AMMONIUM CHLORIDE DECALCIFICATION, AS MODIFIED BY CALCIUM INTAKE: THE RELATION BETWEEN GENERALIZED OSTEOPOROSIS AND OSTITIS FIBROSA

By HENRY L. JAFFE, M.D., AARON BODANSKY, Ph.D., and JOSEPH P. CHANDLER, Ph.D.

(From the Laboratory Division, Hospital for Joint Diseases, New York)

Plates 41 and 42

(Received for publication, July 28, 1932)

Experimental hyperparathyroidism in young and old animals on high and low calcium intakes results in bone decalcification and secondary fibrosis leading to the production of generalized ostitis fibrosa (1–6). We conceived the mechanism of these changes as follows:

> "It may well be that the immediate antecedent of experimental ostitis fibrosa is a condition, perhaps related to the disturbed acid-base equilibrium, which may also be caused by other agents than parathormone. However, it seems essential that the action be continuous, and that the condition which it causes be maintained for long periods without endangering the life of the experimental animal, or a certain minimum of well-being. Parathormone is specific in the sense that it satisfies these requirements" (1).

We recognized, in view of the known effects of parathormone upon the mineral metabolism, that other means of similarly affecting mineral metabolism might produce similar end-results. It was deemed desirable to undertake a quantitative study of the question of experimental bone decalcification, using ammonium chloride to bring about generalized osteoporosis and possibly ostitis fibrosa.

Ammonium chloride has long been known to be an effective agent in producing acidosis, as in its metabolism urea is synthesized from the ammonium radicle and hydrochloric acid is liberated. Ammonium chloride may be administered in considerable quantities for a long time. Haldane demonstrated an increased excretion of bases and phosphates in the experimental acidosis thus produced (7). The

calcium reserves in the bone may be expected to take a considerable part in the neutralization of the acid, and an increased excretion of calcium and phosphorus, especially through the kidneys, has been confirmed experimentally (8).

Increases of serum phosphatase were demonstrated in the course of this study, which were roughly parallel to the degree of bone decalcification (9).

Methods

Four litters of dogs were used, 3, 6, 8, and 18 months old, respectively, at the beginning of the experiment. At the outset the 3 months old puppies averaged about 3 kilos, the 6 months about 8 kilos, the 8 months old dogs about 10 kilos, and the 18 months old dogs about 17 kilos. Each group contained one or more control animals which did not receive ammonium chloride. Two types of control were used, those maintained on a calcium-adequate diet and those on a calcium-inadequate diet. The experiment was continued for 11 weeks.

Diet.—The diet consisted of fresh ground lean horse meat, mixed with 2 per cent cod liver oil and 5 per cent canned tomatoes. This constituted the calcium-poor diet. It was adequate in other respects. The animals receiving the calcium-adequate diet were given a supplement consisting of 2.5 gm. of bone meal and 2.5 gm. calcium lactate (equivalent to about 1 gm. calcium) per kilo of meat mixture. On this diet the control animals grew well and were in excellent condition. The food intake, though measured daily, was given practically *ad libitum*. The animals consumed from 0.5 to 2.5 kilos per day, depending on their size. The youngest dogs on the low calcium diet with ammonium chloride supplement suffered loss of appetite towards the end of the experiment and their food consumption decreased to about 0.5 kilos per day.

Ammonium Chloride Administration.—Ammonium chloride was administered at first in the form of a 1 per cent solution by stomach tube, and later in 1½ per cent and 2 per cent solutions, once a day at the beginning of the experiment, and twice a day when the volume administered would otherwise have been too large to be given at one time. At the beginning of the experiment the amount of ammonium chloride was about 0.08 gm. per kilo of body weight per day, for all the groups. It was increased relatively rapidly in the 18 months old dogs to a final daily dose of 1 gm. per kilo body weight, in the 8 months old dogs to 0.8 gm. per kilo, in the 6 months old dogs to 0.7 gm. per kilo, and in the 3 months old dogs to 0.4 gm. per kilo. The final dose was continued for 17 days before the conclusion of the experiment.

Course of the Experiments

The control animals on the adequate calcium intake continued to grow, including even the 18 months old dogs that had practically com-

pleted their growth at the beginning of the experiment. The animals on a low calcium diet without ammonium chloride gained weight more slowly after a time, although their growth in length seemingly continued. On the other hand, the dogs on the low calcium diet receiving ammonium chloride eventually developed anorexia and gained no weight. This was particularly noticeable in the youngest group where gain in weight ceased after the first 2 weeks on the low calcium diet with ammonium chloride. One animal developed deformities and fractures—a young puppy on low calcium diet, receiving ammonium chloride. The ammonium chloride effect was not as pronounced in the older animals. The animals receiving high calcium intakes and the ammonium chloride grew well.

Gross Pathologic Findings

At autopsy the bones of the control dogs receiving the calcium supplement were normal in every respect.

They cut with usual resistance and the cortices were compact. The spongy trabeculae at the ends of the long tubular bones were entirely normal in appearance, and the internal architecture showed the usual arrangement of the trabeculae. The periosteum covering the long tubular bones stripped normally and the articular cartilages were smooth and glistening. The soft tissues showed no abnormalities in the gross, and the intestinal mucosae were intact throughout.

The animals of all age groups on a low calcium intake showed, on the other hand, readily discernible thinning of their bones, on comparison with their controls.

In Fig. 1 it is plainly visible that the cortex of the femur of the dog on the low calcium diet shows marked cortical thinning when compared with its adequate calcium control. Gross lamellation of the cortex with enlargement of the vessel canals is apparent. The general marrow cavity is enlarged. The spongy bone at the lower end of the femur extends over not quite as great a distance as does the spongy bone of the litter mate controls on the high calcium diet. The individual trabeculae are thinner, and this thinning of the trabeculae appears both in the extreme epiphyseal ends of the bone and in the metaphyses. The spongy trabeculae of the diaphysis have practically disappeared. Thus the effect of a low calcium intake is not limited to the spongy bone, but is reflected also in the cortex.

The macroscopic effects of the ammonium chloride treatment were observed in all the age groups, but were most striking in the younger

animals. In all the age groups the gradations of change were found to be strikingly dependent upon the calcium intake. Those dogs receiving an adequate calcium intake and ammonium chloride showed less bone thinning than those receiving a low calcium diet and ammonium chloride.

Examination of Fig. 1 will show that in the 170 litter, the femoral cortex of the dog on a low calcium diet plus ammonium chloride is thinner than that of the dog on an adequate calcium diet plus ammonium chloride but not very much different from the low calcium control. The femoral cortices of both of these animals are definitely lamellated and the spongy bone of both the low calcium control and the low calcium plus ammonium chloride animals, while fairly abundant, consists of much more delicate trabeculae than observed in the metaphyses and epiphyses of the dogs receiving an adequate calcium intake plus ammonium chloride.

In the youngest age group the added effect of ammonium chloride brought out more striking changes than the low calcium diet alone.

In a dog put in the experiment at 3 months of age, a low calcium diet and ammonium chloride led to fractures and deformities. Less striking changes were observed in the litter mate given ammonium chloride and a high calcium diet. These differences are depicted in Fig. 2. The photograph shows a pronounced osteoporosis of the femur of the dog receiving an adequate calcium intake plus ammonium chloride; the cortex is extremely thin, about one-half to one-third that of its control. The spongy bone which is fine and delicate continues into the diaphysis but not as far as in the control; there are no deformities or fractures; the cortex shows definite gross lamellation. The litter mate on a low calcium intake with ammonium chloride suffered extensive fractures of the ribs, femora, and humeri. At autopsy great care had to be exercised in the removal of the bones to prevent further fractures because of the very marked friability. Healing of the fractures with callus proliferation and deformities was observed. The specimen shown in the figure is from the lower end of a macerated femur. The bone was so friable that some of it disintegrated in the process of maceration. Nevertheless the thin tissue-paper-like cortex is noticeable. The narrow deformed marrow cavity was closed in places by internal callus and the spongy trabeculae of the metaphyseal region were so soft and friable that careful handling had to be exercised to prevent the production of artefacts.

This also holds true for the 130 litter, shown in Fig. 3. The cortex of the femur of the dog on a low calcium intake plus ammonium chloride is very much thinner than that of the dog on an adequate calcium intake plus ammonium chloride and even somewhat thinner than the cortex of the low calcium control. The spongy trabeculae are thinner and the intertrabecular spaces wider in the animal on the

low calcium diet plus ammonium chloride than in the one which received an adequate calcium intake plus ammonium chloride. The spongy bone is even more delicate than in the low calcium control.

It was obvious in the gross as well as microscopically that rickets played no part in these experiments. Indeed our experience in this and in other series of tests has shown our meat diet, with the cod liver oil and tomato juice supplement, entirely adequate for growth and for prevention of deficiency diseases.

Microscopic Anatomy

In the study of the ribs, comparative examinations were made of the various animals within the same group. We were careful to make comparisons on the basis of the severity of changes at equal distances from the costochondral junction.

Three of four ribs from each of the dogs were subjected to histologic examination. Generally the 6th, 7th, 8th, and 9th ribs were taken, and a large piece including the costochondral junction was examined. Fixation in Helly's fluid, decalcification in nitric acid, and staining with hematoxylin and eosin.

18 Months Old Group.—There were slight but readily distinguishable differences in the ribs of all the animals within this group, but these were not very marked. The control showed the most compact bone with the narrowest vessel canals and the fewest osteoclasts. The dogs on the adequate calcium intake plus ammonium chloride showed the least deviation from the normal. In them the vessel canals were already moderately enlarged but on the whole quite smooth, and osteoclasts were few in number. The dog on the low calcium diet and that on the low calcium diet plus ammonium chloride showed, in those ribs examined, the greatest change from the normal. Their ribs displayed the most marked enlargement of the vessel canals, and the greatest amount of subperiosteal and subendosteal resorption. It is, however, striking that the ribs in none of the dogs of this group presented marrow fibrosis.

8 Months Old Group.—In this group, the adequate calcium control had the most compact ribs. The histologic examination showed that maturity had not as yet been reached, and that there was active bone formation at the costochondral junctions. Naturally, the vessel canals of the cortex had not taken their final narrow size, though they were smooth walled and there was, except in the region of the costochondral junction, a minimum of subperiosteal and subendosteal resorption. The other two animals in this group showed a slight but definite deviation from the control, and the one on the low calcium diet with the ammonium chloride probably showed this change more than the one on the adequate calcium plus ammonium chloride. The differences between these two animals receiving

ammonium chloride was evident, although not very pronounced. These deviations from the normal consisted of increased size of the vessel canals, some increase of the connective tissue within these enlarged canals, and some increased subperiosteal and subendosteal resorption. In none of the animals of this group was the marrow fibrosed.

6 Months Old Group.—The histologic examination of the ribs showed a distinct separation of the animals into two classes—those whose bones were normal or approached the normal, and those whose bones were resorbed and considerably fibrosed. The calcium intake was the factor that governed the separation of the animals into these two groups.

The control dogs receiving an adequate calcium intake showed the usual minimal transformation changes, limited especially to the vicinity of the costochondral junctions. These are to be expected in growing transforming ribs in normal animals. The cortices of such ribs were more compact as they receded from the costochondral junctions. The vessel canals were of varying widths, but on the whole their walls were smooth and there were a minimal number of osteoclasts. The dogs receiving the adequate calcium diet plus ammonium chloride showed only the slightest histologic deviation from the control. In these the administration of ammonium chloride caused at the most a slight cortical thinning and some slight osteoclast increase in the enlarged Haversian canals. The marrow was not scarred in either these or the control.

The control receiving a low or inadequate calcium intake showed pronounced changes characterized by a pathologic exaggeration of the normal resorptive and transforming phenomena observed in the bones of growing dogs. The rib cortices throughout the sections were thinned to a very pronounced degree; the vessel canals were increased in diameter and filled with cellular connective tissue; the walls of the vessel canals showed many lacunae containing osteoclasts. There was in addition considerable subperiosteal and subendosteal resorption. The marrow was very extensively fibrosed and the intramedullary trabeculae were thinned, deformed, and showed evidences of active resorption. The proliferating cartilage zones were not widened and there was active resorption and fibrosis immediately beneath the growing zones.

The changes in the ribs of those dogs receiving this low or inadequate calcium intake plus ammonium chloride were entirely in the same direction as those observed in the low calcium control. The changes were of the same nature and distribution, and possibly of slightly greater extent.

3 Months Old Group.—In this group the adequate calcium control showed the expected transformation changes. Normal rib ossification was in progress, resulting in the production of a more compact cortex at distances away from the most active zones of transformation (costochondral junctions). The dog receiving adequate calcium and ammonium chloride showed slight but definite resorption in excess of that present in the normal animal. This was characterized by some subperiosteal and subendosteal resorption and some increased connective tissue

in the vessel canals with increased osteoclasts within these canals. Furthermore there was a very slight tendency to marrow fibrosis near the costochondral junctions.

The animal receiving the low calcium diet plus ammonium chloride showed very extensive resorptive and fibrotic changes. Every rib examined had fractures and infractions in various stages of healing. Some of the long bones showed fractures and deformities in various stages of healing. The effects of this régime caused scarring of the marrow, thinning of the cortices of the ribs, marked enlargement of the vessel canals with increased connective tissue in them. Numerous osteoclasts were observed.

DISCUSSION

These experiments are in accord with the known fact that the administration of ammonium chloride induces porosis of bones. The age correlation is significantly demonstrated, as the effects of ammonium chloride diminish very materially as soon as the animal passes the young puppy stage. Smaller dosage per kilo sufficed to produce in young puppies very much more marked changes than could be produced by much larger doses in the adult.

The effect of ammonium chloride administration should not be considered, as it sometimes is, independently of the calcium intake or the calcium-phosphorus ratio. In this series of experiments, particularly in all animals 6 months and older, the level of calcium intake was most decisive in causing development or prevention of marked degrees of bone decalcification. In these dogs the differences between the low calcium and adequate calcium groups were frequently more obvious than the differences between dogs on an adequate calcium intake with and without ammonium chloride supplement. In the youngest puppies a definite effect of ammonium chloride in the adequate calcium group was evident, but even here the effect of the calcium withdrawal was considerably more drastic. The effects of administration of ammonium chloride with or without an adequate calcium intake led to gross fractures and deformities in only one of the youngest dogs. This animal received no supplementary calcium.

In none of these animals was there any evidence of rachitoid lesions which Bernhardt and Rabl (10) observed in young rats on a diet deficient in calcium and phosphorus, to which 2 per cent ammonium chloride was added as well as a small and, under the conditions of their experiment, possibly inadequate cod liver oil supplement.

The effects of ammonium chloride in causing an increased excretion of calcium are known. Furthermore the conclusion that the calcium comes from the skeleton is obvious. These facts have prompted certain clinicians to utilize ammonium chloride decalcification as a therapeutic vehicle towards the correction of bone deformities due to rickets (11). The ammonium chloride was generally given in doses of about 0.2 gm. per kilo body weight daily, but supplementary procedures, such as Bier's hyperemia daily for 15 to 20 hours, absolute rest in bed, and the application of constant traction and mechanical correction were used. It has been reported that by such non-surgical procedures some degree of correction of rachitic bow-legs is obtainable at a saving of time. It is more difficult to obtain these effects after the period of active rickets, and while osteoporosis may be obtained with ammonium chloride, it is more difficult to correct a deformity except by a long tedious usage of the auxiliary mechanical methods.

The histologic studies, furthermore, revealed a number of features that are helpful in understanding certain of the pathologic aspects of the general subject of osteoporosis. The feeding of an adequate calcium supplement led to a definite reduction in the rate of decalcification in all of our experiments. The older dogs receiving ammonium chloride and supplementary calcium showed a minimal number of osteoclasts and few Howship's lacunae. With an inadequate calcium intake and ammonium chloride, the ribs of such dogs showed increases in lacunae and osteoclasts and also in subperiosteal and subendosteal resorption. The same features held in the younger age groups whose animals receiving an adequate calcium intake tended to show less active resorption. In these animals also a low calcium intake led to the appearance of more osteoclasts and Howship's lacunae and the associated subperiosteal and subendosteal resorption. In the younger animals calcium insufficiency was found to be distinctly responsible for the rapidity with which the histologic pictures of resorption appeared, and for their severity.

When the decalcification was rapid, such degrees of marrow fibrosis and such extensive bone resorption resulted that the histologic picture could be designated as generalized ostitis fibrosa. The youngest animals in our experiments receiving an inadequate calcium intake plus ammonium chloride developed such lesions, while the older ones on

the same régime only developed what could be called simple osteoporosis.

This difference in effect in the various age groups brings up the question of what is to be comprehended by the term ostitis fibrosa, and also the still more important question of relationship to each other of the ostitides fibrosae produced by various procedures. We have previously reported on the production of ostitis fibrosa in guinea pigs and dogs through the administration of parathormone. These lesions are in a broad sense of the same histologic appearance as those that are produced in young actively growing dogs on a low calcium but otherwise adequate diet, although other important differences were noted. The administration of ammonium chloride to a puppy receiving an inadequate calcium diet will lead, when the diet is so protected that none of the vitamin deficiencies will result, to generalized ostitis fibrosa, histologically quite indistinguishable from that which results from an inadequate calcium intake alone. Furthermore, the amounts of calcium adequate for slow growth will, if these puppies are very young and actively growing, produce a histologic picture of ostitis fibrosa, different only in quantitative aspects from that which appears in a rapidly growing puppy on an inadequate calcium intake.

While the ostitides fibrosae produced by the above mentioned procedures lead to basically the same histologic pictures, the gross appearances of the dogs subjected to these varying régimes will be different. Dogs on low calcium intake, receiving parathormone or relatively large doses of ammonium chloride will show severe ostitis fibrosa associated with stunting and deformities. The stunting is due primarily to loss of appetite. It is significant therefore that a severe ostitis fibrosa will develop under the influence of ammonium chloride or parathormone in such animals whose growth has practically stopped. The ostitis fibrosa is here clearly attributable to a drastic decalcification unaided by the demands of rapid growth. In simple calcium deficiency a similar picture of ostitis fibrosa will be produced in an animal in which the calcium deficiency is aggravated not by parathormone nor by ammonium chloride, but by the physiological demands of bone growth.

We must conceive of generalized ostitis fibrosa as a rather inclusive category, embracing not only the clinical generalized form (von Reck-

linghausen's disease), which has a specific parathyroid etiology, and the experimentally produced ostitis fibrosa of similar origin, but also the experimentally produced conditions which are caused by other means leading to very rapid calcium depletion with resulting marked marrow connective tissue proliferation. Thus, either clinically or experimentally, if bone decalcification is very rapid—and this would hold in the young or in the adult—generalized ostitis fibrosa occurs. From this point on there may be modification of both the gross and histologic features of the ostitis fibrosa, due to the special underlying factor inducing the decalcification. Thus it is conceivable that hyperparathyroidism may in the course of the development of ostitis fibrosa favor incidentally very extensive hemorrhage into the marrow, resulting in brown blood cysts and the formation of giant cell tumors. It has been recently shown (12) that generalized ostitis fibrosa may exist without the other classical features of von Recklinghausen's disease (cysts and giant cell tumors) apparently on the basis of a hyperparathyroidism, as at autopsy parathyroid adenomas were found.

On the other hand, a slowly developing decalcification from whatever cause will lead, both in the young and adult, to a less severe lesion which is called osteoporosis, because of the absence of striking fibrosis. The occurrence of osteoporosis in adults under a number of circumstances is well known and one of the most common causes for it is Graves' disease. In this disease there is negative mineral balance, but the patients do not suffer from the effects of diminished calcium intake. The disease is chronic and the decalcification is slow but progressive.

In long tubular bones the same phenomena are observed. Relatively rapid decalcification has been found to occur in the metaphysis—the region of rapid bone growth and metabolism,—and slow decalcification in the diaphysis and epiphysis. Corresponding to these changes are the histologic pictures of ostitis fibrosa and osteoporosis observed in the respective portions of such long bones (6).

The modifying effects of several factors are also illustrated in experimental rickets. It is well known that rickets cannot be produced experimentally in rats by withholding vitamin D unless there is a simultaneous mineral imbalance (disturbance of calcium-phosphorus ratio). In very young rapidly growing rats with curative

vitamin D supplement, but on a low calcium diet, an ostitis fibrosa will develop. On the same régime with 1000 to 2000 rat units of vitamin D, the marrow fibrosis will be essentially prevented and simple osteoporosis will ensue. The same diet with the exclusion of vitamin D will produce rickets.

While these experiments have yielded comparisons of low calcium and high calcium régimes, it is recognized that similar results might have been obtained with a different type of calcium-phosphorus imbalance—namely on low phosphorus diets.

CONCLUSIONS

1. In all age groups the effects of ammonium chloride administration were found to be strikingly dependent upon the calcium intake.

2. Dogs receiving an adequate calcium diet and ammonium chloride showed less decalcification than those receiving a low calcium diet with or without ammonium chloride.

3. In the younger groups the added effect of ammonium chloride to calcium-low diet brought out more striking changes than a low calcium diet alone.

4. When the decalcification was less severe—in the oldest dogs on the low calcium diet with or without ammonium chloride, and in the younger dogs on an adequate calcium intake with ammonium chloride —generalized thinning of the bones without marrow fibrosis resulted (osteoporosis).

5. When the decalcification was rapid and severe—in the youngest dogs on low calcium diet, particularly with ammonium chloride— generalized decalcification and secondary marrow fibrosis resulted (ostitis fibrosa).

6. Generalized ostitis fibrosa is a rather inclusive term and may be applied to the histologic picture which results when clinical or experimental decalcification is rapid, and therefore leads to extensive marrow fibrosis.

7. The special underlying causes of the decalcification may incidentally contribute features to modify the generalized osteoporosis or ostitis fibrosa as in rickets and in von Recklinghausen's disease.

BIBLIOGRAPHY

1. Jaffe, H. L., and Bodansky, A., *J. Exp. Med.*, 1930, **52**, 669.

2. Jaffe, H. L., Bodansky, A., and Blair, J. E., *Arch. Path.*, 1931, **11,** 207.
3. Bodansky, A., Blair, J. E., and Jaffe, H. L., *J. Biol. Chem.*, 1930, **88,** 629.
4. Bodansky, A., and Jaffe, H. L., *J. Exp. Med.*, 1931, **53,** 591.
5. Jaffe, H. L., Bodansky, A., and Blair, J. E., *Arch. Path.*, 1931, **12,** 715.
6. Jaffe, H. L., Bodansky, A., and Blair, J. E., *J. Exp. Med.*, 1932, **55,** 139.
7. Haldane, J. B. S., *J. Physiol.*, 1931, **55,** 265.
8. Bernhardt, H., *Z. klin. Med.*, Berlin, 1924, **100,** 735.
9. Bodansky, A., Jaffe, H. L., and Chandler, J. P., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29,** 871.
10. Bernhardt, H., and Rabl, C. R. H., *Klin. Woch.*, 1925, **4,** 758.
11. Rabl, C. R. H., *Arch. klin. Chir.*, 1925, **137,** 619.
12. von Schupp, H., *Deutsch. Z. Chir.*, 1931, **233,** 195.

EXPLANATION OF PLATES

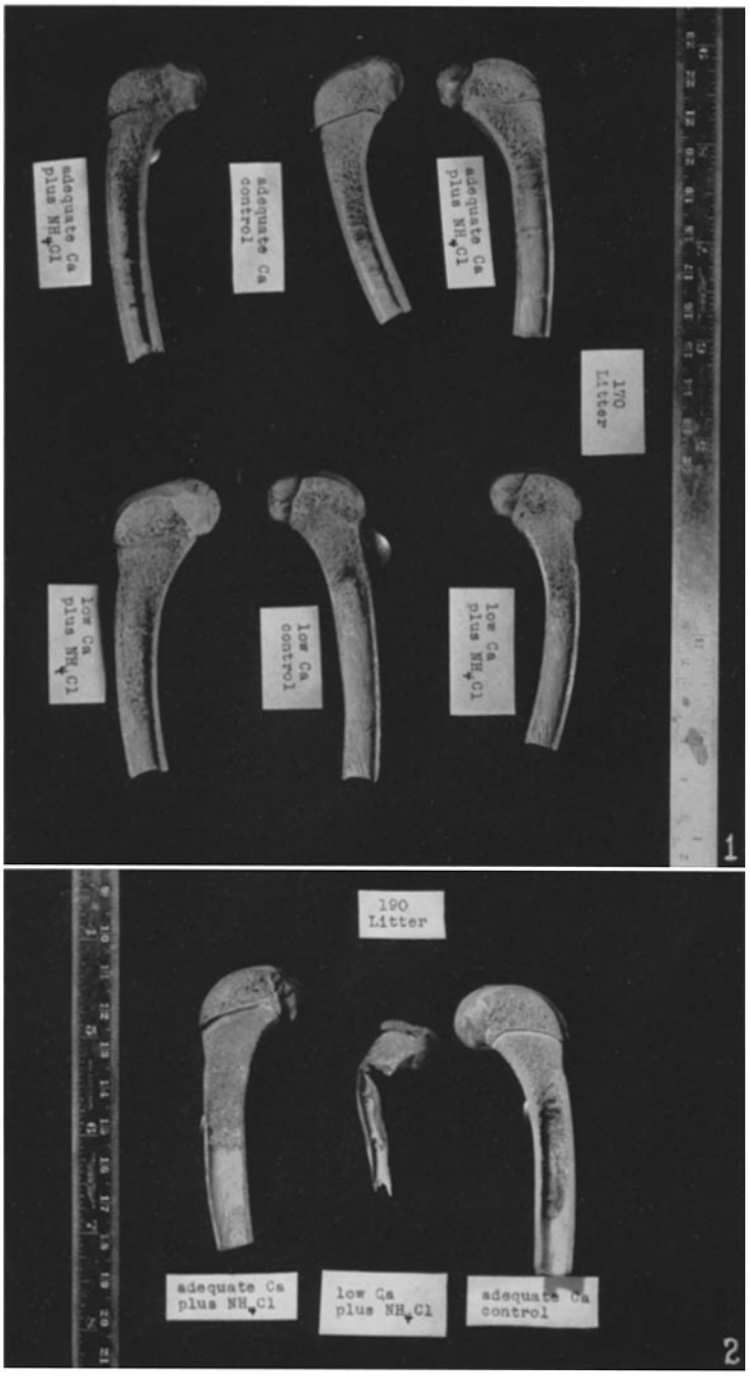
PLATE 41

FIG. 1. Photograph of the distal portions of the femora of the 6 months old dogs. Macerated specimens.

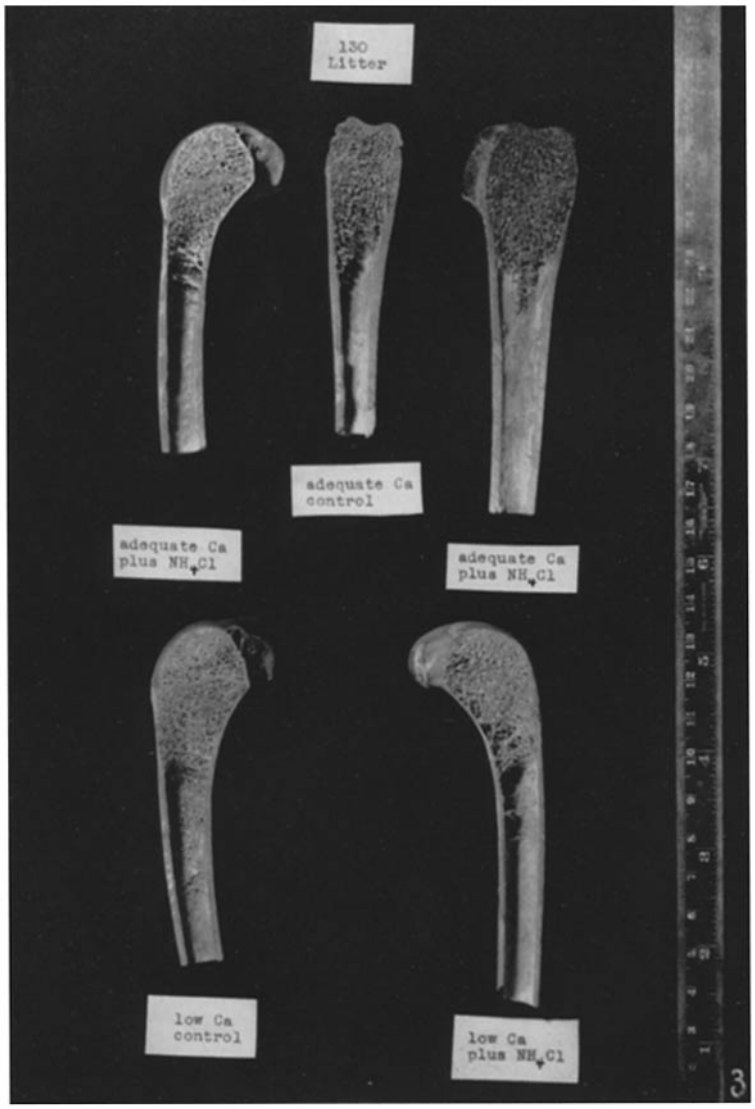
FIG. 2. Photographs of the distal portions of the femora of the 3 months old dogs. Macerated specimens.

PLATE 42

FIG. 3. Photographs of the distal portions of the femora of the 18 months old dogs. Macerated specimens.



(Jaffe *et al.*: Ammonium chloride decalcification)



(Jaffe *et al.*: Ammonium chloride decalcification)