elevating property of DCA for the first 22 days of the experiment. Thereafter the EST increased to a final value intermediate between normal and that of rats treated with DCA alone.

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Alterations in Long Bones of Young Albino Rats Induced by Dihydrotachysterol. (Hytakerol).* (18212)

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In the stages of transformation of ergosterol by ultra-violet radiation into a substance with antirachitic potency, Holtz and Schreiber(2) had determined the presence of two different factors, the antirachitic factor and a separate intermediate factor which they called the "Kalcinosefactor." A year later Windaus(3) reported the isolation of a toxic substance, tachysterol which he thought was a contaminant in highly effective antirachitic agents. Animal experimentation indicated that the dihydro-derivative of tachysterol had marked hypercalcemic effects, which suggested its use as a therapeutic agent in parathyroprivia (4,5). This drug was first used clinically by Holtz and its nature exhaustively restudied by von Werder(6). Albright and Ellsworth (7) had considered the action of parathormone was to raise the level of urinary phosphate excretion, and Albright(8) later demonstrated that the actions of parathormone, vitamin D and dihydrotachysterol were similar but that the action of the latter was more nearly like that of parathormone because it tended to increase urinary phosphorus excretion in contrast to that of vitamin D which increased intestinal absorption of calcium.

The present investigation was undertaken to determine the nature of changes occurring in growing bone resulting from toxic dosage of dihydrotachysterol and to compare these with changes reported, using other factors regulating bone architecture. The only such reference in English that we have found is a statement by McLean(9) that the lesions resulting from administration of vitamin D and dihydrotachysterol were similar and that no fibrous infiltration was observed as seen in hyperparathyroidism.

Methods. A group of 9 rats (A) 1 month old, were given daily subcutaneous injections over a period of from 30 to 35 days, total dosage varying from 3.75 mg to 5.5 mg of the crystalline dehydrotachysterol. Two litter mates were given daily injections of sesame oil and 2 others served as untreated controls (Table I). In 2 rats (Group B), 2 months old, the drug was given daily by stomach tube over a period of 10 days, total dosage being 1.03 mg and 1.16 mg of crystalline product. Again 2 litter mates were given an equivalent amount of sesame oil over the same

* Some of these experiments were presented by title at the American Association of Anatomists, April 5, 1950(1).

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2. Windaus, A., *Proc. Roy. Soc. London.*, Series B., 1931, v108, 568.

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7. Albright, F., and Ellsworth, R., *J. Clin. Invest.*, 1929, v7, 183.

8. Albright, F., *Trans. Am. Assn. Phys.*, 1938, v53, 221.

9. McLean, F. C., *J.A.M.A.*, 1941, v117, 609.

TABLE I. Drug and Oil Administered by Subcutaneous Injection. All animals 1 month old.

Animal	Length of exp. in days	Treatment	Total dosage
A 1 ♀	35	0	Control
2	35	0	,,
3	35	Sesame oil 3 cc	,,
4	35	4.4	,,
5	35	Dihydrotachysterol	3.75 mg cryst.
6 ♂	35	,,	4.375
7 ♀	30	,,	5.5
8	30	,,	3.75
9 ♂	30	,,	4.5
10	30	,,	4.375
11	30	,,	4.375
12	30	,,	4.375
13	30	,,	4.375

TABLE II. Drug and Oil Administered by Stomach Tube. All animals 2 months old. Length of experiment for each animal, 10 days.

Animal	Treatment	Total dosage
B 1 ♀	0	Control
2 ♂	0	,,
3	Sesame oil 10.3 cc	,,
4 ♀	23.3	,,
5	Dihydrotachysterol	1.03 mg cryst.
6 ♂	,,	1.16

TABLE III. Drug and Oil Administered by Stomach Tube. All animals 2 months old.

Animal	Length of exp. in days	Treatment	Total dosage
C 1 ♀	7	0	Control
2	14	0	,,
3	21	0	,,
4	7	Sesame oil 3.1 cc	,,
5	14	7.2	,,
6	21	11.1	,,
7	7	Dihydrotachysterol	.31 mg cryst.
8 ♂	7	,,	.42
9 ♀	14	,,	.81
10 ♂	14	,,	1.15
11 ♀	21	,,	1.67
12 ♂	21	,,	1.68
13	28	,,	2.64
14	42	,, 28 days	
		14 days recovery	2.55

period and 2 others remained as untreated controls (Table II).

A third group of 8 animals (C) were given the drug every second to fourth day by stomach tube over a period of from 1 to 4 weeks, total dosage varying from .31 mg to 2.645 mg of the crystalline product. Controls for this group were similar to those in Groups A and B (Table III).

During the experimental period the animals were fed Rockland Rat Diet supplemented by corn, carrots and green vegetables. Weight charts on individual animals of Group A showed no significant variations between controls and treated animals. In Groups B and C, however, with oral administration the treated animals failed to gain and in most cases, lost weight after 6 to 10 days of treatment. This tendency was observed also in animals given only sesame oil orally. At the end of the experimental period the animals were killed, the long bones removed, fixed in Zenker-formol solution, decalcified, sectioned and stained in routine manner, Masson's trichrome stain being most used. Samples of liver, lung and kidney were also obtained for reference.

Experimental results. Controls. Examination of histological preparations of femurs from untreated controls and those either injected or fed sesame oil gave findings in all cases consistent with those of all rats in the various age groups. In all cases the epiphyseal cartilages were proliferative with regular zones of provisional calcification on the diaphyseal side. Trabeculae in the metaphyses were regularly arranged and covered with a row of osteoblasts. The compact bone of the shaft was regularly and evenly formed with no irregularities on either periosteal or endosteal surfaces.

Group A. In all the animals of Group A, however, subjected to daily subcutaneous injections of dihydrotachysterol, the epiphyseal cartilages were somewhat wider than normal with no apparent alteration in the cartilage matrix. The diaphyseal border was very irregular, some columns of hypertrophic degenerating cartilage cells extending deeply into the metaphysis. On the epiphyseal side of the cartilage the cell groups were somewhat irregular also (Fig. 1). The most marked changes observed in the treated animals of this group were found in the compact bone of the shafts. The thickness of the bone was considerably decreased. Numerous cystic spaces and channels had perforated this compact bone (Fig. 2), in some cases offering

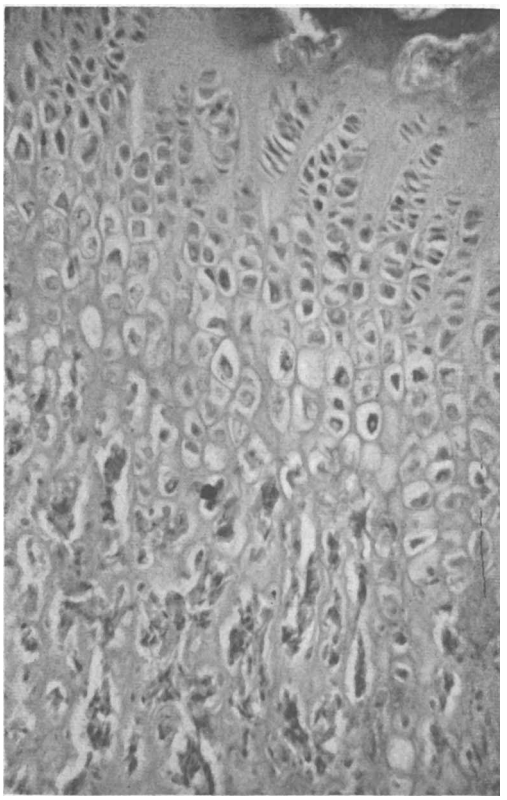

FIG. 1.

Femur, rat A6, treated subcutaneously for 35 days, total dosage 5.5 mg cryst. dihydrotachysterol, showing distortion of cell columns in the epiphyseal cartilage and irregular lacy trabeculae in the metaphysis. ×165.

wide communication between the periosteum and the marrow cavity. Closer examination showed these spaces to be quite variable in size and shape, but limited largely to the inner two-thirds of the bone. In some instances these spaces were filled with hemopoietic tissue. Most of the spaces contained a very vascular connective tissue with numerous fibrocytes with large pale nuclei and slender processes. Around the periphery of some of these cavities particularly the most extensive ones, occasional multinucleated osteoclasts were encountered (Fig. 3). Histological examination of samples of liver, lung and kidney and parathyroid from animals treated with the drug revealed no evidences of calcification, necrosis or other pathological alteration.

Group B. In the 2 animals given dihydrotachysterol daily for 9 and 10 days respectively despite evident disturbance as indicated by weight loss there was little evidence of an alteration in the bony structure. Epiphyseal cartilages, and epiphyseal and metaphyseal trabecular bone were quite similar to that even in oil treated and untreated controls. However, in the compact bone of the shaft the endosteal surfaces were characterized by semicircular depressions. In some places this surface had thin inner layer of more basically stained bone, evidently of recent deposition.

Sections of livers of these animals revealed in one case (a female) a marked fatty change in the liver cells in the central portion of the lobules indicated by the presence of one or more large cytoplasmic vacuoles. Cells in periportal areas exhibited cloudy swelling. A few apparent cases of brightly stained precipi-

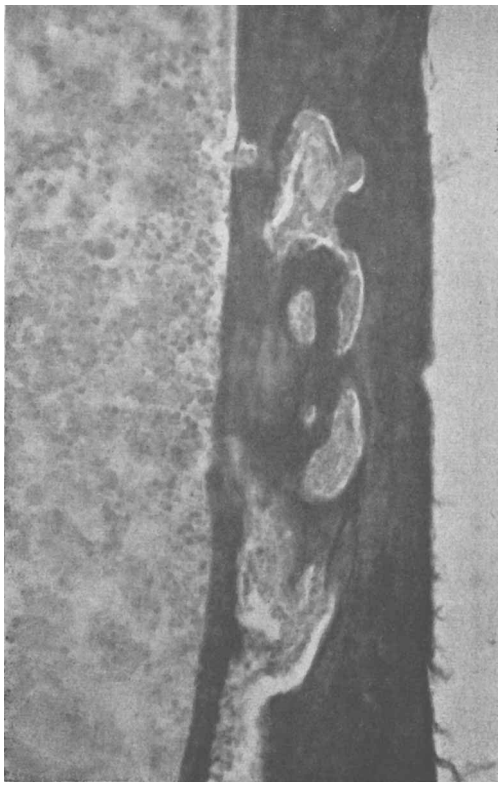

FIG. 2.

Femur, rat A5, treated subcutaneously for 35 days, total dosage 3.75 mg cryst. dihydrotachysterol, showing extensive channels and cystic spaces in the cortex of the shaft resulting from osteolysis. ×165.

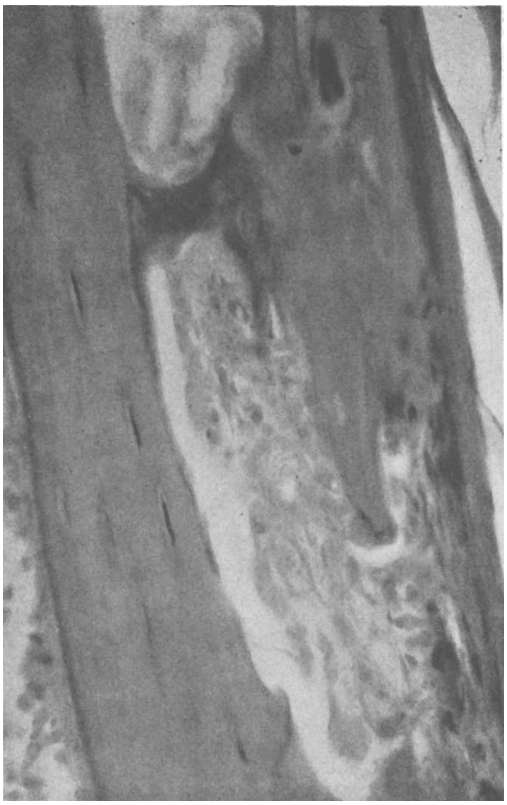

FIG. 3.
Detail of a portion of the cortex of the femoral shaft of rat A6, (same as in Fig. 1) illustrating infiltration of one of the cavities by cellular fibrous tissue. Note the enlargement of the bone lacunae. ×420.

tated material were found in and around nephric tubules, but no areas of definite calcification were seen.

Group C. In this group the sesame oil and dihydrotachysterol were fed by stomach tube every second, third or fourth day, over periods of from 1 to 4 weeks, thus varying the total dosage considerably and allowing greater total dosage without lethal effects. In those animals treated for 1 or 2 weeks, with relatively low total dosage, alterations similar to those described in Group B were encountered in the long bones. As treatment was prolonged 3 or 4 weeks and total dosage correspondingly increased, marked changes were found resembling but reaching greater severity than those described for Group A, injected subcutaneously.

The most obvious changes were a marked decrease in thickness of the compact bone, extensive widening of the existing Havervian canals, and great irregularity of, the endosteal surfaces (Fig. 4). Numerous spaces in bone had become invaded either by fibro-vascular or actively hemopoietic tissue. Some of these extended almost or quite to the periosteal surface (4 weeks) so making it difficult to conceive of the bone as effective in weight bearing, and making histological preparation difficult (Fig. 5). The epiphyseal cartilages were most irregular in width, some groups of swollen cartilage cells being found deep in the metaphyses. The regularity of the cell columns was again much distorted which was to be associated with equal distortion of metaphyseal bone which consisted of a lacy net-

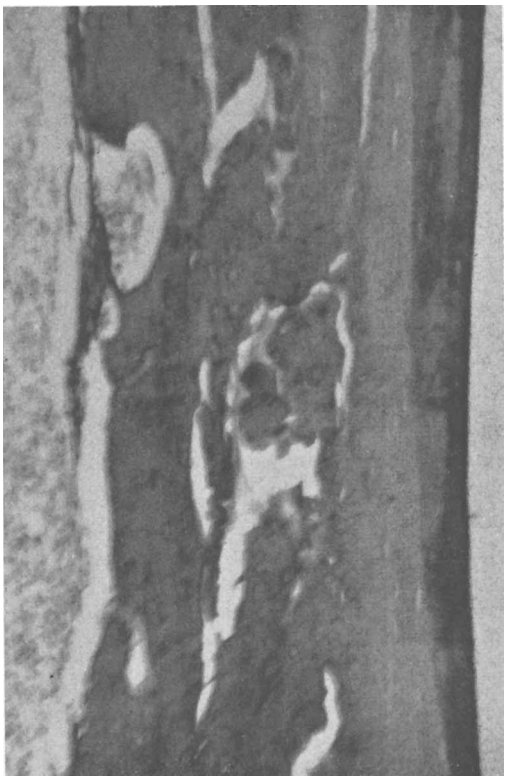

FIG. 4.
Cortex of the femoral shaft of rat C12, with total dosage of 1.68 mg cryst. dihydrotachysterol given by stomach tube over a 3 weeks period. Extensive breakdown of the lamellar pattern, cavitation, and isolation of bone fragments are noted. ×323.

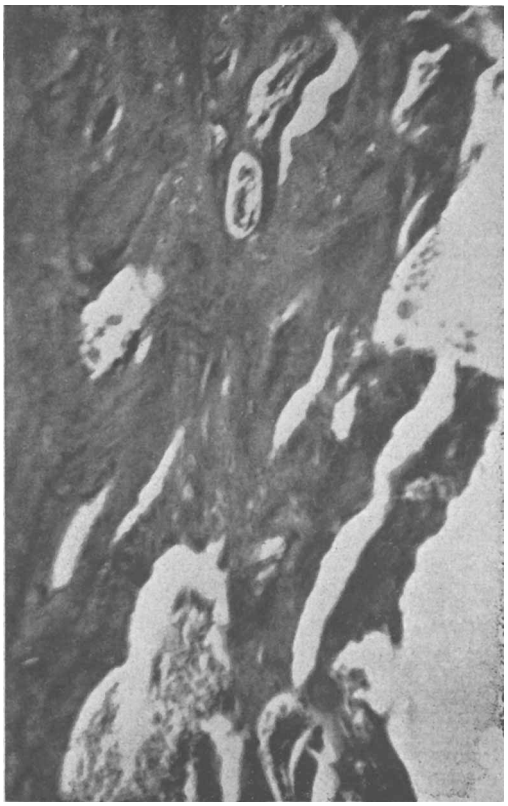

FIG. 5.
Cortex of the femoral shaft of rat C13 treated by stomach tube, over a 4 weeks period, with total dosage of 3,645 mg cryst. drug. Very extensive breakdown, with channeling and cavitation throughout the inner portion of shaft, with some infiltration by marrow tissue. ×323.

work of slender trabeculae. This same distortion was also apparent in the spongy bone of the epiphyses. These changes were of course most marked in animals treated over a 4 week period. In the one animal so treated and allowed 2 weeks for recovery there was no evidence of a recovery in the skeletal architecture.

In some animals following 4 weeks of treatment by feeding particularly the rat allowed a 2 weeks recovery period. The histological structure of the liver and kidneys was considerably altered. After 4 weeks liver cells placed centrolobularly were swollen, granular and vacuolated, many with pycnotic nuclei. After the same treatment and 2 weeks recovery this change, resembling that seen with heavy glycogen deposition, was seen in practically all liver cells.

In all cases after the first week of treatment tubular casts and stromal deposits of heavily stained, apparently precipitated material, were present increasingly as the time and total dosage increased. Tubular block and resultant dilation of proximal tubules and Bowman's capsule became very prominent in those animals receiving greater total dosage. Epithelial degeneration, sluff and underlying regeneration were frequently seen in proximal (?) tubules in the case in which 2 weeks recovery time was allowed. In scattered areas definite calcified deposits were found.

Discussion. The abundant evidence presented here of bone resorption in young rats resulting from chronic dosage with dihydrotachysterol is in partial agreement with findings of Lenel(10) on chronically treated rats. We found considerably less evidence of regenerative activity expressed in newly formed osteoid tissue. The bones of our experimental animals, maintained on a completely adequate diet, did not exhibit any evidence of bone necrosis as shown by Lenel. The series of channels and cystic spaces extending principally into hemopoietic marrow or, at first with cellular fibrous tissue, suggesting lesions of hyperparathyroidism. With the high total dosage given over a 4 week period this resorption of bone salts reached marked proportions. The failure to find evidence of reparative growth in the individual allowed 2 weeks for recovery indicates that the resorptive effect of the drug is cumulative and long lasting and does not support Lenel's findings of an osteosclerotic phase, over long experimental periods.

Our reported findings of distortion occurring in the epiphyseal cartilage and irregularities in the resorption process in the metaphyses of the long bones during and following treatment have not previously been reported. The experiments reported by Eger and Titze (11) were not comparable to those we have presented but do not support our findings of bone resorption, because of difference in dos-

10. Lenel, R., *Frankf. Z. Path.*, 1941, v56, 31.

11. Eger, W., and Titze, G., *Klin. Wochensch.*, 1942, v21, 859.

age. The increasing incidence of calcium (?) casts in the convoluted tubules and deposits in interstitial tissue of the kidney as total dosage increased indicates a direct relationship between the amount of dihydrotachysterol present, the amount of calcium removed from bony tissues, and its excretion by the kidneys.

The similarity between the lesions in cortical bone we have demonstrated and those experimentally produced by overdosage with commercial vitamin D products, as viosterol (*e.g.* Jones and Robson) (12), leads to the suggestion that the osteolytic effects encountered in the latter case were induced by the presence of dihydrotachysterol as a contaminant. It is interesting to note that similar lesions in cortical bone were demonstrated in guinea pigs depleted of vitamin C by Silberberg and Silberberg(13), probably induced in that instance by disappearance of the organic matrix.

It is quite possible that blocks resulting from calculi and interstitial calcification and subsequent dilation of damaged renal tubules in the more advanced stages of the treatment were of sufficient degree to have secondarily significantly altered the capacity of the kidney to excrete calcium and reabsorb phosphates.

Results obtained from administration of the drug by injection and by forced feeding indicate its greater absorption and effectiveness by the latter route as shown by the greater degree of bone resorption in animals of group C as compared with those of group A, treated over similar time periods.

Summary. Groups of young albino rats were treated either by repeated subcutaneous injections or by repeated forced feedings with dihydrotachysterol (Hytakerol) over periods ranging from 7 to 30 days, total dosage of the drug varying from .31 to 5.5 mg. The histological alterations in the long bones resulting from this treatment have been described. Significant changes were induced with relatively lower dosage when the drug was given by stomach tube but with either method were in direct proportion to the total dosage and duration of the experiment. The most striking changes consisted of the appearance of extensive channels, wide passageways and cystic cavities extending varying distances from the endosteal toward the periosteal surfaces of the cortex of the long bones. After prolonged oral administration most of the shaft was reduced to a trabecular meshwork. Some of these spaces were occupied by highly vascular hemopoietic tissue, with some infiltration of fibroblastic cellular tissue. Infrequently at the distal ends of these spaces, large multinucleated osteoclasts were found. With chronic oral treatment of a 10-day period only minor irregularities were encountered on the endosteal surface and a thin layer of osteoid tissue, apparently recently deposited, was present.

Although with lower dosage, no changes were found either in the metaphyseal bone or the epiphyseal cartilage, with great dosages and longer time intervals several variations were noted in the cartilage, and the metaphyseal bony trabeculae were very slender and distorted. We considered all of these changes to be the result of direct action of dihydrotachysterol causing a removal of calcium from bone. The appearance in late stages of renal tubular calculi, blockage, distention and cellular degeneration in proximal tubules were presumed to be the result of hypercalcemia secondary to the osteolysis caused by the drug.

The dihydrotachysterol (Hytakerol) used in the experiments was furnished through the courtesy of Dr. A. Scribner of Winthrop-Stearns, Inc.

12. Jones, J. H., and Robson, G. M., *Am. J. Physiol.*, 1933, v103, 338.

13. Silberberg, M., and Silberberg, R., *Anat. Rec.*, 1948, v102, 141.

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