XXVIII. THE EFFECT OF EXCESSIVE DOSES OF IRRADIATED ERGOSTEROL ON THE CALCIUM AND PHOSPHORUS CONTENT OF THE BLOOD.

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In earlier papers of this series [Harris and Moore, 1928, 1, 2, 1929], a description has been given of some of the effects produced by large overdoses of vitamin D (irradiated ergosterol). Amongst these effects is the extensive deposition of calcium salts in various parts of the body. This finding, together with the well known effects of deprivation of vitamin D, rendered it desirable to investigate the effect of overdosage of the vitamin on the calcium and phosphorus content of the blood. Hess and Lewis [1928], indeed, had noticed several cases of hypercalcaemia and three cases of abnormally high blood-phosphorus in a series of rachitic patients treated with irradiated ergosterol at the rate of 2·5 to 5 mg. *per diem.*

This note is intended to give a preliminary report of our results. Publication at the present juncture is prompted by the appearance of a note by Hess, Weinstock and Rivkin [1928] which has just come to our notice. These authors found that in young rats, on a diet high in phosphorus but containing negligible amounts of calcium, so that the serum-calcium was very low (6·4 mg. per 100 cc.), the serum-calcium was rapidly raised towards a more normal level by daily administration of 1 mg. of irradiated ergosterol. Our own experiments differ from those of Hess and his co-workers in two important particulars—we used adult animals, and made no attempt to restrict the calcium intake below the normal.

The main experiment we have to describe was carried out with adult rabbits which were allowed an unlimited diet of oats and cabbage leaves. Water was offered to the animals, but was never touched. Six rabbits were used and, after a control period during which samples of blood were withdrawn periodically for estimation of the serum-calcium by the method of Kramer and Tisdall [1921] and of the inorganic phosphate of the blood by the method of Briggs [1924], were divided into three groups. The first group received daily 0·5 cc. of arachis oil; the second received 0·5 cc. arachis oil containing 10 mg. non-irradiated ergosterol; the third received 0·5 cc. of arachis oil containing 10 mg. irradiated ergosterol[1]. The oil was administered

[1] The ergosterol was irradiated in alcohol as previously described [Harris and Moore, 1928, 1, 2].

by pipette, lest mixing with the ordinary food should lead to its refusal. The rabbits proved unexpectedly resistant to overdosage of the vitamin, and it was only after nearly a month of this treatment that any change in the blood-Ca or -P was noted. Although no record was kept of the food consumption it was noted, 25 days after the first dose, that the two rabbits receiving irradiated ergosterol had consumed much less than usual. The following day food was again refused, and on that day the blood showed an increased content of inorganic phosphorus, the serum-calcium, however, remaining normal. A fortnight later the blood-phosphorus was still high, about 50 % above normal, and the serum-calcium showed a slight increase. The control animals showed no significant change throughout. (The figures are given in Table I.)

Table I.

P = mg. inorganic phosphorus per 100 cc. blood.
Ca = mg. calcium per 100 cc. serum.

	No ergosterol				Non-irradiated ergosterol				Irradiated ergosterol			
	I		II		III		IV		V		VI	
Date	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca
Dec. 10th	4·20	10·9	4·50	11·7	4·50	9·7	4·60	12·0	5·00	11·4	5·00	11·8
„ 15th	4·45	11·0	4·60	11·4	4·30	9·5	4·00	11·0	4·50	12·4	5·40	11·6
„ 20th	4·00	10·4	4·30	10·3	4·70	10·3	4·50	11·5	5·30	13·0	5·10	12·0
„ 31st	4·12	10·7	4·40	10·9	4·10	10·0	4·00	11·8	5·70	11·8	5·30	11·5
Jan. 3rd	4·30	10·3	4·35	10·4	4·30	10·5	4·40	11·3	5·40	12·0	5·00	11·4
	End of control period. Feeding started											
„ 7th	4·35	10·6	4·28	10·3	4·00	10·2	4·30	11·0	5·10	11·8	5·20	11·7
„ 10th	4·00	10·7	4·12	10·5	4·44	10·3	4·00	11·4	5·72	11·8	5·00	12·0
„ 14th	4·20	11·0	4·40	10·0	4·40	9·9	4·20	10·8	4·70	11·7	5·30	11·8
„ 31st	4·41	11·3	4·17	10·6	5·00	—	4·30	11·1	7·50	11·6	7·50	11·8
Feb. 13th	4·10	11·5	4·35	11·3	4·70	10·1	4·15	11·5	7·65	12·5	8·00	12·6

We hesitate to accept the last estimations on rabbits V and VI as showing a definite rise in the serum-calcium. Certainly they are higher than the preceding ones, but the normal fluctuations in the serum-calcium of rabbits are often as great as the difference between the last and penultimate analyses. Unfortunately at the time we were in ignorance of Hess's work on the administration of vitamin D to rats, and the rabbits were killed at this stage and examined *post mortem* for the characteristic changes.

Post mortem examination showed that the usual deposition of calcium salts had taken place. That in the kidney was visible to the naked eye, and the urine in the bladder was noticeably cloudy and found to be saturated with calcium salts. The rabbits had not lost weight, however, and, though eating less than normally, were by no means starving.

The absence of a definite hypercalcaemia, even if it be admitted that the approaching onset of such a condition is indicated, may be due to the relatively high resistance of the adult animals to overdosage with vitamin D and adequate excretion. A larger dose, or continuation of the experiment for a longer period, might have produced a definite increase in the serum-calcium.

It seems unlikely that the larger amounts of calcium fed to our animals is the cause of the difference between our results and those reported by Hess in cases of experimental hypocalcaemia. In this connection it is interesting to note that Hess, Weinstock and Rivkin [1928] were unable to bring about, in adult rats, the rapid fall in the serum-calcium which, in young animals, was produced by the low calcium-high phosphorus diet which they employed. At the same time, the composition of the diet cannot be ignored as a possible factor in determining the precise effect of overdosage of vitamin D. Deficiency of the vitamin may be followed by a low blood-phosphorus or a low blood-calcium, or both, with of course faulty calcification, according to the diet; it is quite possible that excess of the vitamin may cause high phosphorus or high calcium, or both, again according to the diet. Hess states that he is investigating this point.

In any case, the degree of hypervitaminosis must have some part in deciding the blood-picture, and in an experiment on young rats (100 g.) to which irradiated ergosterol (0·1 % of diet) was administered until, after 14 days, a loss of from 20 to 30 g. in weight had resulted, we have found again a high inorganic phosphorus content of the blood and also a definitely raised serum-calcium. The blood, a mixed sample from two rats, contained 7·0 mg. of inorganic phosphorus per 100 cc., an amount about 50 % above the normal, while the mixed serum contained 12·6 mg. of calcium per 100 cc., about 25 % more than the normal, which in rats is about 10 mg., with a range of 9·5 to 10·5. The animals used in this experiment received a diet containing an adequate amount of calcium and phosphorus in normal ratio.

Further experiments are being carried out to show the effect on the calcium and phosphate content of the blood of different degrees of hypervitaminosis, and an attempt is being made to investigate the significance of the increase in inorganic phosphorus which, according to the preliminary experiments here described, seems to precede the hypercalcaemia.

Summary.

1. In the young rat, the administration of a complete synthetic diet (normal Ca–P ratio) containing 0·1 % of irradiated ergosterol was followed by a 50 % increase of blood-inorganic phosphate and a 25 % increase of serum-calcium.

2. In adult rabbits receiving a normal mixed diet, administration of 10 mg. *per diem* of irradiated ergosterol per animal was followed, after upwards of a fortnight at the normal level, by a 50 % increase in the blood-inorganic phosphate. After six weeks there was still no significant rise in serum-calcium although abnormal deposits of calcium had already appeared in the body.

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