STUDIES ON THE MODE OF ACTION OF IRRADIATED ERGOSTEROL

III. The Effect of Irradiated Ergosterol Administration on the Formation of Bone Trabeculae [1, 2]

By WALTER BAUER and ALEXANDER MARBLE [3]

(From the Medical Clinic of the Massachusetts General Hospital, Boston)

(Received for publication June 6, 1931)

In a previous communication (1) data were presented which showed that the administration of irradiated ergosterol to individuals with calcium deficiency diseases resulted in an increased absorption and retention of calcium and phosphorus. Presumably these retained salts were deposited in the bones. The amount of retention noted during such therapy apparently depended upon the severity of the existing calcium phosphate deficiency. If there existed a calcium phosphate deficiency of the serum as well as of the bones, the retention of calcium and phosphorus was greater than when there was a calcium phosphate deficiency of the bones only.

The present experiments were undertaken in order to obtain direct proof that the calcium and phosphorus retained in calcium deficiency diseases subsequent to irradiated ergosterol therapy was deposited in the bones. It was also hoped that it could be demonstrated that the deposition of calcium phosphate in such cases was more marked when irradiated ergosterol was administered in conjunction with a high calcium diet than when only a high calcium intake was employed.

I. Adult cats previously on a low calcium diet.

Calcium phosphate deficiency of the bones can be produced in cats by keeping them on a diet inadequate in calcium for a period of months (2). This calcium phosphate deficiency is characterized by a depletion of the bone trabeculae. Therefore, these experiments were carried out on cats which had received a diet low in calcium for a period of months and, as a consequence, exhibited signs of calcium phosphate deficiency.

Twelve cats were studied. Ten had been on an inadequate calcium intake for $7\frac{1}{2}$ months, two for 22 months. At the end of such periods, the

[1] This is publication No. 6 of the Robert W. Lovett Memorial for the study of crippling disease, Harvard Medical School, Boston, Massachusetts.

[2] Part of the expense of this investigation was paid by the William W. Wellington Memorial Research Fund.

[3] Medical Resident, Massachusetts General Hospital.

left fore leg of each cat was amputated at the shoulder joint under ether anesthesia. The humerus of each leg was prepared as previously described (2). Each humerus showed the characteristic depletion of the bone trabeculae. The humeri were saved until the cats were sacrificed so that a comparison could be made with the corresponding humerus of the opposite leg. Following the operation, the diet was changed from one inadequate in calcium to one having a high calcium content.

Six of the animals were given varying doses of irradiated ergosterol by mouth. The other six were kept as controls, receiving only the high calcium diet. The animals were grouped into pairs as nearly equal in size and weight as possible, one to serve as a control animal and one to receive irradiated ergosterol. The paired animals were simultaneously sacrificed under ether anesthesia at varying lengths of time following the institution of the high calcium diet. The humeri of the right fore legs were saved for examination. The preparation of all bones was identical. Some animals received irradiated ergosterol for 8 days and others for as long as 57 days. Accurate account was kept of the food intake. As may be seen in Table I, the average calcium intake of the cats receiving ergosterol was less than that of their control mates, in some instances only half. Despite this, the bones of the cats receiving irradiated ergosterol showed definitely more trabeculae than did those of their control mates (see Plates I and II). With the exception of cat 11, the cats receiving irradiated ergosterol showed in every instance a higher serum calcium than their control mates and three of these same cats showed a lowered serum phosphorus. If overdosage is the cause of these findings it did not persist long enough to produce the demonstrable decalcification which has been reported by other workers (3), (4), (5).

In cats 11 and 12, the results obtained were disappointing. Before the experiment these two cats, which had been on a low calcium diet for almost two years, exhibited signs of calcium phosphate deficiency of both the serum and the bones: tetany, a low serum calcium, and a marked decrease in the number of bone trabeculae. Yet the humerus of cat 11, after the high calcium diet—irradiated ergosterol regime, did not show appreciably more trabeculae than did the humerus of cat 12, after the high calcium diet period without irradiated ergosterol therapy. Although the serum calcium of the cat receiving irradiated ergosterol had increased from 8.2 mgm. to 11 mgm., it was not so high as the serum calcium of the control animal (12 mgm. per 100 cc.). The failure to obtain more striking differences as the result of irradiated ergosterol therapy can probably be explained by the short duration of the experiment, and the failure of cat 11 to eat the high calcium diet employed.

These experiments furnish definite evidence that the increased calcium and phosphorus absorbed during the administration of irradiated ergosterol results in the deposition of calcium phosphate in the bones.

TABLE I

Record of the weight, average daily dose of irradiated ergosterol, length of time it was administered, the food intake and the serum calcium and phosphorus on the day the cats were sacrificed

Cat number and sex	Date	Weight	Irradiated ergosterol		Average intake (per day)		Serum			Number of control mate
			Length of the experiment	Per day	Calcium	Phosphorus	Date of termination	Calcium	Phosphorus	
		kgm.	*days*	*mgm.*	*grams*	*grams*		*mgm. per 100 cc.*	*mgm. per 100 cc.*	
I ♂.....	June 11 July 2	3.35 3.20	36	None	6.69	7.99	July 17	10.3	6.78	VI
VI ♂....	June 11 July 2	3.35 3.15	36	1.8	2.69	3.19	July 17	12.5	5.32	I
II ♂.....	June 11	2.20	8	3.0	1.39	1.07				None
III ♂....	June 11 July 2 August 7	2.35 2.40 2.05	57	None	13.08	10.12	August 7	12.0	6.18	V
V ♂.....	June 11 July 2 August 7	3.05 2.50 2.75	57	1.0	5.88	4.97	August 7	17.2	4.46	III
IV ♀....	May 25 June 11	3.05 3.05	8	3.0	1.07	1.85				None
VIII ♀..	June 11 July 2 July 24	2.80 2.75 2.65	43	1.6	10.43	11.80	July 24	14.6	7.39	IX, X
IX ♀....	June 11 July 2 July 24	2.10 1.90 1.70	43	None	8.38	6.48	July 24	11.7	5.09	VIII, X
X ♂.....	June 11 July 2 July 24	4.00 4.20 3.55	43	None	14.95	15.24	July 24	9.8	6.54	VIII, IX
*XI ♀...	July 10 August 7	2.59 1.80	20	1.0	1.96	1.52	July 10 August 7	8.2 11.0	5.81 3.65	XII
*XII ♀..	July 10 August 7	2.39 1.90	20	None	5.24	4.06	August 7	12.1	6.63	XI

* On low calcium diet for 22 months; all other cats on low calcium diet for $7\frac{1}{2}$ months prior to change to adequate calcium diet and (in some cases) irradiated ergosterol.

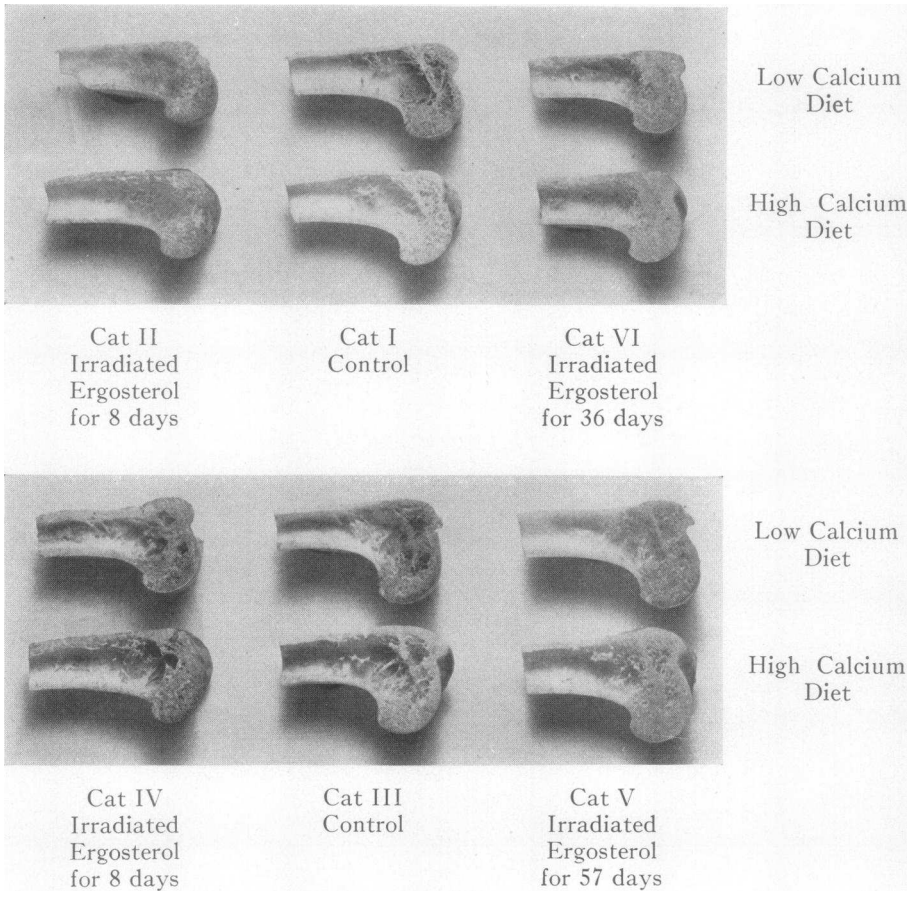

PLATE I. SHOWING THE EFFECT OF IRRADIATED ERGOSTEROL ON THE FORMATION OF BONE TRABECULAE IN CATS PREVIOUSLY ON A LOW CALCIUM DIET

The fact that the number of bone trabeculae was slightly greater in the animals receiving irradiated ergosterol in addition to a high calcium diet is of much greater significance when one remembers that the average calcium intake for these animals was considerably less than the intake of the control animals. The increase in the number of bone trabeculae is not so apparent in the photographs as in the bones themselves because of the presence of high lights and shadows. This comment applies also to Plate II.

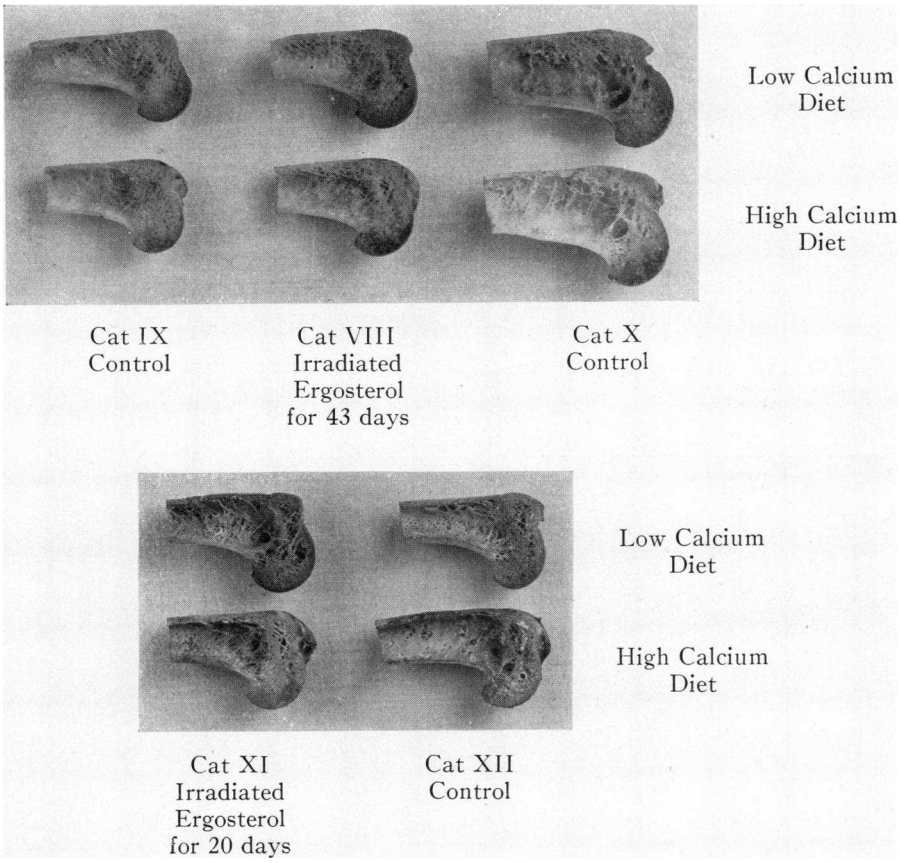

PLATE II. SHOWING THE EFFECT OF IRRADIATED ERGOSTEROL ON THE FORMATION OF BONE TRABECULAE IN CATS PREVIOUSLY ON A LOW CALCIUM DIET

They also demonstrate that this deposition is greater in cases of calcium deficiency when irradiated ergosterol therapy is given in conjunction with a high calcium diet than when only a high calcium diet is employed. The results are of more significance when one realizes that the cats receiving irradiated ergosterol did not eat so much of the high calcium diet as their control mates.

II. Normal kittens

Eight kittens, 6 of them from one litter ($5\frac{1}{2}$ weeks old) and 2 from another litter (3 months old), were given a normal diet, adequate in calcium. Four of them were given irradiated ergosterol by mouth, in doses varying from 0.25 to 1.5 mgm. a day. As set forth in Table II, kitten 13 was

TABLE II

Record of the weight, average daily dose of irradiated ergosterol, length of time it was administered and the serum calcium and phosphorus on the day the kittens were sacrificed

Cat number and sex	Age at the beginning of the experiment	Date	Weight	Irradiated ergosterol		Serum			Number of control mate
				Length of the experiment	Per day	Date of termination	Calcium	Phosphorus	
	months		*kgm.*	*days*	*mgm.*		*mgm. per 100 cc.*	*mgm. per 100 cc.*	
XIII ♀ ..	3	July 18.....	1.20			Aug. 15	10.6	7.77	XIV
		August 15....	1.15	28	0.27				
XIV ♂...	3	July 18.....	1.65			Aug. 15	10.6	8.63	XIII
		August 15....	1.70	28	None				
XV ♀....	$1\frac{1}{2}$	July 18.....	0.6			Oct. 21	10.0	9.42	XVI
		September 14.	1.35	90	None				
XVI ♂...	$1\frac{1}{2}$	July 18.....	0.65			Oct. 21	9.5	8.89	XV
		August 21....	1.20						
		September 14.	1.75	90	0.89				
XVII ♀..	$1\frac{1}{2}$	July 18.....	0.6			Sept. 20			XVIII
		September 14.	1.35						
		September 20.	1.35	63	None				
XVIII ♂.	$1\frac{1}{2}$	July 18.....	0.6			Sept. 20			XVII
		August 21....	1.0						
		September 14.	1.30						
		September 20.	1.35	63	0.65				
XIX ♀...	$1\frac{1}{2}$	July 18.....	0.65			Aug. 15	10.1	8.28	XX
		August 15....	1.10	28	None				
XX ♀....	$1\frac{1}{2}$	July 18.....	0.65			Aug. 15	10.1	5.87	XIX
		August 12....	0.95	28	0.27				

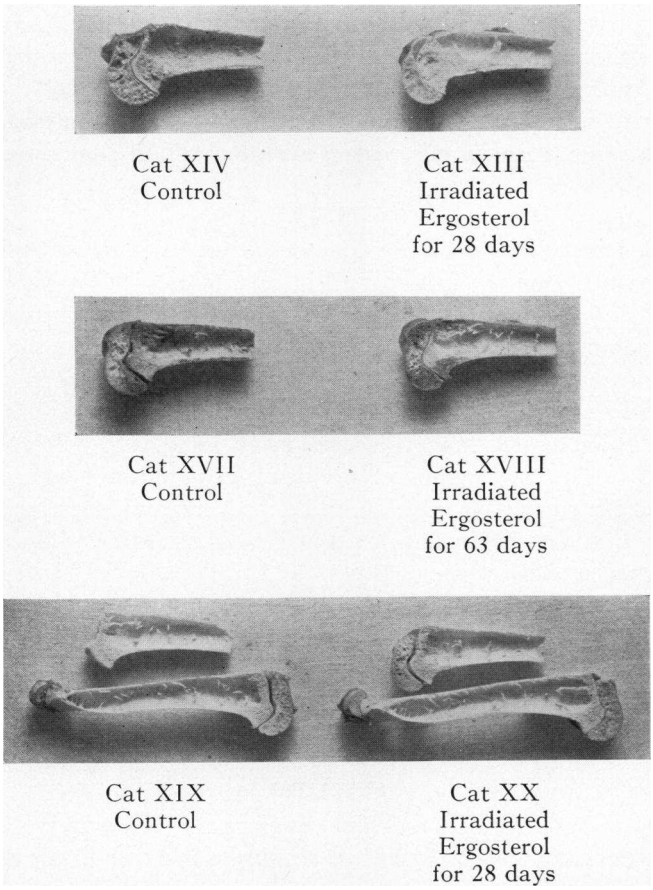

PLATE III. SHOWING THE EFFECT OF IRRADIATED ERGOSTEROL ON THE FORMATION OF BONE TRABECULAE IN NORMAL KITTENS

It will be noted that the changes in these experiments were not so marked as those concerning the adult animals.

paired with kitten 14, 15 with 16, 17 with 18, and 19 with 20. Kittens 13, 16, 18, and 20 were given irradiated ergosterol. They were sacrificed in pairs (except in the case of 17 and 18) as described in the previous experiment, at times varying from 28 to 63 days after the beginning of ergosterol therapy. Here, as before, increased bone trabeculation was found in the kittens receiving irradiated ergosterol (see Plate III). However, no difference was demonstrated in the level of the serum calcium, although the serum phosphorus of the animals receiving irradiated ergosterol was lower.

The findings in these experiments are in accord with those of the other experiments described in this paper, except that the changes are not so marked.

COMMENT

Previous experiments (1) demonstrate that calcium and phosphorus absorption and retention is increased in calcium deficiency diseases when irradiated ergosterol is administered. The present experiments show that this retained calcium and phosphorus is deposited in the bones as calcium phosphate. These experiments also demonstrate that the retention and deposition of calcium phosphate on a high calcium diet is greater in calcium deficiency diseases when irradiated ergosterol is administered. This increased retention of calcium and phosphorus is secondary to increased absorption and deposition and would not occur if a calcium phosphate deficiency of the bones did not exist. These findings are in agreement with those of Brown and Shohl (3) and Light et al. (5). These workers reported an increase in the calcium content of bone and a heavier bone ash when non-toxic doses of irradiated ergosterol were given.

The fact that the number of bone trabeculae in kittens can be increased with irradiated ergosterol therapy indicates why such treatment protects against rickets.

SUMMARY

1. The administration of a high calcium diet to cats with calcium phosphate deficiency of the bones results in an increase in the number of bone trabeculae.

2. This increase in the number of bone trabeculae is greater when, in in addition to a high calcium diet, irradiated ergosterol is given.

BIBLIOGRAPHY

1. Bauer, W., and Marble, A., J. Clin. Invest., 1932, xi, 21. Studies on the Mode of Action of Irradiated Ergosterol. II. Its Effect on the Calcium and Phosphorus Metabolism of Individuals with Calcium Deficiency Diseases.
2. Bauer, W., Aub, J. C., and Albright, F., J. Exp. Med., 1929, xlix, 145. Studies of Calcium and Phosphorus Metabolism. V. A Study of the Bone Trabeculae as a Readily Available Reserve Supply of Calcium.
3. Brown, H. B., and Shohl, A. T., J. Biol. Chem., 1930, lxxxvi, 245. Rickets in Rats. XI. The Alteration of Calcium and Phosphorus Metabolism of Normal and Ricketic Rats Produced by Irradiated Ergosterol.
4. György, P., Klin. Wchnschr., 1930, ix, 102. Die besondere Stellung der Subepiphysären Knochenschicht im Kalkstoffwechsel als eines leicht mobilisierbaren Kalkspeichers.
5. Light, R. F., Miller, G., and Frey, C. N., J. Biol. Chem., 1929, lxxxiv, 487. Studies on the Effects of Overdosage of Vitamin D.