CV. THE EFFECT OF DESICCATED THYROID, IRRADIATED ERGOSTEROL AND AMMONIUM CHLORIDE ON THE EXCRETION OF CALCIUM IN RATS.

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PARHON [1912] observed a marked loss of calcium in rabbits which received lethal doses of thyroid substance by mouth. Aub *et al.* [1929] reported that patients with hyperthyroidism showed an excretion of calcium much greater than that observed on similar diets in hyperparathyroidism or after the administration of acids; he pointed out that the high calcium excretion of hyperthyroidism was unusual in that it occurred chiefly by the intestinal route. Parhon *et al.* [1932] found that demineralisation of the skeleton occurred in dogs during thyroid feeding and was not increased by simultaneous administration of parathyroid extracts. Tibbetts *et al.* [1932] announced that the high faecal excretion of calcium in clinical hyperthyroidism was not influenced by administration of irradiated ergosterol; Watchorn [1930], Pugsley [1932] and others have found however that irradiated ergosterol tends generally to decrease the faecal and increase the urinary excretion of calcium. The present paper reports a study of the effect of desiccated thyroid on the calcium excretion of rats and the influence of irradiated ergosterol¹ thereon, while some experiments on the effect of ammonium chloride acidosis are included for comparison.

METHODS.

The experiments were similar in general plan and in the analytical methods used to those described by Pugsley [1932] in a study of the effects of parathyroid extracts and of irradiated ergosterol on the calcium and phosphorus metabolism of rats. Adult male and female rats of Wistar strain, weighing approximately 240 g., were used. The diet, which contained 0.056 % calcium, was given as a constant weighed ration daily, and was not increased during thyroid treatment in spite of the greater appetite of the animals; the weight of unconsumed food was also recorded. Metabolic rates were found by determination of oxygen consumption in a closed-circuit apparatus, similar to that described by Benedict and Macleod [1929], save that the fall of the oxygen reservoir-spirometer is traced on a rotating smoked drum, the tracing serving also as a record of activity. The metabolic rates are expressed as the percentage increase in oxygen consumption over the oxygen consumption of the individual animals determined in the pre-experimental period.

The desiccated thyroid was supplied by Messrs Ingram and Bell, and was found to contain 0.141 % calcium; it was thoroughly mixed with the food each day, so that each rat received 2 grains daily if, as was almost invariably the case, the entire ration was consumed. Irradiated ergosterol (Mead, Johnson & Co.)

¹ The authors are indebted to Mead, Johnson & Co. for the ergosterol used in these experiments.

was suitably diluted with olive oil and given from a pipette in daily doses of 0.1 ml. Ammonium chloride was given daily at the time of feeding by stomach tube, subcutaneous injection at the dosage required having proved highly toxic.

Results.

The effect of administering desiccated thyroid (2 grains daily) is shown in Fig. 1, which is a composite graph representing the average of six similarly

Fig. 1.

Fig. 2.

Fig. 1. Effect of administering 2 grains desiccated thyroid daily on calcium excretion (average of 6 rats); the dotted line shows the calcium excretion of controls.

Fig. 2. Effect of administering 20,000 units of irradiated ergosterol daily upon the increased calcium excretion induced by thyroid feeding (average of 6 rats). The first arrow indicates the commencement of thyroid treatment, the second the addition of irradiated ergosterol.

Fig. 3. Effect of administering 125 and (later) 250 mg. ammonium chloride daily on the calcium excretion (average of 6 rats).

treated animals. The rats weighed approximately 250 g. at the beginning of the experiment and lost about 70 g. under thyroid treatment. Thyroid medication began on the sixth day, as indicated by the arrow. The dotted lines in the

figures represent the average behaviour of four untreated control rats, in a simultaneous study.

The administration of thyroid soon leads to a marked increase in the faecal excretion of calcium, which becomes approximately constant at the new level, while the basal metabolic rate (B.M.R.) increases progressively. There is also a decided increase in the urinary excretion of calcium (shown, for clearness, on a larger vertical scale) and the ratio between urinary calcium and faecal calcium, initially very small, does not appear to be greatly altered under thyroid treatment.

Fig. 2 represents another experiment on a series of six rats; thyroid treatment was begun as before on the sixth day, but from the twentieth day (second arrow) the animals also received 20,000 international units of irradiated ergosterol daily by mouth. This dosage has been found in similar experiments on normal rats [Pugsley, 1932] to produce a very marked rise in the urinary calcium excretion with a corresponding decrease in the faecal excretion and without any very pronounced effect on the total calcium balance or the general well-being of the animals. It is clear from the figure that in the present experiments the irradiated ergosterol has again largely diverted the calcium excretion from the faeces to the urine, the total excretion of this element (on practically constant intake) being decreased rather than increased, though still far above the levels observed before thyroid feeding.

Smaller doses of irradiated ergosterol have proved to be less effective. Table I summarises an experiment similar in plan to the last, carried out on another series of six rats. The usual effect of thyroid administration was

Table I. *Effect of administration of 1000 and 10,000 units of irradiated ergosterol daily on the increased calcium excretion induced by thyroid feeding.*

Average of four rats.						
Treatment	Day	Urine-Ca mg./day	Faecal Ca mg./day	Ca balance mg./day	Body wt. g.	% increase in O ₂ con- sumption
Control period	2	0.04	4.93	+2.33	224	—
	4	0.06	4.80	+2.76	229	—
	6	0.05	4.59	+2.71	230	0
2 g. desiccated thyroid daily	8	0.04	4.57	+2.63	228	—
	10	0.05	4.60	+1.51	—	—
	12	0.05	5.77	+1.13	221	—
	14	0.03	7.37	-1.40	—	—
	16	0.11	9.25	-3.36	216	—
	18	0.15	10.50	-3.65	—	—
Added 1000 units irradiated ergo- sterol daily	20	0.11	11.50	-4.61	209	112
	22	0.37	11.50	-3.87	—	—
	24	0.50	12.10	-4.48	—	—
	26	0.57	11.47	-3.79	197	—
	28	0.71	13.67	-5.98	—	—
	30	0.48	12.05	-4.13	188	—
	32	0.74	13.00	-5.34	—	—
10,000 units daily; thyroid continued	34	0.70	13.80	-6.10	180	100
	36	1.22	10.90	-3.72	—	—
	38	1.52	9.17	-2.29	168	—
	40	1.47	9.32	-2.39	—	—
	42	2.47	9.10	-3.17	172	—
	44	2.85	5.58	-0.03	163	—
	46	3.70	4.60	+0.10	—	—
	48	4.24	4.46	-0.30	149	156
	50	3.77	5.62	-0.99	—	—
	52	5.00	3.30	+0.10	—	—
	54	6.72	2.60	-0.92	142	—

observed. The administration of 1000 units of irradiated ergosterol daily, from the twentieth day, may have slightly increased the urinary excretion of calcium but had no discernible effect on the faecal or the total excretions. On the thirty-fourth day the dosage was therefore increased to 10,000 units daily; the table shows that this led in the course of a few days to a definite shift of excretion from faeces to urine, with an unmistakable decline in the total calcium excretion, the negative calcium balance being greatly reduced.

Table II summarises another similar experiment on a series of six rats, in which the dosage selected was 2000 units of irradiated ergosterol daily, from the

Table II. *Effect of administration of 2000 units of irradiated ergosterol daily on the increased calcium excretion induced by thyroid feeding.*

Average of four rats.						
Treatment	Day	Urine-Ca mg./day	Faecal Ca mg./day	Ca balance mg./day	Body wt. g.	% increase in O ₂ con- sumption
Control period	2	0.04	4.36	+2.60	231	—
	4	0.05	4.40	+3.17	236	—
	6	0.03	3.77	+3.76	238	0
2 g. desiccated thyroid daily	8	0.06	3.30	+3.64	236	—
	10	0.05	3.00	+3.35	—	—
	12	0.04	4.57	+2.11	231	—
	14	0.02	7.20	-1.04	—	—
	16	0.05	7.30	-1.19	223	—
	18	0.07	8.87	-0.82	—	—
	20	0.02	10.25	-2.21	216	79
Added 2000 units irradiated ergo- sterol daily	22	0.11	10.12	-1.83	—	—
	24	0.13	9.12	-1.01	—	—
	26	0.22	10.30	-2.12	210	—
	28	0.47	10.48	-2.55	—	—
	30	0.54	10.50	-2.65	207	—
	32	0.48	10.51	-2.75	—	—
	34	0.54	10.77	-2.91	206	83
	36	0.67	10.00	-2.27	—	—
	38	0.38	10.00	-1.98	196	—
	40	0.66	10.00	-2.26	—	—
	42	0.24	10.00	-1.84	188	—
	44	0.40	10.90	-2.90	—	—
	46	0.48	10.80	-2.80	165	—
	48	0.45	11.20	-3.25	—	200

twentieth day. Such doses have been found to reduce the faecal excretion of calcium, in normal animals, very decidedly [Pugsley, 1932]; but in the present experiments, although the faecal excretion and negative balance are somewhat lower and the urinary excretion definitely higher than might have been expected in view of the earlier results, there is not convincing evidence that this dose was in any degree effective.

From a series of 12 rats receiving 2 grains of desiccated thyroid daily, blood was collected by the method previously described [Pugsley, 1932] for determination of the serum-calcium by the method of Clark and Collip [1925]. The values obtained fell within the normal range for rats in this colony, the average being 10.3 mg. per 100 ml. (minimum 10.1, maximum 10.4). This agrees with the general opinion [Thomson and Collip, 1932] that there is no simple relation between thyroid function and the level of the serum-calcium.

The effect of ammonium chloride on calcium excretion was studied in six rats, and the results are summarised in Fig. 3. 1 ml. of a 12.5 % solution of the salt was administered daily by stomach tube from the sixth day; as this had little effect, the dose was increased to 2 ml. from the fourteenth day onward.

A marked diuresis naturally resulted, and the excretion of calcium in the urine was considerably increased, the faecal excretion being scarcely affected. This observation confirms the view of Aub *et al.* [1929] that the thyroid hormone does not affect calcium metabolism merely by producing an acidosis.

DISCUSSION.

The work of Aub *et al.* [1929] on the effect of the thyroid hormone on calcium excretion has, curiously enough, stimulated little experimental study. We have entirely confirmed his view that the thyroid hormone stimulates calcium excretion and promotes loss of calcium from the body, and that the channel of excretion is the intestine rather than the kidney (a matter in which this effect differs strikingly from the increased calcium excretion produced by parathyroid hormone, by ammonium chloride or by very large doses of irradiated ergosterol). It seems at present futile to speculate on the factors responsible for directing calcium excretion to one channel rather than another, but it should be emphasised that in our experiments with thyroid feeding the amount of calcium lost in the faeces was often considerably greater than the total calcium intake. Our work also supports the contention of Aub that the action of the thyroid hormone is not to produce acidosis or parathyroid stimulation; and moreover does not seem to be directly correlated with the increase in basal metabolic rate. In experiments not reported herein, we have found that the injection of parathyroid extract into rats receiving desiccated thyroid increases the total loss of calcium from the body. The failure of Tibbetts *et al.* [1932] to observe a reduction in faecal calcium excretion and negative calcium balance, as we did, after administration of vitamin D, may perhaps be ascribed to inadequate dosage; Taylor and Weld [1932] obtained results similar to ours in dogs.

SUMMARY.

The administration of 2 grains of desiccated thyroid daily, by mouth, to adult rats, leads to a marked increase in calcium excretion and establishment of a negative calcium balance. The increase is observed in the faecal rather than in the urinary calcium. The serum-calcium is not elevated.

The administration of irradiated ergosterol, in doses of 10,000 international units or more daily by mouth, to rats receiving desiccated thyroid reduces the faecal and increases the urinary excretion of calcium, and partly or wholly corrects the negative calcium balance. Smaller doses are comparatively ineffective.

The administration of 250 mg. ammonium chloride daily, by mouth, to normal adult rats leads to a marked increase in the urinary excretion of calcium, with little influence upon the faecal excretion.

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