STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM.

V. A Study of the Bone Trabeculæ as a Readily Available Reserve Supply of Calcium.*†

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Plates 13 to 17.

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By far the largest part of the calcium in the body takes part in making up the skeletal structure. Metabolism studies have shown that there are many factors which add or subtract from the body's supply of calcium. Thus pregnancy, lactation, a low calcium intake, ingestion of acids, administration of parathormone[1] and many other influences serve to increase the demand for calcium from the body. The question arises: Is there a special storehouse of calcium, or is the whole body skeleton at the mercy of these various factors? A very significant observation in this connection was made by Hunter and Aub (1). In patients with chronic lead poisoning they observed an increased lead excretion, which accompanied the increased calcium excretion following the administration of parathormone. If these same patients were then put on a high calcium diet so as to store up the amount of calcium lost during the parathormone medication, and were then given a second course of parathormone, they failed to show an increased lead excretion although the calcium excretion was

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[1] Parathormone is the trade name for Collip's parathyroid extract.

elevated as markedly as before.[2] This observation suggests that the first parathormone medication withdrew calcium and the lead deposited with it from some storehouse of reserve calcium; that this store was replenished during the period of high calcium intake; and that finally the second parathormone medication affected only the newly deposited store, and did not affect the skeleton at large, which, of course, still contained considerable lead. It was further noted that following a course of treatment with parathormone, the small daily excretion of lead continued as before. This suggests a constant excretion from some source, such as the cortex of bone, and that this represents the normal catabolism of the skeletal structure.

While working on calcium metabolism in animals, we had noted that the amount of cancellous bone varied considerably in different animals but no note was made whether these variations were associated with any form of therapy or diet. This raised the question: Are the trabeculæ a reserve supply of calcium? Is it possible that the calcium in the bones can be divided into two parts;—a larger portion making up the structural part of the bone, which functions as a mechanism for body support, and a smaller, less stable portion acting as a reserve deposit for use when the body needs calcium or inorganic salts? The anatomical situation of the trabeculæ is in accord with such a theory. They are most numerous at the epiphyseal ends of the bones, where the blood supply is greatest. These delicate, lace-like structures, in close relation to the bone marrow, are well situated to receive calcium deposits in times of calcium excess, and to give up calcium in times of calcium demand. The shafts of the bones are not so well situated for this function.

EXPERIMENTS.

In an attempt to see whether the bone trabeculæ act as a reserve supply of calcium the following animal experiments were planned.

Experiment 1. Effect of Parathormone Administration on the Trabeculæ of Rabbits.

Parathormone administration is one of the most effective ways of increasing the calcium excretion and thus producing a negative

[2] See Hunter, D., and Aub, J. C., *Quart. J. Med.*, 1927, xx, 123, Chart 1.

calcium balance (1, 2). Therefore, we wished to determine its effect on the trabeculæ.

Experiment.—A litter of eight young rabbits was used. At the onset of the experiment they were 5 weeks old. Four were used as controls and four for parathormone injections. They were kept in the same bin, on a normal diet, high in calcium content. Early in the experiment Rabbits 334 (extract) and 274 and 267 (controls) died. The others were kept for a period of 91 days. The animals were given the parathyroid extract in gradually increasing doses until they were all receiving 8 units per day. (See Table I.) This dose they received for from 56 to 74 days of the period.

At the end of the 91 day period (24 hours after the last injection) all animals were anesthetized with ether and blood samples drawn. They were then killed, their blood serum analyzed for calcium (Table I) and their bone structures studied.

TABLE I.

Rabbit	Blood serum calcium	Total No. of units of parathormone injected
	mg. per 100 cc.	
301*	15.5	514
298*	14.2	514
327*	14.3	628
297†	11.6	
314†	10.3	

* Rabbits receiving parathormone.

† Control litter mates.

It is well shown in the x-ray plates (Fig. 1) that the bones of the extract animals (Nos. 298, 301, 327) contain much less calcium than do the bones of their control litter mates (Nos. 297 and 314). This is most marked in the trabeculæ at the epiphyseal end, particularly of the metacarpal bones. Fig. 2 shows the cross-section of the femora from each animal. This photograph clearly demonstrates that there are fewer trabeculæ in the extract animals than in the controls.

Conclusion to Experiment 1.—The trabeculæ are diminished following long continued parathormone administration in rabbits. No gross effect was noted on the cortex of the bone.

Experiment 2. Effect of High and Low Calcium Diets on the Trabeculæ of Adult Cats.

A diet deficient in calcium results in a negative calcium balance (3–5). On a low calcium diet as used in our experiments, we have found that an average sized cat has an average negative calcium balance of approximately .3 gm. per week. In cats starving for a month, Sedlmair (6) was able to demonstrate a calcium loss of 1 per cent of the total body weight, or 2.5 per cent to 11.6 per cent calcium oxide loss from the bones. Wellmann (7) confirmed these observations working with rabbits. The opposite of this (a high calcium diet) results in a positive calcium balance (5, 8, 9). Where does this calcium loss come from and where is the excess deposited?

TABLE II.

Cat	Sex	Type of diet	Days on diet	Total Ca intake for exp.	Daily Ca intake
				gm.	*gm.*
337	♀	Low Ca diet	84	1.29	.016
337		High Ca diet	274	144.70	.528
338	♀	Low Ca diet	84	1.19	.014
338		High Ca diet	156	38.70	.248
339	♀	Low Ca diet	84	1.28	.015
339		High Ca diet	160	68.80	.430
341	♂	Low Ca diet	84	1.19	.014
341		High Ca diet	267	68.20	.257
342	♂	High Ca diet	79	22.60	.286
342		Low Ca diet	269	3.84	.014
343	♂	High Ca diet	79	48.20	.610
343		Low Ca diet	267	3.47	.013
344	♂	High Ca diet	80	31.15	.389
344		Low Ca diet	379	4.66	.012
345	♂	High Ca diet	80	30.06	.382
345		Low Ca diet	161	2.11	.013
346	♀	High Ca diet	79	29.10	.350
346		Low Ca diet	157	1.83	.012

The separate protocols for these animals are appended.

Experiment.—Five cats were placed on a high calcium[3] diet and four on a low calcium diet.[4] At the end of several months the left fore leg was amputated at the shoulder joint under ether anesthesia, then the diets were reversed. After a similar period of time the animals were sacrificed, thus allowing comparison of the humerus representing the high calcium diet period, with the humerus of the low calcium diet period.

[3] High calcium diet consists of milk, and in one animal milk and raw eggs.

[4] Low calcium diet consists of cooked meat, fresh, raw liver and water. Cats stay in excellent condition on this diet even if it is given them for many months.

The bones were all prepared in the same manner, the same length of time being taken for each process. They were cleaned, scraped and cross-sectioned. The marrow contents were removed by burning in an electric furnace. Thus each animal served as his own control and such factors as age, sex, activity and season do not enter into the results obtained. Table II gives in summary form the average daily calcium intake, the total calcium intake for each period and the length of time the animal was kept on each diet.

Figs. 3 to 6 show very well the results obtained from this experiment. Regardless of whether an animal is first placed on a high or low calcium diet, the result is always the same. The bone removed after the period of high calcium feeding contains many trabeculæ and its opposite member representing the period of low calcium diet shows a marked reduction in the number of trabeculæ. From Figs. 5 and 6, it will be seen that these changes are most marked in Cats 343, 337, 345, 342, 339, 346 and 344. These animals had the largest daily calcium intake during the period of high calcium feeding (see Table II). The changes are least marked in Cats 338 and 341 who had the lowest daily calcium intake when on a high calcium diet. The absence of trabeculæ is most marked in Cat 344 (see Fig. 6). It was on a high calcium diet first (80 days) and then on a low calcium diet for 379 days. The bones show almost complete absence of trabeculæ of the bone shaft and a disappearance of the majority at the epiphyseal end (see Figs. 6 and 7).

Conclusions to Experiment 2.—The bony trabeculæ are diminished in an adult cat when kept on a low calcium diet and increased during a high calcium diet. No gross change in cortical thickness was demonstrable.

Experiment 3. The Location of New Calcium Deposits in Adult Cats Shown by Intravital Staining with Alizarin Red.

It has been adequately shown that alizarin red, when administered orally or intramuscularly will stain newly deposited bone (10–14).[5] This affords one an opportunity to show the exact location of newly formed bone.

[5] Since this paper went to press, we have discovered the experiments of John Hunter, done in 1794, in which he used madder in staining growing bone and teeth (The works of John Hunter, with notes, edited by James F. Palmer; Longman, Rees, Orme, Brown, Green, and Longman, London, 1837).

Twelve adult cats were first placed on the same low calcium diet used in Experiment 2. The group protocol is appended. Three died from snuffles early in the experiment. The remaining nine were kept on this low calcium diet for 82 days. It was thought that this was long enough to show a reduction in the number of bony trabeculæ. The cats were then placed on a high calcium diet and alizarin red was injected intramuscularly in order to stain the newly formed trabeculæ. After varying lengths of time on this treatment, the cats were killed. The bones were cleaned, scraped and cross-sectioned. The marrow contents were removed by digestion with pancreatic ferment at pH 8.3 for 24 to 36 hours. Cat 363 was killed on the 5th day and showed very few trabeculæ and these were stained very faint red (see Fig. 8). Cat 356 was sacrificed on the 10th day and as can be seen from Fig. 8, there were only a few trabeculæ but these were stained pink. Cats 362, 359, 360, 356, 357 and 361 were all sacrificed on the 55th day and as can be seen from Fig. 8, they all showed many red stained trabeculæ. Cat 358 was allowed to continue on the high calcium diet for 36 days longer without receiving the dye and showed not only red trabeculæ but also white trabeculæ. Presumably these white trabeculæ represent trabeculæ laid down during the period that the animal received no dye. The alizarin red deposited in all the bones was confined to the trabeculæ except for a small amount deposited around the vessels where they enter the endosteum.

Conclusions.—This group of experiments shows that the calcium stored while on a high calcium diet is deposited in the trabeculæ. The observation that alizarin red is deposited in new formed bone is confirmed.

Experiment 4. Control Experiments to Show that Alizarin Red Is Not Deposited in Bones during Periods of Calcium Equilibrium or Negative Calcium Balance.

Alizarin red is deposited in new formed bone but we have no conclusive proof that it is not deposited in previously formed trabeculæ. In order to investigate this, the following experiments were performed. (The group protocol is appended.)

1. Two cats (Nos. 383 and 384) had received a high calcium diet for months. This diet was continued and they were injected with alizarin red every other day for 21 days. If no new trabeculæ were formed during this period, their bones should show no stained trabeculæ, and the bones from both animals actually showed many trabeculæ but none were stained red (see Fig. 9).

2. Two cats (Nos. 355 and 365) had their left fore leg amputated at the shoulder joint under ether. They were then placed on a low calcium diet. After the 14th day they were injected with 40 units of parathormone daily. Starting

on the 20th day, they also received alizarin red every other day. On the 44th day they were both sacrificed. The photograph of the results (Fig. 6) demonstrates that the bones obtained after the low calcium diet plus parathormone régime have fewer trabeculæ—and none of these were stained red with alizarin.

3. Two cats (Nos. 380 and 381) which received a low calcium diet for about 8 weeks were continued on a low calcium diet and injected with alizarin red intramuscularly every other day. They were killed after they had been on alizarin for 20 days. Their bones showed a marked diminution in the number of trabeculæ and none of the trabeculæ were stained red (Fig. 9).

4. Two cats (Nos. 367 and 368) which had received a low calcium diet for 91 days had their left fore leg removed at the shoulder joint under ether and were then placed on a high calcium diet plus alizarin red. After they had been on this régime for 49 days they were sacrificed, and, as is seen in Fig. 8, the bones representing the low calcium diet showed few trabeculæ—whereas the ones representing the alizarin red—high calcium régime have an increased number of trabeculæ fairly well stained.

Conclusions.—Alizarin red, when administered to animals while in calcium equilibrium or negative calcium balance, does not stain the trabeculæ or previously formed bones. Therefore, we have further proof that alizarin red stains new formed bone.

Experiment 5. The Effect of Growth upon the Trabeculæ in Kittens.

Hamilton has demonstrated that normal infants must have a store of calcium at birth (aside from that contained in the milk ingested), which is sufficient to allow normal bone growth during the first 4 months of life. Could the trabeculæ possibly serve as this calcium supply which is called upon at this time for skeletal growth?

Experiment.—Five kittens were chosen for the experiment to note the effect of growth on the trabeculæ. The kittens were allowed to continue nursing. The first kitten killed at the age of 2 days showed the entire shaft to be filled with trabeculæ (see Fig. 10). The second kitten was killed at the age of 18 days and its bones showed a marked reduction in the number of trabeculæ when compared with those of the first kitten. The remaining kittens were killed on the 26th, 52nd and 67th days. As can be seen from Fig. 10, the older the kitten the fewer were the trabeculæ. This demonstrates that normal bone growth in young animals also results in a reduction of the readily available supply of calcium. Six normal growing kittens (not of the same litter) of varying ages were killed and their bones show very few spicules.

A normal kitten was allowed to continue nursing from its mother. Alizarin red ½ cc. was injected every day between the 20th and 30th days of life, and then every 3 days up to the age of 60 days. The injections were then discontinued. The kitten was sacrificed at the age of 90 days. All the teeth became pink on the 30th day except for the very tips which remained white. Cross-section of the long bones showed the entire cortex to be stained red except near the epiphyseal ends where it was white. Again there were very few red stained spicules present showing that they were not being deposited.

This experiment is very important for comparison with the bones of adult cats on a high calcium diet while receiving alizarin red. The bones from the adult cats show no staining of the cortical bone except at the site of entry of the blood vessels, whereas the entire cortex of the kitten bones was red except for the area already mentioned. This white zone, in all probability, represented newly formed bone after the injections of dye had been stopped. This experiment not only proves that alizarin stains new formed bone but also adds further evidence to our thesis that very little calcium was laid down in the cortex of bones of our adult animals in Experiment 2.

Conclusions.—There is a marked reduction of the trabeculæ in kittens as a result of bone growth. Alizarin red when administered to a growing kitten is deposited in the cortex (shaft) of bones.

Experiment 6. Effect of Parathormone on the Trabeculæ in Growing Kittens and Rats.

Four kittens from the same litter were used, two for controls and two for parathormone injections. The two kittens receiving extract were started on parathormone at the age of 26 days. One control kitten died before the experiment was completed. During the next 56 days each extract kitten received 1270 units of parathormone or an average of 25.4 units per day. At the age of 82 days the three kittens were sacrificed. The serum calcium and phosphorus just before death (18 hours after the last injection) were as follows:

	Calcium *mg. per 100 cc.*	Phosphorus *mg. per 100 cc.*
No. 382, control	9.9	9.4
No. 383, extract	8.1	9.0
No. 384, extract	8.6	6.55

The bones from the extract animals showed no signs of decalcification by x-ray when compared with the normal control litter mate. There was no diminution in the number of trabeculæ present in the extract animals' bones when compared to the controls, but all three showed very few trabeculæ.

This same experiment was tried on two kittens from another litter. The extract was started on the 24th day and was continued for 121 days. During this time the kitten received 1090 units of parathormone. At the time of death the kitten receiving extract had a serum calcium of 10.7 and a serum phosphorus of 7.2. The control kitten had a serum calcium of 11.2 and phosphorus of 6.9. Again no decalcification or diminution of the bone trabeculæ could be demonstrated in the extract animal. The bones from both animals showed almost complete absence of the trabeculæ. Our failure to show signs of decalcification or diminution of trabeculæ in growing cats may be due to the fact that all the readily available calcium is used for normal bone growth.

All of the kittens which received parathormone showed no elevation of serum calcium; in fact it was slightly lower than in the control animals. We have had the same experience with adult cats. We administered as much as 100 units at a time, in eight such animals, and their blood, taken the 6th, 12th, 24th and 48th hours after injection, has never shown any elevation of the serum calcium.

Eight young growing rats from the same litter were all retained on a high calcium diet. At 10 days of age, four were started on parathormone, given intraperitoneally, and this was continued for 110 days. During this time they each received 885 units of parathormone. The serum calciums of the entire group at death were:

Control rats.	*mg. per 100 cc.*	Extract rats.	*mg. per 100 cc.*
No. 389	12.3	No. 389	13.9
No. 385	11.7	No. 385	13.0
No. 386	11.6	No. 386	11.75
No. 387	13.3	No. 390	12.8

X-ray examination of the bones of the animals which had received extract appeared to be much denser and shorter than their control siblings, and on cross-section they also showed many trabeculæ. We are unable to explain these findings.

Conclusions.—Parathormone administration to growing kittens does not result in any greater reduction of the trabeculæ than one observes in the course of normal bone growth. We have never been able to demonstrate a rise in serum calcium in cats or kittens after parathormone administration. Parathormone administration to growing rats results in a diminution in length of bone and an increase in the number of trabeculæ.

DISCUSSION.

The above studies prove that the trabeculæ of bones serve as a source of calcium. Here, as in many other respects, we have an analogy between mineral and protein metabolism. It is the present conception that body protein is composed of structural protein and "deposit protein," and this latter decreases during protein starvation. So here the calcium of the bones is apparently partly structural (cortex) and partly a readily available reserve supply of calcium (trabeculæ). While large amounts of calcium are being used to form new structural elements as in growing animals, the trabeculæ are diminished. Hamilton (15) showed that in normal infants the ratio of calcium to body weight diminishes during the first 4 months. Inasmuch as the skeleton increases proportionately to the increase in weight, he concludes that a normal child has a store of calcium at birth to draw upon during the first 4 months. He further points out that this added store is deposited during the last months of pregnancy so that premature infants do not have this store. This, he believes, is why normal infants do not get rickets until 4 months of age and why premature infants almost invariably get rickets. These facts agree with those of Experiment 5 of our studies in which it was seen that kittens at birth have many trabeculæ which disappear as growth takes place. Autopsy material is now being collected to see whether the same is true of human infants.

A negative calcium balance decreases the trabeculæ and a positive balance increases them. The observation of Hunter and Aub (1) on the failure of parathormone to cause an increased excretion of lead during a second administration of the drug is now apparent. New trabeculæ, uncontaminated by lead, were laid down during the rest period between medication, and consequently a second administration of parathormone caused an elimination of extra calcium without an increased lead excretion. It does not seem unlikely that when the trabeculæ are depleted, the structural part of the skeleton meets demands for calcium, just as in the case of the structural proteins during starvation. But the bone shafts are extremely important in maintaining the body structure. Protected from depletion by this storehouse of readily available calcium, it is only in cases of great need that they are called upon to relinquish calcium. Thus, when the trabeculæ are not present to meet the demand for calcium to form new structural units during growth or stress, it is quite possible that calcium is drawn from the bone shafts, and rickets or osteomalacia results. Such prolonged demand is not common but the appearance of osteomalacia after repeated pregnancies or Graves' disease is very suggestive evidence.

In summarizing the above experiments the following statements would seem justified.

Bone trabeculæ have as their function not only to act as a support against the tension of muscles (Carey) but to serve as the most readily available calcium supply in time of need. They can be influenced by many factors. Thus prolonged administration of parathormone in rabbits will decrease their number. Likewise a prolonged negative calcium balance will diminish these same bony spicules while a high calcium diet increases the amount of cancellous bone. Regardless of whether an animal is kept on a low or high calcium diet for a long period of time, no gross changes are noted in the cortex. The trabeculæ seemingly serve as the labile supply of calcium and the cortex as the stable supply. It would appear that anabolism and catabolism go on at a more or less constant rate in the cortical portion, except at times of unusual demand such as in osteomalacia.

Newly deposited calcium can be observed by intravital staining with alizarin red. By this method it has been established that when an adult animal is depositing calcium, newly formed trabeculæ are all stained red but the cortex remains unstained. This indicates that calcium was not being deposited in the cortex, or if so, it was at too slow a rate to be demonstrated by the deposition of the dye. When calcium equilibrium or a negative calcium balance is being maintained, the administration of alizarin red stains neither the cortex nor the trabeculæ. However, in growing kittens the administration of the same dye results in the entire cortex being stained red. This is further proof that the absence of the dye in the cortex of adult cats signifies that little or no calcium is being deposited in this portion.

The bones of kittens at birth are filled with trabeculæ. These rapidly disappear during the first weeks of life suggesting that they are needed for structural growth. Parathormone administration to cats is without effect; in them there is no resulting rise in the serum calcium and no demonstrable diminution in the number of trabeculæ. Yet its administration to growing rats results in an increased number of trabeculæ, a fact we do not know how to interpret.

CONCLUSIONS.

1. Bone trabeculæ are easily depleted by the prolonged administration of parathormone, long continued negative calcium balance and growth.
2. A long continued high calcium diet results in a rapid accumulation of the trabeculæ.
3. Alizarin red, as has previously been shown in the literature, is deposited in newly formed bone. Its use has made clear that easily mobilizable calcium is not deposited in the shafts of adult animals, but in the trabeculæ of bone.
4. The bone trabeculæ therefore serve as the storehouse of readily available calcium.
5. The shafts have a slow progressive exchange of inorganic salts and are not influenced except in the case of unusual body demands.
6. It is suggested that the absence of trabeculæ in premature infants and their depletion at the end of 4 months in a normal baby might well be an etiological factor in rickets.
7. In our observations, parathormone administration to growing or adult cats has been without effect.
8. Daily injections of parathormone in growing rats results in an increased number of trabeculæ and smaller bones.

Protocols for Experiment 2.

No. 337.—A brown and white, young female cat, weight 2.15 kilos, was placed on a low calcium diet on April 21, 1926. It remained on this diet until July 14, 1926 (84 days). It suffered from snuffles from May 21, 1926, to June 6, 1926. During this period the average daily food intake was: liver 102 gm., meat 99 gm., water 91 cc. The weight at this time was 3.5 kilos. On July 14, 1926, the left fore leg was amputated at the shoulder joint. Ether anesthesia was used. On July 20, 1926, the cat was placed on a known high calcium diet. The weight at this time was 3.0 kilos. The average daily intake for the ensuing 274 days was 440 cc. of milk. On April 15, 1927, the animal was sacrificed so that the bones from the one fore leg on a high calcium diet could be compared with the ones representing the low calcium diet period. At the end of the experiment the cat weighed 2.5 kilos. Autopsy showed no gross pathological changes.

No. 338.—A gray and brown, adult female cat weighing 2.95 kilos was placed on a low calcium diet April 21, 1926. On May 16, 1926, it gave birth to four dead kittens. On May 21, 1926, the cat contracted snuffles from which it recovered on June 9, 1926. It remained on this low calcium diet for 84 days.

During this time the average daily intake was: liver 96 gm., meat 76 gm. and water 80 cc. On July 13, 1926, the cat weighed 2.85 kilos. On this day the left fore leg was amputated at the shoulder joint under ether anesthesia. An uncomplicated postoperative period followed. On July 20, 1926, the weight was 2.45 kilos. The animal was then given a known high calcium diet and took an average of 206 cc. of milk per day for 150 days. At the end of this time it weighed 3.25 kilos. On December 22, 1926, the cat was sacrificed. Complete autopsy showed no gross abnormalities.

No. 339.—A white and gray, adult female cat, weight 3.25 kilos, was placed on a low calcium diet on April 21, 1926. On May 19, 1926, it developed snuffles. Under treatment it recovered on June 6, 1926. On June 16, 1926, the cat gave birth to six normal kittens. It was allowed to nurse these for a period of 2 weeks. The cat's average intake for 84 days was: liver 99 gm., meat 89 gm. and water 63 cc. At the end of this period it weighed 3.00 kilos. On July 14, 1926, the right fore leg was amputated at the shoulder joint under ether anesthesia. On July 20, 1926, the animal was started on a known high calcium diet. At this time it weighed 2.15 kilos. The average daily intake for the next 160 days was 358 cc. of milk per day. On December 29, 1926, the cat weighed 3.15 kilos. At this time it was sacrificed. No macroscopic pathological changes were noted at autopsy.

No. 341.—A gray and white, adult male cat weighing 3.95 kilos was placed on a low calcium diet on April 21, 1926. It consumed an average of 90 gm. of liver, 85 gm. of meat and 32 cc. of water per day for the next 84 days. At the end of this time it weighed 4.75 kilos. On July 14, 1926, the left fore leg was amputated at the shoulder joint under ether anesthesia. The animal was then placed on a milk diet which it took very poorly. On August 25, 1926, the weight had fallen to 2.95 kilos. At this point it was given raw eggs in addition to the milk. For the 267 days postoperative, it had an average daily intake of 78 cc. of milk and 2.43 eggs. At the end of this time the animal weighed 4.2 kilos. It was sacrificed on April 14, 1927.

No. 342.—A tiger-striped, adult male cat weighing 3.35 kilos was placed on a high calcium diet. On May 17, 1926, it contracted snuffles and did not recover until May 28, 1926. The average daily intake for 79 days was 286 cc. of milk. It then weighed 3.05 kilos. On July 13, 1926, the left fore leg was amputated at the shoulder joint under ether anesthesia. 7 days later the animal was placed on a low calcium diet. For 269 days the average daily intake was: 79 gm. of meat, 99 gm. of liver and 50 cc. of water. At this time it weighed 4.2 kilos. On April 14, 1927, it was sacrificed. The autopsy was negative.

No. 343.—A white, tiger-striped adult male cat, weighing 3.0 kilos, was started on a high calcium diet on April 24, 1926. On May 23, 1926, it showed signs of snuffles. These disappeared after May 30, 1926. The cat consumed an average of 508 cc. of milk during the next 79 days and weighed 3.40 kilos. On July 13, 1926, the left fore leg was removed at the shoulder joint under ether anesthesia.

On July 20, 1926, the animal was started on a low calcium diet and kept on it for 267 days. The average daily intake for this period was: meat 60 gm., liver 100 gm. and water 50 cc. At the end of this period it weighed 3.75 kilos. On April 13, 1927, it was sacrificed. Autopsy showed no gross pathology.

No. 344.—A brown and white, adult male cat, weighing 2.85 kilos, was placed on a high calcium diet on April 25, 1926. It had signs of snuffles on May 23, 1926. The nose became cold and all signs of snuffles disappeared on June 6, 1926. The average daily intake for a period of 80 days was 324 cc. of milk. The weight at this time was 2.45 kilos. On July 13, 1926, the left fore leg was amputated at the shoulder joint under ether anesthesia. On July 20, 1926, the cat was placed on a low calcium diet. It remained on this diet in excellent physical condition until it was sacrificed August 2, 1927, a period of 379 days. During this time the average daily intake of food was: liver 100 gm., meat 50 gm. and water 52 cc.

No. 345.—An all black, adult male cat, weighing 2.80 kilos, was put on a high calcium diet on April 25, 1926. The average intake for the next 80 days was 318 cc. of milk. At the end of this time it weighed 3.1 kilos. On July 13, 1926, the left fore leg was amputated at the shoulder joint under ether anesthesia. On July 20, 1926, the cat was placed on a low calcium diet. The average daily intake for 161 days was: liver 100 gm., meat 62 gm. and water 80 cc. At the end of this period it weighed 3.90 kilos. On December 27, 1926, the animal was sacrificed. Autopsy showed no gross pathological changes.

No. 346.—An adult, black and white female cat weighing 2.95 kilos was placed on a high calcium diet on April 25, 1926. On May 22, 1926, the cat developed snuffles. All signs of snuffles were absent on May 30, 1926. On June 22, 1926, three fat, normal appearing kittens were born and were not weaned until they were 2 weeks of age. During this 79 day period the cat had an average daily intake of 296 cc. of milk. The weight at this time was 2.70 kilos. On August 14, 1926, the left fore leg was amputated at the shoulder joint under ether anesthesia, 6 days later the cat was placed on a low calcium diet. For a period of 157 days, the cat had an average daily intake of: liver 98 gm., meat 48 gm. and water 28 cc. On December 22, 1926, the cat was sacrificed. At this time it weighed 3.55 kilos. The autopsy findings were negative.

Protocols for Experiment 3.

On January 18, 1927, twelve cats were placed on the same low calcium diet. On this régime they all received approximately 13 mg. of calcium per day. Three cats (Nos. 355, 365 and 366) contracted snuffles on January 28, 1927, were isolated and later killed. The nine remaining cats were kept on the low calcium diet for 82 days. On April 10,1927, they were all placed on a high calcium diet. They also received 2 cc. of alizarin red intramuscularly the next 4 days. On the 5th day Cat 363 was killed and the bones were prepared and saved for comparison. They were then given 1 cc. of alizarin intramuscularly every day. On June 3, 1927, Cats 357, 358, 359, 361 and 364 were found to have fluctuating areas at the site of injection of the alizarin red. They were all incised under ether and red stained material was obtained, no frank pus. Starting on April 18, 1927, each cat received 1 cc. of alizarin every other day. On April 30, Cat 356 was killed and the bones were prepared and saved for comparison.

From this date they received 1 cc. of alizarin every 3 days. On June 3, the following cats were killed: Nos. 356, 357, 360, 361, 362 and 364. The bones were all prepared in the same manner. Cat 358 was given no more injections but was kept on a high calcium diet. It was sacrificed on July 9, 1927. The bones were prepared in the same manner. None of the cats showed any gross pathological changes at autopsy.

Protocols for Experiment 4.

Two normal cats (Nos. 380 and 381) which had been on an unlimited high calcium diet for weeks were placed on a known high calcium diet on June 13, 1927. On July 11, 1927, they were started on alizarin red ½ cc. every other day. On July 31, 1927, they were both sacrificed. They had both gained weight during the period of observation. No gross pathological changes were noted at autopsy.

Two cats (Nos. 355 and 365) that had previously been on an unlimited high calcium diet had their left fore leg amputated at the shoulder joint on June 15, 1927, under ether anesthesia. On June 16, No. 355 weighed 2.25 kilos and No. 365 weighed 2.70 kilos. On this same day they were placed on a low calcium diet. From July 2 on they were both given 40 units of parathormone per day. On July 7 they were also started on alizarin red ½ cc. every other day. They were sacrificed on July 30 for comparison of the bones and to see if the spicules had been stained by the dye. At the time of death, No. 355 weighed 2.45 kilos and No. 365 weighed 3.1 kilos. There were no gross pathological findings at autopsy.

Two cats (Nos. 383 and 384) which had been on a low calcium diet for 49 days were placed in cages on June 13, 1927, and the same diet continued. Starting on July 11, 1927, they were given ½ cc. of alizarin red every other day. On July 31, 1927, they were sacrificed to see if the spicules were stained red. Their weights had remained stationary. The autopsies were negative.

Two cats (Nos. 367 and 368) which had been on a low calcium diet for 91 days had their left fore leg amputated at the shoulder joint under ether anesthesia. On June 15, 1927, No. 367 weighed 3.25 kilos and on June 16, No. 368 weighed 2.15 kilos. On June 30 they were placed on a high calcium diet and alizarin red. They were both sacrificed on August 18, 1927, for comparison of the bones. Their weights at this time were: No. 367—3.45 kilos; No. 368—2.60 kilos. The autopsies were negative.

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EXPLANATION OF PLATES.

Plate 13.

Fig. 1. Shows the effect of long continued administration of parathormone upon the bones of rabbits. The bones of Rabbit 301 (extract) show marked signs of decalcification as compared to its control litter mate, No. 314.

Plate 14.

Fig. 2. The effect of parathormone on the cancellous bones. The femurs of Rabbits 301, 298 and 327 show fewer trabeculæ than do the femurs of their control litter mates, Nos. 297 and 314.

Fig. 3. The effect of diet on the amount of cancellous bone. Cat 342. The upper humerus represents the high calcium diet period and contains many more trabeculæ than its opposite member (the lower humerus) representing the low calcium diet period.

FIG. 4. The effect of diet on the amount of cancellous bone. Cat 339. The upper humerus represents the high calcium diet period and contains many more trabeculæ than its opposite member (the lower humerus) representing the low calcium diet period.

PLATE 15.

FIG. 5. A photograph showing effect of diet upon the amount of cancellous bone. The humerus representing the high calcium diet period is placed above its opposite member representing the low calcium diet period. The bones are arranged in rows according to the dietary régime and each pair is from a different animal. The epiphysis is missing from the humerus representing the high calcium diet period of Cat 343, and also from the humerus representing the low calcium diet of Cat 345.

FIG. 6. The effect of diet and parathormone upon the amount of cancellous bone. The humerus representing the high calcium diet period is placed in the upper row and its opposite member representing a low calcium diet is placed in the lower row. The changes are most marked in Cat 344, which did not receive parathormone. (Refer also to Experiment 4 in text.)

FIG. 7. The effect of diet on the trabeculæ. The upper femur (Cat 344) shows the absence of trabeculæ after a long period on a low calcium diet, whereas the femur from Cat 339 shows many trabeculæ as a result of a period on a high calcium diet.

PLATE 16.

FIG. 8. Showing the location of new calcium deposits in adult cats by the injection of alizarin red while the animals were on a high calcium diet. The bones representing Cats 356, 357, 358, 359, 360, 361, 362, 363 and 364, have been fully described under Experiment 3 (see text). The bones from Cats 367 and 368 are so arranged that the bone above the number represents the low calcium diet period and that below represents the high calcium diet plus alizarin red period. (For further details, see Experiment 4, Cats 367 and 368.)

PLATE 17.

FIG. 9. The effect of diet on the trabeculæ. The bones from Cats 367, 383 and 384 show many trabeculæ after being on a high calcium diet. Cats 368, 380 and 381 show a diminution in the number of trabeculæ as a result of long continued negative calcium balance.

FIG. 10. The effect of growth on the trabeculæ. The bones of the kittens described in Experiment 5 are shown here arranged in the order of age. They show a diminution in the number of trabeculæ as the kittens grow older.

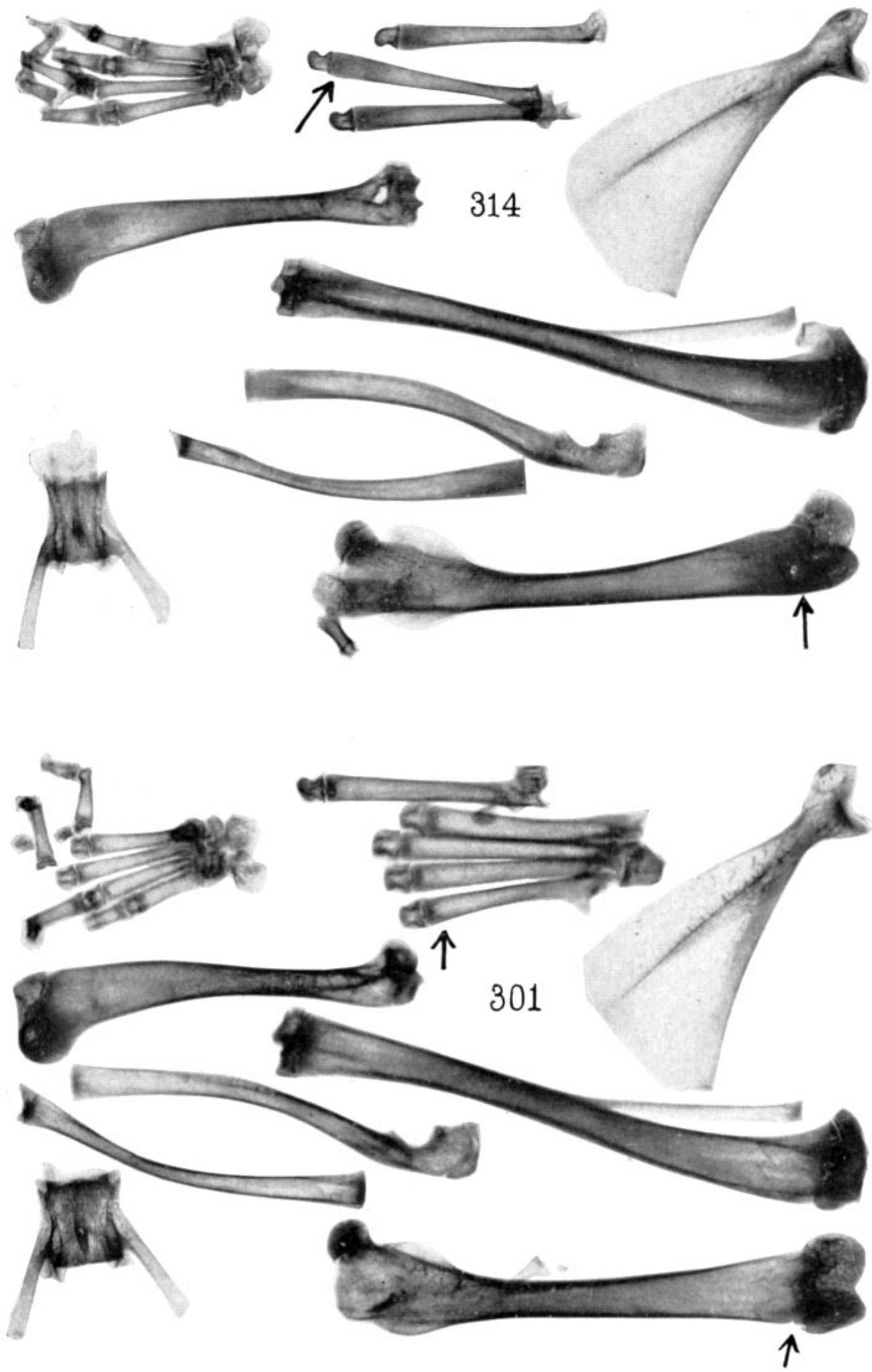

FIG. 1.

(Bauer, Aub, and Albright: Calcium and phosphorus metabolism. V.)

FIG. 2.

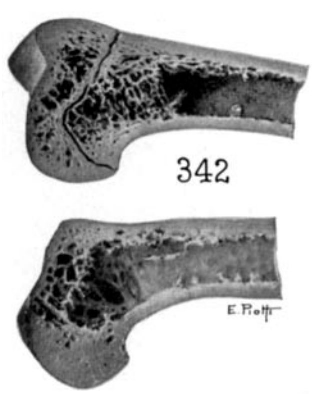

FIG. 3.

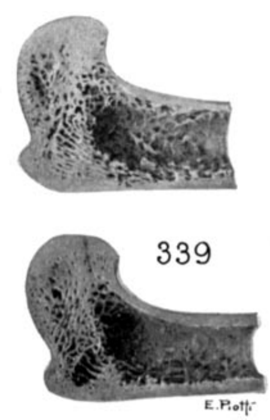

FIG. 4.

(Bauer, Aub, and Albright: Calcium and phosphorus metabolism. V.)

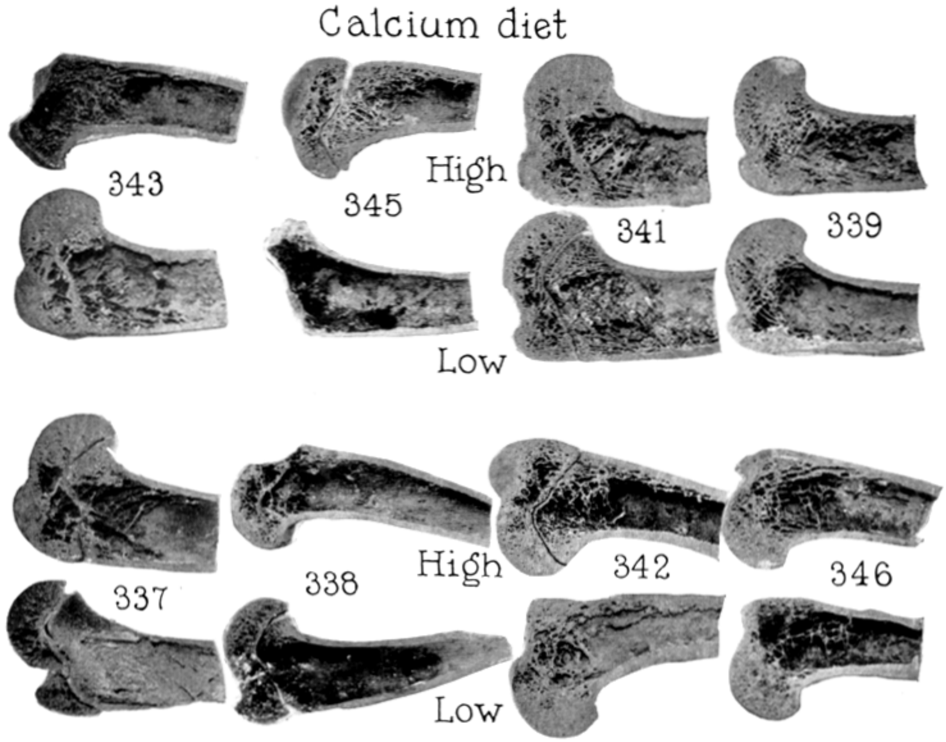

FIG. 5.

FIG. 6.

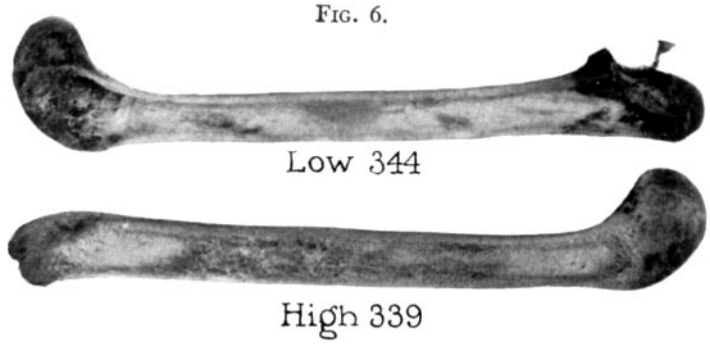

FIG. 7.

FIG. 8.

(Bauer, Aub, and Albright: Calcium and phosphorus metabolism. V.)

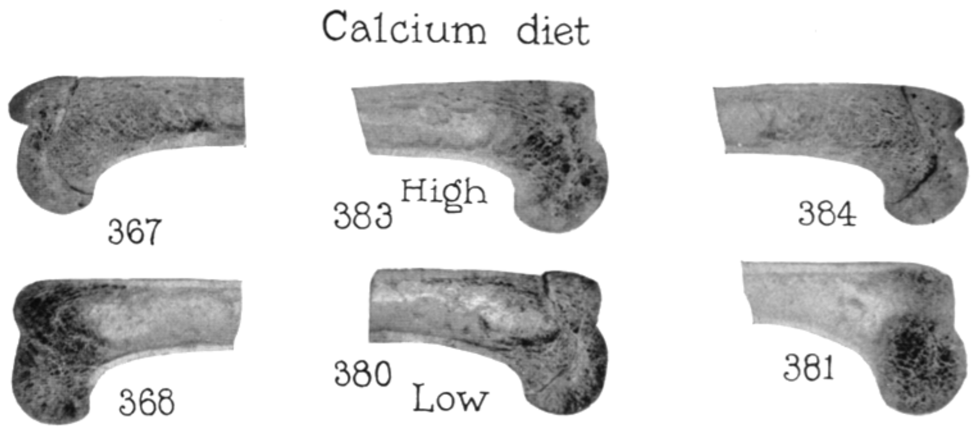
Calcium diet
367
383 High
384
368
380 Low
381

FIG. 9.

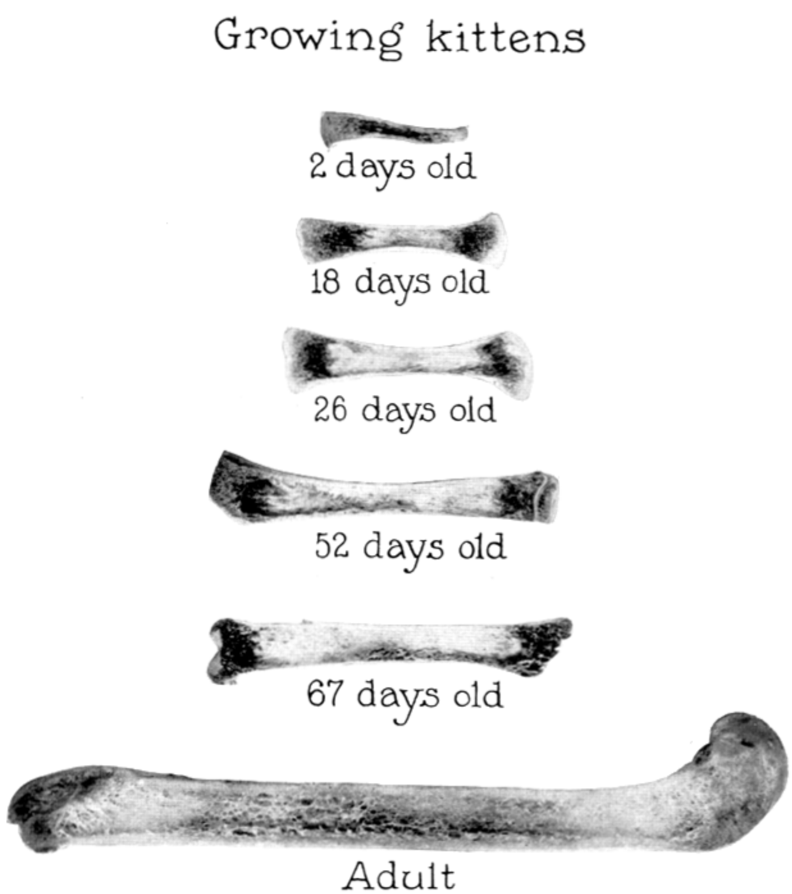
Growing kittens
2 days old
18 days old
26 days old
52 days old
67 days old
Adult

FIG. 10.