INFLUENCE OF CALCITONIN IN PREVENTING SOFT TISSUE CALCIFICATION BY DIHYDROTACHYSTEROL IN RATS

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ABSTRACT

INFLUENCE OF CALCITONIN IN PREVENTING SOFT TISSUE CALCIFICATION BY DIHYDROTACHYSTEROL IN RATS

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Three experiments, using a total of 60 rats, were conducted to determine the influence of calcitonin administration in preventing or diminishing the clinical signs and tissue calcification associated with dihydrotachysterol (DHT), a vitamin derivative.

Daily oral administration of 50 μg. of DHT in corn oil produced clinical signs of progressive emaciation, muscular weakness and death. Kyphosis and enteric disturbances were evident. They were gross lesions of mineralization on the surface of the myocardium and kidneys. Microscopically there was extensive calcification in the aorta, heart, kidneys, stomach and to a lesser extent in the small intestine and lungs. Rats given the same amount of DHT and 20 MRC units/kg. of calcitonin had the same clinical signs and lesions as those given only the DHT. The degree of soft tissue calcification was dose-related. Decreasing the interval of calcitonin administration had no influence in decreasing the soft tissue calcification. Calcification of soft tissue by DHT was not prevented by calcitonin.

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To my wife, Olga
and
my children Ana and Martin

INTRODUCTION

Calcitonin and hypervitaminosis D have opposite effects in the animal body. Calcitonin inhibits the removal of mineral from bone, reduces the level of calcium and phosphate in the blood and apparently promotes the deposition of new bone. On the other hand, vitamin D has a hypercalcemic effect by facilitating the absorption of calcium from the gastrointestinal tract. Massive doses of vitamin D promote a syndrome characterized by metastatic calcification of the soft tissues.

As long as the end product of vitamin D intoxication, the calcification of soft tissues, is common to several diseases of known or unknown etiology, it was of interest to investigate if calcitonin were able to prevent or diminish the clinical signs and tissue structural changes associated with hypervitaminosis D.

The objective of this research was to determine whether calcitonin has any prophylactic effect against the signs and lesions caused by chronic massive doses of dihydrotachysterol in adult rats.

LITERATURE REVIEW

Calcitonin

Existence. It was believed until recently that regulation of plasma calcium was achieved by appropriate alteration in the secretion rate of the parathyroid hormone. The stimulus for the production of the hormone was a decrease in plasma calcium (McLean and Urist, 1955), and this promoted osteolysis and mobilization of calcium from bones. The control of hypercalcemia was attributed to the suppression of parathyroid hormone production. Nevertheless, by using dogs devoid of parathyroid glands, Sanderson et al. (1960) found that the control of induced hypercalcemia was still inefficient. Copp et al. (1962) carried perfusion experiments in the dog upon the parathyroid-thyroid gland mass as a unit. Generalized depression of blood calcium resulted when the glands were perfused with blood high in calcium; but when thyroparathyroidectomy was performed, the blood calcium increased beyond normal limits, thus providing evidence that some substance produced by the glands had a hypocalcemic effect. They postulated the existence of a new calcium lowering hormone which they called "calcitonin" and deduced erroneously that it was produced by the parathyroid gland. The existence of calcitonin was soon confirmed by further perfusion experiments using a different

technique (Kumar et al., 1963; MacIntyre et al., 1965) but without distinguishing between the parathyroid source claimed by Copp et al. and an origin from the thyroid gland.

Source. Foster et al. (1964) carried out further perfusion experiments in the goat, since in this species the thyroid and parathyroid glands could be perfused separately. They reported that calcitonin is of thyroid origin and is secreted by the thyroid when this gland is perfused with high-calcium blood. Hirsch et al. (1963) reported that a factor liberated from the rat thyroid gland was effective in causing a reduction in blood calcium. In their experiments, the parathyroid tissue was separated from the thyroid tissue and the thyroid gland was identified as the source of the hypocalcemic factor. They named the hormone "thyrocalcitonin". Although doubts were expressed at first (Copp, 1969), it now seems generally agreed that calcitonin and thyrocalcitonin are synonymous.

The cells in the mammalian thyroid which secrete calcitonin have been called C cells by Pearse (1966). The cells are parafollicular in the dog but in other species, such as the pig, they occupy epifollicular and follicular positions. Bussolati and Pearse (1967) identified calcitonin in the C cells in the dog and the pig by immunofluorescence techniques. The thyroid gland of such animals as the chicken does not produce

calcitonin (Kraintz and Puil, 1967). The hormone in that species is produced by the ultimobranchial bodies, which are distinct organs in birds, fish, amphibians and reptiles (Copp et al., 1967). In mammals, the ultimobranchial tissue normally fuses with the thyroid gland.

Mode of action. The effects of calcitonin and parathyroid hormone appear to be antagonistic. Whereas parathyroid hormone promotes resorption of bone and thus hypercalcemia, calcitonin inhibits bone resorption (Aliapoulios et al., 1966) and causes a decrease in blood calcium levels (Gudmundsson et al., 1966). Calcitonin can cause hypocalcemia in the absence of the parathyroid gland or parathormone (Hirsch et al., 1963) and can act independently of the pituitary gland (Milhaud and Moukhtar, 1965), the kidney and the gastrointestinal tract (Munson et al., 1968). Both calcitonin and parathormone decrease serum phosphate and their effects are additive (Milhaud and Moukhtar, 1966b). Both cause phosphaturia (Robinson et al., 1966) but their effects on blood calcium levels tend to cancel out each other.

Soliman et al. (1967) pointed out that removal of the kidney did not alter the plasma calcium-lowering response to calcitonin injection and therefore, that effect is not mediated by a renal mechanism. Furthermore, they stated that the plasma calcium lowering effect was not accompanied by an increase in soft tissue calcium. They concluded that bone was the most likely site of action of calcitonin.

Reports conflict as to whether calcitonin exerts an effect upon intestinal calcium absorption. Krawitt (1967) found that calcium absorption and lumen-to-plasma flux were slightly decreased in calcitonin treated animals. Milhaud and Moukhtar (1966a), on the other hand, reported that calcitonin increased the amount of calcium absorbed during digestion and enhanced the utilization of dietary calcium by the intestines.

That calcitonin inhibits osteolysis and thus calcium resorption was first demonstrated by Aliapoulios *et al.* (1966). They utilized organ cultures of calvarian bones from young mice and showed that calcitonin was effective in controlling the osteolytic effect of added parathormone. Their findings were confirmed by Friedman and Raisz (1965), Gaillard (1967) and Reynolds (1967) who also utilized *in vitro* techniques.

In vivo studies were conducted by Martin *et al.* (1966) to provide further evidence of direct inhibition of bone resorption by calcitonin. They found a significant reduction in urinary hydroxyproline excretion after calcitonin administration. Klein and Talmage (1968) also concluded that repeated administration of calcitonin results in an inhibition of all phases of bone resorption. This was reflected in the diminished hydroxyproline levels in urine, measured as a reliable index of collagen breakdown.

Giraud *et al.* (1967) indicated that calcitonin increased metaphyseal bone mineral and reduced the number of osteoclasts in the affected area. The increase in fully mineralized

metaphyseal bone was associated with an increase in partially mineralized osteoid. They explained the ability of the hormone to inhibit bone resorption to an increase in the rate of bone formation, a reduction of the rate of bone demineralization, or to an interference with the resorption phase of unmineralized collagen. Any of these mechanisms would result in a decrease in the movement of calcium from bone to blood and a lowered serum calcium concentration. That calcitonin acts independently of parathyroid hormone in preventing bone resorption was reported by Foster et al. (1966b). They observed reduced osteoclast counts and an accumulation of trabecular bone in the tail bones of parathyroidectomized rats treated with massive doses of calcitonin for 28 days.

More detailed isotopic studies by Johnston and Deiss (1966), Mazzuloli et al. (1966) and Robinson et al. (1967) left little doubt that inhibition of bone resorption is an adequate explanation of the acute effects of calcitonin.

In addition to inhibition of resorption, there is the possibility that calcitonin may also increase accretion of bone. Wase et al. (1966a) found that when calcitonin was continuously infused intravenously into rats along with ^{45}Ca for about 1 hr., increased amounts of radioactivity were found in bone. Wase et al. (1966b) demonstrated that the subcutaneous

administration of calcitonin to young male rats for 21 days resulted in significantly greater cortical thickness in the tibia, femur and humerus, as measured from a tetracycline marker, than in control rats. Kumar et al. (1968) approached the problem in the opposite manner, by using rats with chronic calcitonin deficiency. Bone changes were compared in thyroid-intact and thyroidectomized rats (with thyroxine replacement), both groups having functional parathyroid transplants. Significantly less bone formation occurred in the femurs of the thyroidectomized rats than in those of thyroid-intact rats. On the other hand, Milhaud and Moukhtar (1966a), using kinetic analysis, found that administration of calcitonin resulted in a decrease in bone anabolism as well as in bone catabolism in both intact and thyroparathyroidectomized rats. Based on histology and tetracycline labeling in thyroparathyroidectomized rats, Baylink et al. (1969) also concluded that calcitonin inhibited both bone resorption and bone formation.

Protection against hypercalcemia. According to Hirsch and Munson (1969), the same experiments involving hypercalcemic perfusion of the thyroid gland that led to the discovery of calcitonin, also provided evidence that the thyroid gland, by releasing calcitonin, could protect against hypercalcemia. In their opinion, other experiments in which hypercalcemia was produced by injection of a calcium salt or by administration of parathyroid hormone or vitamin D, further support that

possibility. The latter claim that the effect of the thyroid gland in protecting against hypercalcemia is more prominent when the hypercalcemia is produced by parathyroid hormone or large doses of vitamin D, than when hypercalcemia is produced by injection of calcium. Hypercalcemia induced by parathormone or hypervitaminosis D apparently stimulates bone resorption, whereas the injection of calcium decreases bone resorption by inhibiting release of parathormone. Other reports also suggest a marked effect of the thyroid gland in reducing the hypercalcemic effect of parathyroid hormone in rats (Hirsch and Munson, 1966; Gittes and Irvin, 1965). Melancon and DeLuca (1969) recently reported that rats which were hypercalcemic due to large doses of vitamin D had an additional transient hypercalcemia following thyroparathyroidectomy. Parathyroidectomy alone did not duplicate that response. However, thyroidectomy 8 hours after parathyroidectomy, also gave rise to an additional hypercalcemia. The secondary serum calcium elevation was attributed to the removal of the endogenous calcitonin, since the injection of exogenous calcitonin prevented hypercalcemia.

The ability of exogenous calcitonin to reduce or prevent vitamin D-induced hypercalcemia has been established by Mittleman et al. (1967). They showed that in hypercalcemia produced by the administration of a single dose of 10^5 USP

units of vitamin D_3, thyroparathyroidectomized rats had a significant decrease in the plasma concentration of calcium during calcitonin administration. A similar response was achieved when the rats were given a 10-fold greater dose of vitamin D_3. In collateral studies by the same authors on the release of ^{85}Sr from the skeleton, thyroparathyroidectomy decreased, and vitamin D_3 administration markedly increased, the ratio of urinary ^{85}Sr to tibial ^{85}Sr. On the other hand, the administration of calcitonin produced decreases in the excretion of ^{85}Sr.

Protection against soft-tissue calcification. Gabbiani et al. (1968), at the University of Montreal, Canada, found that either calcitonin or thyroxine could partially prevent the calcification of the kidney and heart and the appearance of osteitis fibrosa produced by administration of parathyroid extract to thyroparathyroidectomized rats. When adequate doses of both calcitonin and thyroxine were administered, development of the lesions was completely prevented. In a similar study, Rasmussen and Tenenhouse (1967) observed nephrocalcinosis in thyroparathyroidectomized rats infused for 16 hours with parathyroid hormone. When calcitonin and parathyroid hormone were infused together over a period of 70 hours nephrocalcinosis did not develop.

On the other hand, according to Hirsch and Munson (1969), there is a possibility that endogenously secreted calcitonin might aggravate soft-tissue calcification. They cited Bajusz et al. (1963), who reported that in rats with experimental lesions of the coronary artery there was more myocardial calcification in parathyroidectomized rats than in rats with intact glands. This increased calcification of soft tissues was not observed in thyroparathyroidectomized rats whether they were untreated or given thyroxine. They postulated the existence of a thyroid principle other than thyroxine that was responsible for the greater severity of the lesions in parathyroidectomized rats. Because this study was conducted before the discovery of calcitonin was published, the possible association of calcitonin with the effect was not discussed.

Vitamin D Intoxication

Selye (1932) demonstrated that intoxication of young rats with an impure vitamin D preparation (irradiated ergosterol) produced osteitis fibrosa-like bone lesions with multiple spontaneous fractures. In older rats, hypervitaminosis D tended to produce calcification in various soft tissues, especially in the arteries, the heart and the kidneys. Numerous additional investigations have since shown that purified vitamin D_2,D_3 and AT-10 (Dihydrotachysterol, a vitamin derivative) can

elicit essentially similar lesions. A suitable review of hypervitaminosis D was made by Mulligan (1947). A complete description of the syndrome and the lesions produced by dihydrotachysterol was reported by Selye (1957) and Selye et al. (1963 and 1965).

MATERIALS AND METHODS

Source and Maintenance of Animals

Adult female Sprague-Dawley rats were used throughout, except for the 6 immature rats used in Experiment 2. They were housed in groups of 2 or 3 in galvanized steel cages with the front and bottom of wire screen. Water was provided from glass bottles with stainless steel tubes. Rats were fed commercial feed*. During the second half of Experiment 1 the feed was ground to enable the rats to be able to consume it.

Chemicals

The calcitonin was of porcine origin and provided through the courtesy of Dr. J. P. Aldred of Armour Pharmaceutical Company, Chicago, Ill. The potency of the hormone was 9 MRC Units/mg. in Experiment 1 and 60 MRC Units/mg. in Experiment 3. One MRC Unit is equivalent to approximately 4 µg. of pure porcine calcitonin (Copp, 1969). Calcitonin solutions were prepared immediately before use. The vehicle used was 16% gelatin in Experiment 1 and 5% gelatin in Experiment 3. The vehicle was maintained at 37 C before and after incorporation of the calcitonin.

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*Purina Laboratory Chow

A solution of dihydrotachysterol* (DHT) in 50 ml. of corn oil was prepared weekly and stored at 4 C.

Experimental Procedure

Experiment 1. Twenty-four female, adult rats ranging in weight from 245 to 265 g. were randomly divided into 4 groups of 6 each. They were weighed daily and examined for the presence of lesions or other signs of abnormality. The experiment lasted 30 days.

Each rat in Group 1 was given 0.5 ml. of corn oil by stomach tube and were given subcutaneous injections of 0.2 ml. of 16% gelatin, once a day, during the experimental period.

Rats in Group 2 were treated daily with 50 µg. of DHT in 0.5 ml. of corn oil by stomach tube. Dosage was lowered to 25 µg. after 2 weeks of treatment due to loss of weight.

Group 3 rats were treated daily with 50 µg. of DHT in 0.5 ml. of corn oil by stomach tube. Calcitonin diluted to 16% gelatin vehicle was injected daily at the dose of 20 MRC Units/kg. of body weight subcutaneously. The dose of DHT was lowered to 25 µg. at the same time as Group 2.

Rats in Group 4 were given daily injections of calcitonin in 16% gelatin vehicle at the rate of 20 MRC Units/kg. during the experimental period.

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*Mann Research Laboratories, Div. of Becton-Dickinson & Co., New York, N.Y.

Necropsy procedures. At the end of the treatment period the rats were anesthetized with ethyl ether and killed by exsanguination. Blood samples were obtained by cardiac puncture with 20 gauge, 1 inch needles. Heparinized 2 ml. vials were used to collect blood for hemoglobin concentration and packed cell volumes. Nonheparinized 10 ml. tubes were used to collect blood for content of calcium, phosphorus and alkaline phosphatase.

A gross necropsy examination was performed on each rat. Weights of the liver, kidneys and heart plus lungs were recorded.

Histologic techniques. Samples from the aorta, coronary artery, myocardium, lungs, thyroid gland, stomach, duodenum, liver and kidneys were collected and fixed in 10% formalin solution containing sodium acetate as a buffer. Portions of the rib cage, tibia and tail were collected and decalcified before embedding in paraffin. Sections were cut at 6 μ and stained routinely with hematoxylin and eosin. In selected cases, the Von Kossa stain for calcium salts was applied.

Hematologic determinations. Hemoglobin concentrations were determined by the cyanmethemoglobin method and packed cell volumes by the microhematocrit method (Coles, 1968). Serum levels for calcium (Patton and Reeder, 1956) and phosphorus (Fiske and Subbarow, 1929) were determined by use of a

spectrophotometer*. Alkaline phosphatase concentrations were obtained by using the Monitor method**.

Experiment 2. This experiment was undertaken to provide further information on the effect of DHT given at different doses to immature and mature rats and to determine the most appropriate dosage at which DHT could induce detectable lesions without killing the rats.

Six young female rats averaging 145 g. and 6 adult female rats with a mean weight of 258 g. were used. One young and one adult rat were assigned to each of 6 pairs and all rats were given DHT by stomach tube daily for 14 days. Individual dosages were: 5 µg. for Pair 1, 10 µg. for Pair 2, 20 µg. for Pair 3, 30 µg. for Pair 4, 40 µg for Pair 5 and 50 µg. for Pair 6. Body weight was recorded daily and, at the end of the experiment, rats were killed and the usual necropsy and histologic procedures were performed. Blood samples were not obtained.

Experiment 3. Twenty-four female, adult rats ranging in weight from 250 to 305 g. were randomly divided into 4 groups of 6 each, as in Experiment 1. Experiment 3 differed from

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*Coleman Jr., Coleman Instruments Corp., Maywood, Ill.

**American Monitor Corporation, Indianapolis, Ind.

Experiment 1 in that the daily dosage of calcitonin (20 MRC/kg.) was divided into thirds and given at 8 hour intervals. The dose of DHT was 25 µg. instead of 50 µg. and the experiment lasted 15 days instead of 30 days.

Histologic techniques and hematologic determinations were similar to those in Experiment 1. Weights of organs were not taken. Except for this, necropsy procedures were also similar.

RESULTS

Experiment 1

Body weight. Differences in body weight among the groups were recorded at the end of the experiment (Table 1). Group 1 gained 11% with respect to initial weight. Group 2 and Group 3 decreased 35% and Group 4 gained 20%. Weight changes followed a similar pattern in Groups 1 and 4 and in Groups 2 and 3.

Clinical signs. There were no distinct clinical signs in Groups 1 and 4 other than a steady increase in weight. In Groups 2 and 3 the outstanding signs were progressive emaciation and muscular weakness. The skin became dry and inelastic, forming numerous wrinkles. The bones and particularly the ribs were visible through the skin and there was marked kyphosis in the lower thoracic and upper lumbar region (Fig. 1). Five rats died, 1 in Group 2 and 4 in Group 3. Extreme loss of weight was a consistent feature in these rats. Persistent diarrhea was also seen in these rats a few days before death but otherwise scant and hard feces were common features in rats in Groups 2 and 3.

Weight of organs. Table 1 gives the weights of the organs in the different groups and the percentages of body weights that the organs represent. There were not significant differences in the weights of the liver, lungs and heart between Groups 1 and 4

Table 1. Treatments, Average initial and terminal weights and selected organs weights of rats in Experiment 1.

Group no. and treatment	No. rats at end of expt.	Aver. initial wgt. (g)	Aver. wgt. at necropsy (g)	Liver Wt. (g)	Liver % body wt.	Kidneys Wt. (g)	Kidneys % body wt.	Heart and Lungs Wt. (g)	Heart and Lungs % body wt.
1 Corn oil and gelatin	6	257.6	290.8	11.2	3.84	1.0	0.36	4.83	1.65
2 DHT*	5***	261.1	171.0	7.4	4.32	0.9	0.54	3.06	1.78
3 DHT and calcitonin**	2***	250.0	167.5	8.1	4.82	1.0	0.64	3.33	1.92
4 Calcitonin	6	258.3	310.0	11.8	3.79	1.0	0.34	4.50	1.45

* DHT was given daily by stomach tube to rats in Groups 2 and 3 at the rate of 50 µg. for 15 days and 25 µg. for the remaining 15 days of the experiment.

** Calcitonin was given subcutaneously as a single daily injection at the rate of 20 MRC Units/kg. to rats in Groups 3 and 4.

*** One of 6 rats from Group 2, and 4 of 6 rats from Group 3, died during the experiments.

Figure 1

Extreme loss of weight, emaciation, dry and inelastic skin and kyphosis in rat treated with DHT. Similar signs were seen in rats treated with DHT plus calcitonin. Rat 8, Group 2, Experiment 1.

and between Groups 2 and 3. Organs from Groups 1 and 4 were generally heavier than the ones from Groups 2 and 3. The weights of the kidneys did not vary appreciably; hence the ratio of kidney weight to body weight was increased in Groups 2 and 3.

Gross lesions. At necropsy, rats in Groups 2 and 3 lacked the normal amount of subcutaneous, perirenal and omental fat. The muscles appeared atrophic and curvature of the spinal column was pronounced. The skin appeared inelastic with atrophy of the dermal tissue and of the cutaneous musculature. There was mineralization on the surfaces of the myocardium and kidneys. Gross lesions were not observed in rats of Groups 1 and 4.

Microscopic lesions. Lesions of hypervitaminosis D were noticed in rats of Groups 2 and 3 (Table 2). Calcification was most extensive in the aorta, heart and coronary vessels, kidney and stomach and, to a lesser extent, in the small intestine and lungs. A few arteries in the region of the thyroid and parathyroid gland were calcified but the glandular tissue appeared to be spared (Fig. 2).

The media of the proximal aorta was almost totally calcified in the majority of the rats in Groups 2 and 3 (Fig. 3). The coronary arteries were involved in almost every rat, especially the internal elastic membrane and the smooth muscle fibers of the media (Fig. 4). In the myocardium, the calcified muscle cells were distributed irregularly but frequently around the

Table 2. Grading of microscopic soft tissue calcification in selected tissues from rats in Experiment 1.

Group No.	Rat No.	Aorta	Heart	Kidney	Stomach	Duodenum	Liver	Lung
1	1	0	0	2	0	0	0	0
	2	0	0	2	0	0	0	0
	3	0	0	2	0	0	0	0
	4	0	0	0	0	0	0	0
	5	0	0	2	0	0	0	0
	6	0	0	0	0	0	0	0
2	7*	3	3	3	3	3	0	3
	8	3	3	3	3	3	0	0
	9	3	3	3	2	2	0	0
	10	3	3	3	3	0	0	0
	11	3	1	3	1	0	0	0
	12	3	3	3	3	3	0	0
3	13	3	3	3	3	1	0	0
	14*	3	3	3	3	1	0	1
	15*	3	3	3	3	3	0	2
	16	3	3	3	3	3	0	3
	17*	3	3	3	3	3	0	0
	18*	3	3	3	3	3	0	2
4	19	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
	21	0	0	1	0	0	0	0
	22	0	0	2	0	0	0	0
	23	0	0	1	0	0	0	0
	24	0	0	2	0	0	0	0

*Died during the course of the experiment.

Grade 0 = no lesion
1 = minimal
2 = moderate
3 = maximal

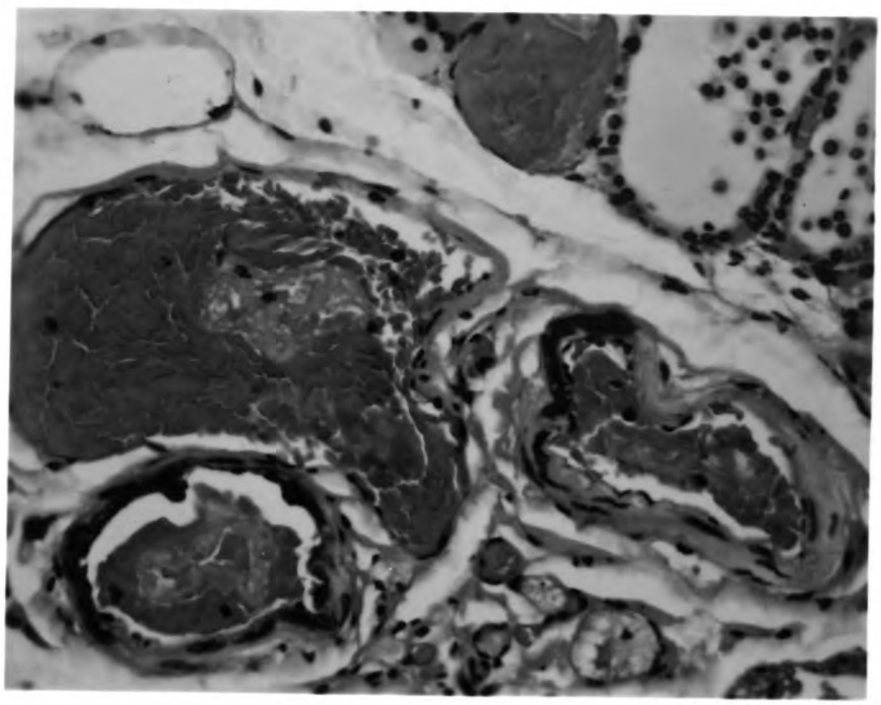

Figure 2

Small arteries in the vicinity of the thyroid gland with calcification in the media. Rat 16, Group 3, Experiment 1 - H & E stain x 560.

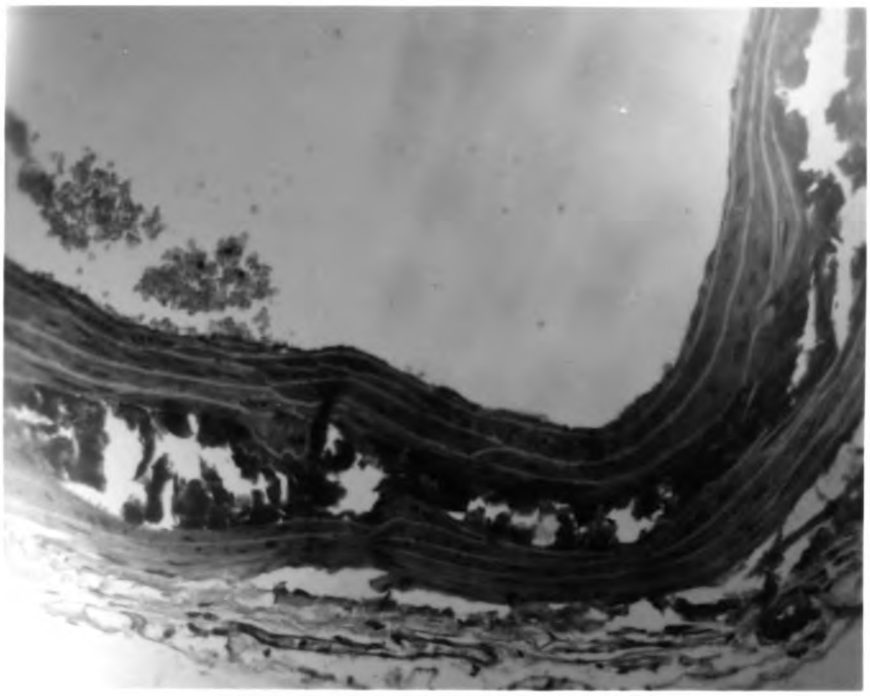

Figure 3

A cross-section of the aorta showing uniform impregnation with calcium salts in the media. Note absence of inflammatory reaction. Rat 10, Group 2, Experiment 1 - H & E stain x 560.

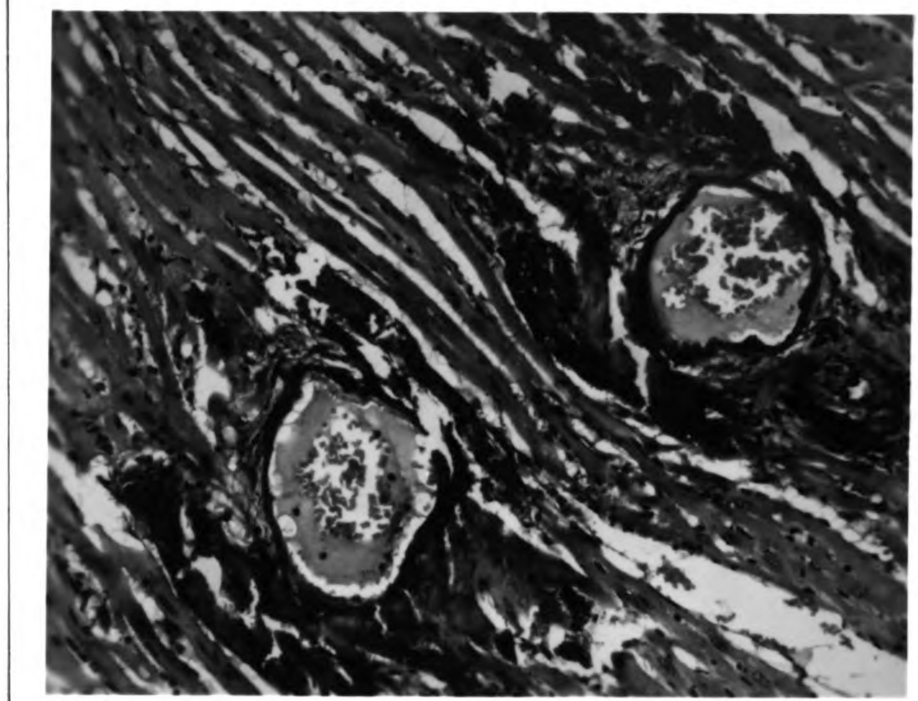

Figure 4

Involvement of coronary arteries with calcification of the wall and heavy calcium salt deposits between the myocardial fibers. Rat 10, Group 2, Experiment 1 - H & E stain x 562.

coronary arteries or in the subendocardial region (Fig. 5). In some instances, the deposition of calcium was so extensive that it was detectable in coronary arterial branches of all dimensions (Fig. 6). The endocardium, especially of the left atrium, was occasionally severely calcified. Valves were normal in all cases.

Kidneys were the only organs that presented some degree of calcification in rats of all groups. However, the degree of mineralization was much less in Groups 1 and 4. There were only isolated areas of mineralization situated in the vicinity of the cortico-medullary junction. These were interpreted as a common finding for adult rats and were not considered as atypical calcification in the statistical treatment of the different groups. In Groups 2 and 3, the kidneys were the commonest site of severe calcification in all the rats. Calcification usually involved extensive areas in the subcapsular zone and localized areas in the medullary portion (Fig. 7). The cells of the epithelium of the convoluted tubules were frequently impregnated with calcium. Sometimes the basement membranes were heavily calcified, the same being true of branches of the renal artery in the hilus of the kidneys (Fig. 8).

The stomach was also a common site of severe calcification in the rats of Groups 2 and 3. Prominent changes were in the wall of arteries, the tunica muscularis and muscularis mucosae (Fig. 9). When mucosal changes occurred, they were restricted

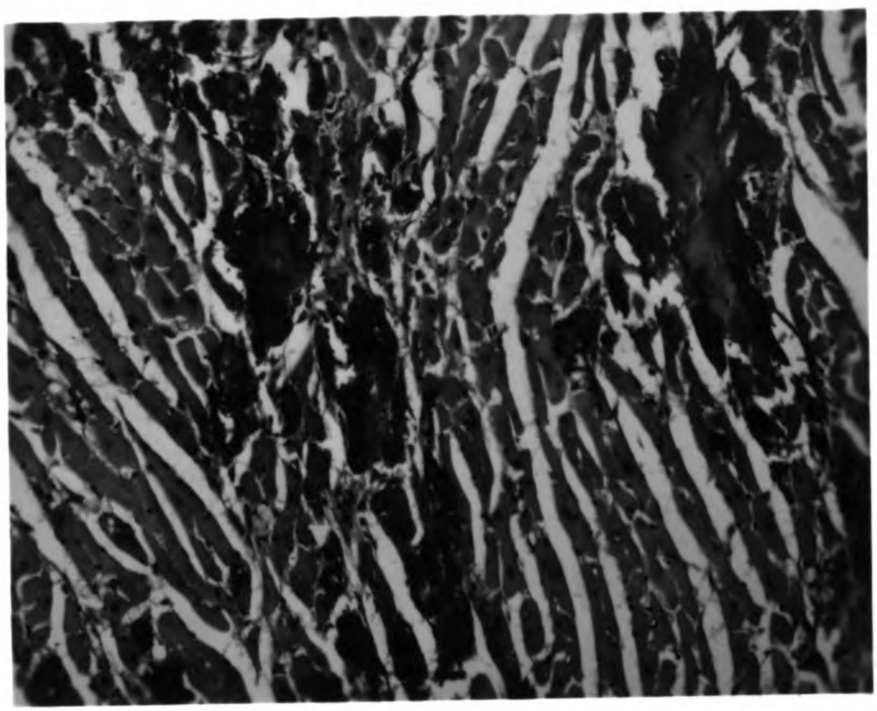

Figure 5

Myocardial calcification. The deposition of calcium was frequently found around coronary arteries or in the subendocardial region. Rat 13, Group 3, Experiment 1 - H & E stain x 560.

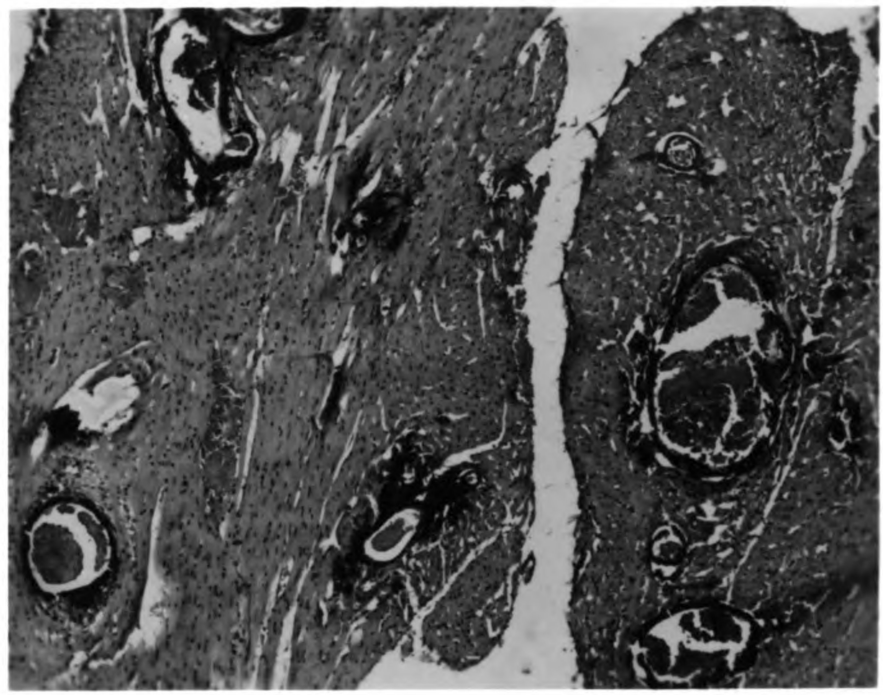

Figure 6

Heavy deposition of calcium salts in coronary arterial branches of different dimensions. Rat 17, Group 3, Experiment 1 - H & E stain x 562.

Figure 7

Disruption of the architecture of the renal tissue with deposition of large masses of calcium salts in the cortical area. Rat 9, Group 2, Experiment 1 - H & E stain x 560.

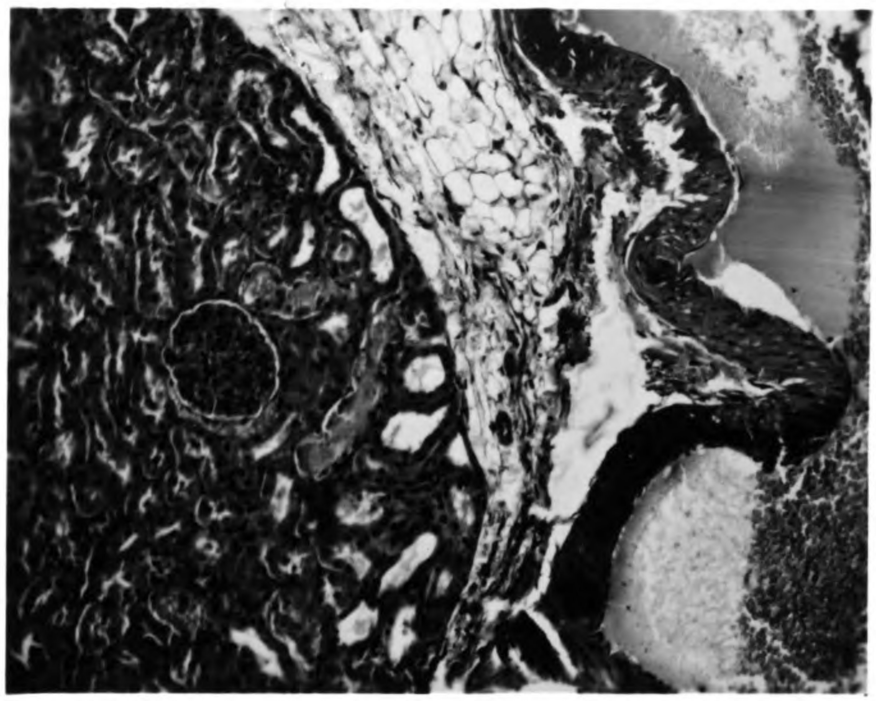

Figure 8

Branch of the renal artery in hilus of kidney with mineralization in the media and disruption of the muscle fibers. Rat 10, Group 2, Experiment 1 - H & E stain x 560.

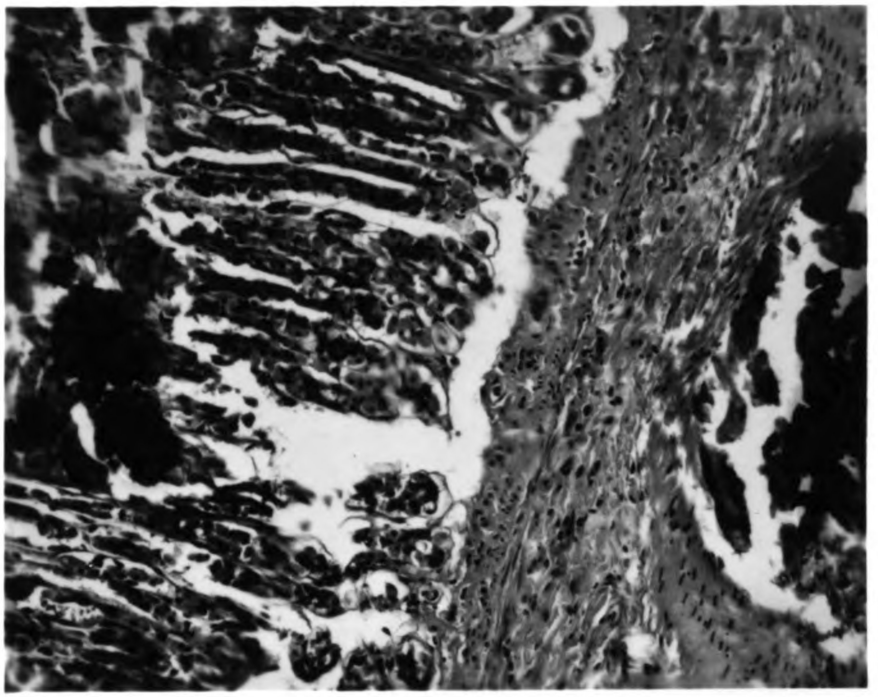

Figure 9

Tunica muscularis, acid-secreting mucosa and lamina propria of stomach with severe cellular destruction and massive deposition of calcium salts. Rat 16, Group 3, Experiment 1 - H & E stain x 560.

to the acid-secreting mucosa and lamina propria. Occasionally, cells lining the glands were calcified and at times calcified concretions were in the lumens.

The duodenal mucosa, tunica muscularis and muscularis mucosa appeared normal, whereas calcium deposits often appeared in the walls of small arteries and arterioles (Fig. 10).

Calcification of the lung tissue was not common. In some rats of Groups 2 and 3 calcification occurred in the walls of the alveolar ducts and alveoli. Sometimes the pulmonary elastic tissue and the walls of interalveolar capillaries were calcified (Fig. 11).

Bone changes were evident in Groups 2 and 3. Increased density of trabecular bone was the principal feature. This was noticeable near the epiphyseal plate of the tibia (Fig. 12). Narrowing of the marrow spaces, irregularity of the epiphyseal plate and failure of osteoid to calcify properly were among the more priminent changes (Fig. 13).

Statistical interpretation of microscopic lesions. Samples of aorta, heart, kidney, liver, stomach, intestine and lungs were graded numerically as to the degree of mineralization and the different groups were compared. The results are given in Table 2. The 95% confidence limits for observations consisting of 6 animals are: (1) for Groups 1 and 4, in which no atypical calcifications were noted, 0 to 39.3; (2) for Groups 2 and 3

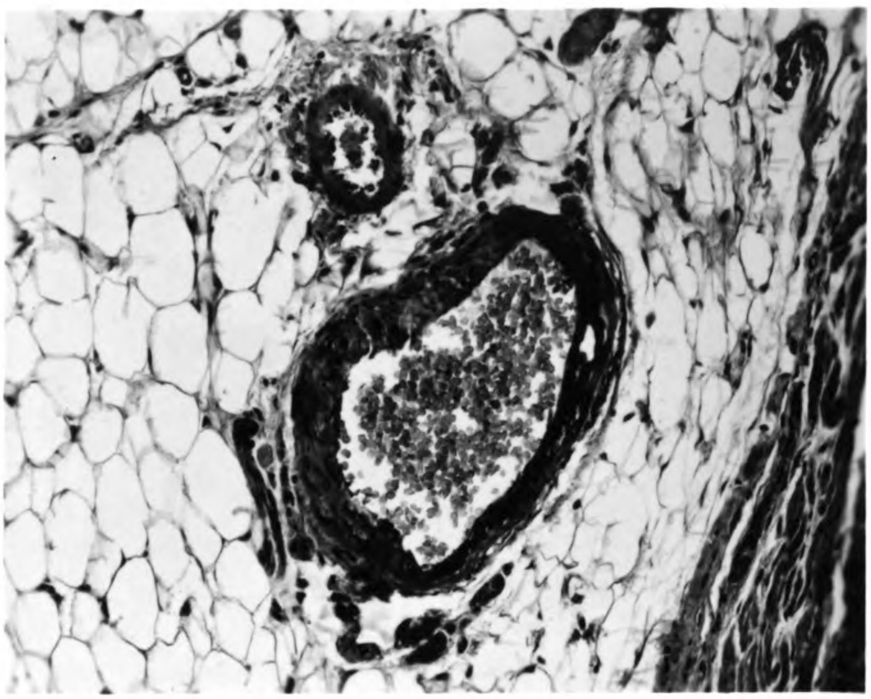

Figure 10

Wall of small artery of the duodenum with mineralization of the media and disruption of muscle fibers. Rat 12, Group 2, Experiment 1 - H & E stain x 560.

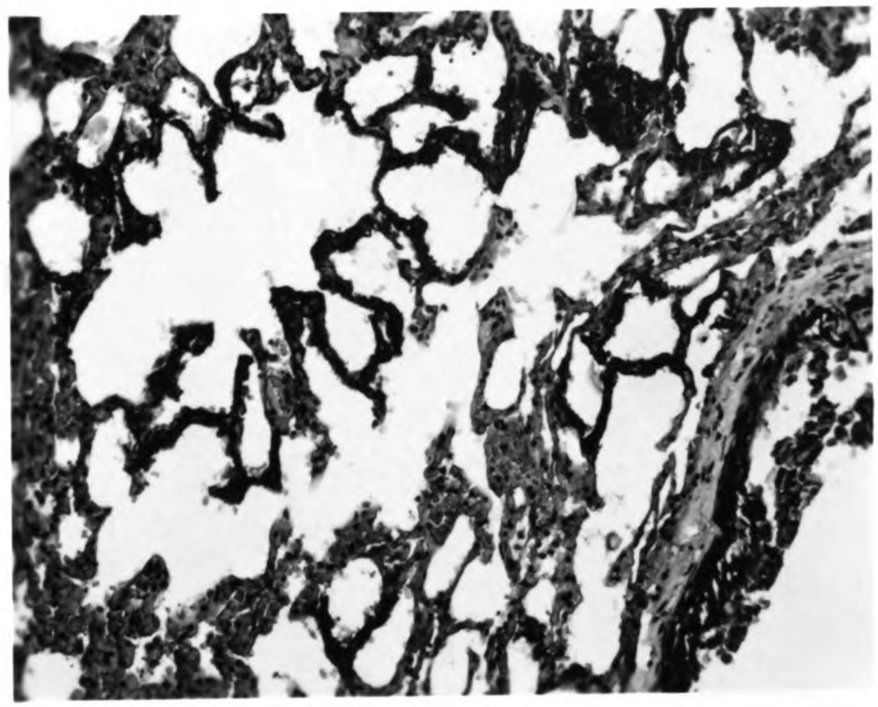

Figure 11

Lung. Notice calcification in the alveolar and peribronchial tissue. Rat 16, Group 3, Experiment 1 H & E stain x 560.

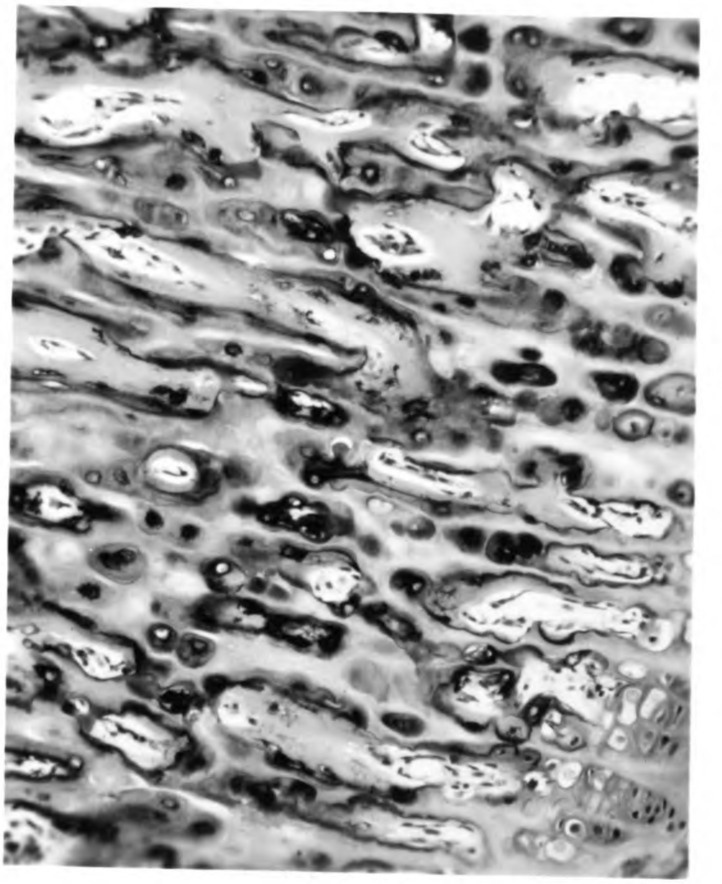

Figure 12

Tibia. Increased density of trabecular bone. Bone marrow spaces are narrow and hematogenous marrow is almost absent. Rat 12, Group 2, Experiment 1 - H & E stain x 560.

Figure 13

Tibia. Notice irregularity of epiphyseal plate, few marrow spaces and absence of hematogenous marrow. Rat 13, Group 3, Experiment 1 - H & E stain x 560.

in which all the subjects showed atypical calcifications, 61 to 100 percent. Since there is no overlap in these confidence limits, the probability of observing these effects by chance is considerably less than 0.05 percent. Therefore, these observations are statistically valid. The same conclusion was drawn when the data were analyzed by the chi-square method applying the Yates allowance (Lewis, 1966).

Hematologic determinations. The results are given in Table 3. Packed cell volumes and hemoglobin concentration decreased significantly ($P < 0.05$) in Groups 2, 3 and 4 with respect to the controls. Calcium values increased significantly in Group 2 ($P < 0.05$). Hemolysis occurred in some of the blood samples and therefore the values given for inorganic phosphorus are not sufficient to warrant any conclusions in Groups 3 and 4. For the same reason, the constant K that represents the averages of the products of calcium and phosphorus is not valid for Groups 3 and 4. Hemolysis of the samples also prevented the analysis for alkaline phosphatase in many samples. The few values obtained are not included in Table 3.

Experiment 2

Due to the limited objective of this experiment, the effects of different doses of DHT when given to immature and mature rats, only changes in body weight and microscopic lesions were used as bases for comparison.

Table 3. Terminal hematologic determinations from rats in Experiment 1.[#]

Group No.	No. of rats	Initial daily dose	Average values and range				
			Packed cell volume	Hemoglobin (Gm./100 ml.)	Calcium (mg./100 ml.)	Inorganic phosphorus (mg./ 100 ml.)	Constant K (calcium x phosphorus)
1	6	Vehicle	39.5 (31.0-46.0)	14.0 (12.4-15.0)	9.8 (8.7-10.5)	7.6 (6.3-9.1)	74.4 (64.8-95.5)
2	5	DHT* 50 µg.	32.6** (27.2-37.3)	12.6** (12.0-14.2)	12.7** (11.8-13.5)	6.9 (6.4-7.9)	87.6** (82.9-93.2)
3	2	DHT* 50 µg. Calcit. 20 MRC U/kg.	34.0** (29.1-39.0)	12.7** (11.2-14.2)	10.5 (9.7-11.3)	***	***
4	6	Calci-tonin 20 MRC U/kg.)	31.5** (24.2-38.3)	12.8** (9.6-14.2)	10.3 (9.7-11.2)	***	***

* Dosage was reduced to 25 µg. per rat during last 15 days of the experiment.

** Significantly different ($P < 0.05$) from control values.

*** Insufficient number of samples for analysis.

\# Experiment 1 lasted 30 days.

Body weight. The changes in body weight related to the different doses of DHT are given in Table 4. In young rats there was no decrease in weight at any dose level. Gains in weight were recorded in young rats treated daily with 5, 10, 20, 30 and 40 µg. of DHT. No change in weight occurred in the rate given the 50 µg. dose. In adult rats there was a slight weight gain in rats treated with 10 or 20 µg. of DHT. Rats treated with either 30, 40 or 50 µg. steadily decreased in body weight.

Microscopic lesions. The microscopic lesions were evaluated by using the same numerical scale as in Experiment 1. The results are given in Table 5. The relationship between microscopic lesions and body weight changes were clear-cut in adult rats. Lesions were more severe in adult rats, especially in those given 30, 40 or 50 µg. of DHT.

Experiment 3

Body weight. Differences in body weight between the groups at the end of the experiment were not significant. Group 1 gained 4% with respect to initial weight. Group 2 lost 9%. Group 3 lost 6% and Group 4 gained 4%. The rate of gain or loss followed a similar pattern in Groups 1 and 4 and in Groups 2 and 3.

Table 4. Changes in body weight of rats treated with different doses of DHT in Experiment 2.

Dosage of DHT (μg.)	Age of rats	Initial weight (g.)	Final weight (g.)	Difference (g.)	Gain or loss (%)
5	Young	150	180	+30	+20
	Adult	260	260	0	0
10	Young	160	205	+45	+28
	Adult	275	285	+10	+4
20	Young	140	185	+45	+32
	Adult	250	255	+5	+2
30	Young	135	175	+40	+30
	Adult	240	225	-15	-6
40	Young	155	190	-35	-22
	Adult	250	225	-25	-10
50	Young	130	130	0	0
	Adult	275	220	-55	-20

Table 5. Microscopic assessment of degree of calcification and percentage of changes in body weight in rats in Experiment 2.

Dosage of DHT (μg)	Age of rats	Aorta	Heart	Kidney	Stomach	Duodenum	Liver	Lung	Final % weight change
5	Young	0	0	2	0	0	0	0	+20
	Adult	0	0	0	0	0	0	0	0
10	Young	0	0	0	0	0	0	0	+28
	Adult	0	1	2	0	0	0	0	+4
20	Young	0	0	2	0	1	0	0	+32
	Adult	1	1	0	0	1	0	0	+2
30	Young	1	1	1	0	0	0	1	+30
	Adult	2	2	2	0	0	0	1	-6
40	Young	2	0	2	0	1	0	0	+22
	Adult	1	1	1	0	3	0	0	-10
50	Young	0	1	1	0	1	0	0	0
	Adult	3	2	2	0	2	0	0	-20

Grade
0 = no lesion
1 = minimal
2 = moderate
3 = maximal

Clinical signs. There were no distinct clinical signs in Groups 1 and 4. In Groups 2 and 3 there were progressive emaciation and muscular weakness but to a lesser degree than in Experiment 1. Loss of weight was not pronounced. Deaths did not occur. Toward the end of the experimental period the skin appeared somewhat dry and inelastic and kyphosis was noticeable in the majority of the rats in Groups 2 and 3.

Gross lesions. At necropsy, rats in Groups 2 and 3 had the same type of lesions described in Experiment 1. Nevertheless, the intensity of the lesions was of a lesser degree. There were no gross lesions in rats of Groups 1 and 4.

Microscopic lesions. Typical lesions of hypervitaminosis D were noticed in rats in Groups 2 and 3. The numerical assessment of the lesions is given in Table 6. The location of lesions followed the same pattern as described in Experiment 1, but the intensity of the lesions was less. In some rats the muscular layer of the stomach was fragmented (Fig. 14) and the myocardium was somewhat hyalinized, but calcification was not a prominent feature.

Statistical interpretation of microscopic lesions. The results of the analysis of data in Experiment 3 were similar to the results obtained in Experiment 1. Groups 1 (controls) and 4 (calcitonin) differed significantly from Groups 2 (DHT) and 3 (DHT and calcitonin) with respect to the presence or absence of typical lesions.

Table 6. Grading of microscopic soft tissue calcification in selected tissues from rats in Experiment 3.

Group No.	Rat No.	Aorta	Heart	Kidney	Stomach	Duodenum	Liver	Lung
1	1	0	0	3	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	2	0	0	0	0
	5	0	0	2	0	0	0	0
	6	0	0	3	0	0	0	0
2	7	0	1	2	0	0	0	0
	8	1	0	2	2	0	0	0
	9	3	2	2	2	0	0	0
	10	3	1	1	3	0	0	0
	11	3	2	0	2	1	0	0
	12	2	1	3	3	1	0	0
3	13	3	3	3	3	0	0	0
	14	2	2	1	2	0	0	0
	15	1	2	1	0	0	0	0
	16	2	2	3	1	0	0	0
	17	2	1	3	1	0	0	0
	18	3	0	3	0	0	0	0
4	19	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
	21	0	0	2	0	0	0	0
	22	0	0	2	0	0	0	0
	23	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0

Grade: 0 = no lesion
1 = minimal
2 = moderate
3 = maximal

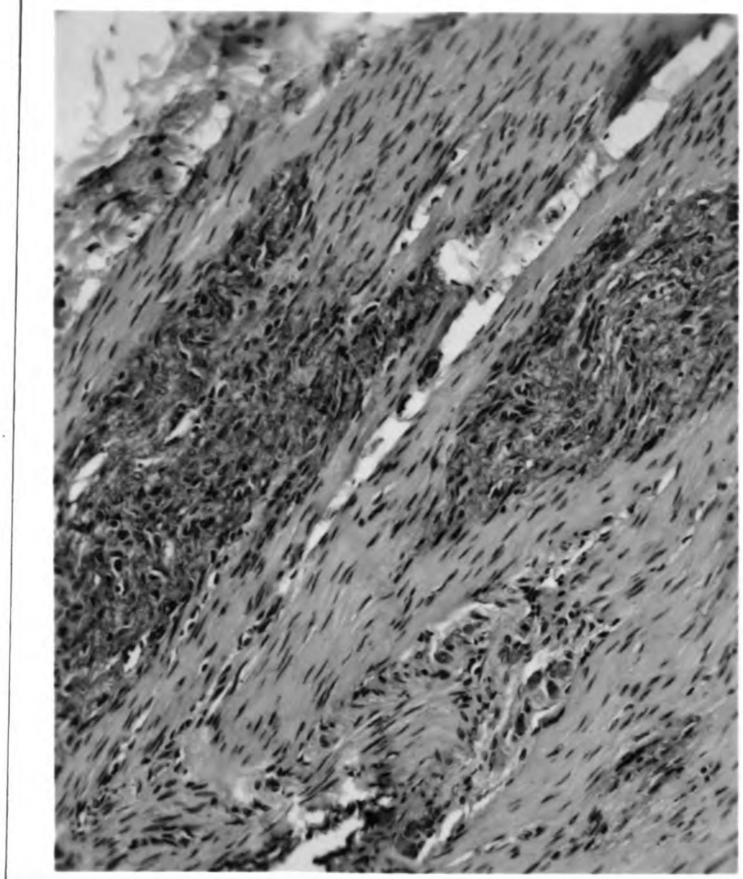

Figure 14

Muscular layer of stomach. Observe necrosis and fragmentation of the muscle cells. Rat 13, Group 13, Experiment 3, H & E Stain x 560.

Hematologic determinations. The results of the hematologic determination on the terminal blood samples from treated and control rats are given in Table 7. Packed cell volumes were highest in Group 3. Hemoglobin concentration was similar in all groups. Calcium levels were significantly higher in Groups 2 and 3 ($P < 0.05$). Phosphorus levels in the blood were not significantly altered in Groups 2 and 3. Values for inorganic phosphorus in Group 4 are not necessarily representative of the group, due to hemolysis of the majority of the samples. The constant K value was significantly higher ($P < 0.05$) in Groups 2 and 3 as compared to the controls, even though the values for inorganic phosphorus were essentially the same in Groups 1, 2 and 3. Values for the constant K in Group 4 are not meaningful due to the hemolysis mentioned previously. Alkaline phosphatase values are not included for the same reason.

Table 7. Terminal hematologic determination from rats in Experiment 3.#

Group No.	No. of rats	Initial daily dose	Average values and range				
			Packed cell volume %	Hemoglobin (Gm./100 ml)	Calcium (mg./100 ml)	Inorganic phosphorus (mg./100 ml)	Constant K (calcium x phosphorus)
1	6	Vehicle	32.5 (26.0-35.1)	12.8 (9.1-13.3)	9.9 (9.3-10.4)	7.8 (6.7-9.5)	77.2 (62.3-98.8)
2	6	DHT* 25 ug.	34.3 (27.3-41.2)	13.7 12.0-14.8)	12.1* (10.8-13.7)	8.1 (8.6-8.7)	98.0* (82.0-119.1)
3	6	DHT, 25 ug. Calcitonin, 20 MRC U/kg.	36.0* (33.1-38.9)	13.8 (13.4-14.2)	12.2* (10.8-13.1)	8.1 (7.6-9.0)	98.8* (84.2-117.9)
4	6	Calcitonin 20 MRC U/kg.	31.4 (30.3-40.0)	13.4 (11.8-14.7)	9.6 (8.8-10.0)	**	**

*Significantly different ($P < 0.05$) from control values
**Insufficient number of samples for analysis.
#Experiment 3 lasted 15 days.

DISCUSSION

These experiments indicate that calcitonin is unable to prevent soft tissue calcification produced by DHT in adult rats. Calcification was not prevented even by simultaneously reducing the length of the experiment, lessening the dose of DHT and reducing the intervals of administration of calcitonin. Hence, under the conditions of these experiments, calcitonin administration did not have a protective or prophylactic action.

There are many factors either singly or in combination that could have affected the results already described. The following might be the more likely:

Dosage of calcitonin. The dose level of calcitonin utilized in the experiments described here was 20 MRC Units/kg. and was similar to the dosage utilized by Farnell (1969). This author was able to prevent calcification in magnesium-deficient rats by using calcitonin. As a basis of comparison, Foster et al. (1966) utilized 40 MRC Units/kg. four times daily or 80 MRC Units/kg. once a day for 28 days to produce measurable effects on bone density in parathyroidectomized rats.

Depletion of endogenous calcitonin. A depletion of calcitonin in the thyroid gland is a possibility pointed out by certain workers. Gittes et al. (1968) demonstrated that when marked hypercalcemia was induced by repeated calcium chloride

administration for 6 hours there was a 30 to 60 percent reduction in the amount of calcitonin in the thyroid gland in rats. Albrecht (1968) also reported depletion of endogenous calcitonin after a treatment with daily injections of 5,000 I.U. of Vitamin D_3 for 7 days. Logically, in the present experiments the release of endogenous calcitonin should have been slight since the hypercalcemic factor was given for 15 to 30 days. Furthermore, if endogenous calcitonin was not being produced, the exogenous calcitonin that was administered might have served only to compensate for this lack of secretion. If, in turn, the dose of exogenous calcitonin was too low, the osteolytic effect of parathormone would not be neutralized. In this hypothetical condition, then, 2 hypercalcemic mechanisms could possibly be working together (hypervitaminosis D and hyperparathyroidism) without an adequate compensatory mechanism.

Compensatory parathormone secretion. Due to the experimental design it is not known if the hypercalcemia noted in the terminal analysis of blood was a feature throughout the experiments. If hypercalcemia were constant during the entire experiment, parathormone secretion would not be expected. But if this were not the case the action of calcitonin could have resulted in a compensatory secretion of parathormone. Foster et al. (1966) was unable to produce changes in bone in intact rats treated with calcitonin and concluded that parathormone

secretion was responsible since parathyroidectomized rats responded to calcitonin treatment by marked changes in bone.

DHT action in adult rats. Another factor to consider is that the action of DHT on adult rats is extremely severe. Selye (1957) reported the different sensitivity of adult and young rats. Young rats had lesions less severe than older rats and usually the lesions occurred only in the cardiovascular system. In Experiment 2 none of the younger rats lost weight at any dose level of DHT, and the lesions that occurred were not severe. Adult rats, on the other hand, were more susceptible to weight loss and lesions were more prominent.

Calcitonin action in adult rats. On the contrary, adult rats have been found less responsive to calcitonin. The evidence for this has been provided by several authors. Milhaud et al. (1967) reported that calcitonin was most effective in the young, rapidly growing rat. The rate of hypercalcemia was also found to be slower in the old rats than in the young rats (Orimo, 1967). This suggests a less vigorous endogenous calcitonin response with advancing age. Similar results have been obtained by Cooper et al. (1967) in rats and by Care and Duncan (1967) in sheep and lambs. Rasmussen and Tenenhouse (1967) pointed out that a change from a 125-g. rate to a 250-g. rat usually leads to 1- to 2-fold decrease in

sensitivity to parathyroid hormone, but at least an 8- to 10-fold decrease in responsiveness to calcitonin. Pechet et al. (1967) anticipated that calcitonin "will be found to play a more prominent role in younger animals with high rates of bone growth than in older animals, since the greater the rate of bone turnover, the greater the need for the regulation of bone resorption promoted by calcitonin".

The above considerations regarding the action of both DHT and calcitonin on adult rats led to the conclusion that the age of the rats utilized in these experiments is probably the most important single factor in explaining the failure of calcitonin to prevent soft tissue calcification.

"Escape phenomenon". Possibly what Raisz et al. (1967) described as an "escape phenomenon" could have occurred. This is mainly a reduced effect of calcitonin after repeated injections. Bone in culture failed to respond to further additions of calcitonin after 48 hours. In an in vivo experiment, the hypocalcemic effect of calcitonin was less in rats that had been previously treated with calcitonin than in control rats with no prior treatment with calcitonin.

Diet. Workers in several laboratories have mentioned that dietary calcium and phosphate has an influence on the response to exogenous calcitonin. Hirsch and Munson (1969) pointed out

that the dietary intake of calcium and phosphate and, especially, the calcium:phosphorus ratio influence calcium absorption and excretion, the level of serum calcium and hence the secretion of parathyroid hormone, the rate of bone resorption and, most likely, the secretion of calcitonin. The hypocalcemia produced by calcitonin in rats fed a diet containing 0.05% calcium and 0.3% phosphorus (standard Harvard low-calcium diet) was much greater than that produced in rats fed a commercial diet which was low in phosphate and calcium. This probably indicates that the response to calcitonin administration could have been considerably increased by adding phosphate to the diet used in our experiment.

SUMMARY

Three experiments, using a total of 60 rats, were conducted to determine the influence of calcitonin administration in preventing or diminishing the clinical signs and tissue calcification associated with administration of dihydrotachysterol (DHT), a vitamin derivative.

Daily oral administration of 50 µg. of DHT in corn oil produced clinical signs of progressive emaciation, muscular weakness and death. Kyphosis and enteric disturbances were evident. There were gross evidences of mineralization on the surface of the myocardium and kidneys. Microscopically there was extensive calcification in the aorta, heart, kidneys, stomach, and, to a lesser extent, the small intestine and lungs. Rats given the same amount of DHT and 20 MRC Units/kg. of calcitonin had the same clinical signs and lesions as those given only the DHT. The degree of soft tissue calcification was dose-related. Decreasing the interval of calcitonin administration had no influence in decreasing the soft tissue calcification. Calcification of soft tissue by DHT was not prevented by calcitonin.

BIBLIOGRAPHY

Albrecht, G.J.: Der Einfluss von Vitamin D_3 auf die Acktivität des Thyrocalcitonins in Rattenschilddrüssen. Z. Physiol. Chem., 349, (1968):779-783.

Aliapoulios, M. A., Goldhaber, P., and Munson, P. L.: Thyrocalcitonin inhibition of bone resorption induced by parathyroid hormone in tissue culture. Science, 151, (1966): 330-331.

Bajusz, E., Jasmin, G. and Mongeau, A.: Thyroid-parathyroid interrelationship in processes governing myocardial calcification. Endocrinology, 73, (1963): 92-96.

Baylink, D., Morey, E. and Rich, C.: Effect of calcitonin on the rates of bone formation and resorption in the rat. Endocrinology, 84, (1969): 261-269.

Bussolati, C., and Pearse, A. G. E.: Immunofluorescent localization of calcitonin in the "C" cells of pig and dog thyroid. J. Endocrinol. 37, (1967): 205-210.

Care, A. D., and Duncan, T.: Age as a factor in the response to thyrocalcitonin secretion. J. Endocrinol., 37 (1967): 107-108.

Coles, E. H.: Veterinary Clinical Pathology. W. B. Saunders Company. Philadelphia and London. 1967., p. 83.

Cooper, C. W., Hirsch, P. F., Toverud, S.U. and Munson, P.L.: An improved method for the biological assay of thyrocalcitonin. Endocrinology, 81, (1967): 610-616.

Copp, D. H., Cameron, E. C., Cheney, B., Davidson, A. G. F., and Henze, K. G.: Evidence for calcitonin-a new hormone from the parathyroid that lowers blood calcium. Endocrinology, 70, (1962): 638-649.

Copp, D. H., Lockcroft, D. W., and Kueh, H.: Ultimobranchial origin of calcitonin. Hypocalcemic effect of extracts from chicken glands. Canad. J. Physiol. and Pharm., 45, (1967): 1095-1099.

Copp, D. H.: Calcitonin and parathyroid hormone. Ann. Rev. of Pharmacology, 9, (1969): 327-344.

Farnell, D. R.: Influence of porcine thyroicalcitonin in magnesium deficient and normal rats. Ph.D. Thesis. Michigan State University. 1969.

Fiske, C. A., and Subbarow, Y.: The colorimetric determination of phosphorus. J. Biol. Chem., 66, (1925): 375-380.

Foster, G. V., Baghdiantz, A., Kumar, M. A., Slack, E., Soliman, H. A., and MacIntyre, I.: Thyroid origin of calcitonin. Nature (Lond.), 202, (1964): 1303-1305.

Friedman, J., and Raisz, L. G.: Thyrocalcitonin: inhibitor of bone resorption in tissue culture. Science, 150, (1965): 1465-1467.

Gaillard, P. J.: Bone culture studies with thyrocalcitonin. Cited by MacIntyre, I. (1967), p. 178.

Giraud, V. F., Doyle, F. H., Bordier, P., Matrajt, H., and Tun-Chot, S.: Roentgenologic and histologic changes in bone produced by thyrocalcitonin. Am. J. Med., 43. (1967): 691-695.

Gittes, R. F., Toverud, S. U., and Cooper, C. W.: Effects of hypercalcemia and hypocalcemia on the thyrocalcitonin content of rat thyroid glands. Endocrinology, 82, (1968): 83-90.

Gittes, R. F., and Irvin, G. L.: Thyroid and parathyroid roles in hypercalcemia:evidence for a thyrocalcitonin-releasing factor. Science, 148, (1965): 1737-1739.

Gudmundsson, T. V., MacInytre, I., and Soliman, H. A:: The isolation of thyrocalcitonin and a study on its effects in the rat. Proc. Roy. Soc. B., 164, (1966): 460-477.

Hirsch, P. F., Gauthier, G. F., and Munson, P. L.: Thyroid hypocalcemic principle and recurrent laryngeal nerve injury as factors affecting the response to parathyroidectomy in rats. Endocrinology, 73, (1963): 244-252.

Hirsch, P. F., and Munson, P. L.: Importance of the thyroid gland in the prevention of hypercalcemia in rats. Endocrinology, 79, (1966): 655-658.

Hirsch, P. F., and Munson, P.L.: Thyrocalcitonin. Physiol. Reviews, 49, (1969): 548-622.

Johnston, C. C. Jr., and Deiss, W. P. Jr.: An inhibitory effect of thyrocalcitonin on calcium release *in vivo* and on bone metabolism *in vitro*. Endocrinology, 78, (1966): 1139-1143.

Klein, D. C., and Talmage, R. V. Thyrocalcitonin suppression of hydroxyproline release from bone. Proc. Soc. Exp. Biol. and Med., 127, (1968): 95-98.

Kraintz, L., and Puil, E. A.: Absence of hypocalcemic activity in chicken thyroid. Canad. J. Physiol. and Pharm., 45, (1967): 1099-1103.

Krawitt, E. L.: Effect of thyrocalcitonin on duodenal calcium transport. Proc. Soc. Exptl. Biol. Med., 125, (1967): 1084-1086.

Kumar, M. A., Foster, G. V., and MacIntyre, I.: Further evidence for calcitonin, a rapid acting hormone which lowers plasma-calcium. Lancet, II, (1963), 480-482.

Kumar, M. A., Sturtridge, W. C., Jowsey, J., and Wase, A. W. Cited by Hirsch and Munson (1969), p. 573.

Lewis, A. E. Biostatistics. Reinhold Publishing Co., New York. 1966, p. 186.

MacIntyre, I., Foster, G. V., and Kumar, M. A.: The thyroid origin of calcitonin. In: The parathyroid glands. (P. J. Gaillard, R. V. Talmage and A. M. Budy, eds.), Chicago Univ. Press, Chicago, Ill., 1965, p. 89.

MacIntyre, I., : Calcitonin:A general review. Cal. Tiss. Res., 1, (1967): 173-182.

McLean, F. C., and Urist, M. R.: Bone. The Univ. of Chicago Press, Chicago, Ill., 1955.

Martin, T. J., Robinson, C. J., and MacIntyre, I.: The mode of action of thyrocalcitonin. Lancet, I, (1966): 900-902.

Mazzuoli, G. F., Terrenato, L., Coen, G., and Antonozzi, I.: Effect of thyrocalcitonin on bone and renal excretion of calcium 45 and strontium 85 in the rat. Folia Endocrinol (Pisa), 19, 1966): 7-13.

Melancon, M. J., and De Luca, H. F.: Interrelationships between thyrocalcitonin, parathyroid hormone and vitamin D: Control of serum calcium in hypervitaminosis D. Endocrinology, 85, (1969): 704-710.

Milhaud, G., and Moukhtar, M. S.: Hypophysectomie et thyrocalcitonine. C. R. Acad. Sci., Paris, 260, (1965): 3179-3182..

Milhaud, G., and Moukhtar, M. S.: Thyrocalcitonin: effects on calcium kinetics in rats. Proc. Soc. Exp. Biol. Med., 123, (1966a): 207-209.

Milhaud, G., and Moukhtar, M. S.: Antagonistic and synergistic actions of thyrocalcitonin and parathyroid hormone on the levels of calcium and phosphate in the rat. Nature, 211, (1966b): 1186-1187.

Milhaud, G., Perault-Staub, A. M., and Moukhtar, M. S.: Thyrocalcitonine: effet de l'age sur l'hypocalcemie et l'hypophosphatemie. Compt. Rend., 264, (1967): 110-113.

Mittleman, R., Chausmer, A., Bellavia, J., and Wallach, S.: Thyrocalcitonin activity in hypercalcemia produced by calcium salts, parathyroid hormone and vitamin D. Endocrinology, 81, (1967): 599-604.

Mulligan, R. M.: Metastatic calcification. Arch. Path., 43, (1947): 177-230.

Munson, P. L., Hirsch, P. F., Brener, H. B., Reisfeld, R. A., Cooper, C. W., Wasthed, A. B., Orimo, H., and Potts, J.T., Jr.: "Thyrocalcitonin", in Recent Prog. in Horm. Res., 24, (1968): 589-650.

Patton, J., and Reeder, W.: New indicator for titration of calcium with (Ethylenedinitrilo) tetraacetate. Anal. Chem. 28, (1956): 1026-1028.

Pearse, A. G.E.: The cytochemistry of the thyroid C cells and their relationship to calcitonin. Proc. Roy. Soc. B., 164, (1966): 478-487.

Pechet, M. M., Bobadilla, E., Carroll, E. L., and Hesse, R. H.: Regulation of bone resorption and formation. Am. J. Med., 43, (1967): 694-710.

Raisz, L. G., Au, W. Y. W., Friedman, J., and Niemann, I.: Thyrocalcitonin and bone resorption. Studies employing a tissue culture bioassay. Am. J. Med., 43, (1967): 684-690.

Rasmussen, H., and Tenenhouse, A.: Thyrocalcitonin, osteoporosis and osteolysis. Am. J. Med., 43, (1967): 711-726.

Reynolds, J. J.: Inhibition of thyrocalcitonin of bone resorption induced *in vitro* by vitamin A. Proc. Roy. Soc. (London), Ser. B., 170, (1968): 61-69.

Robinson, C. J., Martin, T. J., and MacIntyre, I.: Phosphaturic effect of thyrocalcitonin. Lancet, II, (1966): 83-84.

Robinson, C. J., Martin, T. J., Matthews, E. W., and MacIntyre, I.: Mode of action of thyrocalcitonin. J. Endocrinol., 39, (1967): 71-79.

Sanderson, P. H., Marshall, F., and Wilson, R. E.: Calcium and phosphorus homeostasis in the parathyroidectomized dog; evaluation by means of EDTA and calcium tolerance tests. J. Clin. Invest., 39, (1960): 662-670.

Selye, H.: On the stimulation of new bone-formation with parathyroid extract and irradiated ergosterol. Endocrinology, 16, (1932): 547-558.

Selye, H.: Effect of various hormones upon the syndrome of dihydrotachysterol intoxication. Acta Endocrinol., 25, (1957): 83-90.

Selye, H., Strebel, R., and Mikulaj, L.: A progeria-like syndrome produced by dihydrotachysterol and its prevention by methyltestosterone and ferric dextran. J. Am. Geriatr. Soc., 11, (1963): 1-16.

Selye, H., Tuchweber, B., and Jacqmin, M.: Protection of various anabolic steroids against dihydrotachysterol induced calcinosis and catabolism. Acta Endocrinol., 49, (1965): 589-602.

Soliman, H. A., Robinson, C. J., Foster, G. V., and MacIntyre, I.: Mode of action of calcitonin. Cal. Tiss. Res., 1, (1967): 242-243.

Wase, A. W., Peterson, A., Rickes, E., and Solewski, J.: Some effects of thyrocalcitonin on calcium metabolism of the rat. Endocrinology, 79, (1966a): 687-691.

Wase, A. W., Solewski, J., Rickes, E., and Seidenberg, J.: Action of thyrocalcitonin on bone. Nature, 214, (1966b): 388-389.

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