HYPERVITAMINOSIS*

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THE strong, specific, anti-rachitic action of irradiated ergosterol was first shown by Holtz,[19] Gyöorgy[13] and Beumer and Falkenheim[3] and has since been often demonstrated. It appears that the feeding of normal individuals with irradiated ergosterol has led to somewhat different results in relation to the calcium and phosphorus content of blood. In healthy adults, Howard and Hoyle[21] found that the administration did not lead to an appreciable change in the inorganic phosphorus content of the blood, whereas Kroetz[32] has found an increase in inorganic phosphorus. Hess, Lewis and Rivkin[16] found that the administration of irradiated ergosterol to normal children led to an enhancement alike of the calcium and phosphorus content of their blood. With various chronic ailments in adults which were treated with increasing doses of viosterol an increase of calcium in the serum has been shown.

It is also a remarkable result of ergosterol treatment that in cases of osteomalacia a decided improvement was observed by Starlinger.[53] Treatment by fat restriction and large doses of viosterol was found by Linder and Harris[35] to be effective in cases of chronic diarrhœa with tetany. In artificially produced fractures in rats, Bors[5] claimed a speedy healing was obtained. Inspired by Bors' investigation, Knoflach[29] studied the clinical effect of viosterol in 51 cases of fractures of long bones, 31 other cases being reserved for controls. In children and in adults up to fifty-five years of age, no material influence upon the period of healing was noted, although callus formation was more rapid and abundant. In persons over fifty-five years old, however, a remarkable difference was observed. In 9 such cases, consolidation of the fractures occurred apparently more speedily than in young patients. Feriz[12] obtained good results with viosterol in the treatment of delayed union of fractures.

The pathological action of overdoses of ergosterol was first mentioned by Pfannenstiel[41] and further investigated by Kreitmair and Moll[31] in the same year. They produced in animals which were fed large amounts of ergosterol a strongly toxic action and an extreme medial calcification. Since then Kreitmair and Hintzelmann,[30] Wentzel[56] and Duguid,[11] and many others have shown a moderately complete picture of so-called hypervitaminosis. It results in calcification of the blood vessels, the stomach and the kidney. In rabbits, Baumgartner, King and Page[2] found a softening of the bones. Kidney concretions have been described by Dixon and Hoyle[10] (1928), (Hoyle[20]), and Spies and Glover.[52] Schmidtman[47] (1928), Reber and Walkoff,[45] Rabl,[44] Heubner[18] and Harris,[14, 15] Simmonet and Tanret,[50] all confirm in general these pathological findings with different preparations of irradiated ergosterol. Putschar[43] described marked calcification of the tubules and interstitial tissue in the kidneys of an atrophic child which had received viosterol (Vigantol) for three months prior to its death. Poucher,[42] however, claims that no ill effects have followed the feeding of as much as twenty-one to fifty-two times the prophylactic daily dose of viosterol.

Schwenholz[48] claimed that in the case of pregnant rats, fed 10 mg. of ergosterol a day, the young were resorbed *in utero*, were prematurely aborted, or were born dead. In a very few cases they lived, but were very small and under-developed and did not survive long. Comel[7] found that with bitches under intensive ergosterol treatment, enough ergosterol passes into the milk of the mother to provide toxic symptoms in the pups. Numerous other papers, mostly quite uncritical, have dealt with the effects of extraordinary doses. In many cases

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the authors failed to determine the potency of the product they employed, or at least did not state it, giving only the quantity of irradiated ergosterol in mg.

Harris and Moore,[14] working with a standardized product, demonstrated that 1,000 times the curative dose for rats was not harmful, that 10,000 times was definitely toxic, and 100,000 times was rapidly fatal. Bills and Wirick[4] found that they had to give 4,000 times the curative dose to get definite injurious effects in rats; 40,000 times the dose was strongly toxic. Harris and Moore[14] showed, further, that ergosterol itself, unirradiated, that is, the precursor of vitamin D, was harmless even in these large amounts; and that it was the active principle, the vitamin D which resulted on irradiation, which was the toxic substance. Over-irradiation, which destroys the anti-rachitic factor, resulted in a non-toxic product. Windaus,[57] on the other hand, has claimed that preparations of vitamin D, formed by irradiation of ergosterol, also contain a toxic substance which is probably distinct from the anti-rachitic factor, although the preparations most active in curing rickets are at the same time most toxic. Schultz and Meyer[49] claim that there is no parallelism between the anti-rachitic factor and the toxic factor in irradiated ergosterol.

The marked calcification of the vessels and organs and the apparent softening of the bones of animals fed ergosterol has led several investigators to look for an increased calcium and phosphorus content of the serum. The calcium is usually definitely increased, but the phosphorus is often inconclusive, showing variations which are not much greater than in the normals. Klein[28] and Smith and Elvove[51] (1929), in investigations into the effect of large doses of ergosterol on the calcium and phosphorus values of serum and tissue, found that the calcium was nearly always high but the phosphorus only slightly so. Taylor, *et al.*,[54] working with dogs, found, however, that the serum phosphorus showed a very marked rise just preceding the death of the animal. De Toni[9] was able to show a rise in the inorganic phosphorus of the serum of cattle, horses and human beings when irradiated ergosterol was added to it.

The hypercalcæmias produced by administration of parathormone and ergosterol are in many respects similar. The animals lose weight and appetite and some have diarrhœa. The daily administration of 10 mg. of irradiated ergosterol to normal dogs produces, according to Jones, Rapoport and Hodes,[25] a marked hypercalcæmia within two weeks, which is often higher than that produced by parathormone. A marked hypercalcæmia can be rapidly produced in parathyroidectomized dogs by the administration of large doses of irradiated ergosterol. Dogs from which the parathyroids have been removed can be kept alive indefinitely and apparently in good health by repeated doses of ergosterol. Demoli and Christ[8] found that giving irradiated ergosterol to dogs before parathyroidectomy prevents the onset of tetany. Pappenheimer[39] considered the action of ergosterol in producing hypercalcæmia and calcification to be independent of the parathyroid system, since he was able to get the same ergosterol effects in rats from which he considered he had removed all traces of parathyroid tissue. Taylor *et al.*,[54] however, consider that vitamin D exerts its action on blood calcium through stimulation of the parathyroid function. These workers found that after removal of the parathyroids by the usual method, administration of viosterol relieved the resulting tetany and raised the blood calcium, but if the operation was made to remove all parathyroid tissue from the neck of the dog, the animals were very resistant to viosterol treatment, tetany was not relieved and blood calcium remained low. (For an excellent review of the work on the possible relation of the effects of ergosterol to parathyroid function see the paper by Taylor, *et al.*[54]).

The question of the source of extra calcium in the serum under viosterol treatment has been a matter of some controversy. Hess, Weinstock and Rivkin,[17] Baumgartner, King and Page,[2] Light, Miller and Frey,[34] Brown and Shohl,[6] Watchorn,[55] Harris and Innes[15] and others have considered it to be drawn in whole or in part from the skeleton. On the other hand, Jones, Rapoport and Hodes[25] consider that the extra calcium comes from the food by increased absorption, and Jones and Robson,[26] failing to find any decrease in the bone ash after ergosterol treatment, conclude that "irradiated ergosterol in extremely toxic doses has no specific action which results in actually withdrawing

calcium salts from bone comparable to its ossifying action when given in therapeutic doses.''

In a series of experiments conducted on chickens we have found, as in the rabbits, a definite increase of the serum calcium, but only inconclusive variations in the phosphorus. This has also been the finding of Massengale and Nussmeier[37] for chickens. The birds, unlike rabbits and rats, however, have not shown the same definite calcification of the vessels. In a former investigation we were unable to discover any calcification in either vessels or organs, but in the experiments to be described we have now succeeded in producing marked calcification in the kidneys of most of the birds by a more prolonged period of feeding with an excess of ergosterol. As far as we are aware, this is the first record of definite calcification being produced in hypervitaminosis in chickens. Kreitmair (1928) considered chickens as being among the most refractory to ergosterol treatment of all the animals he tried. With doses which would produce severe calcification in cats, rabbits and rats, he was unable to demonstrate any changes in chickens. Their general condition, however, has been much the same as that of rabbits and rats. They have lost weight during the latter stages of feeding and have developed a marked leg weakness. It was expected that this weakened condition of the bone would be accompanied by a demineralization of the skeleton. If such withdrawal of calcium salts took place, however, it was not sufficiently large to show on analysis of the bones, as the ash, calcium and phosphorus of the dried bones of the chickens receiving excess of ergosterol were practically normal. It was found, however, that administration of parathormone to a similar group of chickens over a similar period of time also produced no appreciable change in the mineral constituents of the bone. Since parathormone is thought to produce its well known effect of raising the serum calcium by withdrawing calcium from the skeleton (see, for instance, Lambie, Kermack and Harvey,[33] Bauer, Aub and Albright[1] and Jaffe and Bodansky[24] [1930]) it was felt that the extra calcium produced in the serum by both ergosterol and parathormone probably came both in part from the bone and in part from increased absorption from the gut. In the experiments to be described there was ample calcium in the diet, and hence, increased absorption may here have been the predominating factor in producing the extra calcium. The apparent softened condition of the bone, together with the normal ash content, presents, however, an anomalous situation which it is hoped to elucidate by further experiments, using animals on diets containing both high and low calcium content.

Accompanying the softened condition of the bones of their rabbits, Baumgartner, King and Page[2] observed a decrease in the bone phosphatase (the enzyme discovered in ossifying cartilage and bone by Robison[46] and thought to be responsible for bone formation and maintenance). King and Hall[27] confirmed this finding, and in the present investigation a similar decrease in bone phosphatase under the action of ergosterol feeding has been observed. The enzyme content of the bones of the birds receiving parathormone, on the other hand, has remained quite normal; this is the reverse of the finding of Page[38] for the action of parathormone.

EXPERIMENTAL

On February 12, 1931, twenty-four five months' old barred Plymouth Rock pullets were divided into four pens of six birds each.

Each bird in pen 1 received 3 c.c. (1500 D) of viosterol* daily; those in pen 2 received 6 c.c. daily. Each bird in pen 3 received 5 units of parathormone† (¼ c.c.) daily, while the birds in pen 4 received 10 units of parathormone (½ c.c.) daily. The viosterol was given by means of a rubber catheter per os. The parathormone was administered by intramuscular injection.

The birds were weighed individually each week for six weeks, after which time, if alive, they were sacrificed and necropsies performed.

1. GROWTH AND DEVELOPMENT‡

For pen No. 2 we can consider this a regular decrease when we omit the first value. It is best to consider pen No. 1 in the same way. The values of pens 3 and 4 can be considered as growth or increase data. The increase for the

* We wish to record our thanks to the Winthrop Chemical Company, who kindly supplied us with this concentrated solution of ergosterol.

† The ''parathormone'' was kindly supplied to us by the Eli Lilly Company.

‡ We are indebted to Dr. R. C. Moffatt, of the Ontario Agricultural College, for this analysis of the growth rates.

first week in pens 1 and 2 can be considered as a "hang over" from previous natural growth, as the birds were still growing when changed from normal diet to this treatment.

The marked changes in the physical condition of the birds with comparatively small changes in the weights necessitated a rather detailed study of the growth data.

TABLE I.

AVERAGE WEIGHTS OF BIRDS AT WEEKLY INTERVALS IN OUNCES

Pen No.	*Feb. 14*	*Feb. 21*	*Feb. 28*	*Mar. 7*	*Mar. 14*	*Mar. 21*
1	57.0	57.2	57.8	56.2	55.4	48.6
2	56.0	61.8	59.4	55.2	51.6	47.8
3	66.2	69.5	69.8	70.8	69.3	67.2
4	48.0	49.3	52.7	53.7	55.7	57.5

The equation for all growth data is $W=Ae^{kt}$ where W is the weight, A is a constant, and e is the base for natural logarithms, 2.71828. k is a growth constant, which when multiplied by 100 gives the instantaneous percentage rate of growth and t is the time measured in units—in this case one week—from the beginning of the period for which the data are used.

In the case of pens 1 and 2, which give decay data and follow the law of decay, the value of k will evidently be negative.

Taking logarithms of the equation $W=Ae^{kt}$ we get the equation $\log W = \log A + 0.4343\ kt$ or $\log W = B + 0.4343\ kt$ which will be a straight line between the variables log W and t and the lines will slope upward from left to right.

For decay curves, as for pens 1 and 2, we have $W = Ae^{-kt}$ or $\log W = B - 0.4343\ kt$, which will be a straight line slanting downward from left to right. This can be shown to advantage on semi-logarithmic paper by plotting weights against time. (See Graphs I and II).

Using the method of least squares to find the equation of best fit for the above type, the following equations were found:

Pen 2 $W = 62.6e^{-0.066t}$ using last 5 values only

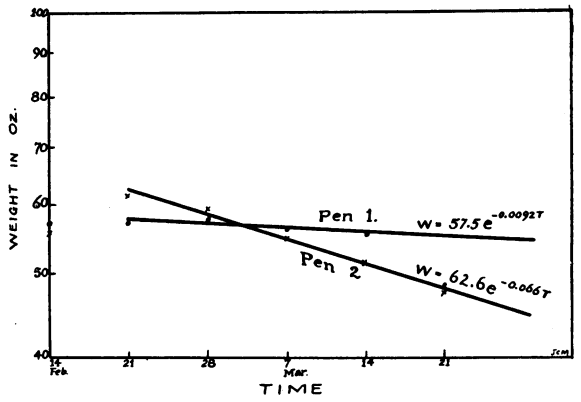
Pen 1 $W = 57.5e^{-0.0092t}$ using last 5 values only

Pen 3 $W = 68.4e^{0.0025t}$ using all data

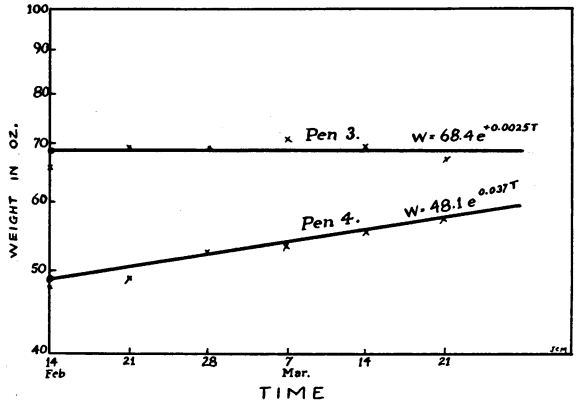
Pen 4 $W = 58.1e^{0.037t}$ using all data

For pen 2, the constant 62.6 is the most probable value of the weight corresponding to zero time or the weight at the beginning of the period for which the data were taken to work out this equation — in this case February 21. Similar interpretations can be made for the other equations. Pen 1 decreased from this

GRAPH I



GRAPH II.



period at the rate of only 0.92 per cent. However, pen 2 decreased at the rate of 6.6 per cent per week. Pens 3 and 4 increased at the rate of 0.25 per cent and 3.7 per cent respectively.

Geometric mean method (Moffatt).—In this method the rate of growth is based on the geometric mean of the values used (pen 2)

$$\text{or } k = \frac{-10.2 \times 100}{3\sqrt{61.8 \times 51.6}} = -6.05 \text{ per cent}$$

RATES OF GROWTH USING GEOMETRIC MEAN:

Pen 1 −1.05 per cent
Pen 2 −6.05 per cent
Pen 3 +1.14 per cent
Pen 4 +3.62 per cent

Using the same values as previously.

The values obtained by this method agree very closely with the logarithmic method, which is the accepted method where the growth is very definite.

This detailed analysis of the growth data shows that there is a definite decrease in the rate of growth of the birds receiving 3 c.c. of viosterol daily (–1.05 per cent) and a corresponding greater decrease for those receiving 6 c.c. of viosterol daily (–6.05 per cent). At the same time, the birds receiving parathormone show definite increase in the rate of growth—those receiving 5 units daily increasing at the rate of +1.14 per cent and those receiving 10 units daily at the rate of +3.6 per cent. A superficial examination of the weekly weights of these birds does not show any such striking changes.

The birds in pen 2 (6 c.c. viosterol daily) and No. 459 (pen 1—3 c.c.) after three weeks of experimental feeding began to show very definite symptoms of disturbance. The first symptom noticed was a continual shaking of the head (which later was noticed in all the birds in pen 2) and in a few days the birds would walk with a stagger. Later, complete loss of equilibrium was noticed in a few cases. The bird, when attempting to walk, would sway and then pitch forward on its head, fall on its side and be quite unable to assume the standing position. This condition suggested a lesion of the cerebellum or spinal cord, but at necropsy and on histological examination nothing definite was found.

In order to study this peculiar condition, two birds, Nos. 450 and 452 were retained on the treatment after the other birds had been sacrificed. From March 24th to 30th, these birds received no viosterol, when it was noticed that the condition of unsteady gait and shaking of the head had improved.

On March 31st each bird received 1 c.c. of 10,000 D.* viosterol. This was continued for 3 days, when the unsteadiness reappeared. No viosterol was given then for 4 days. On April 7th each bird received 1 c.c. of 1,500 D. viosterol and the treatment was discontinued. No. 450 died on April 10th. No. 452 continued to decrease in weight and died on April 24th, weighing only 24 oz.

2. BONE ANALYSIS

Total ash. — After necropsy, the tibia and tarsus of the left leg of each bird were removed for analysis. The bones were freed from adhering tissue, longitudinally divided, freed from marrow and dried at 80° C. They were then broken up by means of bone forceps, transferred to a Whatman's thimble and subsequently extracted with ether in a Soxhlet fat extractor for twelve hours. The fat-free bone was then ground to a fine powder by passing it through a small grinding mill, and a sample of one to two grams was transferred to a weighed crucible. The percentage of ash was determined in the ordinary way in an electric muffle furnace.

Bone calcium.—A sample of 0.5 grams of the finely ground bone was transferred into a 100 c.c. digestion tube. A few c.c. of concentrated nitric acid were added and the tube gently rotated. In order to destroy the organic material 2 c.c. of perchloric acid were added and the material digested on a coil heater until heavy white fumes of perchloric acid were given off. When the solution on cooling became colourless it was transferred and made up to 500 c.c. with distilled water. Two c.c. of this solution (accurately measured with an Ostwald-Folin pipette) were transferred to a pyrex centrifuging tube and the calcium determined by the oxalate method, as outlined in a preceding paper (Hall and King[22, 23]).

Bone phosphorus.—One c.c. of the above solution was transferred to a 15 c.c. volumetric flask and the phosphorus was determined colorimetrically by Martland and Robinson's[36] modification of the Briggs' colorimetric method.

The results of the bone analysis are recorded in Table II. There appear to be no signficant variations from the normal values for bone ash, calcium, phosphorus or $\frac{Ca}{P}$ ratio in any of these pens, as compared with the values found for these in normal birds of the same age (Hall and King).

Bone phosphatase.—We have already shown (1931) that the phosphatase content of the bone varies considerably with the condition of the bone; the bone phosphatase is consistently high in rachitic bones and subnormal in hypervitaminosis bones. From this it was felt that, when taken in conjunction with other important assays, the phosphatase content of the bone is a valuable index of the condition of the bone. For this reason, the amount of phosphatase present in the right tibia and tarsus of every bird

* Kindly supplied to us by the Mead, Johnson Company.

was determined in the manner already described by us. The values are tabulated in Table II.

TABLE II

PEN 1. (3 c.c. 1500 D. Viosterol daily)

No.	*Per cent Ash*	*Per cent Ca.*	*Per cent P.*	*Ca./P.*	*Bone phosphatase (Units)*
454	54.7	20.0	9.3	2.15	1.52
455	54.0	19.2	9.2	2.09	0.30
456	53.8	18.9	9.0	2.10	2.32
457	53.4	19.8	9.3	2.13	0.34
458	53.1	19.7	10.0	1.97	1.14
459	51.5	19.2	9.1	2.11	0.58
Av.	53.4	19.4	9.3	2.09	1.03

PEN 2. (6 c.c. 1500 D. Viosterol daily)

448	54.0	19.6	9.3	2.11	0.34
449	55.4	21.1	9.3	2.27	0.06
450	56.4	19.7	9.7	2.03	0.24
451	55.4	19.8	9.8	2.00	0.34
452	52.4	19.6	8.9	2.20	0.08
453	53.4	20.9	9.7	2.15	1.04
Av.	54.5	20.1	9.4	2.13	0.35

PEN 3. (5 units Parathormone daily)

460	52.4	19.8	9.5	2.09	6.20
461	51.1	19.1	9.5	2.01	2.90
462	52.8	19.1	8.8	2.17	5.00
463	54.0	20.3	10.2	1.99	2.68
464	52.2	18.3	9.1	2.01	4.26
465	50.3	17.9	9.3	1.93	2.06
Av.	52.1	19.1	9.4	2.03	3.85

PEN 4. (10 units Parathormone daily)

442	55.4	20.2	9.6	2.10	
443	51.4	18.2	9.2	1.98	3.80
444	54.2	21.4	9.8	2.19	2.20
445	50.5	20.4	9.4	2.17	3.04
446	52.5	20.3	9.4	2.16	4.68
447	52.3	18.8	10.0	1.88	1.40
Av.	52.7	19.9	9.5	2.08	3.02

An analysis of these data shows that the average for the birds receiving 3 c.c. viosterol daily is 1.03 units. The average value found for 12 normal birds of the same age was 3.47 units (Hall and King). Three of the six birds show very low values, while three are only moderately low. On the other hand, the birds which received 6 c.c. viosterol daily, with one exception, show very low phosphatase values, having an average of only 0.32 units. Our findings would appear to indicate that the phosphatase values are roughly indicative of the severity of the condition of hypervitaminosis.

Contrasted with the phosphatase values for the hypervitaminosis chickens are those as exhibited by the birds in pens 3 and 4 receiving parathormone. The birds in pen 4 (10 units of parathormone daily) have an average of 3.02 units of phosphatase, which is within the range of values obtained with normal birds. Those birds which received 5 units of parathormone daily show an average value of 3.85 units.

Serum calcium.—Blood was drawn from the wing vein of each bird every week and calcium was estimated on 2 c.c. of serum by the standard oxalate titration method.

The average values obtained for pens 1 and 2 are shown in Table III. The individual variations in the values obtained for the birds in pens 3 and 4 (parathormone) were so great that no conclusion could be made from them, and they are therefore not included in the Table. This was probably due, in part at least, to the fact that throughout part of this experiment the birds in pens 3 and 4 came into egg production—a condition which seriously upsets the serum calcium values. During the laying period Parhon[40] found that the serum calcium of five pullets ranged between the values of 20.6 and 26.6 mg. per 100 c.c., while during moulting the values lay between 9.0 and 11.3 mg.

TABLE III.

PEN No. 1. WEEKS

No.	*1*	*2*	*3*	*4*	*5*	*6*	*7*
454	14.7	..	15.9	24.4	22.1	19.9	25.2
455	9.8	14.0	14.0	20.1	..	..	17.1
456	9.7	11.9	12.6	14.8	17.5	25.1	23.8
457	14.4	11.1	22.6	25.0	..	17.1	17.3
458	10.3	11.7	11.3	20.0	..	21.1	17.0
459	11.1	13.4	14.6	17.4	*	..	..
Av.	11.7	12.4	15.2	20.3	19.8	20.8	20.1

PEN No. 2.

448	11.1	21.7	17.6	13.5	13.7	15.1	16.9
449	10.1	11.0	13.3	17.2	18.5	..	16.5
450	11.6	22.5	16.6	16.5	16.6	17.2	..
451	10.2	12.3	16.9	*	..	..	..
452	11.7	13.4	16.0	17.0	..	..	21.4
453	11.0	9.2	10.5	15.3	22.3	24.0	18.9
Av.	10.9	15.0	15.1	15.9	17.8	18.8	18.4

*Died or were killed.

Histological examination.—The most valuable information of the experiment was obtained from the histological study of various tissue sections made immediately following necropsy.

Bone sections were made in the mid sagittal plane of the distal end of the femur. The histological appearance of the sections from the birds which received viosterol was practically identical. The cortices of the shafts were compact and of moderate thickness. The nature of the marrow varied somewhat, some shafts being filled with red marrow exclusively, and in some fatty marrow predominated. In all there were bony trabeculæ similar in structure to the compact bone of the shaft, but in addition there were irregular, continuous masses of bony matrix, the background of which had an affinity for hæmatoxylin. These suggested incompletely calcified areas.

The bone sections of the birds receiving parathormone were quite different. The trabeculæ were surrounded by enveloping masses of dense

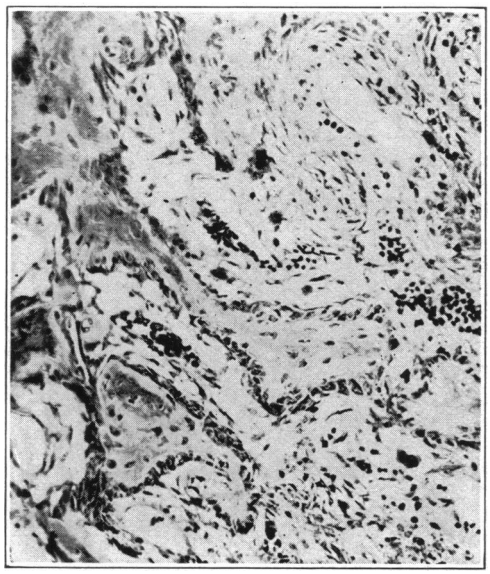


FIG. 1.—The distal end of normal femur showing normal bony trabeculæ surrounded by hyperplastic osteoblasts.

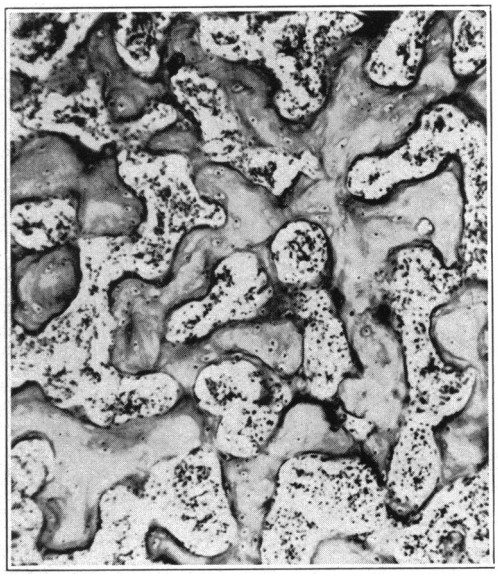


FIG. 2.—The distal end of femur showing dense trabeculation, with the trabeculæ still cartilaginous rather than bony (456—3 c.c. 1,500 D. viosterol daily for six weeks).

FIG. 3.—The distal end of femur, (higher magnification), showing rather more bony structure of the trabeculæ than that shown in Fig. 2. (449—6 c.c. 1,500 D. viosterol daily for six weeks).

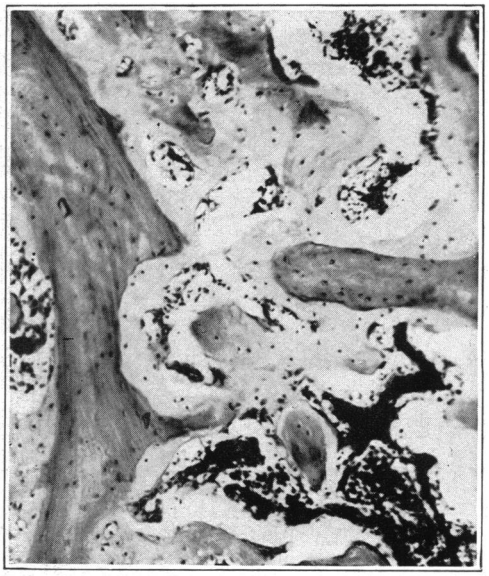


FIG. 4.—The distal end of femur, showing bony trabeculæ surrounded by thick "periosteal" fibrosis. (445-10 units parathormone daily for six weeks).

fibrous tissue. All the sections showed this marked condition of fibrotic hyperplasia or osteitis fibrosa, and indicated that this fibrous tissue had developed from the bony trabeculæ, but had failed to receive a deposit of calcium salts and had developed to a virtual periosteum.

The kidneys of the birds receiving parathormone (pens 3 and 4) were apparently normal in all respects and no calcareous deposit was seen. The kidneys of the birds receiving viosterol (pens 1 and 2) on the other hand, showed marked changes. The glomeruli seemed reduced in number and those remaining were highly cellular and contracted from Bowman's capsules. The epithelium of the secretory tubules was swollen but generally intact. Scattered throughout the sections were masses of blue staining material, interpreted as calcareous deposits. In general, they were

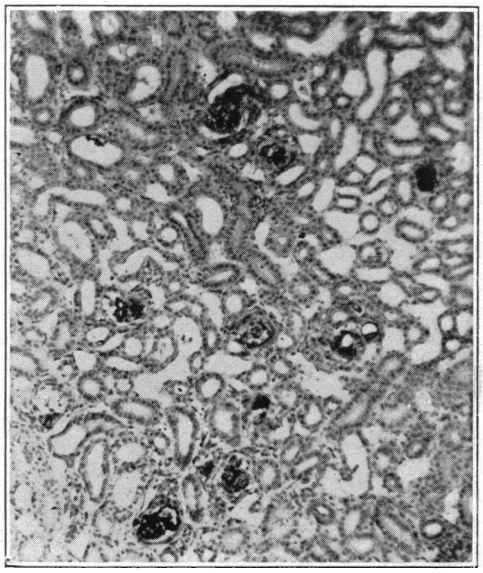


FIG. 5.—Section of chicken kidney, showing the extent of calcareous deposit within the kidney substance. (455—L.P.—3 c.c. 1,500 D. viosterol daily).

FIG. 6.—Section of chicken kidney (higher power) showing calcareous deposits which were apparently being developed within the epithelial cells of a secretory tubule. (455).

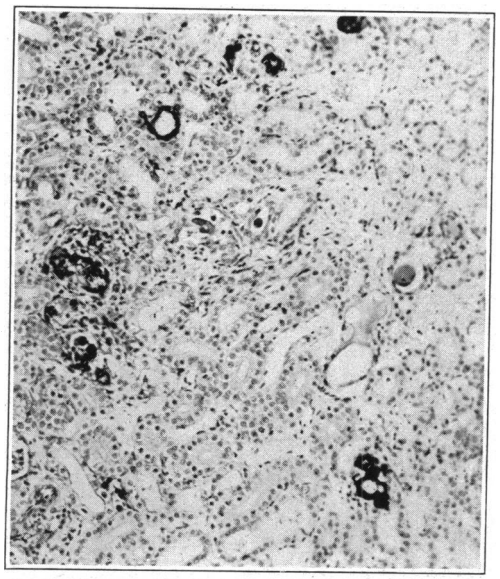


FIG. 7.—Chicken kidney (low power) showing the extent of calcareous deposit within kidney substance. At the upper left there is a ring of deposit which appeared to have been formed in the epithelium. At the middle right is a cast which in the original showed very distinctly the arrangement of concentric laminæ. (449—6 c.c. 1,500 D. viosterol daily).

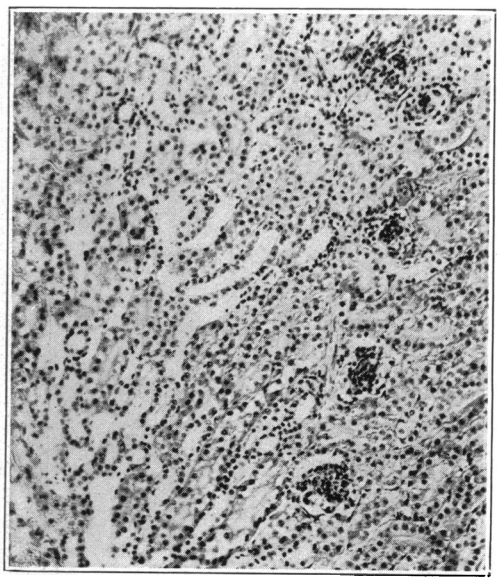


FIG. 8.—Chicken kidney; a representative section to show normal structure and the absence of calcareous deposit. (461—5 units of parathormone daily).

globular in contour and varied in size from that of an epithelial cell to that of a glomerulus. Some of the sections showed small globules of calcareous material within the cells of the secretory tubules. In a few instances calcareous casts were seen within the secretory tubule itself and surrounded by intact epithelium. The structure and position of these suggested that they were deposited within the cells of the secretory tubules and were laid down as successive concentric laminæ. In some cases the masses were large enough to occlude the lumen. In a few instances there were found calcareous masses which appeared to be supplanting glomerular tufts. The absence of the epithelium, however, made precise identification of the location impracticable. The finding of globules within the epithelial cells of the secretory tubules suggested strongly that precipitation of calcium was incited at this site and continued in concentric laminæ until the lumen was occupied by a cancellous "cast" and the epithelium was destroyed by pressure necrosis. Tubules which contained casts within intact epithelium were presumed to be simply receptacles for casts which had been formed at a site more proximal to the glomerulus. An alternative explanation would be that these had been due to precipitation of calcium from urine within the tubule. The presence of a cast occluding a tubule would be likely to cause necrosis of the proximal portion, including the glomerulus, and this would explain the apparent reduction in the number of glomeruli.

Sections of the aorta, small intestine and liver from these birds did not show abnormal histological appearances.

SUMMARY

1. The administration of massive doses of viosterol to chickens produced a condition of anorexia, loss of weight, extreme emaciation and finally death.

2. Hypercalcæmia resulted, and on histological examination heavy deposits of calcium were observed in the secretory tubules of the kidneys.

3. Histological examination of the femurs showed the matrix of the trabeculæ to be normal, but suggested a low amount of calcareous deposit within it. The percentage of ash, calcium and phosphorus of the bones, however, was normal. The bone phosphatase appeared to be present in less than the normal amount.

4. The daily administration of parathormone appeared to produce no ill effects in chickens, comparable to those produced by viosterol.

5. Histological examination of the femurs showed productive fibrosis of the bony trabeculæ without evident deposition of lime salts in the hyperplastic tissue. The mineral constituents and the phosphatase of the bone were present in normal amount.

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