anesthetic activity on peripheral nerves. Perhaps intraventricular TT acts as a local anesthetic on periventricular structures concerned with volitional control of skeletal muscle activity. Differences between effects of procaine and the toxin may be related to the extent of penetration into the periventricular substance and to the rate of inactivation of the drugs at their site of action. Nevertheless, the central neurotoxic effects of TT cannot easily be explained by a simple local anesthetic action since intracisternal and intrathecal injections of TT were ineffective in mimicking the action of procaine.

Summary. Tetrodotoxin injected into the lateral cerebral ventricle of cats produced a reversible loss of volitional control. This syndrome differs from general anesthesia in that the EEG showed a waking pattern which could be "activated" by sensory stimulation throughout the course of motor dysfunction. The behavioral effects of lateral ventricular procaine mimicked those of tetrodotoxin, but procaine interfered with EEG activation due to sensory stimulation.

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Effect of Dietary Calcium Levels on Production and Reversal of Experimental Osteoporosis in Cats.* (29272)

JENIFER JOWSEY† AND J. GERSHON-COHEN
Albert Einstein Medical Center, Philadelphia, Pa.

It has been established from animal experiments that a diet deficient in calcium will cause loss of mineral from the skeleton(1-3), producing the radiologic appearance of osteoporosis(4,5). In young growing animals, in animals deficient in vitamin D, or in animals in which there is an excessive loss of mineral from the skeleton(6), this change is accompanied by rickets and the presence of osteoid tissue. The present study describes the osteoporotic condition produced in adult cats by a diet deficient only in calcium and determines whether the porosis so produced can be reversed by a high-calcium diet.

Methods. Experimental animals. Ten adult female cats were kept for 2 months on an adequate diet to standardize the animals. During this period, or during the first 2 months of the experimental period, each cat had a litter of 3 kittens which were suckled for 6 weeks. Throughout the experiment the animals were kept in a large pen which permitted them exercise. Six of the cats were then fed a diet deficient in calcium,‡ consisting of raw beef heart and distilled water supplemented with vit. D and A and trace amounts of iodine(7); this diet was fed *ad libitum*. Four of the cats were kept as controls and fed on the same diet as the experimental animals, except that they had milk instead of water and tricalcium phosphate was added to the beef heart to supply each cat approximately 40 mg of Ca per kg per day (this was the high-calcium diet).

At the end of 10 weeks the 6 experimental animals showed radiologic evidence of decreased mineral density; 3 of these 6 cats were then fed the high-calcium diet for 10 weeks, while the remaining 3 were maintained

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† Present address: Mayo Clinic, Rochester, Minn.

‡ Analysis, wet weight basis: nitrogen, 2.3%; phosphate 0.18%; and calcium, 0.007%.

TABLE I. The Effect of Dietary Calcium Levels on Blood Calcium Appearance of Parathyroids and Skeletal Weight in Adult Cats.

Diet	Cat	Blood calcium,* mg/100 ml serum	Gross appearance of parathyroids	Skeletal wt, % of eviscerated body wt
High calcium,	1	10.5	—	25.9
20 wk	2	9.7	Pale	27.8
(controls)	3	9.5	Pale and small	31.7
	4	—	Pale	27.9
				Mean 28.3
Low calcium,	5	10.1	Brownish-red	17.2
20 wk	6	10.0	Large, brownish-red	16.4
	7	10.1	Large, pale brown	16.3
				Mean 16.6
Low calcium,	8	9.9	Whitish, half of one was brown	21.3
10 wk;	9	10.0	—	16.9
high calcium,	10	10.1	Pale, almost colorless	18.9
10 wk				Mean 19.0

* All values ± 2 mg/100 ml.

on the low-calcium diet for this period.

During the experiment, tetracycline was given orally to each animal at monthly intervals to mark the amount of new bone formation taking place.

Preparation of material. At the end of the experimental periods, samples of blood were taken from each cat and blood levels of calcium were determined.

Each cat was then killed by injection of sodium pentobarbital (Nembutal sodium) and body weight was determined; the parathyroids were examined and their appearance noted. The viscera were removed and the carcass was then reweighed. The left femur and second lumbar vertebra were removed from each animal and fixed in 70% alcohol.

Skeletal weight. From each carcass, the spinal column with the pelvic girdle, the ribs, and the major bones of one forelimb and both hindlimbs were removed. These bones were cleaned, and weighed to constant weight at room temperature. This dry, defatted weight was expressed as a percentage of the eviscerated body weight.

Radiographs. One femur from each animal was cut in half transversely and the lower end was divided into 2 longitudinally. Each vertebra was trimmed so that the body of the vertebra measured 1.2 cm. Radiographs were made of the lower half of one femur and one vertebra from each cat to give an overall picture of the bone structure of the animals in the different groups.

Microradiography. The pieces of bone that were used for the roentgenograms were then cut into 100 μ thick sections and microradiographs were prepared and used to evaluate the microscopic structure of the bone.

Results. Radiology. The cats fed the low-calcium diet for 10 weeks showed decreased density of the spinal column and some slight change in the trabecular pattern of the end of the long bones. These changes were more marked in the cats fed the low-calcium diet for 20 weeks. On the other hand, the animals fed the high-calcium diet for the second 10-week period showed some slight improvement.

Blood calcium levels. Levels of calcium in the blood, shown in the Table, were not significantly different from one group of animals to another.

Parathyroids. In contrast to the control animals in which the parathyroids were flat and colorless, parathyroids of the animals maintained for 20 weeks on the low-calcium diet were enlarged and were a distinct brownish-red with a well-developed vascular supply, suggesting hyperplasia (Table).

Skeletal weight. The values in the Table express the loss of bone mass by the skeleton in the experimental groups. The adult cats that had been on the low-calcium diet for 20 weeks showed a greatly reduced percentage weight of the skeleton when compared with

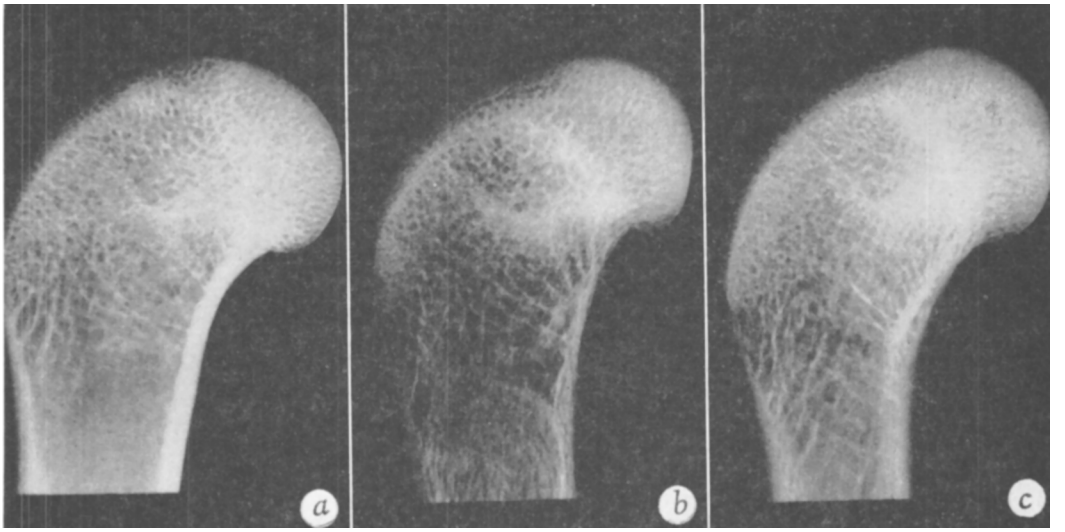

FIG. 1. Roentgenograms of half of lower end of femurs from cats (×3). *a*. Control cat fed a high-calcium diet for 20 weeks. *b*. Cat fed a low-calcium diet for 20 weeks. There is a decrease in density, contributed by loss and thinning of trabeculae, and cortex has become filled with holes. *c*. Cat fed a low-calcium diet for 10 weeks and then a high-calcium diet for 10 weeks. There is a return toward the density of bone of control cat.

control animals, while the 3 animals that had been on the low-calcium diet and then on the high-calcium diet showed values between the control and low-calcium levels.

Radiographs. The radiographs of the trimmed bone slices from the femurs and vertebrae of cats in the different groups showed that, in the animals on the low-calcium diet, the cortex of the shaft of the femur and of the vertebra was porous with only a narrow band of cortical bone remaining periosteally (Fig. 1). The spongiosa was reduced in density, the result of thinning of the trabeculae rather than a reduction in their number; this is substantiated by the microradiographic data.

Microradiography. The microradiographs showed that, in the control group, the cortical bone of the femurs and the trabecular bone of the vertebrae showed very little formation or resorption and the bone was relatively evenly mineralized.

In the animals fed the low-calcium diet there was an increase in the bone surfaces, both in trabecular and in cortical bone that was undergoing resorption (Fig. 2). In the cortical bone, there were a large number of resorption cavities, particularly endosteally, and also a large number of osteones of low mineral density that were labelled by tetracycline fluorescence (Fig. 3). The latter must, therefore, have been formed during the 20-week experimental period.

In the group of cats which were fed the low-calcium diet for 10 weeks and then the high-calcium diet for 10 weeks, although the cortex appeared slightly thinner, there were few resorption cavities throughout the cortex; there were a large number of newly formed osteones labelled with tetracycline.

The trabecular bone from the vertebrae showed similar results. In the animals fed the low-calcium diet, there was a decrease in the number of trabeculae resulting from increased resorption of bone, and, at the same time, an increase in bone formation when compared with the control group. The group on the low and then the high-calcium diet had bones of relatively normal porosity but which consisted of much recently formed bone.

Discussion. The adult cat appears to be unable to adapt to a low calcium intake. On a diet adequate in everything except calcium and with a high bone turnover level stimulated by lactation, the skeleton decreases in mass; this decrease is seen both radiologically and in the smaller proportion of body

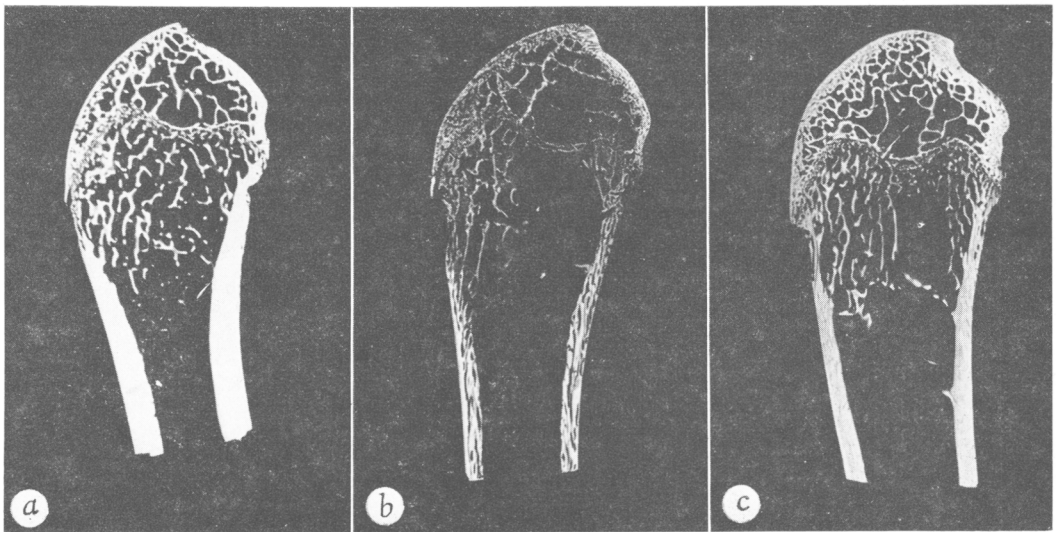

FIG. 2. Microradiographs of longitudinal sections of lower end of femurs of cats showing in detail the reduction in density shown in the roentgenograms (×3). *a*. Control cat fed a high-calcium diet for 20 weeks. *b*. Cat fed a low-calcium diet for 20 weeks. *c*. Cat fed a low-calcium diet for 10 weeks, then a high-calcium diet for 10 weeks.

weight contributed by the skeleton. Radiography and microradiography of specific bones show that this decrease in mass is the result of increased bone resorption which results in (1) a reduction in the width of bone trabeculae and (2) appearance of resorption cavities in the cortical bone. The increased resorption is accompanied by an increase in number of newly formed osteones, suggesting that increased bone resorption alone accounts for the porosity of the bones in the low-calcium animals and that there is no contribution by a failure of bone formation. This appears to be the cause of bone porosity in human senile osteoporosis(8).

Experience in clinical osteoporosis has produced evidence not that the condition is remediable but rather that it is irreversible (9). However, this may be due to a limitation of the technic used for measurement: roentgenography is not a very sensitive method of evaluating bone density. Using microradiographic methods, it is evident that, at least in cats, there can be a reversal of the mechanism producing osteoporosis and that, under the conditions described here, bone density can be restored. The effect of the high-calcium diet appears to be repression of resorption of bone, since the resorption cavities disappear, filled in by new bone, and trabeculae increase in thickness. The result is a return toward normal bone density, thus demonstrating a consistent relationship between the calcium levels in the diet and bone density. This has been shown in rats by Bell, Cuthbertson and Orr(10).

There is some evidence in previous experimental work that low calcium levels in the diet will affect the appearance of the parathyroid glands. Scott and associates(5) have shown that, in growing kittens, a low-calcium diet will cause hyperplasia of parathyroid tissue. Harrison and Fraser(11), working with rats, have reported a correlation between calcium levels in the diet and parathyroid hyperplasia. Copp and Suiker(12) observed increased cellularity of parathyroid tissue in rats fed a low-calcium diet, thus supporting Engfeldt's(13) experiments. In the present report on adult cats, the hyperplasia of the parathyroids that took place as a result of the low calcium levels in the diet perhaps may be directly related to the increase in the resorption process in the bones. The failure to demonstrate a decreased blood calcium in the experimental animals and the absence of abnormal amounts of osteoid tissue precludes the presence of osteomalacia.

There is indirect evidence of a correlation between dietary osteoporosis in animals and

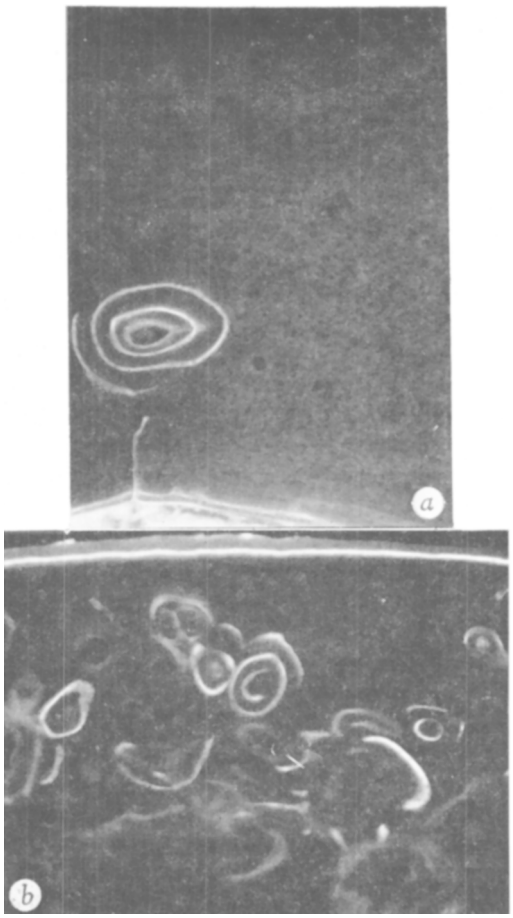

FIG. 3. Appearance under ultraviolet light of sections through middle of shaft of femur of cats. The bone is labelled with monthly doses of tetracycline which indicates sites and rate of bone formation (×50). *a*. Control cat on a high-calcium diet for 20 weeks. *b*. Cat on a low-calcium diet for 20 weeks.

the cause of osteoporosis in animals and the cause of osteoporosis in an aging human population. Malm(14) has shown a relationship between calcium balance and dietary levels of calcium in normal adult men: on a low-calcium diet, adaptation took place in the larger proportion of the subjects and the balance became less negative; when there was a failure to adapt to the low calcium levels, the resulting negative calcium balance eventually produced osteoporosis. Smith and associates(15) and Nordin(7), among others, have indicated the importance of the incidence of osteoporosis and have suggested that patients with osteoporosis often have a low calcium intake, and Whedon(16) has shown that they will often retain calcium avidly over a long period if given a high-calcium diet.

Other factors besides dietary calcium undoubtedly are important in the etiology of osteoporosis and will also lend themselves to experimental investigation. This report has described an experimental approach to one possible factor in the development of osteoporosis and suggests a mechanism of production of porosis of bone.

Summary. Experiments on adult female cats have demonstrated, as measured by roentgenographic appearance, microradiographic data, and tetracycline labeling, the influence of dietary levels of calcium on the structure of bone. It is shown that a low-calcium diet will produce osteoporosis in the adult cat and that this can be reversed by a high-calcium diet.

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