CLXXII. IRRADIATED ERGOSTEROL AND CALCIUM-FREE DIET: EFFECT ON CALCIUM AND PHOSPHORUS METABOLISM.

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BROWN AND SHOHL [1930] and Watchorn [1930] have shown that large doses of irradiated ergosterol lead to a decreased retention of calcium in rats, in spite of the calcification of certain tissues which takes place at the same time. It has been noted also by Brown and Shohl [1930], Duguid, Duggan and Gough [1930] and by Harris [1930] that the severity of the symptoms of hypervitaminosis D corresponds with the amount of calcium in the diet, and that in cases where the intake of calcium is very low, the symptoms are difficult or impossible to produce. Although in the absence of calcium from the diet, irradiated ergosterol in doses of 5 or 10 mg. appears to be non-toxic¹, it remained to be seen whether the metabolism of calcium and phosphorus was affected or not in these circumstances. Total balance experiments therefore have been carried out and are briefly described below.

EXPERIMENTAL.

Steenbock's rachitogenic diet [Steenbock and Black, 1925] was used as the basal diet, salts being added as required. It was found to be practically free from calcium, and to contain 60 mg. of phosphorus per 10 g. dry weight of food. This, though organic in form, can be utilised by the rat, and made it impossible to compare a high Ca : low P with a low Ca : high P diet as was at first hoped.

Adult albino rats of 160–200 g. were used, divided into two groups A and B. To the basal diet of Group A was added calcium carbonate, so that 10 g. of food (dry weight) contained 49 mg. of calcium. For Group B inorganic phosphate was added bringing the total amount to 121.7 mg. P per 10 g. dry weight of food. No calcium was added. Group A served as a control to Group B.

The general technique, including methods of analysis, was identical with that previously described [Watchorn, 1930]. The experimental period consisted of 4 weeks, one preliminary without excess vitamin, two weeks when 0.05 % irradiated ergosterol was added to the food, and one final "curative" or

¹ With still larger doses of irradiated ergosterol given with a calcium-free diet it has been found that ill effects of a different character are produced (Innes and Harris, personal communication).

recovery week. In each group controls receiving two drops of cod-liver oil daily but no irradiated ergosterol were run for the same length of time. The diet was given for about 10 days before beginning the collection of excreta.

The effects of irradiated ergosterol upon Group A were similar to those obtained when the basal diet consisted of caseinogen, rice starch, sugar and fat. The rats lost weight rapidly, the food intake was greatly reduced, the retention of calcium and phosphorus decreased rapidly and the urinary calcium was increased. (Tables I and II A.) Figures for the 1st, 3rd, and 4th weeks only are given, as those for the second week were but slightly different from those for the first). In the present instance however, the urinary phosphorus was also increased by the irradiated ergosterol, whereas on the caseinogen diet it was unaffected. The retention of phosphorus was restored more quickly than that of calcium during the "curative" week.

Table I.

No. of rat	Ca intake Periods			Faecal Ca Periods			Urinary Ca Periods			Total Ca excretion Periods			Ca retention Periods		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
F 26	70.4	17.7	54.1	56.9	17.4	31.1	1.7	11.5	19.0	58.6	28.9	50.1	11.8	-11.2	4.3
F 28	67.8	35.2	32.6	45.6	11.4	31.4	0.8	14.1	6.3	46.4	25.5	37.7	21.4	9.7	-5.1
F 30	53.9	24.5	58.1	45.9	19.5	31.9	Nil	11.0	11.5	45.9	30.5	43.4	8.0	-6.0	14.7
Control rat*															
F 25	61.8	65.5	71.1	41.6	33.8	44.9	0.7	4.9	3.8	42.3	38.7	48.7	19.5	26.8	22.4

Figures represent mg. per rat per day.

Period I. Preliminary.

" II. 2nd week of irradiated ergosterol intake.

" III. "Curative" week.

* Second control developed pneumonia and had to be killed.

Table II.

No. of rat	P intake Periods			Faecal P Periods			Urinary P Periods			Total P excretion Periods			P retention Periods		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Group A															
F 26	86.4	21.7	66.3	37.9	18.5	31.2	2.9	6.9	4.0	40.8	25.4	35.2	45.6	-3.7	31.2
F 28	83.1	43.1	32.6	31.3	15.6	27.5	0.3	10.5	2.8	31.6	26.1	30.3	51.5	17.0	2.3
F 30	66.2	30.3	71.1	15.5	19.4	30.7	1.9	6.4	2.8	17.4	25.8	33.5	48.8	4.5	37.6
Control rat															
F 25	75.9	80.3	84.2	28.7	26.2	30.9	0.7	1.4	2.8	29.4	27.6	33.7	46.5	52.7	50.5
Group B															
F 32	146	161	163	25	26	25	75	79	86	100	115	111	46	45	52
F 33	116	140	145	18	20	16	64	80	101	82	100	117	34	40	28
F 34	145	177	194	16	21	18	94	105	114	110	126	132	35	51	62
F 36	127	154	170	14	11	14	67	88	103	81	99	117	46	55	53
Controls															
F 31	115	176	164	18	17	14	73	112	96	91	129	110	24	47	53
F 35	137	181	163	15	16	12	74	102	119	89	118	131	48	63	32

Figures represent mg. per rat per day.

Period I. Preliminary.

" II. Irradiated ergosterol intake.

" III. "Curative" week.

The results in Group B (Ca-free diet) were strikingly different. The animals continued to gain weight. Phosphorus retention was unaffected by the ir-

radiated ergosterol, for, though apparently the retention was improved, a similar improvement took place in the controls, and was presumably due to better adaptation of the animals to the diet (Table II B). There was no excretion of calcium in the urine or faeces in the absence of excess vitamin D from the diet, but dosage with the irradiated ergosterol at once produced an elimination of calcium by the kidney. There was an immediate improvement when the excessive intake of vitamin ceased. The figures for urinary calcium given in Table III represent also the average daily negative balance of calcium, as there was no intake and no faecal excretion of this element. Two control animals run for the same period failed to excrete any calcium either by kidney or gut during the experimental period.

Table III.

No. of rat	Urinary calcium, mg. per rat per day.			
	Week 1	Week 2	Week 3	Week 4
F 32	Nil	—	4.48	1.74
F 33	"	2.00	6.50	0.64
F 34	"	2.53	5.90	0.73
F 36	"	0.11	5.90	1.68

DISCUSSION.

The first outstanding difference between the rats which received calcium and those which did not was the absence of any effect of large doses of irradiated ergosterol upon the phosphorus metabolism of the latter. These rats were gaining in weight whereas those receiving calcium were not, and this result seems to confirm the suggestion previously made [Watchorn, 1930] that the ill effects upon phosphorus metabolism were at least partly due to loss of weight and low food intake. In spite however of the apparent health and gain in weight of the rats the effect of the irradiated ergosterol was to cause a renal elimination of calcium, thus producing a marked negative balance. The amounts of calcium lost in the urine were considerable, as may be seen by comparing them with the figures previously given [Watchorn, 1930] where the intake of calcium was appreciable. Such a loss of calcium, in the absence of calcium from the diet, can only mean that it is being lost from the bones or tissues. This action of large doses of irradiated ergosterol has been suggested from time to time by various workers and apparently exists whether calcium is present in the diet or not. But for the calcification of tissues the large doses of vitamin D are in themselves insufficient, and need to be combined with a fairly high calcium intake. The action of irradiated ergosterol in the latter circumstance clearly requires further investigation.

SUMMARY.

1. Calcium and phosphorus retention in rats on Steenbock's rachitogenic diet containing both calcium and phosphorus is decreased by excessive doses of irradiated ergosterol.
2. Both the urinary and the faecal phosphorus are increased.

3. The faecal phosphorus is also increased in comparison with the intake, but not the faecal calcium.

4. On a similar basal diet, but calcium-free and richer in phosphorus, the irradiated ergosterol does not affect the phosphorus metabolism but causes elimination of calcium by the kidney.

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