	- 58	29	765	940	-175
6	708	758	- 50	30	733	945	-212
7	771	583	+188	31	738	758	- 20
8	753	583	+170	32	725	768	- 43
9	718	663	+ 55	33	825	614	+211
10	730	674	+ 56	34	842	613	+229
11	730	527	+203	35	833	811	+ 22
12	714	530	+184	36	835	802	+ 33
13	711	649	+ 62	37	829	767	+ 62
14	702	651	+ 51	38	845	768	+ 77
15	710	581	+129	39	850	851	- 1
16	722	580	+142	40	799	850	- 51
17	728	651	+ 77	41	812	637	+175
18	759	654	+105	42	795	637	+158
19	736	527	+209	43	821	702	+119
20	736	505	+231	44	784	700	+ 84
21	726	663	+ 63	45	815	919	-104
22	734	667	+ 67	46	825	918	- 93
23	743	758	- 15	Mean	760	701	+ 59
24	753	751	+ 2				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART A. CONTINUED, PRIMATE 545

Restraint Period (13 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	700	276	+433	66	628	426	+202
48	384	270	+114	67	622	433	+189
49	391	311	+ 80	68	638	677	- 39
50	91	296	-205	69	638	679	- 41
51	464	617	-153	70	635	439	+196
52	568	550	+ 18	71	627	442	+185
53	610	543	+ 67	72	626	409	+217
54	598	855	-257	73	644	304	+340
55	607	830	-223	74	609	500	+109
56	609	625	- 16	75	614	496	+118
57	334	557	-223	76	619	448	+171
58	596	554	+ 42	77	628	469	+159
59	603	543	+ 60	78	630	615	+ 15
Mean	504	524	- 20	79	629	626	+ 3
				80	627	339	+288
Recovery Period (28 days)				81	629	332	+297
				82	638	263	+375
Day	Intake	Excretion	Balance	83	630	268	+362
				84	640	582	+ 58
60	598	937	-339	85	628	578	+ 50
61	624	932	-308	86	629	581	+ 48
62	627	637	- 10	87	668	584	+ 84
63	608	642	- 34				
64	617	1128	-511	Mean	627	567	+ 60
65	612	1116	-504				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART B. PRIMATE 708

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	721	707	+14	25	740	640	+100
2	735	667	+68	26	735	644	+ 91
3	705	525	+180	27	751	677	+ 74
4	736	546	+190	28	747	674	+ 73
5	736	705	+ 31	29	733	626	+107
6	731	707	+ 24	30	716	611	+105
7	767	708	+ 59	31	733	629	+104
8	749	710	+ 39	32	753	640	+113
9	701	558	+143	33	838	680	+158
10	713	574	+139	34	830	668	+162
11	719	904	-185	35	838	690	+148
12	710	901	-191	36	808	709	+ 99
13	717	508	+209	37	826	796	+ 30
14	716	500	+216	38	842	816	+ 26
15	698	656	+ 42	39	839	817	+ 22
16	723	652	+ 71	40	795	802	- 7
17	725	459	+266	41	812	588	+224
18	744	438	+306	42	809	578	+231
19	740	758	- 18	43	776	760	+ 16
20	718	744	- 26	44	800	753	+ 47
21	744	589	+155	45	787	662	+125
22	740	589	+151	46	818	660	+158
23	747	642	+105	Mean	757	664	+ 93
24	745	671	+ 74				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART B. CONTINUED, PRIMATE 708

Restraint Period (40 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	746	335	+411	71	470	512	- 42
48	381	356	+ 25	72	630	519	+111
49	227	432	-205	73	620	462	+158
50	113	363	-250	74	520	464	+ 56
51	310	352	- 42	75	618	375	+243
52	559	334	+225	76	623	590	+ 33
53	617	337	+280	77	641	594	+ 47
54	605	622	- 17	78	616	558	+ 58
55	606	638	- 32	79	632	561	+ 71
56	618	503	+115	80	630	601	+ 29
57	292	502	-210	81	632	571	+ 61
58	592	486	+106	82	652	623	+ 29
59	604	501	+103	83	646	624	+ 22
60	612	587	+ 25	84	653	529	+124
61	618	582	+ 36	85	384	576	-192
62	592	519	+ 73	86	628	555	+ 73
63	593	550	+ 43	X	X	X	X
64	612	570	+ 42	X	X	X	X
65	609	510	+ 99	X	X	X	X
66	602	485	+117	X	X	X	X
67	597	523	+ 74	X	X	X	X
68	610	494	+116	X	X	X	X
69	608	538	+ 70	Mean	564	508	+ 55
70	624	508	+116				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART C. PRIMATE 547

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	728	1036	-308	25	736	770	- 34
2	733	1032	-299	26	743	762	- 19
3	706	474	+232	27	725	469	+256
4	714	469	+245	28	755	470	+285
5	722	857	-135	29	754	687	+ 67
6	751	856	-105	30	710	672	+ 38
7	751	633	+118	31	735	688	+ 47
8	727	631	+ 96	32	737	688	+ 49
9	699	444	+255	33	819	624	+195
10	709	444	+265	34	824	629	+195
11	725	942	-217	35	831	569	+262
12	724	955	-231	36	794	555	+239
13	707	579	+128	37	818	826	- 8
14	716	583	+133	38	833	810	+ 23
15	717	693	+ 24	39	822	643	+179
16	725	694	+ 31	40	813	644	+169
17	739	668	+ 71	41	784	676	+108
18	738	668	+ 70	42	796	690	+106
19	711	735	- 24	43	784	838	- 54
20	737	730	+ 7	44	784	340	+444
21	726	656	+ 70	45	792	787	+ 5
22	748	659	+ 89	46	813	788	+ 25
23	748	728	+ 20	Mean	753	683	+ 68
24	739	729	+ 10				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART C. CONTINUED, PRIMATE 547

Restraint Period (39 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	717	269	+448	71	444	452	- 8
48	88	228	-140	72	613	549	+ 64
49	150	346	-196	73	601	544	+ 57
50	77	246	-169	74	636	456	+180
51	226	401	-175	75	616	453	+163
52	472	298	+174	76	621	600	+ 21
53	615	310	+305	77	620	601	+ 19
54	553	529	+ 24	78	344	476	-132
55	593	515	+ 78	79	635	500	+135
56	407	595	-188	80	630	584	+ 46
57	322	554	-232	81	630	599	+ 31
58	428	552	-124	82	640	492	+148
59	593	554	+ 39	83	641	511	+130
60	611	322	+289	84	638	381	+257
61	606	323	+283	85	439	397	+ 42
62	491	662	-171	X	X	X	X
63	602	654	- 52	X	X	X	X
64	600	502	+ 98	X	X	X	X
65	602	512	+ 90	X	X	X	X
66	592	489	+103	X	X	X	X
67	605	485	+120	X	X	X	X
68	593	799	-206	X	X	X	X
69	603	731	-128	Mean	526	485	+ 41
70	623	436	+187				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART D. PRIMATE 745

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	732	376	+356	25	749	532	+217
2	751	390	+361	26	735	542	+193
3	700	840	-140	27	756	500	+256
4	739	752	- 13	28	739	491	+248
5	570	710	-140	29	757	690	+ 67
6	716	707	+ 9	30	715	676	+ 39
7	753	535	+218	31	747	654	+ 93
8	752	570	+182	32	754	634	+120
9	716	498	+218	33	820	499	+321
10	709	542	+167	34	829	535	+294
11	728	547	+181	35	834	730	+104
12	723	538	+185	36	829	742	+ 87
13	695	595	+100	37	844	586	+258
14	699	604	+ 95	38	841	567	+274
15	708	575	+133	39	825	610	+215
16	711	588	+123	40	794	634	+160
17	725	489	+236	41	799	570	+229
18	733	493	+240	42	808	539	+269
19	743	628	+115	43	785	655	+130
20	743	572	+171	44	791	660	+131
21	740	559	+181	45	804	643	+161
22	743	536	+207	46	818	656	+162
23	749	618	+131	Mean	754	592	+162
24	723	635	+ 88				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART D. CONTINUED, PRIMATE 745

Restraint Period (21 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	587	289	+298	X	X	X	X
48	342	235	+107	X	X	X	X
49	0	238	-238	X	X	X	X
50	97	307	-210	X	X	X	X
51	397	253	+144	X	X	X	X
52	548	148	+400	X	X	X	X
53	358	156	+202	X	X	X	X
54	590	434	+156	X	X	X	X
55	432	464	- 32	X	X	X	X
56	232	487	-255	X	X	X	X
57	367	521	-154	X	X	X	X
58	585	455	+130	X	X	X	X
59	483	481	+ 2	X	X	X	X
60	430	461	- 31	X	X	X	X
61	515	451	+ 64	X	X	X	X
62	484	677	-193	X	X	X	X
63	300	702	-402	X	X	X	X
64	431	410	+ 21	X	X	X	X
65	601	473	+128	X	X	X	X
66	520	424	+ 96	Mean	395	400	- 5
67	0	335	-335				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART E. PRIMATE 757

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	728	833	-105	25	745	634	+111
2	731	822	- 91	26	740	619	+121
3	665	469	+196	27	754	602	+152
4	719	460	+259	28	735	601	+134
5	720	957	-237	29	721	590	+131
6	706	939	-233	30	737	566	+171
7	704	663	+ 41	31	728	691	+ 37
8	745	661	+ 84	32	726	704	+ 22
9	700	637	+ 63	33	842	635	+207
10	717	639	+ 78	34	834	636	+198
11	717	720	- 3	35	823	766	+ 57
12	722	750	- 28	36	796	773	+ 23
13	707	525	+182	37	837	718	+119
14	707	526	+181	38	838	718	+120
15	699	570	+129	39	836	609	+227
16	724	574	+150	40	799	607	+192
17	751	585	+166	41	808	736	+ 72
18	745	615	+130	42	793	736	+ 57
19	740	553	+187	43	790	682	+108
20	734	549	+185	44	817	680	+137
21	764	669	+ 95	45	805	599	+206
22	714	665	+ 49	46	808	597	+211
23	740	750	- 10	Mean	753	660	+ 93
24	735	724	+ 11				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART E. CONTINUED, PRIMATE 757

Restraint Period (41 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	724	330	+394	71	446	445	+ 1
48	106	299	-193	72	649	385	+264
49	327	292	+ 35	73	632	390	+242
50	154	327	-173	74	645	248	+397
51	488	470	+ 18	75	624	232	+392
52	555	470	+ 85	76	628	725	- 97
53	609	470	+139	77	638	724	- 86
54	621	527	+ 94	78	627	510	+117
55	614	527	+ 87	79	637	513	+124
56	621	490	+131	80	630	562	+ 68
57	254	512	-258	81	638	566	+ 72
58	615	512	+103	82	638	623	+ 15
59	622	491	+131	83	652	634	+ 18
60	659	630	+ 29	84	645	719	- 74
61	624	636	- 12	85	560	712	-152
62	609	470	+139	86	633	718	- 85
63	602	517	+ 85	87	652	716	- 64
64	532	363	+169	X	X	X	X
65	605	367	+238	X	X	X	X
66	606	492	+114	X	X	X	X
67	616	504	+112	X	X	X	X
68	582	549	+ 33	X	X	X	X
69	621	565	+ 56	Mean	575	504	+ 71
70	624	429	+195				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART F. PRIMATE 758

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	730	630	+100	25	759	535	+224
2	738	629	+109	26	749	535	+214
3	700	730	- 30	27	730	630	+100
4	721	720	+ 1	28	744	632	+112
5	728	738	- 10	29	727	698	+ 29
6	696	737	- 41	30	727	699	+ 28
7	710	625	+ 85	31	751	640	+111
8	766	626	+140	32	764	642	+122
9	717	592	+125	33	825	724	+101
10	731	591	+140	34	829	725	+104
11	721	655	+ 66	35	829	646	+183
12	742	654	+ 88	36	829	648	+181
13	712	589	+123	37	825	622	+203
14	726	592	+134	38	840	622	+218
15	718	613	+105	39	832	850	- 18
16	706	616	+ 90	40	784	849	- 65
17	746	612	+134	41	798	582	+216
18	729	612	+117	42	805	582	+223
19	747	600	+147	43	812	539	+273
20	711	598	+113	44	808	540	+268
21	724	705	+ 19	45	780	915	-135
22	723	705	+ 18	46	809	916	-107
23	734	716	+ 18	Mean	756	660	+ 96
24	747	714	+ 33				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART F. CONTINUED, PRIMATE 758

Restraint Period (26 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	722	570	+152	71	481	615	-134
48	555	561	- 6	72	644	619	+ 25
49	386	537	-151	X	X	X	X
50	165	627	-462	X	X	X	X
51	514	422	+ 92	X	X	X	X
52	547	388	+159	X	X	X	X
53	593	378	+215	X	X	X	X
54	596	523	+ 73	X	X	X	X
55	590	513	+ 77	X	X	X	X
56	633	702	- 69	X	X	X	X
57	267	788	-521	X	X	X	X
58	597	765	-168	X	X	X	X
59	611	740	-129	X	X	X	X
60	595	512	+ 83	X	X	X	X
61	619	430	+189	X	X	X	X
62	621	604	+ 17	X	X	X	X
63	587	623	- 36	X	X	X	X
64	501	579	- 78	X	X	X	X
65	618	543	+ 75	X	X	X	X
66	611	548	+ 63	X	X	X	X
67	610	542	+ 68	X	X	X	X
68	621	753	-132	X	X	X	X
69	621	621	0	Mean	558	581	- 22
70	614	599	+ 15				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART G. PRIMATE 695

Ambulatory Period (46 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
1	726	521	+205	25	745	817	- 72
2	748	522	+226	26	743	806	- 63
3	410	667	-257	27	738	684	+ 54
4	740	678	+ 62	28	743	679	+ 64
5	754	840	- 86	29	730	425	+305
6	698	834	-136	30	740	423	+317
7	712	589	+123	31	728	730	- 2
8	733	547	+186	32	754	731	+ 23
9	711	487	+224	33	827	694	+133
10	721	500	+221	34	841	695	+146
11	723	463	+260	35	834	406	+428
12	714	458	+256	36	826	420	+406
13	716	586	+130	37	842	730	+112
14	706	591	+115	38	829	731	+ 98
15	702	721	- 19	39	840	698	+142
16	725	729	- 4	40	791	698	+ 93
17	733	661	+ 72	41	779	845	- 66
18	759	666	+ 93	42	804	845	- 41
19	756	716	+ 40	43	787	544	-243
20	734	715	+ 19	44	819	543	+276
21	734	903	-169	45	830	848	- 18
22	740	900	-160	46	829	846	- 17
23	745	714	+ 31	Mean	752	663	+ 88
24	745	673	+ 72				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART G. CONTINUED, PRIMATE 695

Restraint Period (25 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
47	717	255	+462	71	476	387	+ 89
48	154	242	- 88	X	X	X	X
49	381	232	+149	X	X	X	X
50	529	226	+303	X	X	X	X
51	584	467	+117	X	X	X	X
52	341	459	-118	X	X	X	X
53	609	469	+140	X	X	X	X
54	602	366	+236	X	X	X	X
55	598	360	+238	X	X	X	X
56	199	473	-274	X	X	X	X
57	303	474	-171	X	X	X	X
58	605	478	+127	X	X	X	X
59	601	403	+198	X	X	X	X
60	615	483	+132	X	X	X	X
61	604	486	+118	X	X	X	X
62	603	536	+ 67	X	X	X	X
63	511	540	- 29	X	X	X	X
64	504	488	+ 16	X	X	X	X
65	596	473	+123	X	X	X	X
66	602	443	+159	X	X	X	X
67	604	445	+159	X	X	X	X
68	597	588	+ 9	X	X	X	X
69	603	572	+ 31	Mean	527	429	+ 97
70	630	388	+242				

TABLE II, CONTINUED

DAILY PHOSPHORUS BALANCE THROUGHOUT THE STUDY

(milligrams)

PART H. PRIMATE 787

Restraint Period (33 days)							
Day	Intake	Excretion	Balance	Day	Intake	Excretion	Balance
50	115	362	-247	74	418	350	+ 68
51	478	460	+ 18	75	447	345	+102
52	416	311	+105	76	483	287	+196
53	452	324	+128	77	219	285	- 66
54	486	433	+ 53	78	473	333	+140
55	467	490	- 23	79	433	335	+ 98
56	404	378	+ 26	80	505	574	- 69
57	254	431	-177	81	220	579	-359
58	561	403	+158	82	278	757	-479
59	356	405	- 49	X	X	X	X
60	600	441	+159	X	X	X	X
61	547	726	-179	X	X	X	X
62	583	621	- 38	X	X	X	X
63	470	618	-148	X	X	X	X
64	411	459	- 48	X	X	X	X
65	474	452	+ 22	X	X	X	X
66	550	299	+251	X	X	X	X
67	414	311	+103	X	X	X	X
68	481	417	+ 64	X	X	X	X
69	422	408	+ 14	X	X	X	X
70	463	367	+ 96	X	X	X	X
71	356	378	- 22	X	X	X	X
72	595	895	-300	Mean	434	459	- 25
73	477	909	-432				

TABLE III

SPECIFIC ACTIVITY OF Ca^{45} OF ASHED BONE IN THE ULNA

(counts per minute per gram of ash)

Primate Number	Days after Injection	Proximal End	Shaft 1	Shaft 2	Shaft 3	Distal End
775	2	69,866	29,078	31,505	42,219	154,281
745	9	50,045	50,685	50,414	36,750	160,554
695	13	95,264	32,328	22,404	49,391	185,294
758	14	48,919	31,893	16,774	33,603	120,635
787	24	244,129	127,197	126,165	197,111	486,607
547	28	43,932	20,920	27,221	34,045	136,830
708	29	106,161	66,928	87,484	151,520	408,824
545	30	32,718	21,492	27,783	40,528	127,657
757	30	133,645	61,424	63,152	120,014	364,858
Mean		91,631	49,105	50,322	78,353	248,393
Ratio*		1.8	1.0	1	1.6	4.7

*Ratio = Mean specific activity of each segment to the mean specific activity of Shaft 2.

TABLE IV

SPECIFIC ACTIVITY OF Ca^{45} OF ASHED BONE IN THE RADIUS

(counts per minute per gram of ash)

Primate Number	Days after Injection	Proximal End	Shaft 1	Shaft 2	Shaft 3	Distal End
775	2	118,557	40,630	31,404	38,064	166,558
745	9	124,704	52,668	33,446	36,166	111,443
695	13	107,518	60,995	28,789	70,030	91,792
758	14	110,482	58,709	23,180	22,737	81,582
787	24	457,848	261,265	123,073	150,456	468,678
547	28	114,939	41,870	23,202	37,438	154,833
708	29	251,836	109,105	79,118	101,235	296,947
545	30	105,766	31,644	23,400	24,516	121,914
757	30	318,365	146,414	60,501	79,943	281,818
Mean		190,001	89,256	47,346	62,287	197,285
Ratio*		4.0	1.9	1	1.3	4.2

*Ratio = Mean specific activity of each segment to the mean specific activity of Shaft 2.

TABLE V

SPECIFIC ACTIVITY OF Ca^{45} OF ASHED BONE IN THE TIBIA

(counts per minute per gram of ash)

Primate Number	Days after Injection	Proximal End	Shaft 1	Shaft 2	Shaft 3	Distal End
775	2	289,651	62,482	22,971	32,296	147,626
745	9	157,034	39,829	19,105	27,587	105,143
695	13	108,209	26,959	17,912	24,873	73,758
758	14	82,622	27,662	15,017	11,834	38,946
787	24	550,761	181,241	67,606	78,742	253,003
547	28	82,904	21,253	12,543	11,983	53,299
708	29	275,983	82,005	27,078	43,896	224,639
545	30	93,235	31,416	12,797	19,424	79,547
757	30	281,042	86,423	25,232	27,212	142,725
Mean		213,493	62,141	24,473	30,872	124,298
Ratio*		8.7	2.5	1	1.3	5.1

*Ratio = Mean specific activity of each segment to the mean specific activity of Shaft 2.

TABLE VI

SPECIFIC ACTIVITY OF Ca^{45} OF ASHED BONE

IN THE FIBULA

(counts per minute per gram of ash)

Primate Number	Days after Injection	Proximal End	Shaft 1	Shaft 2	Shaft 3	Distal End
775	2	519,753	97,000	37,380	43,328	174,534
745	9	345,786	60,948	31,694	35,242	196,875
695	13	176,122	36,006	19,778	27,128	104,191
758	14	178,438	33,100	19,975	16,437	43,080
787	24	516,038	190,683	71,888	91,383	309,502
547	28	151,956	22,474	16,100	17,157	59,677
708	29	490,594	70,064	47,684	66,271	193,261
545	30	175,799	61,560	36,869	23,971	98,840
757	30	390,000	58,005	26,616	34,023	157,749
Mean		327,165	69,982	34,220	39,438	154,189
Ratio*		9.6	2.0	1	1.2	4.5

*Ratio = Mean specific activity of each segment to the mean specific activity of Shaft 2.

TABLE VII

STATISTICAL COMPARISON BETWEEN AMBULATORY AND RESTRAINT PERIODS OF THE INTAKE OF CALCIUM BY SEVEN PRIMATES

(mg./24 hours)

Populations Compared	Means	Standard Deviation	t Value	Probability
Primate 695 Ambulatory Restraint	1161 673	126 201	12.1355	$P < 0.001$
Primate 708 Ambulatory Restraint	1169 682	96 181	15.4593	$P < 0.001$
Primate 757 Ambulatory Restraint	1163 707	102 164	15.4274	$P < 0.001$
Primate 745 Ambulatory Restraint	1162 508	106 239	15.0735	$P < 0.001$
Primate 758 Ambulatory Restraint	1166 711	98 174	13.7521	$P < 0.001$
Primate 547 Ambulatory Restraint	1160 648	95 202	14.9841	$P < 0.001$
Primate 545 Ambulatory Restraint	1174 678	104 231	10.6381	$P < 0.001$
All 7 Primates Combined Ambulatory Restraint	1165 658	5 64	17.8083	$P < 0.001$

TABLE VIII

STATISTICAL COMPARISON BETWEEN AMBULATORY AND RESTRAINT PERIODS OF THE CALCIUM EXCRETION OF SEVEN PRIMATES

(mg./24 hours)

Populations Compared	Means	Standard Deviation	t Value	Probability
Primate 695				
Ambulatory	1001	191	7.4823	$P < 0.001$
Restraint	637	189		
Primate 708				
Ambulatory	948	132	10.0720	$P < 0.001$
Restraint	653	133		
Primate 757				
Ambulatory	1044	145	8.7798	$P < 0.001$
Restraint	706	204		
Primate 745				
Ambulatory	873	113	6.9058	$P < 0.001$
Restraint	580	223		
Primate 758				
Ambulatory	1015	120	5.1414	$P < 0.001$
Restraint	827	180		
Primate 547				
Ambulatory	1025	204	7.6326	$P < 0.001$
Restraint	690	189		
Primate 545				
Ambulatory	1074	220	2.9317	$P < 0.01$
Restraint	836	329		
All 7 Primates Combined				
Ambulatory	997	62	6.1605	$P < 0.001$
Restraint	705	88		

TABLE IX

STATISTICAL COMPARISON BETWEEN AMBULATORY AND RESTRAINT PERIODS OF THE CALCIUM BALANCE OF SEVEN PRIMATES

(mg./24 hours)

Populations Compared	Means	Standard Deviation	t Value	Probability
Primate 695				
Ambulatory	160	207	2.3735	$P < 0.02$
Restraint	37	291		
Primate 708				
Ambulatory	221	112	5.3673	$P < 0.001$
Restraint	29	221		
Primate 757				
Ambulatory	119	141	2.8496	$P < 0.01$
Restraint	1	230		
Primate 745				
Ambulatory	288	151	6.3382	$P < 0.001$
Restraint	77	304		
Primate 758				
Ambulatory	151	149	5.3960	$P < 0.001$
Restraint	116	259		
Primate 547				
Ambulatory	136	207	3.4674	$P < 0.001$
Restraint	42	253		
Primate 545				
Ambulatory	100	216	2.9372	$P < 0.01$
Restraint	159	403		
All 7 Primates Combined				
Ambulatory	167	60	5.3013	$P < 0.001$
Restraint	47	69		

TABLE X

STATISTICAL COMPARISON BETWEEN AMBULATORY AND RESTRAINT PERIODS OF THE INTAKE OF PHOSPHORUS BY SEVEN PRIMATES

(mg./24 hours)

Populations Compared	Means	Standard Deviation	t Value	Probability
Primate 695				
Ambulatory	752	67	8.9086	$P < 0.001$
Restraint	527	139		
Primate 708				
Ambulatory	757	43	9.3676	$P < 0.001$
Restraint	564	129		
Primate 757				
Ambulatory	753	46	8.4729	$P < 0.001$
Restraint	575	131		
Primate 745				
Ambulatory	754	51	12.1328	$P < 0.001$
Restraint	395	178		
Primate 758				
Ambulatory	756	42	9.9669	$P < 0.001$
Restraint	558	117		
Primate 547				
Ambulatory	753	39	9.1455	$P < 0.001$
Restraint	526	159		
Primate 545				
Ambulatory	760	45	9.1341	$P < 0.001$
Restraint	504	159		
All 7 Primates Combined				
Ambulatory	756	4	7.3845	$P < 0.001$
Restraint	539	67		

TABLE XI

STATISTICAL COMPARISON BETWEEN AMBULATORY AND RESTRAINT PERIODS OF THE PHOSPHORUS EXCRETION OF SEVEN PRIMATES

(mg./24 hours)

Populations Compared	Means	Standard Deviation	t Value	Probability
Primate 695				
Ambulatory	663	137	7.3014	$P < 0.001$
Restraint	429	100		
Primate 708				
Ambulatory	664	101	7.4072	$P < 0.001$
Restraint	508	87		
Primate 757				
Ambulatory	660	103	6.0030	$P < 0.001$
Restraint	504	133		
Primate 745				
Ambulatory	592	119	5.2299	$P < 0.001$
Restraint	400	145		
Primate 758				
Ambulatory	660	89	3.2750	$P < 0.01$
Restraint	581	108		
Primate 547				
Ambulatory	685	150	6.4000	$P < 0.001$
Restraint	485	129		
Primate 545				
Ambulatory	701	129	3.7473	$P < 0.001$
Restraint	524	186		
All 7 Primates Combined				
Ambulatory	661	32	5.4104	$P < 0.001$
Restraint	496	61		

TABLE XII

STATISTICAL COMPARISON BETWEEN AMBULATORY AND RESTRAINT PERIODS OF THE PHOSPHORUS BALANCE OF SEVEN PRIMATES

(mg./24 hours)

Populations Compared	Means	Standard Deviation	t Value	Probability
Primate 695				
Ambulatory	88	154	0.4988	N.S.
Restraint	97	153		
Primate 708				
Ambulatory	93	96	1.5515	N.S.
Restraint	55	124		
Primate 757				
Ambulatory	93	107	0.8013	N.S.
Restraint	71	147		
Primate 745				
Ambulatory	162	104	4.2298	$P < 0.001$
Restraint	5	206		
Primate 758				
Ambulatory	96	93	3.6888	$P < 0.001$
Restraint	22	171		
Primate 547				
Ambulatory	68	151	0.6042	N.S.
Restraint	41	163		
Primate 545				
Ambulatory	59	121	1.7718	$P < 0.10$
Restraint	20	185		
All 7 Primates Combined				
Ambulatory	94	31	2.3638	$P < 0.05$
Restraint	42	39		

TABLE XIII

STATISTICAL COMPARISON BETWEEN GROUPS OF PRIMATES

(mg./24 hours)

Populations Compared	Means	Standard Deviation	t Value	Probability
Calcium Intake				
Primates 695, 787, 708, 757	652	181	0.5249	N.S.
Primates 745, 758, 547, 545	638	220		
Calcium Excretion				
Primates 695, 787, 708, 757	650	197	2.4927	$P < 0.02$
Primates 745, 758, 547, 545	722	238		
Calcium Balance				
Primates 695, 787, 708, 757	-6.3	239.2	2.2258	$P < 0.05$
Primates 745, 758, 547, 545	-83.9	292.4		
Phosphorus Intake				
Primates 695, 787, 708, 757	529	139	1.2803	N.S.
Primates 745, 758, 547, 545	504	165		
Phosphorus Excretion				
Primates 695, 787, 708, 757	481	128	0.8864	N.S.
Primates 745, 758, 547, 545	497	150		
Phosphorus Balance				
Primates 695, 787, 708, 757	54.4	153.8	2.1787	$P < 0.05$
Primates 745, 758, 547, 545	6.7	180.1		