EXPERIMENTAL FIBROUS OSTEODYSTROPHY (OSTITIS FIBROSA) IN HYPERPARATHYROID DOGS

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Ostitis fibrosa has been reported to occur spontaneously in animals other than man. Unsuccessful attempts have also been made to reproduce this condition by various experimental means. We have now produced the changes of true generalized ostitis fibrosa in guinea pigs and dogs (1-4). The experimental part of the present report describes in detail our results with dogs.

Spontaneous Occurrence of Ostitis Fibrosa in Animals

The bone changes of spontaneous ostitis fibrosa resemble in many ways, but frequently do not duplicate those observed in the fibrous osteodystrophies of man.

With regard to the appearance of osteoid tissue in the lesions observed in the spontaneous fibrous bone diseases of animals, Ingier (5) and Pick (6) stated that such lesions can be called ostitis fibrosa, even in the absence of osteoid tissue. Peculiar to some animals with fibrosed bones is the common development of obstructions of the nasal and oral cavities, as these are encroached upon by thickening of their walls. As a result such animals may have sniffing respiration. However, fibrous bone lesions exist in animals not afflicted with sniffing respiration. Christeller (7), Rehn (8), Hintze (9), Busolt (10) and Ingier (5) described swine, goats, cattle and horses whose bones showed either true generalized ostitis fibrosa, or a local form of true ostitis fibrosa. In most instances only skulls were studied, but in a small number of cases complete examination of the skeleton was made. In these cases generalized ostitis fibrosa was found.

Koch (11) and Christeller (7) described spontaneous ostitis fibrosa in monkeys, Koch pointing out that this condition had been previously confused with rickets. White (12) detailed three cases of ostitis deformans in monkeys. Her cases were typical examples, both clinically and pathologically, of Paget's disease.

Dogs are also subject to spontaneous ostitis fibrosa. Christeller described a dog whose bones were thin and porotic. The long tubular bones showed curva-
ture and diminished longitudinal growth. In these bones the lesions were most prominent in the upper and lower portions of the diaphysis, where there was fibrous replacement of the marrow, transformation of the existing bone, new bone formation, bone resorption with numerous Howship’s lacunae and osteoclasts. The haversian canals of the cortex were widened and invaded by fibrous tissue. The epiphyseal cartilage plates were present and relatively normal endochondral ossification was still in progress. The entire skeleton was involved, but the ribs showed relatively the greatest alterations.

Schmey (13) and Pick (14) described an atrophic deforming generalized bone disease in senile dogs with a predominance of osteoporosis, but with little fibrosis. Hager (15) described a case of ostitis fibrosa in a dog whose upper and lower jaws were tremendously enlarged. Weber’s (16) dog, 8½ months of age, had spontaneous fibrous osteodystrophy with thickened porotic bones, cysts and giant-cell tumor formations. The jaw of his dog was also enlarged and cystic. The parathyroids were normal, and calcium metastases were not observed.

Attempts at Experimental Production of Ostitis Fibrosa

Many attempts have been made to reproduce experimentally all or some of the lesions of ostitis fibrosa—that is, cysts, hemorrhages, giant-cell tumors, marrow fibrosis, bone resorption and osteoid tissue.

Traumatization of the marrow has been extensively tried with the view of producing bone cysts or local ostitis fibrosa, but Lexer (17), Lotsch (18), and Nissen (19) have shown conclusively that injury, and the resultant hemorrhage into the marrow, do not lead to local cyst formation. Contrary to the opinion of Konjetzny (20) and Pommer (20, it seems logical to conclude that before an intramedullary hemorrhage results in local cyst formation there must be a generalized or an extensive local disease of the bone. The injection of fluids or the introduction of foreign bodies into the marrow cavity, injury of the bone or marrow by X-ray, by radium, by toxins developed in parabiotic animals, or by toxins of injected bacteria have led neither to true generalized ostitis fibrosa nor to true local ostitis fibrosa. The scarring, the marrow degeneration, or the cyst formation that may result from such treatment are only local reactive manifestations, having nothing to do with genuine ostitis fibrosa.

One-sided diets were among the first means employed in attempts at the experimental production of dystrophic bone diseases in dogs. Calcium deficiency was generally the underlying principle of the treatment. In most of the experiments the dogs received horse meat, fat or carbohydrates, and distilled water. The lesions produced in the various experiments were quite uniform. The older reports lacked detailed histological descriptions (22), and designated the changes produced as rickets or a ricket-like disease. Dibbelt (23) did not make a diagnosis. It remained for Korssakoff (24) to point out that the condition produced in dogs on such a diet was osteoporosis and not rickets. Miva and Stoeltzner (25) studied
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in great detail the changes produced by this diet and came to the same conclusion. They described marked osteoporosis with fibrosis of the marrow, but indicated that lacunar resorption was not severe enough to establish the lesion as ostitis fibrosa.

On a calcium-poor diet we, like some of the authors mentioned, have produced osteoporosis in dogs. These were fed lean meat but were protected against vitamin deficiency. On the other hand, in dogs which we maintained for a long period in a state of chronic hyperparathyroidism—both on adequate and calcium-poor diets—we produced generalized ostitis fibrosa. The clinical course, the evolution of the bone lesions, the gross and microscopical appearances of the bone and marrow were quite different in the two groups of animals, and indicated the specificity of the parathyroid extract treatment as the cause of experimental ostitis fibrosa.

The Relation of the Parathyroid Glands to Bone Dystrophies

Askanazy (26) was the first to suggest the etiological significance of a parathyroid adenoma found by him in a case of ostitis fibrosa. After his finding, many other instances of parathyroid enlargement were reported in association with ostitis fibrosa deformans, ostitis fibrosa cystica, osteomalacia, and rickets. Parathyroid enlargement has also been noted in rats suffering from experimental rickets.

Recently Barr and Bulger (27) summarized from the literature the cases with parathyroid tumors. They added 29 cases to those previously collected by Hoffheinz (28), making a total of 74 cases with pathologically enlarged parathyroid glands. These included both malignant and benign tumors, as well as enlargements due to simple hyperplasia. Authentic malignant growths of the parathyroids are extremely rare, and it is significant that none of them have been associated with evidence of functional derangement of the parathyroid glands as reflected in bone lesions. The majority of enlarged parathyroids reported are probably due to functional hyperplasia. The association of many diseases of bones with parathyroid hyperplasia or with benign tumor of the parathyroid glands is striking. Barr and Bulger state that 60 per cent of all cases of parathyroid enlargement had evidence of bone lesions. Clinically the most frequent as well as the most striking association is with the generalized form of ostitis fibrosa cystica—that is, the so-called v. Recklinghausen's disease.

The consensus of opinion until recently was that the parathyroid enlargement observed in association with these bone diseases was of a secondary nature, and appeared as a result of a compensatory hypertrophy due to the bone deficiency. In 1926 Mandl (29) brought clinical evidence to dispute this view. He removed a parathyroid tumor in a case of ostitis fibrosa cystica and reported rapid clinical
improvement of his patient, with decrease of urinary calcium excretion. He was the first to suggest, on the basis of his tests, that the skeletal changes in clinical cases were caused by a disturbed parathyroid function associated with the enlargement of the parathyroid gland.

Mandl's observations were soon confirmed and his conclusions widely accepted. A rapidly increasing series of reported cases supports the hypersecretion hypothesis. Gold (30), Barr, Bulger and Dixon (31), Barr and Bulger (27), Wilder (32), Boyd, Milgram and Stearns (33), Snapper (34), Richardson, Aub and Bauer (35), and Compère (36) reported cases showing a varying amount of improvement following extirpation of enlarged or even normal-sized parathyroids when hyperparathyroidism was diagnosed.

Hypercalcemia, and negative calcium balance when this has been demonstrated, may be attributed to the increased secretion of its active principle by the enlarged parathyroid gland. The case reported by Richardson, Aub and Bauer (35), by Bauer, Albright and Aub (37), by Hannon, Shorr, McClellan and DuBois (38), and by McClellan and Hannon (39) is important. In this case, which presented a clinical picture of hyperparathyroidism, no parathyroid tumor was found, and two normal appearing parathyroid glands were removed. The improvement in the condition of the patient was so marked that it indicated to these authors that hyperparathyroidism may result from hyperfunctioning of glands of normal size and appearance. The possibility exists that some undiscovered or abnormally active accessory parathyroid tissue may produce hyperparathyroidism in clinical cases where a tumor is not found after exploration.

A review of the symptoms in reported cases diagnosed as suffering from hyperparathyroidism, and of the clinical and chemical findings, reveals certain conditions common to all. Among these are: progressive muscular weakness, general lassitude and hypotonia; pain in and bowing of the lower extremities; resorption of the skeletal bones, frequently shown to be associated with hypercalcemia and with a negative mineral balance. Following operation the calcium balance, when this was studied, generally became positive. Fractures which occurred during the course of the disease healed very slowly (30, 31, 40) but soon after parathyroidectomy a fractured femur united firmly (34). Following removal of the parathyroid tumor, X-ray showed increased density of the bones in some of the cases (30, 31, 32, 34, 35). No positive evidence of healing of the bone cysts was included in the reported cases. Giant-cell tumors of the bones were noted in the cases of Wilder and of Barr, Bulger and Dixon, and these were found to be healed within a few months after removal of the parathyroid tumor.

Some of the reports cited above, available to us when our work began, indicated clearly that parathyroid adenomas or parathyroid hypersecretion were etiologically related to ostitis fibrosa cystica. We therefore felt that injections of parathyroid extract into experimental animals might produce bone lesions similar or analogous to
those found in clinical osteitis fibrosa, provided the animals could be maintained in a state of hyperparathyroidism without succumbing.

It is interesting to note that although a number of investigators injected parathormone into experimental animals for long periods, and although increased excretion of calcium at the expense of the bones is well established, no investigator has studied the bones of such animals histologically.

Experimental work with parathormone by Collip (41), Greenwald (42) and others had demonstrated that its injection led to hypercalcemia and to increased excretion of calcium and phosphorus, particularly in the urine; to lowered serum phosphorus and hypotonicity. Overdosage phenomena had also been described by Collip, Hueper (43) and Learner (44) in experimental animals (vomiting, diarrhea, hematuria, hemorrhagic gastro-enteritis, kidney impairment and hyperphosphatemia, and extensive metastatic calcification in the soft tissues, especially the kidneys, lungs and gastro-intestinal tract).

A careful study of the effects of daily injections of parathormone on rabbits, kittens and rats was made by Bauer, Aub and Albright (45). They were primarily interested in the question of whether the trabeculae serve as a reserve supply of calcium. After continued parathormone administration to rabbits for 91 days, during the major part of which they received 8 units daily, the trabeculae were reported as diminished in number, but no effect was noted on the cortex of the bone either in the gross examination or by X-ray. In growing kittens the administration of parathormone, on the average of 25.4 units daily for 56 days, did not result in any greater reduction of the trabeculae than observed in the course of normal bone growth. In the growing rat the administration of parathormone, 885 units over 110 days, resulted in diminution of the length of the bones and in an increase of the number of trabeculae. The serum calcium of the treated rabbits was higher than in the controls, while the serum calcium of the treated kittens was reported lower than in the controls, but there were no changes in the rats. They concluded, in spite of the fact that their own data do not seem to support their view, that bone trabeculae are easily depleted by prolonged administration of parathormone, and that these trabeculae serve as the source of readily available calcium.

Ingier (5), Christeller (7), Stenholm (46), Pick (6), Schmorl (47) and others emphasize the necessity of making histological studies of bone even in human cases, but especially in animal bone, before drawing conclusions regarding bone changes. Our own experience confirms
them. We have been frequently surprised at finding, upon histological examination, extensive resorption of cortical bone in our experimental animals, when the gross examination did not show anything obviously unusual. We believe that the results of Bauer, Aub and Albright, without the evidence of an histological examination, cannot be accepted as proving the absence of cortical resorption. Aub, Bauer and Albright based some of their conclusions on absence of hypercalcemia and on negative gross examinations. That these are not a proof of lack of parathormone effects is shown by our results in guinea pigs, which while receiving doses not large enough to produce hypercalcemia, still showed cortical bone resorption microscopically (1, 2, 48). In dogs as well (Nos. 3 and 4, see protocols), cortical resorption was produced on 2 units of parathormone daily for 20 days, although hypercalcemia was never observed.

Experimental Methods

We started with puppies 6 to 9 weeks of age, weighing 1 to 2 kg. The diet consisted of lean meat fed daily in amounts of about 15 per cent of body weight. Later, when active growth ceased, the amount fed was reduced to 10 per cent of body weight. These amounts corresponded actually to feeding ad libitum; the meat was frequently not consumed in full. Records of meat consumption were kept. The meat was supplemented daily with 1 cc. of cod-liver oil and at least 50 gm. of canned tomato. During various phases of the experiment known supplements of calcium were given in the form of calcium lactate by stomach tube, or calcium lactate and bone meal were added to the meat. The dogs were kept under carefully controlled hygienic conditions.

Serum calcium and phosphorus determinations were done at frequent intervals. The Clark-Collip modification of the Kramer-Tisdall method was employed in the calcium analyses, and the Benedict-Theis method in the phosphorus analyses. If the animal was receiving parathormone, the blood was generally examined about 18 hours after the last injection, the animals being fasted during that interval. The parathormone was injected subcutaneously in daily doses, which were increased as indicated by the course of the experiment.

The tissues and bones were fixed in neutral formalin or in Helly's fluid. The bones fixed in formalin were decalcified in Mueller's fluid plus 5 per cent glacial acetic acid. Those fixed in Helly's fluid were decalcified in 5 per cent nitric acid. When tissues were stained for calcium, they were fixed in 95 per cent alcohol and the v. Kossa method was used. Paraffin sections were always made. The details of these methods as employed in this laboratory have been described (49).
Experimental Results

After having gradually increased the daily dose of parathormone, we found that the dog could tolerate relatively large doses of the extract without showing hypercalcemia. We attribute the production of experimental ostitis fibrosa in dogs to the increased parathormone effects obtained by prolonged administration of relatively large doses of the extract. Otherwise the production in dogs of ostitis fibrosa would be difficult, because doses of parathyroid extract large enough to produce rapid resorption of the bone and injury to the marrow, lead in the dog to fatal hypercalcemia before there is much fibrous repair.

The parathormone was injected in a daily dose, at first usually of 2 units per kilogram, which was raised gradually in some dogs to as high as 5 units per kilogram towards the end of the experimental period of 5 to 6 months. Repeated small daily injections of parathormone at first usually raised the serum calcium to about 15 or 16 mg. per 100 cc. It was sometimes necessary to interrupt the parathormone treatment because of loss of appetite and other symptoms of overdosage. As treatment was continued, the serum calcium gradually fell to normal values or lower, and larger doses could be given with only a temporary elevation of serum calcium. This condition was produced most rapidly in one dog on a low calcium intake. In that dog (No. 8), serum calcium indeed dropped to as low as 7.8 mg. per 100 cc., and seemed to be associated with a tissue calcium lowered sufficiently to account for the tremors and tetany observed during the experiment (see protocol).

Immunity acquired as a result of previous parathormone treatment cannot be inferred from the normal serum calcium values in dogs treated for a long time with parathormone (Nos. 9 and 10), and from the hypocalcemia observed in one instance (No. 8). For when the calcium reserves were allowed to replenish after discontinuing parathormone for a short period, parathormone injections had their usual effect of raising the serum calcium (see protocols).

We produced, depending upon the dosage and the length of time under parathormone, all degrees of change from mild to severe bone resorption, and from slight fibrous replacement to degeneration of the marrow with hemorrhage, when the animals died from overdosage. Finally, we produced typical ostitis fibrosa cystica in several dogs. The most pronounced lesions were observed in three dogs that were
injected for 5 to 6 months. At the end of their experimental periods they were receiving 20 units daily.

One of the most marked clinical features in all these young growing dogs was the stunting of their growth, which was referable to a cessation of bone formation, and therefore of growth at the epiphyseal cartilage plates. After prolonged treatment the bones were deformed, and the forelegs showed lateral bowing. Such animals had pronounced hypotonicity of the muscles and developed plantigrade stance. In addition there was frequently torsion of the tubular bones on the long axis. The bone changes were generalized and lesions were found in the jaw bones, the skull, and all the long tubular bones studied. The ribs in the vicinity of the costochondral junctions offered the best material for study. In the long tubular bones, the fibrous changes were present only in the diaphysis and metaphysis. The epiphyses showed only a mild degree of simple resorption of the trabeculae and the marrow remained entirely free of fibrous changes. It is important to emphasize that none of our dogs had swellings at the epiphyseal cartilage plates, nor were there any indications of widened metaphyses or costochondral junctions.

In view of the frequent confusion in interpretation of the histological picture found in clinical or experimental bone dystrophies diagnosed as osteomalacia, osteoporosis and osteitis fibrosa, it is important to emphasize the criteria which must be satisfied by the lesion to which the term generalized osteitis fibrosa is applied. These are (a) resorption of the existing spongy and cortical bone, (b) invasion of the enlarged haversian spaces and of the marrow canal by fibrous tissue, and (c) the presence of Howship's lacunae, containing osteoclasts, on the walls of the haversian spaces, on the inner and outer surfaces of the compacta, and on the surfaces of the spongy trabeculae. New bone formation (osteoid tissue), as a substitute for the original lamellar bone, and cysts and hemorrhages in the marrow cavity, while frequently found, are in our opinion not essential for the diagnosis of osteitis fibrosa. Osteoid tissue appears as a result of the reparative process going on in bones subject to osteitis fibrosa, and may be absent, slight or extensive, depending on the functional need for reinforcement by osteoid repair.

Upon histological examination all the stated essential criteria of
ostitis fibrosa were found to be present in the dogs suffering from chronic hyperparathyroidism.

The fibrous bone changes were produced in dogs on a high or low calcium intake. In one dog (No. 7), receiving 650 mg. of calcium daily in the form of calcium lactate administered by stomach tube, considerable fibrous replacement of the bone was observed on a dose of 8 units of parathormone daily during the last 30 days of the parathormone treatment. The production of ostitis fibrosa was enhanced in the dogs by depleting their calcium stores more rapidly through the diminution of the calcium intake, which permitted giving larger doses of parathormone without danger of fatal hypercalcemia. When this condition is realized, an essential prerequisite exists for the production of ostitis fibrosa by parathyroid extract.

That the changes observed could not be attributed to calcium deficiency alone is evident from examination of bones of control dogs kept for months on a low calcium diet. These animals developed osteoporosis, which is histologically quite different from ostitis fibrosa cystica. Furthermore, by the use of parathormone, we have produced ostitis fibrosa in guinea pigs that were receiving a normal diet of adequate calcium content (1, 2).

PROTOCOLS

In the following protocols of 10 dogs on parathyroid treatment, only those data have been included which seemed necessary to a clear understanding of the means employed in the experimental production of ostitis fibrosa and of the results observed in the bones and tissues. The procedure had necessarily to be suited to each animal and was necessarily modified by developments. The central object was to preserve the life of the animal long enough to elicit the effects of experimental hyperparathyroidism on bone. A detailed presentation and discussion of the chemical data obtained will be published elsewhere, but sufficient data are given here to throw light on the clinical course of experimental hyperparathyroidism. The serum phosphorus data are given only when they indicate an exceptional condition. Some dogs died during the course of the experiments. With others, the experiments were terminated by administration of ether.
Dog 1.—Initial weight, 2.3 kg. Weight at the conclusion of the experiment, 11 days later, 2 kg. Calcium supplement, 600 mg. in the form of bone meal and calcium lactate. Four units of parathormone daily for 6 days and 8 units for 4 days. The dog ate all food until the 9th day, when complete anorexia appeared. The dog died on the morning of the 11th day. Serum calcium, 17.0 mg. per 100 cc. on the 10th day.

Autopsy showed loss of abdominal fat, involution of the thymus, and decrease of the external parathyroids to about one-half of the usual size. An hemorrhagic area was present in the left upper lobe of the lung; the kidneys were pale; the bones broke easily. Histological examination showed bone resorption, but the marrow changes formed the most striking feature. There was extensive necrosis of the marrow of all the bones examined. The marrow injury was most marked at the metaphyses and costochondral junctions. It was least pronounced or absent in the epiphyses. Fragmentation of the nuclei of the marrow cells was a prominent feature. The megakaryocytes were very badly degenerated and had entirely disappeared from some sections. Numerous mature neutrophilic and eosinophilic leukocytes appeared in the marrow. The erythrogenic cells were also degenerated. The connective tissue reticulum of the marrow was proliferated. There was diapedesis of red blood cells, particularly in the metaphysis, extensive dilatation of blood vessels, and considerable free marrow hemorrhage. Small collections of fibrin were observed in the hemorrhagic areas, and phagocytes also appeared in the marrow. The osteoclasts were large and numerous and for the most part not degenerated, indicating that they were reactive cells. In addition to the very extensive degeneration of the marrow, the osseous tissue of the ribs and tubular bones showed evidences of very rapid decalcification. The trabeculae and the walls of the haversian canals were nearly completely stripped of cells. The cartilage cores in the areas of endochondral ossification were either nearly bare or bare by the resorption of the previously deposited fiber-bone. New endochondral bone formation had ceased. This bone resorption was undoubtedly due mainly to vascular processes. Osteoclasts and Howship's lacunae were present, but mostly under the periosteum. Embedded and ground-disk cross-sections from the middle of the diaphyses of the long tubular bones showed enlargement of the haversian canals with Howship's lacunae containing osteoclasts. Examination of the soft tissues demonstrated extensive degeneration of the centers of the Malpighian corpuscles of the spleen, with congestion and leukocytic infiltration of the pulp; involution atrophy of the thymus; congestion of the lymph nodes; and congestion, leukocytic infiltration and pigmentation of the liver. The kidneys showed some calcium casts in the tubules. There was evidence of pneumonia, and the suprarenals were congested, with leukocytic infiltration. The tissues were not fixed or stained especially for calcium.

Summary.—This dog died of acute hyperparathyroidism. One of the most striking changes was in the bone marrow, which was ex-
tremely degenerated and hemorrhagic. The bones showed extensive
decalcification. There had as yet been little marrow repair. The
lymphoid tissue of the spleen was also degenerated.

**Dog 2.**—Initial weight, 1.65 kg. Weight at conclusion of experiment, 14 days
later, 1.4 kg. No supplementary calcium was added to the diet. Four units of
parathormone were given for 6 days, and 6 units for 3 days. On the 9th day the
dog lost its appetite. On the 10th day it had bloody stools, and its serum calcium
was 16.5 mg. per 100 cc. The dog died on the 14th day. Autopsy showed involution
of the external parathyroids to about one-half the usual size and bilateral
pneumonia and fibrinous pleurisy on the left side. Histological examination
showed numerous degenerated kidney tubules containing calcium casts. Some
glomeruli contained calcium deposits. The spleen was scarred, and showed evi-
dence of active phagocytosis and leukocytic infiltration. The lungs contained
patches of pneumonia and granules of deposited calcium. The bones were not
studied. The parathyroids showed simple involution atrophy.

**Summary.**—This dog died of acute hyperparathyroidism complicated
by bronchopneumonia. The soft tissues showed metastatic calci-

**Dog 3.**—Initial weight, 1.95 kg. Weight at the termination of the experiment,
21 days later, 2.3 kg. Calcium supplement, 200 mg. daily, in the form of bone
meal and calcium lactate. Injected 2 units of parathormone daily for 20 days.
Serum calcium was 12.4, 11.7, and 12.8 mg. per 100 cc., respectively on the 11th,
18th and 21st days. The dog was killed to terminate the experiment. Autopsy
showed a good state of nutrition. The organs and bones showed nothing unusual
in the gross. The epiphyseal cartilage plates were thin. The thick cortex of the
shafts of the long bones appeared compact in the gross. But when compared with
a normal litter control, ground disks made from sections taken from the middle of
the diaphysis of the long tubular bones showed definite microscopical evidence of
resorption even of the apparently compact cortex. The Schwalbe and haversian
canals were enlarged, but in stained sections no excessive connective tissue in-
vasion of the canals was observed. In the stained sections of the long tubular
bones no disturbance of endochondral ossification and no fibrous replacement of
the marrow were found. The ribs, however, even with the small doses of para-
thalmone received by this dog, showed degeneration of the blood-forming cells
of the marrow, with destruction of the megakaryocytes which were being phago-
cytosed. There was fibrosis of the marrow, with the appearance of numerous
leucocytes and eosinophiles. At the costochondral junctions rapid decalcification
had taken place, and the more compact bone showed evidence of lacunar resorption
with osteoclasts. The soft tissues showed nothing very unusual. Some kidney
tubules contained calcium casts.
Summary.—Pronounced effects on the bone and marrow were observed even when parathormone was given for a short period in doses not sufficient to induce a hypercalcemia. The bone resorption was generalized and quite evident in the cortex of the diaphysis. The marrow changes were strikingly present in the ribs.

Dog 4.—Initial weight, 1.7 kg. Weight at the conclusion of the experiment, 21 days later, 1.95 kg. Calcium supplement, 600 mg. daily (bone meal and calcium lactate). Two units of parathormone were injected daily for 20 days. The serum calcium was 14.2, 12.4, 12.2, and 12.0 mg. per 100 cc., respectively, on the 11th, 12th, 18th and 21st days. The animal was killed to terminate the experiment when it was in excellent condition. Grossly neither the bones nor the soft tissues showed anything unusual. In ground disks of sections from the middle of the shafts of the long tubular bones resorption, as evidenced by enlarged vessel canals, was observed. Stained longitudinal sections of the long tubular bones showed some resorption of the compact and spongy bone, but connective tissue scarring was absent. The ribs, as in Dog 3, showed destruction of marrow cells, rapid decalcification at the costochondral junctions, osteoclasts and Howship's lacunae. There were calcium metastases in the kidneys. Otherwise the soft tissues showed no unusual features.

Summary.—In this dog, as in Dog 3, bone resorption and marrow fibrosis occurred after small doses of parathormone.

Dog 5.—Initial weight, 1.3 kg. Weight at conclusion of the experiment, 38 days later, 2.5 kg. Calcium supplement, 280 mg. daily for 5 days (in the form of bone meal), and 100 mg. (bone meal and calcium lactate) to the end of the experiment. The animal ate all its food until the 37th day, when it suddenly developed complete anorexia. The dog received 2 units of parathormone daily for the first 7 days, then 4 units daily for 17 days, 8 units daily for 4 days, 12 units daily for 6 days, and finally 16 units daily for 2 days. On the 37th day the parathormone was discontinued. At this time the dog was not obviously moribund. It was found dead on the 38th day. On the 12th day the serum calcium was 12.0 mg. 100 cc.; on the 28th, 11.6 mg; and on the 34th day 11.0 mg. The blood obtained post-mortem showed a serum calcium of 13.6 mg. The dog probably died about 30 hours after the last injection. The urine obtained from the bladder at autopsy was acid (total titratable acidity equivalent to 660 cc. N/10 hydrochloric acid per liter). The autopsy showed that the dog was well nourished. There was considerable oedema of the subcutaneous tissues of the neck. The external parathyroids were reduced to from one-third to one-half their usual size. The lungs showed hemorrhagic and oedematous areas. The rest of the soft tissues presented nothing unusual in the gross. The bones were soft and brittle, and broke easily; the skull bones were easily cut with the scissors. Histological examination of the
bones showed rapid decalcification. The subepiphyseal cartilage plate trabeculae were reduced to cores of cartilage. New bone formation had ceased at the epiphyseal cartilage plates and costochondral junctions. The marrow was necrotic in places. Much of the cellular marrow had disappeared from the diaphysis. The marrow was fibrosed and contained leucocytes. The resorption of the bone was shown by the naked trabeculae and by the enlarged haversian canals. Numerous osteoclasts in Howship’s lacunae were observed, but the hemorrhages and the vascular dilatations indicated that much of the resorption was vascular. While there was extensive resorption even in the shafts of the tubular bones, no osteoid tissue was observed in the marrow. The thymus was involuted. The lungs showed bronchitis and broncho-pneumonia and irregular collections of calcium were observed in the inflammatory exudate. The spleen was somewhat fibrotic and there was considerable necrosis of splenic cells. The parathyroids showed simple involutionary changes; the cells were to a great degree spindle-shaped.

**Summary.**—The dog undoubtedly died of hypercalcemic shock due to overdosage. It showed the acute effects of parathormone on the bone and marrow, superimposed upon the chronic effects of the previous treatment. A striking feature was the absence of osteoid tissue, which apparently does not appear until there is pronounced decalcification necessitating repair.

*Dog 6.*—Initial weight, 1.1 kg. The dog attained a weight of 2.1 kg. about 2 weeks before its death, which occurred 65 days after the beginning of the experiment. At autopsy it weighed 1.8 kg. The calcium supplement consisted of 280 mg. calcium in the form of bone meal for 5 days; of 100 mg. of calcium (bone meal and calcium lactate) for the next 36 days; it was increased to 1200 mg. for 5 days; no calcium was given for 2 days, and finally 650 mg. were given in the form of 50 cc. of a 10 per cent calcium lactate solution by stomach tube daily for 17 days. The dog received 2 units of parathormone for the first 7 days, 4 units for 5 days, the serum calcium rising to 14.8 mg. per 100 cc. on the 12th day of the experiment. Parathormone was discontinued for 7 days and was again begun on the 19th day of the experiment at 2 units daily. This dose was continued for 6 days, followed by 4 units daily for 4 days, 6 units daily for 6 days, and 10 units daily for 7 days. The serum calcium on the 27th, 33rd and 41st days was 12.2, 11.2 and 10.6 mg. per 100 cc., respectively. There was a progressive drop in spite of increased parathormone dosage, which was probably due to the fact that the dog was getting only about 100 mg. calcium daily during this period. The last serum calcium value was obtained just before the calcium supplement was increased to 1200 mg. daily on the 41st day. On the 43rd day, the dog still being on 10 units of parathormone daily, the serum calcium rose to 15.3 mg. per 100 cc. The parathormone was discontinued for 4 days. Treatment was resumed on the 47th day, with 6 units daily. The calcium intake was reduced.
to 650 mg. by stomach tube. On the 51st day of the experiment serum calcium of 19.0 mg. per 100 cc. was found, and 18.5 mg. on the following day. On continuing the treatment, the serum calcium dropped to 12.6 mg. by the 58th day and on the 61st day it was 11.3 mg. The dog showed pronounced loss of appetite on the 57th day and left almost all of its food from the 62nd day on. It died on the 65th day. 2 hours before death the serum calcium was 15.8 mg. and the serum phosphorus 11.9 mg. per 100 cc. (The coincidence of these high values is a common observation in fatal hyperparathyroidism.)

At autopsy the external parathyroids seemed to be slightly smaller than usual. There were patches of pneumonia in the left lower, and right lower and middle lobes of the lung. The heart muscles were pale and oedematous. The kidneys were yellowish, the markings indistinct, and throughout the kidney white specks, apparently calcium deposits, were seen. There was an intense gastro-enteritis. The stomach wall was thickened to about five times normal. The stomach mucosa was extremely injected, and showed numerous mottled light-yellow areas, apparently containing calcium deposits. The injection of the intestines extended to the end of the ileum; the sigmoid and colon were free. The bones were softened, and broke easily. There were no swellings at the costochondral junctions.

The ribs on histological examination showed marrow fibrosis, but some lymphoid marrow was still present. The cortical bone was resorbed, and there were numerous Howship's lacunae and osteoclasts. Endochondral ossification was still in progress, the new formed trabeculae being lined by osteoblasts. Sections of the long tubular bones also showed marrow fibrosis and bone resorption, but to a lesser degree than the ribs. In ground-disk sections from the middle of the diaphysis enlarged vessel canals were found. The lungs were consolidated and calcium deposits were present in the inflammatory exudate. The stomach and intestines were congested, necrotic in places, and calcium deposits were found in the mucosa. The kidney tubules contained calcium casts. The parathyroid sections showed involutional atrophy of the parenchymatous cells.

**Summary.**—On a moderate calcium intake parathormone dosage was increased rapidly without adverse effects. When the calcium intake was increased, the serum calcium rose at first, but soon declined to normal values. However, the dog was apparently in a state of severe hyperparathyroidism for about 1 week before its death while it showed normal serum calcium values. There was terminal hypercalcemia and hyperphosphatemia. At autopsy the severe effects of acute hyperparathyroidism were observed in the soft tissues, while the bones showed the effects of chronic hyperparathyroidization. It is noteworthy that osteoid tissue formation was not prominent.

**Dog 7.**—Initial weight, 1.5 kg. Killed accidentally 106 days later, when it weighed 4.35 kg. The diet was supplemented with 1400 mg. of calcium in the form
of bone meal for the first 5 days. During the next 64 days 600 mg. of calcium (bone meal and calcium lactate) were added daily. Following that, for 37 days the dog received daily 50 cc. of 10 per cent calcium lactate by stomach tube (equivalent to 650 mg. of calcium), and was killed accidentally on the 106th day of the experiment by suffocation when the stomach tube was inserted in the trachea. Two units of parathormone were injected daily for the first 7 days, then 4 units for 17 days, 8 units for 4 days. The serum calcium values were 11.9 on the 12th day, and 11.3 on the 28th. Parathormone was increased to 12 units for 2 days, but was discontinued for the next 4 days because anorexia developed; no serum calcium determination was made. Before parathormone injections were resumed, serum calcium was 11.4 mg. per 100 cc. (on the 34th day). It rose to 15.6 mg. on the 39th day of the experiment, after parathormone had been given for 4 days (10 units daily). This was the first time hypercalcemia was observed in this dog, although signs of hyperparathyroidism had been present (anorexia). Parathormone was discontinued for 2 days, and then resumed, 8 units being given daily for 21 days until the 61st day of the experiment. The serum calcium was 12.2 mg. per 100 cc. on the 42nd day, 12.6 mg. on the 45th, 13.7 mg. on the 53rd, and 11.1 mg. on the 60th day of the experiment. Parathormone injections were increased to 14 units daily on the 62nd day. On the 63rd day the serum calcium rose to 14.5 mg. On the 67th day the serum calcium was 17.1 mg. but serum phosphorus was not raised. The animal refused food though he was active. Parathormone was discontinued for 2 days, and then resumed at 6 units daily. After 8 days it was increased to 8 units daily, which dose was continued from the 77th day to the 106th day. The serum calcium showed a regular drop from 11.5 to 9.8 between the 74th and 97th day of the experiment, in spite of the daily parathormone injections.

The animal at autopsy was well nourished. There was considerable abdominal fat. The soft tissues showed no gross pathological lesions. The dog was stunted in growth, the bones were short, and the cortex of the long tubular bones was thin. The ribs showed fibrosis of the marrow. The lymphoid marrow had been largely replaced by loose connective tissue containing numerous eosinophiles and a sprinkling of marrow cells. The blood vessels were dilated. Near the costochondral junctions and underneath the periosteum there were numerous osteoclasts with evidence of active osteoclastic resorption. A few small cysts were seen throughout the marrow. These were lined by flattened endothelioid cells and probably represented blood vessels dilated as a result of circulatory stasis. The haversian spaces of the compacta were enlarged, but in spite of the fibrosis of the marrow and the resorption of the bone most of the trabeculae were lined by osteoblasts and active endochondral ossification was in progress. There was metaplastic new-bone formation. Sections of the long tubular bones showed essentially the same changes as the ribs, but in somewhat lesser degree. The ground disks of sections from the diaphysis of the long tubular bones also showed resorption, the vessel canals being much enlarged. The parathyroid showed involutionary changes and metastatic calcification of the kidney tubules and stomach mucosa was found.
**Summary.**—On parathormone therapy with an intake of at least 600 mg. of calcium daily there was bone resorption and marrow fibrosis, but there was also new-bone formation. The bone was deposited on cartilaginous trabeculae in the course of endochondral ossification. Having succeeded in maintaining this dog for a longer period than No. 6, we obtained a more severe degree of bone resorption and marrow and bone fibrosis.

**Dog 8.**—Initial weight, 1.1 kg. Killed to terminate the experiment 168 days later, at which time it weighed 2.9 kg. No supplementary calcium was given except between the 52nd and 60th days, when the dog received 650 mg. of calcium daily in the form of calcium lactate by stomach tube.

This dog received at first 2 units of parathormone daily; from the 8th to the 24th day, 4 units daily; from the 25th to the 29th day, 6 units daily; from the 30th to the 34th day, 12 units daily; from the 35th to the 39th day, 16 units daily; from the 40th to the 48th day, 6 units daily, and from the 49th to the 51st day, 10 units daily. Between the 52nd and the 107th day parathormone was discontinued. On the 108th and 109th days 3 units were given daily; then 8 units from the 110th to the 158th day, and 20 units daily to the 168th day, when the dog was killed to terminate the experiment.

Blood calcium studies showed strikingly the progressive fall that occurred as the animal was decalcified. The serum calcium was 13.0 mg. per 100 cc. on the 12th day, 11.4 on the 28th, 10.9 on the 34th, 9.6 on the 42nd, and 8.1 on the 49th day. The dog at this time was rigid, dull, and had respiratory difficulties. On the 53rd day it had tremors and tetany, the serum calcium being 7.8 mg. per 100 cc. The tetanic symptoms may be taken as an indication of extreme exhaustion of calcium from the bones and soft tissues. At this time the forelegs were bowed outward, and the animal was weak and had difficulties in walking. It was decided to discontinue the parathormone and administer 650 mg. of calcium daily in order to preserve the animal's life. Between the 53rd and 60th day, when the dog was receiving the large calcium supplement, its clinical condition improved rapidly, the serum calcium rising to 9.0 mg. On the 60th day the calcium was discontinued and the serum calcium began to show a consistent drop reaching 7.8 mg. per 100 cc. by the 97th day. On the 108th day parathormone was again started at 8 units daily. The serum calcium remained fairly constant at about 8.0 mg. per 100 cc. or less. On the 168th day, when the experiment was terminated, and after the dog had been on 20 units for 10 days, the serum calcium was 7.8 and the serum phosphorus 6.4 mg. per 100 cc., although at first the animal had responded to the increased dose by a serum calcium as high as 10.4 mg.

The clinical condition of the dog makes an interesting study. On the 40th day its abdomen was distended, apparently as a result of hyperparathyroid hypotonia. Between the 48th and 53rd days there was difficulty in breathing, dullness and rigidity, with frequent tetanic seizures with spasmodic defecation and urina-
tion when the dog was handled. On the 53rd day respiratory failure developed during a tetanic seizure. At this time a serum phosphorus value of 10.7 mg. per 100 cc. was found. Artificial respiration restored the dog in several minutes, after which the muscles were relaxed. High phosphorus values were observed frequently, particularly after tetanic seizures, which varied in severity during the period that followed, which lasted for about 8 weeks, while the dog was not receiving any parathormone. There was rapid improvement after calcium therapy was initiated. The appetite improved, and the animal walked about the cage. The calcium was discontinued after about a week, but parathormone therapy was not resumed for 7 weeks longer, until the 107th day. The tremors reappeared together with spasmodic defecation, grunting respiration, and atony of the abdominal wall. Greater deformities of the forelegs developed, the plantigrade stance becoming worse. Shortly before the dog was killed, bloody stools and bloody diarrhea appeared, probably due to parathormone overdosage (without hypercalcemia).

Weight at autopsy was 2.9 kg. The dog had attained its maximum weight of 3.58 kg. 3 weeks previously. The animal had been greatly stunted, both humeri were rotated on their long axes, as observed in osteomalacia, and even on gross examination marked softening of the bones was evident. The internal organs showed nothing unusual in the gross.

On histological examination the ribs showed very extensive replacement of the marrow by cellular connective tissue. Embedded in it were a few islands of lymphoid marrow. In these islands the blood forming cells were for the most part well preserved, and red and white blood cells were maturating. The megakaryocytes were few and degenerated. The spindle connective tissue replacing the marrow was in places oedematous, and it contained a sprinkling of lymphocytes, polymorphonuclear leucocytes, some macrophages and plasma cells. A few lymphoid marrow cells could still be observed scattered through the connective tissue, which was quite vascular. Some osteoclasts were also observed in the marrow connective tissue. Very little remained of the original cortical bone, which had been resorbed, as evidenced by the enlarged vessel canals filled with fibrous tissue. One of the ribs examined showed a fracture through the cortex, and there was an attempt at repair by the formation of subperiosteal and endosteal callus. Throughout the fibrosed marrow a large number of osteoid and lamellar-bone trabeculae were observed. Many of these osteoid trabeculae were covered by osteoclasts, while the lamellar bone trabeculae were generally naked, and showed osteoclasts in considerable numbers in or near Howship's lacunae. At the costochondral junctions there was considerable osteoid-bone, and the intertrabecular connective tissue contained numerous osteoclasts. Many small cyst-like spaces were observed throughout the scarred marrow, but were most numerous at the costochondral junctions. They were lined by very flat endothelioid cells, and many were surrounded by lymphoid marrow. Some of these cysts were definitely blood-vascular spaces, containing red blood cells. These spaces were apparently related to stasis of marrow circulation. Some were possibly of
lymphatic origin. However, it seems to be impossible to trace definitely the origin of all of the cyst-like spaces.

Examination of longitudinal sections of the upper end of the humerus showed that the articular cartilage was normal. A very striking finding was the preservation of the marrow of the epiphysis, which was cellular and showed no fibrosis. The bone trabeculae of the epiphyses were thin, but not transformed. The epiphyses were therefore entirely free of ostitis fibrosa. The metaphyses and diaphyses, however, showed cortical resorption, osteoid-bone formation, numerous osteoclasts in Howship's lacunae, fibrosis and marked congestion of the marrow and enlargement of the vessels with marrow hemorrhage. There had been a pathological fracture of the humerus. The femur showed similar changes, and even to a greater degree. They were confined to the metaphysis and diaphysis. The femur also had a pathological fracture. The sections of several other tubular bones showed similar changes, but to a lesser degree.

Cross-sections from the diaphyses of the long tubular bones showed extremely enlarged vessel canals containing fibrous marrow. Some Howship's lacunae and osteoclasts were also seen. The haversian canals were lined by osteoblasts. Ground disks from the same regions also showed marked resorption.

The kidneys on the whole were well preserved, showing in places nests of lymphoid cells, a few sclerosed glomeruli, and calcium in some tubules. Numerous liver cells were replaced by fat. The other tissues showed nothing unusual. The specimens were not fixed or stained specially for calcium.

Summary.—This dog showed extensive resorption and fibrous replacement of the bone and marrow with considerable new bone formation (osteoid tissue). The fibrosed marrow was congested and contained cyst-like spaces. Deformities of the legs developed and histologically there was evidence of numerous pathological fractures. In spite of many evidences of severe hyperparathyroidism, the serum calcium showed consistent decline, apparently accompanied by lowered tissue calcium, leading eventually to tetany. The development of all of these consequences of hyperparathyroidism was no doubt enhanced by the calcium-poor diet, but cannot be attributed, of course, to the diet as such.

Dog 9.—Initial weight, 2.25 kg. The dog gained weight slowly but continuously to the termination of the experiment, 167 days later, when it weighted 5.35 kg. Calcium supplement, 200 mg. daily for the first 23 days (bone meal and calcium lactate), and 1300 mg. from the 24th to 27th day (calcium lactate). From the 28th to the 88th day the dog received daily 650 mg. of calcium (50 cc. of a 10 per cent solution of calcium lactate by stomach tube). Between the 89th and the 143rd day no supplementary calcium was given, then 100 mg. of calcium (bone meal and calcium lactate) were added and continued to the end of the experiment.
Four units of parathormone were injected during the first 6 days, and 8 units from the 7th to the 10th day. The serum calcium was 16.4 mg. per 100 cc. on the 10th day. Parathormone was omitted from the 11th to the 16th day, the serum calcium falling to 11.3 mg. on the latter day. From the 17th to the 20th day 6 units were given daily and the serum calcium rose to 12.4 mg. On the 21st and 22nd days the dog received no parathormone; on the 23rd and 24th, 8 units daily; none on the 25th and 26th. The serum calcium was 11.4 mg. per 100 cc. on the 24th and 11.0 on the 27th. From the 27th to the 43rd day the dog received 8 units daily; the serum calcium rising to 15.0 mg. on the 35th day and 15.5 on the 36th. The treatment being continued, serum calcium fell to 10.9 mg. on the 42nd day. On the 44th day the parathormone was increased to 12 units daily. The serum calcium was 12.1 mg. on the 45th day. On the 51st day, after some loss of appetite, the parathormone was decreased to 6 units daily, raised to 8 units daily on the 59th day, and continued at this dose to the 138th day. The serum calcium remained constant at normal values while the animal received 650 mg. of calcium daily (to the 88th day). During the period when no calcium supplement was given (88th day to 143rd day) the serum calcium dropped consistently. It was 10.0 mg. per 100 cc. on the 100th, 9.6 on the 103rd, 9.6 on the 112th, 8.5 on the 133rd, and 8.8 on the 140th day. On the 140th day the parathormone was raised to 20 units a day and continued at this dose until the termination of the experiment of the 167th day. The serum calcium rose to 9.9 mg. per 100 cc. on the 147th day, and was 10.1 on the 150th, 10.8 on the 154th day, 10.7 on the 161st day, and 10.6 on the 167th day.

Clinically this animal showed hypercalcemia and hyperparathyroidism on several occasions. The appetite was generally good and there was a progressive gain in weight. There was marked stunting of growth, the forelegs were bowed outwards, the bones were thin. The dog was well nourished at autopsy and otherwise showed nothing unusual in the gross.

On histological examination, the ribs showed extensive fibrous replacement of the marrow, but considerable lymphoid marrow still remained. The fibrous tissue of the marrow was oedematous. Marked resorption of the existing bone was evident, and numerous osteoclasts were observed in Howship's lacunae in the marrow connective tissue. Numerous trabeculae of osteoid-bone were observed near the costochondral junction. Some cyst-like spaces were present. The changes were of the same order, but somewhat less pronounced than in Dog 8. The long tubular bones also showed resorption of the compacta, enlargement of the vessel canals, which were filled with fibrous tissue. There was subepiphyseal cartilage osteoid-bone formation, and some fibrosis of the marrow. The epiphyses were free of these changes. Stained cross-sections and ground disks made of bone taken from the middle of the diaphyses of tubular bones showed enlarged vessel canals, and subperiosteal and subendosteal resorption. In the stained sections numerous osteoclasts were seen in the enlarged vessel canals, as well as some fibrous tissue and osteoid.
In a section of the lower jaw bone resorption, fibrosis, osteoclasts and cyst-like spaces were found, while a section from the calvarium showed similar changes and some osteoid-bone formation.

Nothing unusual was observed in the kidney, and no calcium deposits were found in sections stained by the v. Kossa method. None of the other soft tissues showed anything striking, and calcium deposits were found only in the stomach. The parathyroid glands seemed to have undergone simple involutional atrophy.

**Summary.**—This dog also showed the typical bone changes of chronic hyperparathyroidism. The histological picture satisfies the criteria established as necessary for the diagnosis of ostitis fibrosa. The absence of metastatic calcification in the tissues of this dog, in spite of the prolonged treatment, is significant. It seems to indicate that while metastatic calcifications are to be expected in acute hyperparathyroidism with hypercalcemia, the absorption of the calcium deposits is likewise to be expected in the stage of chronic hyperparathyroidism without hypercalcemia. (See also Dog 10.)

**Dog 10**—Initial weight, 1.05 kg. Weight at termination of the experiment, 185 days later, 4.8 kg. Greatest weight, 5.3 kg., was attained 19 days before the dog was killed. Calcium supplement, 560 mg. calcium daily for the first 5 days (bone meal and 200 mg. calcium daily from the 6th to the 105th day (bone meal and calcium lactate). Calcium was discontinued until the 162nd day, when 100 mg. of calcium daily was added and continued to the 185th day. The dog was injected with 2 units of parathormone daily for the first 7 days, 4 units daily to the 11th day, at which time the serum calcium was 15.2 mg. per 100 cc. From the 12th to the 24th day the dose was reduced to 2 units daily. The serum calcium on the 28th day was 12.7 mg. On the 30th day parathormone was increased to 8 units daily, but injections were discontinued from the 33rd to the 40th day because of loss of appetite. They were resumed on the 41st day at 6 units daily. The serum calcium was 14.7 mg. on the 42nd day, 12.5 on the 45th, 12.3 on the 53rd, and 11.5 mg. on the 60th day. On the 61st day parathormone was increased to 12 units daily. The serum calcium rose to 12.2 mg. on the 63rd, and 13.0 mg. on the 67th day, with associated loss of appetite. After omitting one dose of parathormone, it was decreased on the 69th day to 6 units daily until the 78th day, at which time the serum calcium was 11.6 mg. On the 79th day the injection of 8 units of parathormone was begun and this dose was continued to the 157th day. Serum calcium was 11.5 mg. on the 81st day, 10.7 on the 88th, and 11.6 on the 96th day. On the 106th day the supplementary calcium was discontinued, the parathormone dose being maintained at 8 units daily. The serum calcium was 10.8 mg. on the 118th day, 12.5 on the 112th, 10.7 on the 130th, 11.1 on the 151st, and 10.8 on the 158th day. On the 160th day the dose of parathormone...
was increased to 20 units daily, but the serum calcium remained unchanged at
11 mg. on the 165th day, 11.2 on the 168th, 11.0 on the 172nd, 11.2 on the 179th,
and 11.2 on the 185th day, when the dog was killed to terminate the experiment.

On autopsy the dog was well nourished. Otherwise the autopsy showed no-	hing unusual in the gross, except that there was slight bowing of the bones. The
dog was markedly stunted. The changes in the bones of this dog were essentially
the same as in No. 9. Histological examination of the soft tissues revealed nothing
unusual. The splenic pulp was somewhat fibrosed and some of the liver cells
were fatty. Tissues appropriately fixed and stained showed a striking absence
of calcium. This was in significant contrast with our observations of metastatic
calcifications in dogs dying of acute hyperparathyroidism. Only a few kidney
tubules showed some calcium in the lumina.

Summary.—The tissue changes and their significance were the
same as in Dog 9. Bone lesions characteristic of osteitis fibrosa were
produced as a result of prolonged treatment with parathormone.

DISCUSSION

We have produced osteitis fibrosa in young growing puppies. We
believe that the period of active skeletal growth is more suitable for
the development of this condition. During the period of growth the
demand for calcium is great, but even on a liberal calcium intake para-
thalmone interferes with calcium utilization, and with the formation
of normal bone, the calcium balance being negative on appropriate
dosage. An analogy is found in the well-known requirement in the
standardized procedure for the production of experimental rickets,
the development of which is favored by rapid growth. The changes
that we have observed in experimental hyperparathyroidism are not
of the type that are caused by low calcium diets. On such diets ani-
mal are not stunted, provided the diets are not deficient in vitamins;
no marrow destruction and fibrosis are found. These lesions seem to
be specific effects of parathormone treatment. The decalcification
in hyperparathyroidism is not only more rapid than in simple calcium
deprivation, but perhaps because of the greater rate of decalcification
the secondary effects develop which serve to distinguish the two
conditions.

That parathormone affects the lymphoid marrow cells directly, in
addition to its effects on the bone, was observed in all dogs dying of
acute hyperparathyroidism. We believe that during acute hyper-
parathyroidism destructive changes appear also in other lymphoid tissues. There seems to be no doubt that the severe marrow fibrosis is in part an expression of the healing of the injured marrow during prolonged parathormone therapy.

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.

In acute experimental hyperparathyroidism, metastatic calcifications are generally observed, associated with hypercalcemia. We have confirmed this observation. In some of our animals, the stage of acute hyperparathyroidism was succeeded by chronic hyperparathyroidism, without hypercalcemia. In these animals, when they came to autopsy metastatic calcifications were absent or nearly absent. We suggest that metastatic calcifications had been present in these animals earlier, and were resorbed during the chronic stage of hyperparathyroidism.

It has been widely realized that parathormone effects are to be judged by calcium excretion, particularly in the urine, rather than by a rise of serum calcium. And yet, absence of parathormone effects has been frequently inferred from the absence of hypercalcemia. It therefore remains necessary to emphasize that parathormone effects on bone may be observed, as our protocols prove, even when the calcium values are normal or lower than normal.

The lesions produced in these young animals were of the order observed in v. Recklinghausen's disease (ostitis fibrosa cystica, or the hypostotic-porotic form of osteodystrophia fibrosa). It is well known that clinically most of the cases of ostitis fibrosa cystica of v. Recklinghausen come to notice before middle age, the condition probably having developed for many years without clinically prominent symptoms. Hirsch, basing his conclusions on X-ray experience, believes that most of these cases begin early in life (50).

One of the very striking features observed in our young dogs whose
epiphyses were as yet not fused with the diaphyses, and in whom epiphysial cartilage plates were still present, was the absence of fibrous osteodystrophy in the epiphyses. The epiphyses at most showed some simple bone atrophy, but marrow fibrosis and active bone resorption, with Howship's lacunae and osteoclasts, were absent. The lesions were most prominent in the metaphyses, where active new bone formation would normally take place. It seems quite definite that bone injury and marrow fibrosis observed by us in these experiments are not dependent upon vascular changes, as the epiphyses are also exceedingly vascular during the growing stage and would share in the changes if the vascular factor were decisive.

The presence of osteoid tissue as a requisite for establishing the diagnosis of ostitis fibrosa seems to have been given too much significance. In advanced clinical cases with considerable deformity, it appears almost always. Its appearance, we believe, is related to the physiological need of reinforcing bones subject to decalcification. In our dogs we observed osteoid tissue formation when decalcification was most severe, and when there was definite need for repair, as for instance in response to a gross fracture. We conceive that rapid decalcification, when gross fractures do not occur, leads to a weakening of the bone at innumerable points, especially if the animal is active. In view of the stresses and strains exerted at these points, microscopical fractures may be conceived, in the repair of which osteoid is produced. Osteoid may also be formed on greatly thinned bone as an expression of the need to repair.

In regard to the presence of cysts in our dogs with advanced fibrous lesions, these are, we believe, and as many pathologists have observed in clinical material, due for the most part to circulatory stasis that results from the distortion and scarring of the marrow incident to the development of the ostitis fibrosa.

In dogs dying of acute hyperparathyroidism we observed reduction in the size of the external parathyroids to about one-half their usual size. Microscopically, the reduction in size was found related to a diminution in the size of the parenchymatous cells and to their distortion. We interpret this picture as evidence of functional involutional atrophy due to substitution therapy.
SUMMARY

These experiments have shown that parathyroid extract (parathormone Collip) can be injected into puppies in increasing amounts for long periods without fatal results. Thus time is allowed for bone changes to develop. Long continued injection leads to progressive decalcification and resorption of the existing bone, to fibrous replacement of the marrow, and to the production of the other features characteristic of ostitis fibrosa. Deformities eventually appear. It is safe to assume that the bone changes produced by hyperparathyroidization have the same pathogenesis as those observed in clinical cases believed to be instances of hyperparathyroidism—that is, cases with a negative mineral balance and decalcification of the skeleton.

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EXPLANATION OF PLATES

PLATE 35

FIG. 1. Rib of Dog 9 showing costochondral junction. The marrow is fibrosed, some islands of lymphoid marrow are still present. A few cysts are observed. Magnification 18×. Stained with hematoxylin and eosin.

FIG. 2. Rib of Dog 10. The marrow is fibrosed and contains numerous osteoclasts. Cysts are present. Magnification 20×. Stained with hematoxylin and eosin.

FIG. 3. Rib of Dog 8. The marrow is fibrosed and numerous cysts are seen. Considerable newly formed trabeculae are present. In places there is disappear-
ance of the cortical bone. Magnification 10×. Stained with hematoxylin and
eosin.

Fig. 4. Rib of Dog 8 showing remaining islands of lymphoid marrow, many of
which surround cysts. Fibrosis of the marrow, thinning and transformation of
the cortex are more evident than in Fig. 3. Magnification 20×. Stained with
hematoxylin and eosin.

Fig. 5. A higher power picture from same rib showing marrow fibrosis, cysts,
and newly formed fiber-bone. Magnification 150×. Stained with hematoxylin
and eosin.

Fig. 6. Shows part of the epiphysis (above) and metaphysis (below) the epi-
physeal cartilage plate of femur of Dog 8. There is no endochondral-bone forma-
tion at the epiphyseal cartilage plate. Lymphoid marrow is observed in the epi-
physis, where marrow fibrosis is absent. In the metaphysis marrow fibrosis and
numerous cysts are seen. Magnification 16×. Stained with hematoxylin and
eosin.

PLATE 36

Fig. 7. The diaphysis of the femur of Dog 8 showing considerable marrow
fibrosis with resorption of bone. Magnification 20×. Stained with hematoxylin
and eosin.

Fig. 8. Osteoid tissue in the fibrosed marrow of the femur of Dog 10. Magni-
fication 60×. Stained with hematoxylin and eosin.

Fig. 9. Fiber-bone formation in the fibrosed marrow. Magnification 300×.
Stained with hematoxylin and eosin.

Fig. 10. Shows extensive osteoclastic resorption below the epiphyseal cartilage
plate of the humerus of Dog 9. Magnification 85×. Stained with hematoxylin
and eosin.

Fig. 11. Shows resorption and fibrosis of bone from calvarium of Dog 10.
Magnification 85×. Stained with hematoxylin and eosin.