UCRL-1464
Unclassified-Biology Distribution

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

UNCLASSIFIED

THE EFFECT OF AGE AND LOW PHOSPHORUS RICKETS ON CALCIFICATION AND THE DEPOSITION OF CERTAIN RADIOACTIVE METALS IN BONE

D. H. Copp, J. G. Hamilton, D. C. Jones, D. M. Thomson and C. Cramer

September, 1951

Berkeley, California

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

THE EFFECT OF AGE AND LOW PHOSPHORUS RICKETS ON CALCIFICATION AND THE DEPOSITION OF CERTAIN RADIOACTIVE METALS IN BONE.*

D.H. Copp,** J.G. Hamilton, D.C. Jones, D.M. Thomson, and C. Cramer

From the Department of Physiology, Faculty of Medicine, University of British Columbia, Vancouver, B.C. and the Divisions of Physiology, Medical Physics, Experimental Medicine and Radiology, and Crocker Laboratory, Radiation Laboratory, University of California; Berkeley and San Francisco, California.

Radioactive isotopes have made possible new techniques for study of metabolic turnover in the skeleton. In addition, many of the radioactive elements involved in nuclear fission are deposited in the skeleton, where the chronic effects of their radiations may produce bone atrophy or bone tumors. The hazard to health, quite similar to that from chronic radium poisoning, has been emphasized by the growing importance of atomic fission from an industrial and military point of view. It was this problem that first led us to investigate the deposition of metals in bone. (1)

In metabolic behavior, most of these metals appear to fall into one of the following groups (2):

(a) Alkaline earths, such as calcium, strontium, barium and radium, which are localized almost exclusively in the skeleton, and which are generally affected by the same conditions which affect calcium metabolism.

(b) Metals such as yttrium and plutonium which show some distribution in soft tissues, but which are chiefly localized in bone.

---

*This paper is based on work carried out under Contract No. W-7405-eng-48A with the Atomic Energy Commission.

**Presented paper before the Third Conference on Metabolic Interrelations, sponsored by the Josiah Macy, Jr., Foundation, New York City, January 9, 1951.

(c) Metals such as cerium, lanthanum, americium and curium, which are initially taken up by the liver in large amounts, as well as by bone. These metals disappear from the liver within a few months, but remain quite firmly fixed in the skeleton.

I. EFFECT OF AGE AND LOW PHOSPHORUS RICKETS ON THE RETENTION OF CERTAIN RADIOACTIVE METALS IN BONE. (Ca^{45}, $Sr^{89,90}$, Ba^{140}, Y^{88}, and Ce^{144})

The retention of these metals in bone was compared in the following groups of animals:

(a) Mature adult rats in which bone growth had practically ceased.

(b) Young growing rats, in which there was active growth of new bone.

(c) Young rats with low phosphorus rickets, in which new osteoid matrix was forming, but deposition of bone salt did not take place.

All the animals used were female rats of the Long-Evans strain. The adult rats were 6-12 months old, and showed no appreciable weight gain. The young normal animals were weaned at 21 days to the stock diet, and were 7-10 weeks old when used for these experiments. The rachitic rats had been weaned at 21 days and reared on a synthetic diet (3) very low in phosphorus (0.008-0.015% P), although adequate in calcium (0.43-0.46% Ca). The animals were 7-10 weeks of age when used and were x-rayed to determine if florid rickets was present.

The following radioactive isotopes were used:

Ca^{45} - 5 microcuries of Ca^{45} with 1.25 mg. Ca as calcium chloride, injected intravenously. (In the later experiments Ca^{45} with a higher specific activity - 0.01 mg per microcurie - was used.)

$Sr^{89,90}$ - 5 microcuries of carrier-free radiostrontium (produced by nuclear fission) injected intraperitoneally.

Ba^{140} - 5 microcuries of carrier-free radiobarium (produced by fission), injected intraperitoneally.

Y^{88} - 5 microcuries of carrier-free radioyttrium (produced in the cyclotron) as chloride in isotonic saline at pH 4, injected intramuscularly.

Ce^{144} - 5 microcuries of carrier-free radiocerium (produced by fission), as chloride in isotonic saline at pH 4, injected intramuscularly.

Following injection of the respective radioactive isotope, the animals were placed in individual metabolism cages, and urine and feces were collected separately. They were sacrificed 8 days later, and tissues and excreta were analyzed for radioisotope. The results obtained are given in Table 1. The values given for Ca^{45}, $Sr^{89,90}$, and Ba^{140} are expressed as per cent of the administered dose; for Y^{88} and Ce^{144}, they represent the per cent of the isotope adsorbed from the site of intramuscular injection. Each figure represents the average of values from 5-8 animals.

OBSERVATIONS

Ca^{45} A significant part of the dose of radiocalcium was retained in the skeleton of the adult animals. Since very little new bone salt is being formed in these animals, this is probably accounted for by ion exchange with calcium already present in the bone. In young growing animals, the retention is much greater, and may represent radiocalcium actually incorporated in the newly formed bone salt. A similar effect of age on the retention of P^{32} (as phosphate) in bone has been observed by Falkenheim in mice (4) and by Weissberger and Harris in rats (5). There was very little radiocalcium remaining in the rachitic animals when sacrificed, and this may have been due to inability to form new bone salt. The radiocalcium was lost primarily in urine, as had been observed by Weissberger and Harris (6) when radiostrontium was given to rachitic rats.

TABLE I

EFFECT OF AGE AND LOW PHOSPHORUS RICKETS ON THE BONE UPTAKE OF Ca, Sr, Ba, Y & Ce IN THE RAT.

	Per Cent of the Absorbed Dose in			
Ca^{45} (1.25 mg. Ca)	Skeleton	Urine	Feces	Liver
Normal-Adult	31.7	4.7	43.2	
Normal-Young	73.4	12.7	7.2	
Rachitic-Low P.	13.4	74.5	1.7	
$Sr^{89,90}$ (Carrier-free)				
Normal-Adult	29.1	42.7	26.9	
Normal-Young	71.9	16.4	12.8	
Rachitic-Low P.	20.4	69.4	7.5	
Ba^{140} (Carrier-free)				
Normal-Adult	37.3	23.2	31.8	
Normal-Young	61.8	12.4	25.3	
Rachitic-Low P.	33.9	52.4	11.4	
Y^{88} (Carrier-free)				
Normal-Adult	70.5	18.6	5.2	5.7
Normal-Young	76.8	15.1	4.6	3.5
Rachitic-Low P.	74.1	8.4	6.7	6.2
Ce^{144} (Carrier-free)				
Normal-Adult	42.6	8.2	5.9	43.5
Normal-Young	61.5	5.7	12.0	21.8
Rachitic-Low P.	72.4	4.3	5.0	18.3

$Sr^{89,90}$ The retention of radiostrontium in bone was very similar to that of Ca^{45} in these three groups of animals. However, the adult animals excreted almost ten times as much of the $Sr^{89,90}$ in urine and this was associated with a smaller proportion in the feces.

Ba^{140} Although qualitatively similar to radiocalcium and radiostrontium, there was a greater relative retention of radiobarium in adult and rachitic animals. This may be due to the decreased solubility and shift in ion exchange.

Y^{88} Neither age nor rickets appeared to have any significant effect on the retention of radioyttrium in bone, suggesting that its metabolism must be quite different from that of the alkaline earths. This was confirmed by the difference in the distribution in radioautographs described below.

Ce^{144} Age and rickets also appear to have very little effect on the bone fixation of radiocerium. The smaller proportion in the skeleton of the adult animals is accounted for by the larger amount in the liver. The proportion of the dose in the latter is extremely variable, and appears to depend on form and methods of administration.

RADIOAUTOGRAPHS

Radioautographs were prepared from thin undecalcified sections of bone by the technique of Axelrod (7). The bone sections and corresponding radioautographs are shown in Figs. 1-6.

Fig. 1 shows the distribution of radiostrontium in the femur from a normal young rat. The isotope is found throughout the bone, with particularly heavy deposits in the new bone which is forming below the epiphysis. In Fig. 2, the distribution in the femur of a rachitic rat is shown. While considerable radiostrontium is found in the thin mineralized shaft of the bone, there is none in the uncalcified osteoid matrix beneath the epiphysis. Radioautographs

of bones from animals injected with Ca^{45} are very similar. The radiocalcium is found only in areas in which bone salt is present, and none is found in the uncalcified osteoid matrix of rachitic bone.

This is in contrast to the behavior of radioyttrium (Fig. 3) and radiocerium (Fig. 4). These two elements, in common with plutonium zirconium and many other heavy metals (1) show a marked concentration in the uncalcified osteoid matrix of rachitic bone. There appears to be a specific affinity of these metals for bone protein, even in the absence of bone salt. This is further evidence that their deposition in bone is not analogous to that of calcium.

Radiocerium has a peculiar spotty distribution throughout the dense cortex of bone, which is also evident in the radioautograph of the distribution of americium in adult bone (Fig. 5). A high power magnification of the latter (Fig. 6) shows the radioactive material in the neighborhood of the small blood vessels which run in the cortex. Although the cause of this perivascular distribution in cortical bone is not known, it is commonly associated with the lanthanide earths and americium and curium, which are usually in the trivalent state and have very similar physical and chemical properties. These elements are also taken up by the liver in large amounts initially (2).

II. EFFECT OF AGE AND LOW PHOSPHORUS RICKETS ON THE TURNOVER OF CALCIUM AND STRONTIUM IN THE SKELETON.

The marked effect of age and rickets on the retention of radiocalcium and radiostrontium prompted a more detailed investigation to study the changes responsible for these differences. The original work was carried out with radiostrontium (8), but was repeated later using Ca^{45} (9) since the latter is the element normally involved in bone metabolism.

EXPERIMENTAL

Isotopes: The radiostrontium used was carrier-free, and consisted of a mixture of Sr^{89} and Sr^{90} formed by nuclear fission. Since Sr^{90} decays to a radioactive daughter, Y^{90}, with a 60-hour half-life, all samples were held for 24 days before counting so that equilibrium might be attained, and any Y^{90} present at the time of the experiment would have decayed. A dose of approximately 5 microcuries of radiostrontium in 0.25 ml. neutral isotonic saline was injected intraperitoneally into each animal.

The dose of radioactive calcium used contained 5 microcuries of Ca^{45} with 1.25 mg. Ca as calcium chloride in 0.25 cc of solution. This was administered by intravenous injection. The amount of carrier calcium present was found to be sufficient to raise the level of serum calcium as much as 25%, but later experiments with high specific activity calcium (0.01 mg. Ca per microcurie) indicated that this amount of carrier had no significant effect on the distribution and excretion of the isotope.

Animals: The adult animals were mature female rats of the Long-Evans strain, from 6-12 months old. These were raised and maintained on the regular stock diet. Skeletal growth was at a minimum in these animals.

The animals with low phosphorus rickets were prepared according to the method described by Coleman et al (3). These were weaned at 21 days, and fed a diet high in calcium (0.43%) and very low in phosphorus (0.03%) for 5-6 weeks. They were 9 weeks old and weighed approximately 90-100 grams at the time of the injection of radiocalcium or radiostrontium.

The young normal animals were fed a complete diet from weaning until they were 7-9 weeks old, at which time they weighed approximately 200 grams. They were then injected with the radiocalcium or radiostrontium.

Procedure: After injection of the radioactive isotope, the animals were placed in individual metabolism cages, and urine and feces were collected separately. The rats were sacrificed at various time intervals from a few minutes up to 16 days after the injection. In all, 273 animals were used.

At autopsy, the femur was removed and analyzed for radiostrontium or radiocalcium; the total amount in the skeleton was estimated according to the method described by Jones and Copp (8). An aliquot of serum was analyzed, and the total serum $Sr^{89,90}$ or Ca^{45} was estimated using the value of 2.4% of body weight reported by Berlin et al (10).

The results are shown graphically in Figs. 7-12. Each point represents the average of values from 4-8 animals.

A. EFFECT ON THE METABOLISM OF RADIOCALCIUM.

Serum Fig. 7 shows the amount of radiocalcium present in the serum during the first 4 hours following intravenous injection. The fall is rapid in all three groups, but is relatively slower in the adult animals, and remains at a consistently higher level. This reflects the slower removal by skeleton and kidney in the adults.

Skeleton The radiocalcium present in the skeleton during the first four hours is shown in Fig. 8, and for the first 16 days is shown in Fig. 9. It is apparent that the initial uptake by the bones of both normal and rachitic young animals is very rapid. However, while the isotope appears to remain fixed in the skeleton of the normal animals, it is rapidly lost from the bones of the animals with low phosphorus rickets. This agrees with the findings with radiostrontium, and suggests a very labile calcium fraction in bone which rapidly reaches equilibrium with the serum radiocalcium, and declines as the isotope is excreted in the urine and the serum level falls. The ratio of Ca^{45} in bone to that in serum indicates that some 15% of the

bone calcium is in this "labile" fraction. The significance of this fraction in the calcification mechanism merits further investigation. In the adult animals, the uptake by the skeleton is much slower, reaching a maximum at 2-4 hours as has been observed by Norris et al (11). It then declines slowly. The uptake probably reflects ion exchange with the calcium already present in bone salt, since there is little or no new bone formation in these animals. Less than 0.5% of the bone calcium appears to be involved in this reaction.

Urine (Fig. 10) The excretion of radiocalcium was similar in both young and adult normal animals, but was tremendously increased in the rats with low phosphorus rickets. "Renal Serum Clearances" were calculated 12 hours after injection by dividing the rate of urinary excretion at this time by the total serum radiocalcium, and expressing the result as per cent of the total serum in the body "cleared" of isotope by urinary excretion per minute. For normal young animals, the average value was 0.6% per minute; for the normal adults it was 2.0% per minute; while for the group with low phosphorus rickets the clearance was 14%, or 0.16 cc/100 sq. cm. body surface/min.

This may be compared with the values for inulin clearance or glomerular filtration rate of 0.23 cc/100 $cm.^2$/min. reported by Friedman et al (12). It suggests that there is very little reabsorption of calcium by the renal tumules in these animals.

The high urinary excretion and renal serum clearance in these rachitic rats, despite a normal level of blood calcium, suggests that there is a direct effect on calcium clearance by the kidney. This may be associated with the low level of inorganic blood phosphate in these animals.

Feces (Fig. 11) The fecal excretion of radiocalcium was low in the young normal animals, and almost insignificant in the rachitic group. However, it was relatively high in the adult animals, and accounts for most of the Ca^{45} lost from the skeleton.

Conclusions: Ion exchange is undoubtedly an important factor in the uptake of radiostrontium and radiocalcium by the skeleton, and is perhaps the only one of significance in the adult animals. However, in the young animals, it does not appear to be the only mechanism. The very rapid initial uptake in the normal young animals, with fixation and very slow loss argues against an exchange process, and in these animals much of the radiocalcium is probably incorporated in new bone salt. In the rachitic rats, the initial uptake is equally rapid, but there is no fixation, and the loss from the skeleton is also rapid. This suggests a small and very labile calcium fraction in these animals.

This labile calcium, which amounts to some 15% of the total calcium in rachitic bone, may represent exchangeable calcium on the surface of the bone crystals. Radioautographs of femurs taken 1 hour after injection, when the Ca^{45} in rachitic bone is at a maximum, show the radiocalcium only in areas in which bone salt is present, with none in the uncalcified osteoid matrix. As the serum level of Ca^{45} continues to fall, as the isotope is poured out in the urine, the Ca^{45} in the labile bone fraction will fall with it, since they are in equilibrium.

Of particular interest is the relatively high renal clearance of calcium in the rachitic animals. Since previous experiments have indicated active absorption of radiostrontium from the intestinal tract of these animals, it would appear that high urinary excretion is a more important factor in the negative calcium balance in these animals than is

impaired absorption from the intestinal tract.

B. EFFECT ON THE METABOLISM OF RADIOSTRONTIUM

The results of these experiments with radiostrontium have been reported by Jones and Copp (8). Following intraperintoneal injection of $Sr^{89,90}$, the plasma level rises during the first 15 minutes, as the radiostrontium is absorbed from the peritoneal cavity. It then falls rather rapidly in the young and rachitic animals; more slowly in the adults. Indeed, the plasma radiostrontium in the latter remains 5-10 times the value in the younger animals. This may be explained by slower removal by skeleton and kidney.

The level of $Sr^{89,90}$ in the skeleton is shown in Fig. 12. As was observed by Norris and Kisielski (11), radiostrontium rapidly concentrates in the skeleton. The uptake was continuous in the adult animals up to 2 hours, and reached a maximum value at 2-4 hours. This may be accounted for by ion exchange with bone salt (13). In young animals, the initial rate of uptake was five times as great, and the maximum was reached within 30 minutes. The radiostrontium remained fixed in the skeleton, and very little loss was observed even 4-8 days after injection. It is probable that this rapid uptake and fixation is associated with incorporation of the strontium in the newly formed bone salt.

The same initial rapid uptake was observed in the rachitic animals, and the maximum reached was similar. However, this was followed by active removal from the skeleton, so that at the end of 24 hours, less than 1/3 of the radiostrontium originally present at 1 hour was still left in the bones. This was associated with a tremendous excretion of radiostrontium in the urine. New bone salt is not formed in these rachitic animals, so that radiostrontium cannot be incorporated in this form, although ion exchange with existing bone

salt may take place as it does in the adult. The evidence suggests that the initial rapid uptake is due to a labile combination with bone, from which the radiostrontium is readily released in the rachitic animal. This may be due to exchange with labile calcium on the surface of the crystals of bone salt, or may be an initial step in the calcification process. As with radiocalcium, radioautographs showed that even at the time of maximal uptake, radiostrontium was deposited only in bone salt, with none in uncalcified osteoid matrix.

In the young animal, very little radiostrontium was lost in the urine in contrast to the rachitics, in which a large part of the dose was eliminated by this route within 24 hours. Plasma clearances were calculated by dividing the excretion rate (determined graphically) by the radiostrontium level in the plasma. Since there was great divergence in the weights of the animals in the different groups, consistency was obtained by expressing the clearance as the per cent of the total blood plasma "cleared" of radiostrontium by urinary excretion per minute, rather than the more usual cc of plasma "cleared" per minute.

Plasma clearance was similar in both young and adult normal animals, with approximately 1 per cent of the blood plasma "cleared" per minute. In the rachitic rats, the plasma clearance was 10-15 times greater than in the normals, indicating a direct effect of this condition on the excretion of radiostrontium by the kidney. This may be associated with the low level of inorganic phosphate in the blood of these animals (5).

SUMMARY

1. Radiocalcium and radiostrontium are removed from the plasma much more slowly in the adult animals than in the other two groups.
2. The uptake of radiocalcium and radiostrontium by adult bone is continuous

for the first 2 hours, and reaches a maximum within 4 hours.

3. Skeletal uptake of radiocalcium and radiostrontium is much more rapid in the young animals, reaching a maximum within 30 - 60 minutes. The deposited isotope appears to remain fixed in the bone.

4. In rachitic rats, the rapid initial uptake was similar to that in the normal young animals, but was followed by active loss from the skeleton, so that only 1/3 was left at 24 hours, and less than 1/5 at 16 days. This suggests a labile combination with bone, possibly due to exchange with labile calcium on the surface of the crystals of bone salt.

5. A large part of the dose of radiocalcium and radiostrontium was excreted by the rachitic rats within the first 24 hours, and the plasma clearance was 10-15 times as great as in the normal animals, approaching the glomerular filtration rate. This appears to be due to a direct effect of low phosphorus rickets on excretion by the kidney.

III. EFFECT OF A LOW PHOSPHORUS DIET ON THE EXCRETION OF RADIOCALCIUM AND RADIOSTRONTIUM.

Day and McCollum (14) observed marked bone resorption and negative calcium balance in rats reared on a diet deficient in phosphorus. They felt that the bone resorption was necessary to provide phosphorus for the essential needs of the soft tissues. So severe was the deficiency that the animals died after 8-10 weeks from collapse of the softened rib cage and respiratory failure.

The marked reduction in the retention of radiocalcium and radiostrontium in rachitic bone has already been discussed. In the following experiments the isotopes were injected into normal young 21 day old rats, or into mature adult rats, and the animals were then changed to a

diet low in phosphorus.

The previous experiments have shown that a large part of the dose of Ca^{45} or $Sr^{89,90}$ is deposited in the skeleton of the normal animals within 2-4 hours after injection. Following this period, half the animals in each group were changed to a diet very low in phosphorus (0.01%) while the remainder were retained on the complete control diet. The animals were placed in metabolism cages and the urine and feces were collected separately at daily intervals for 30 days and analyzed for Ca^{45} or $Sr^{89,90}$. At the end of this time, the animals were sacrificed. The carcass was analyzed for residual Ca^{45} or $Sr^{89,90}$.

From these values, the quantity of radiocalcium or radiostrontium remaining in the skeleton at different times was calculated and plotted on semi-log paper against time. The curves are shown in Figs.13-15.

Fig. 13 shows the effect of the low phosphorus diet on the Ca^{45} retained by young rats. By the end of the second day, the excretion of radiocalcium is higher in the animals fed the low phosphorus diet, and it continues at a much higher rate for the duration of the experiment. After two weeks, the biological half-life of the Ca^{45} is 37 days in the animals on the low phosphorus diet as compared to 236 days in the controls fed the complete diet. The actual excretion was 5-6 times as great in the animals on the experimental diet.

Similar curves for the adult animals are given in Fig. 14. The excretion during the first week was much greater than in the young, as was observed earlier. However, the low phosphorus diet did enhance the excretion of Ca^{45}, and the biological half-life was reduced from 164 to 62 days.

The curves for $Sr^{89,90}$ in young animals are shown in Fig. 15.

The curves are quite similar to those for Ca^{45}, and show a marked effect of the low phosphorus diet on the excretion of radiostrontium. The excretion is increased almost immediately, and the biological half-life is reduced from 350 to 42 days.

The first effect observed when an animal is restricted to a phosphorus deficient diet is a fall in the level of phosphate in serum, and its disappearance from the urine. The prompt increase in the urinary excretion of radiocalcium and radiostrontium suggests that this is related to the fall in serum phosphate and may be due to the increased renal clearance mentioned earlier. The increased removal from bone suggests that exchange, at least of the recently deposited Ca^{45}, may be affected by these changes in the phosphate level.

A similar experiment was carried out in which, after injection of radiocalcium, the animals were changed to a diet adequate in phosphate but very low in calcium. In these animals, the excretion of Ca^{45} was actually reduced as compared to the controls. This suggests that a low calcium diet would have little value for increasing removal of such radioactive alkaline earth metals as calcium, strontium or radium from the skeleton.

SUMMARY

1. Many of the radioactive metals formed in nuclear fission are deposited in skeleton. The metabolism of isotopes of alkaline earth metals such as calcium, strontium and barium is markedly affected by age and low phosphorus rickets, while the metabolism of other heavy metals such as yttrium and cerium is largely unaffected.

2. Radioautographs of rachitic femur show that $Sr^{89,90}$ and Ca^{45} are deposited only in the presence of bone salt, while Y^{88} and Ce^{144} and other heavy metals are also laid down in uncalcified osteoid matrix.
3. Kinetic studies of skeletal uptake and excretion of radiocalcium and radiostrontium show considerable mineral exchange, even in adult rats. Skeletal uptake is more rapid and greater in growing animals, and the radioactive isotopes remain fixed in the skeleton, presumably by incorporation in new bone salt.
4. In rachitic rats, the initial uptake by bone is rapid, but the Ca^{45} or $Sr^{89,90}$ is then rapidly lost from the skeleton and excreted in urine. The "labile" fraction amounts to some 15% of the bone calcium, and may represent calcium on the surface of the crystals of bone salt.
5. Renal clearance of Ca^{45} and $Sr^{89,90}$ is 10-15 times as great as in the normal animals, and approaches the glomerular filtration rate. This could be due to a direct effect of low phosphorus rickets on the kidney.
6. When normal animals are injected with Ca^{45} or $Sr^{89,90}$, and are then fed a diet low in phosphorus, the excretion of the radioactive isotopes in urine is increased, and removal from the skeleton is accelerated.

ACKNOWLEDGMENT

The authors wish to acknowledge the valuable criticism and assistance of Professor C. W. Asling of the Department of Anatomy and Institute of Experimental Biology, University of California, Berkeley. They would also like to thank Mrs. Ruth Lerner and Mrs. Sybil Cole for important technical assistance.

REFERENCES

1. Copp, D.H.; Axelrod, D.J.; and Hamilton, J.G.: The Deposition of Radioactive Metals in Bone as a Potential Health Hazard, Am.J.Roentgenol., 58:10-16 (1947)

2. Hamilton, J.G.: The Metabolic Properties of the Fission Products and the Actinide Elements, Rev. Mod. Phys. 20:718-728 (1948)

3. Coleman, R.D.; Becks, H.; Kohl F.vanN.; and Copp, D.H.: Skeletal Changes in Severe Phosphorus Deficiency of the Rat, Arch.Path. 50:209-232 (1950)

4. Falkenheim, M.: The Influence of Growth on the Phosphorus Metabolism of the Mouse and the Effect of Thyroxin at Various Ages, Am.J.Physiol. 138: 175-179 (1942)

5. Weissberger, L.H.; and Harris, L.S.: Effect of Tocopherols on Phosphorus Metabolism, J.Biol.Chem. 151:543-545 (1943)

6. Weissberger, L.H.; and Harris, L.S.: A Possible Vitamin D Assay Technique with Radiostrontium, J.Biol.Chem. 144:287 (1942)

7. Axelrod, D.J.: An Improved Method for Cutting Undecalcified Bone Sections and its Application to Radioautography, Anat.Rec. 98:19-25 (1947)

8. Jones, D.C.; and Copp, D.H.: The Metabolism of Radioactive Strontium in Adult, Young and Rachitic Rats, J.Biol.Chem. (In Press)

9. Thompson, D. M.; and Copp, D.H.: (unpublished data)

10. Berlin, N.I.; Huff, R.L.; Van Dyke, D.C.; and Hennessy, T.G.: The Blood Volume of the Adult Rat as Determined by Fe^{59} and P^{32} Labelled Red Cells, Proc. Soc. Exp.Biol. & Med. 71:176-178 (1948)

11. Norris, W.P.; and Kisielski, W.: Comparative Metabolism of Radium, Strontium and Calcium, Cold Spr.Harbor Symp.Quant.Biol. 13:164-172 (1948)

12. Friedman, S.M.; Mackenzie, K.R.; and Friedman, C.L.: Renal Function in the Adrenalectomized Rat, Endocrinol. 43: 123-125 (1948)

13. Hodge, H.C.; Gavett, E.; and Thomas, I.: The Adsorption of Strontium at Forty Degrees by Enamel, Dentin, Bone, and Hydroxyapatite as shown by the Radioactive Isotope, J.Biol.Chem. 163:1-6 (1946)

14. Day, H.G.; and McCollum, E.V.: Mineral Metabolism, Growth and Symptomatology of Rats on a Diet Extremely Deficient in Phosphorus, J.Biol. Chem. 130: 269-283 (1939)

FIGURE CAPTIONS

Fig. 1 Femur from a young normal rat injected with $Sr^{89,90}$ and sacrificed 1 week later. Note the radiostrontium deposited in the shaft and the calcified areas below the epiphysis. (From Hamilton, Rev. Mod. Phys. 20, 718 (1948) courtesy of American Institute of Physics, Publishers).

Fig. 2 Femur from a six week old rat with lor phosphorus rickets, injected with $Sr^{89,90}$ and sacrificed 1 week later. Note that the radiostrontium is deposited in calcified areas only, with none in the uncalcified osteoid matrix below the epiphysis. (From Copp, Axelrod and Hamilton, J. Roentgenol. 58, 10 (1947)).

Fig. 3 Femur from a 9 week old rat with low phosphorus rickets, injected with Y^{88} and sacrificed 1 week later. Note the superficial deposition of radio-yytrium in the shaft, and the heavy deposits of the metal in the uncalcified osteoid matrix below the epiphysis. (From Copp, Axelrod and Hamilton, Am. J. Roentgenol. 58, 10 (1947)).

Fig. 4 Femur from a six week old rat with low phorphorus rickets, injected with Ce^{144} and sacrificed 1 week later. Note the superficial deposition of radiocerium in the shaft and the heavy deposits in the uncalcified osteoid matrix with the shaft. (From Copp, Axelrod and Hamilton, Am. J. Roentgenol. 58, 10 (1947)).

Fig. 5 Femur from an adult rat injected with Am^{241} and sacrificed 16 days later. Note spotty distribution of the americium within the shaft, similar to the distribution of radiocerium. (From Hamilton, Rev. Mod. Phys. 20, 718 (1948)).

Fig. 6 Higher power magnification of the section of femur and americium radioautograph shown in Fig. 5. Note the deposition of americium in the region around the small blood vellels of the cortex of the shaft. (From Hamilton, Rev. Mod. Phys. 20, 718, (1948)).

Fig. 7 Percent of the administered dose of Ca^{45} in serum following intervenous administration.

Fig. 8 Percent of the administered dose of Ca^{45} in the skeleton following intravenous administration.

Fig. 9 Percent of the administered dose of Ca^{45} in the skeleton (estimated) following intravenous injection.

Fig. 10 Cumulative excretion of Ca^{45} in urine (expressed as percent of the administered dose) following intravenous injection.

Fig. 11 Cumulative excretion of Ca^{45} in feces (expressed as percent of the administered dose) following intravenous injection.

Fig. 12 Percent of the administered dose of radiostrontium in the skeleton (estimated) following intraperitoneal injection.

Fig. 13 Percent of the administered dose of Ca^{45} retained by young rats fed a normal diet and those fed a diet low in phosphorus.

Fig. 14 Percent of the administered dose of Ca^{45} retained by adult rats fed a normal diet, and by those fed a diet low in phosphorus.

Fig. 15 Percent of the administered dose of $Sr^{89,90}$ retained by young rats fed a normal diet, and by those fed a diet low in phosphorus.

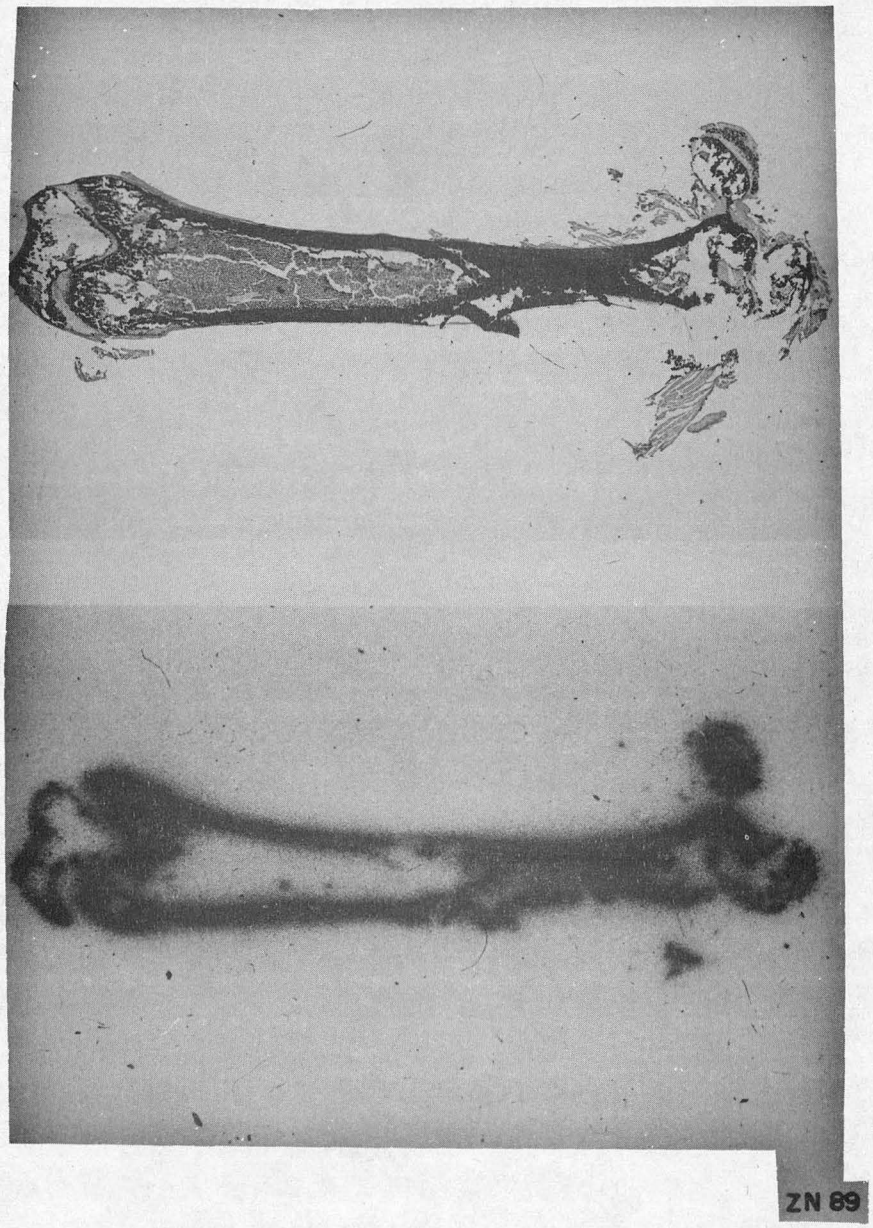


Fig. 1

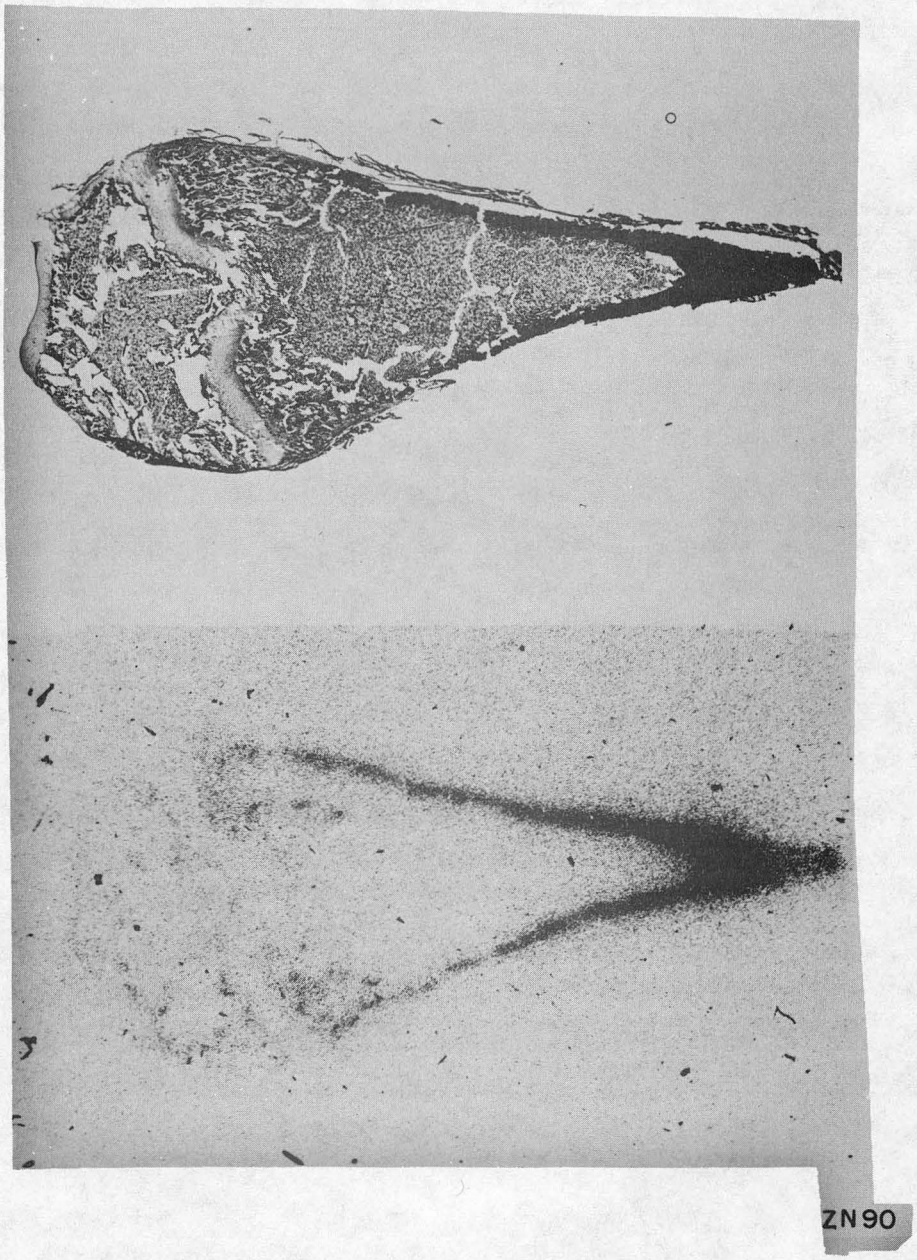


Fig. 2

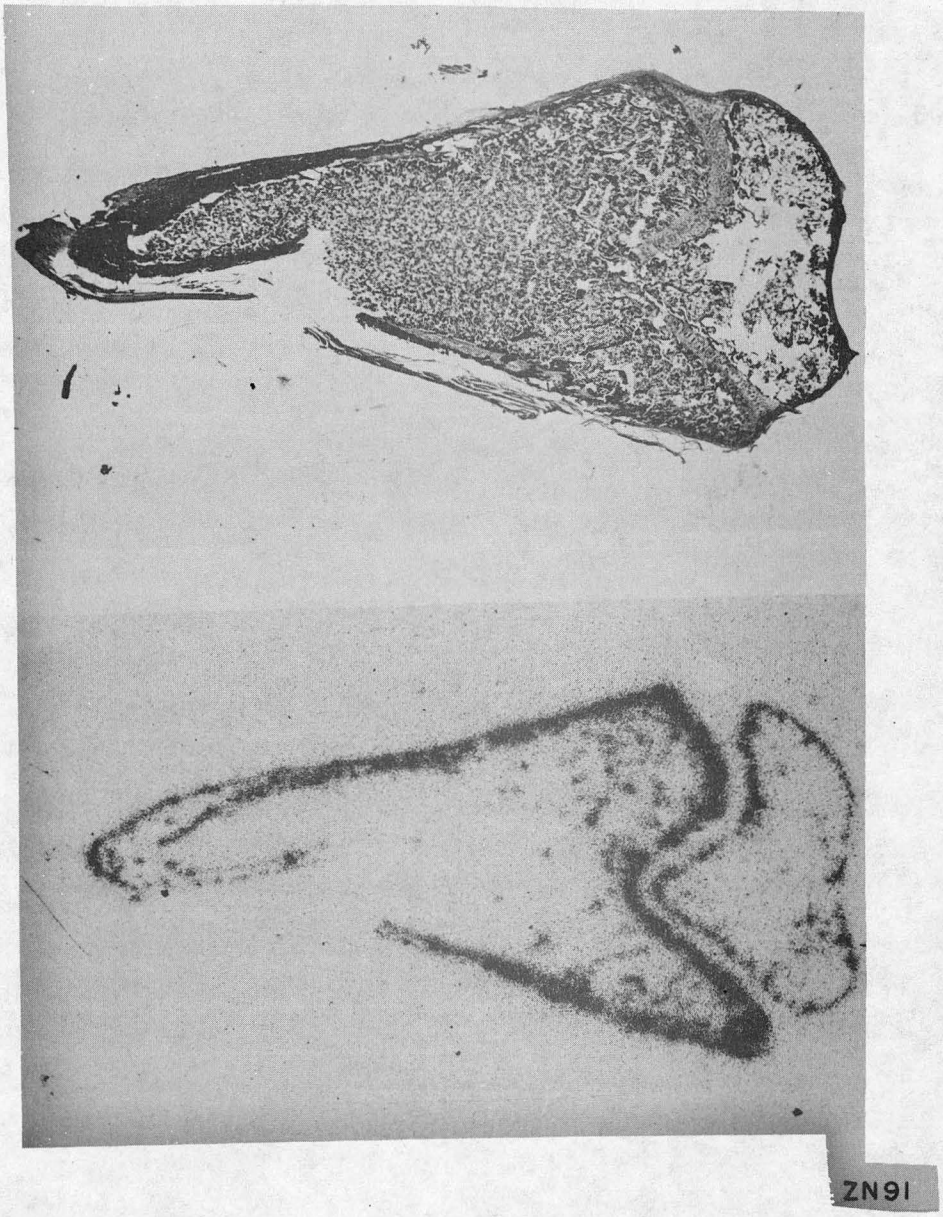


Fig. 3

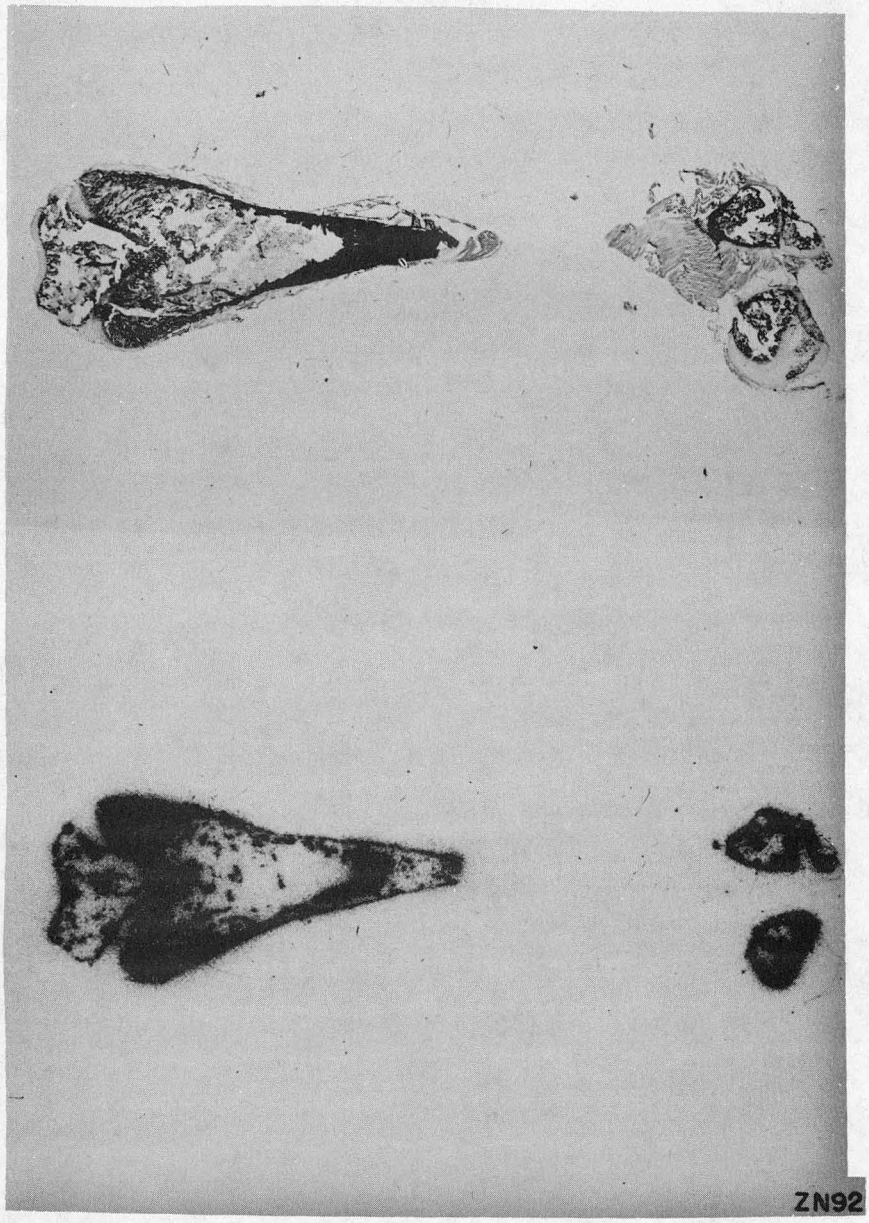


Fig. 4

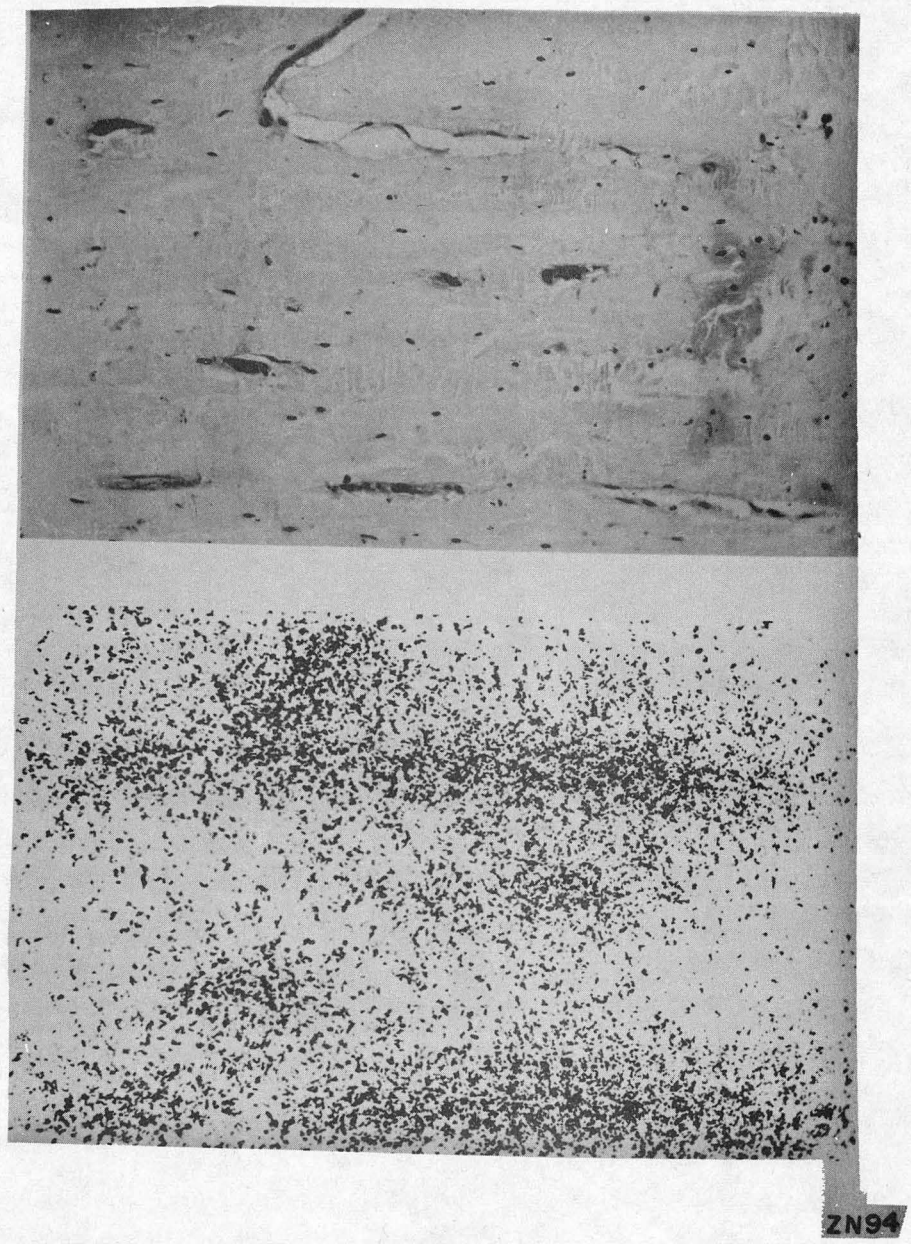


Fig. 5

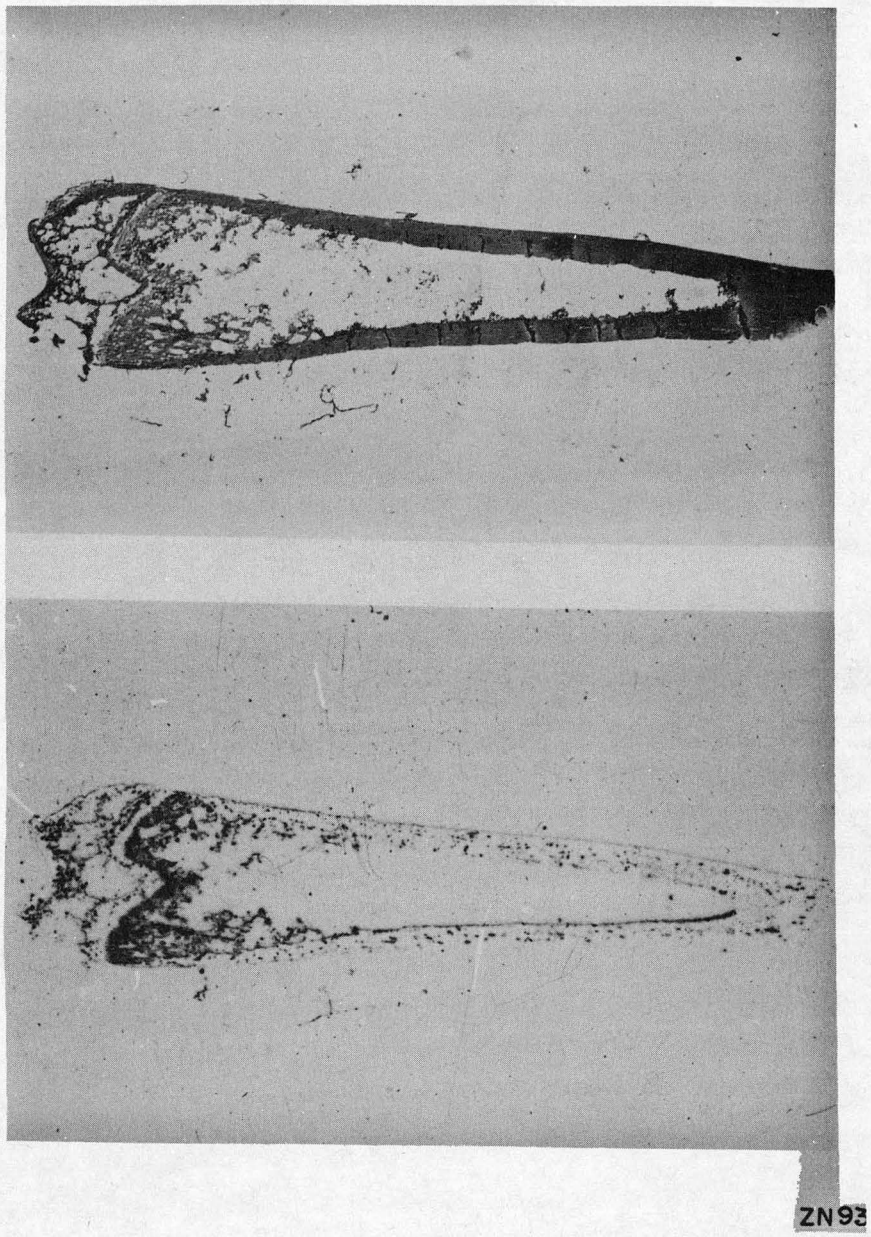


Fig. 6

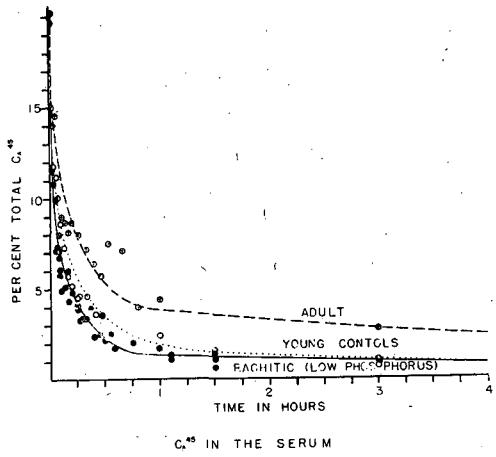

PER CENT TOTAL Ca^{45}
15
10
5
ADULT
YOUNG CONTOLS
RACHITIC (LOW PHOSPHORUS)
1
2
3
4
TIME IN HOURS
Ca^{45} IN THE SERUM

Fig. 7

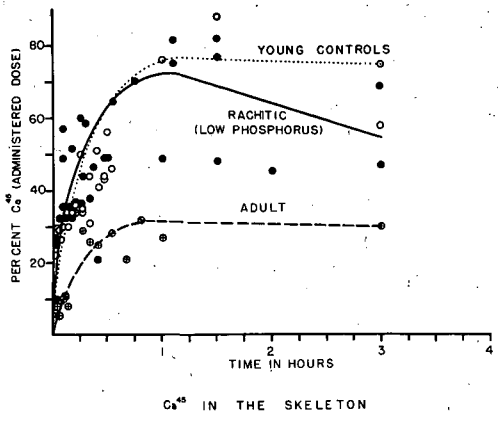
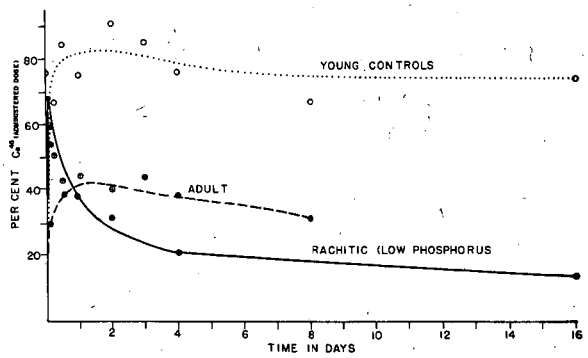
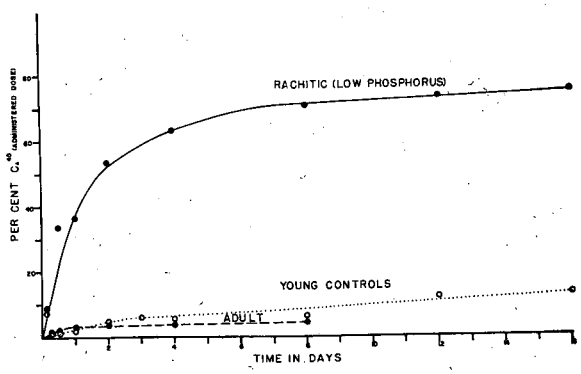

PER CENT Ca^{45} (ADMINISTERED DOSE)
80
60
40
20
YOUNG CONTROLS
RACHITIC
(LOW PHOSPHORUS)
ADULT
1
2
3
4
TIME IN HOURS
Ca^{45} IN THE SKELETON

Fig. 8



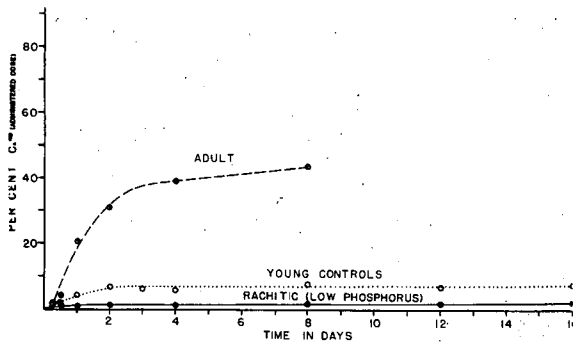
Ca^{45} IN THE SKELETON

Fig. 9



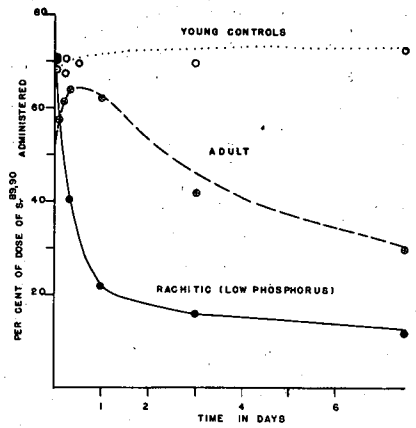
CUMULATIVE EXCRETION OF Ca^{45} IN URINE

Fig. 10



CUMULATIVE EXCRETION OF C_{14} IN FECES

Fig. 11



$Sr^{89,90}$ IN THE SKELETON

Fig. 12

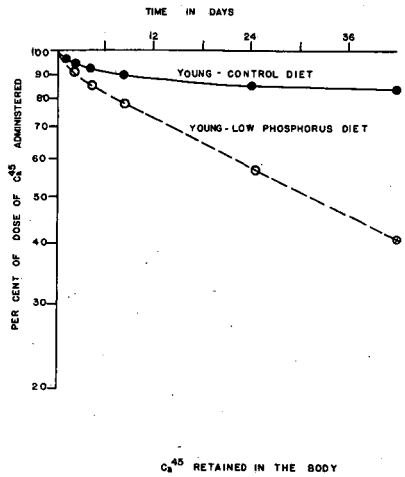

TIME IN DAYS
12
24
36
100
90
80
70
60
50
40
30
20
PER CENT OF DOSE OF Ca^{45} ADMINISTERED
YOUNG - CONTROL DIET
YOUNG - LOW PHOSPHORUS DIET
Ca^{45} RETAINED IN THE BODY

Fig. 13

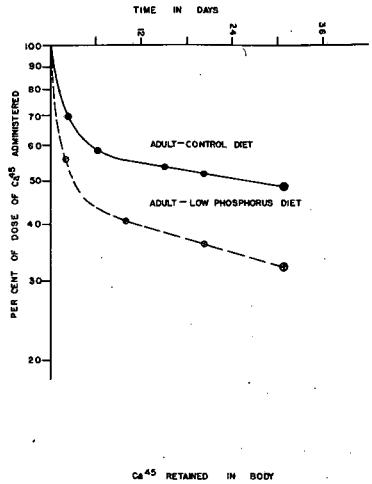

TIME IN DAYS
12
24
36
100
90
80
70
60
50
40
30
20
PER CENT OF DOSE OF Ca^{45} ADMINISTERED
ADULT-CONTROL DIET
ADULT-LOW PHOSPHORUS DIET
Ca^{45} RETAINED IN BODY

Fig. 14

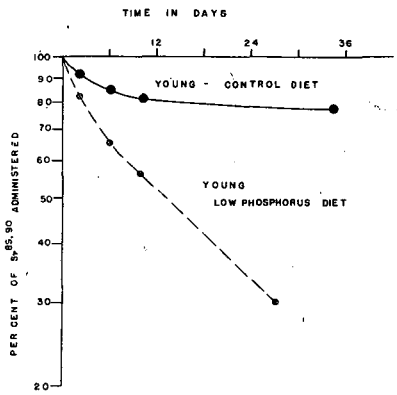

TIME IN DAYS
12
24
36
100
90
80
70
60
50
40
30
20
PER CENT OF Sr85,90 ADMINISTERED
YOUNG - CONTROL DIET
YOUNG
LOW PHOSPHORUS DIET

Fig. 15