CCLXIV. THE EFFECT OF 2:4-DINITROPHENOL UPON CALCIUM, CREATINE AND CREATININE EXCRETION IN THE RAT.¹

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It has been shown by a number of workers that nitrated phenolic compounds have a marked stimulating effect upon metabolism. The literature has recently been extensively reviewed by Dodds [1934] and Tainter and Cutting [1934]. The effect seems in no way to be attributable to thyroid stimulation and in general is decidedly different from the effect of thyroid administration. It has been shown by Pugsley *et al.* [1934] that the administration of desiccated thyroid or thyroxine causes a marked creatinuria and an increased faecal excretion of calcium in rats. This is also true of the thyrotropic hormone of the anterior pituitary [Collip, 1934]. The observed increased excretion of calcium confirms the clinical studies of Aub *et al.* [1929] and Hansman and Wilson [1934] on hyperthyroidism.

In view of these facts it seemed of interest to investigate the effect of 2:4-dinitrophenol upon calcium excretion and simultaneously to study the effect upon creatine and creatinine excretion.

Methods.

The diet, feeding of the animals, and analytical methods were the same as those previously reported by Pugsley *et al.* [1934]. During the experimental period the dry 2:4-dinitrophenol was intimately mixed with the diet. Some difficulty was encountered at first in getting the rats to consume their usual amount of food; however, with the increased metabolic rate which ensued this obstacle was soon overcome. The rats did not appear as ravenous as in the previous work on feeding desiccated thyroid. The creatine and creatinine excretions were followed on the same rats as the calcium studies.

Results.

The effect of feeding 25 mg. of dinitrophenol per rat daily upon the calcium excretion, calcium balance and metabolic rate is shown in Table I. The values represent the average of 8 rats. After a preliminary period of 8 days the dinitrophenol was given for 26 days and then withdrawn and the rats allowed to recover. The faecal calcium was increased to approximately twice its control level within 10 days and remained at this high level during the feeding of the drug. The calcium excretion in the urine was not significantly altered, except on withdrawal of the dinitrophenol when a slight increase occurred. The calcium balance is shifted from a positive value of 1 mg. daily during the control period

¹ A preliminary account of this work was reported at the meeting of the Royal Society of Canada, May 1935.

Table I. *The effect of dinitrophenol upon the calcium excretion of adult rats (8 animals).*

Treatment	Days	Urine Ca mg./day	Faecal Ca mg./day	Ca balance mg./day	% increase in O ₂ con- sumption
Control period	2	0.03	2.64	+0.93	—
	4	0.03	2.57	+1.00	—
	6	0.02	2.65	+0.93	—
	8	0.03	2.36	+1.21	0
25 mg. dinitrophenol daily from the 8th day until the 34th day	10	0.08	2.64	+0.46	—
	12	0.04	2.97	-0.41	—
	14	0.03	4.10	-0.83	—
	16	0.02	5.59	-2.07	+85
	18	0.01	4.96	-2.23	—
	20	0.01	4.71	-1.97	—
	22	0.02	6.24	-3.64	+77
	24	0.02	5.57	-2.97	—
	26	0.03	5.15	-2.36	—
	28	0.04	5.10	-2.66	—
	30	0.03	5.00	-2.21	+30
	32	0.02	5.50	-3.24	—
	34	0.01	5.75	-3.72	—
Dinitrophenol discontinued	36	0.03	4.45	-1.72	—
	38	0.35	2.76	+0.49	—
	40	0.40	2.62	+0.58	—
	42	0.40	2.62	+0.58	—

to a negative value of 2-3 mg. daily during the experimental period. On withdrawal of the dinitrophenol the calcium balance becomes positive and a decided decrease in the faecal calcium is observed. The metabolic rates were determined every week and are expressed as the percentage increase in oxygen consumption over that found in the control period. The variations are probably due to the fact that the rats had not consumed as much of the dinitrophenol before the test as they had in some of the other determinations. Magne *et al.* [1932] report a rapid habituation to the drug in large non-lethal doses and such may have occurred here. The metabolic rates were determined at approximately the same hour on each occasion.

Table II. *The effect of dinitrophenol upon the creatine and creatinine excretion of adult rats (8 animals).*

Treatment	Days	Creatinine mg./100 g. body wt. daily	Creatine mg./100 g. body wt. daily	Body wt. g.
—	2	2.62	0.60	266
	4	2.65	0.63	262
	6	2.57	0.53	266
	8	2.66	0.62	266
25 mg. dinitrophenol daily from 8th to 32nd day	10	3.84	2.84	261
	12	3.96	3.16	253
	14	3.84	3.18	249
	16	3.84	3.10	239
	18	3.74	4.10	230
	20	3.88	3.30	225
	22	3.20	3.36	223
	24	3.22	3.68	218
	26	3.46	3.96	210
	28	3.43	4.47	201
	30	3.46	4.75	197
	32	3.28	5.25	187

Note. The creatine is expressed as creatinine.

In a preliminary study in this investigation the dinitrophenol was given by subcutaneous and intraperitoneal injections of the sodium salt; here the results were not very satisfactory, owing to the short duration of the effect on the metabolic rate and the rapid excretion of the dinitrophenol. No definite rise in the calcium excretion was elicited by this technique even when the dose was divided and administered 3 times daily. Intraperitoneal injections of dinitrophenol dissolved in olive oil were also not very satisfactory in producing a definite increase in calcium excretion.

The results of feeding 25 mg. of dinitrophenol per rat daily on the body weight, creatinine and creatine excretion are shown in Table II. These results are in agreement with those previously reported for drugs which produce a loss of body weight, a decrease in glycogen stores and an increased excretion of creatine and creatinine. The creatinine excretion is increased to approximately 45% above the control level and the creatine excretion is 4 times as great as during the control period. The loss of body weight is approximately 3 g. daily. A creatinuria of about the same extent was produced by intraperitoneal injections of the sodium salt in a dosage of 5 mg. 3 times daily.

DISCUSSION.

It appears from these results that substances which produce a prolonged elevation of the metabolic rate are capable of causing an increased excretion of calcium in the faeces. In cases where a transient increase in metabolic rate occurs, as with intraperitoneal injections of dinitrophenol, the effect is not so clearly shown. This increased excretion of calcium in the faeces would probably not occur with therapeutic amounts of dinitrophenol, since the dosage used in these experiments is proportionately very much larger than that usually prescribed for human beings. Although Aub *et al.* [1929] were unable to find any increase above the normal level in clinical cases with increased metabolic rate due not to hyperthyroidism but to subacute infections, it appears from these results that a time factor is involved in producing the effect.

Rabinowitch and Fowler [1934] reported an increased blood creatinine in patients receiving dinitrophenol. Most workers agree that the urinary nitrogen is unchanged or slightly increased with toxic doses of the drug [Magne *et al.*, 1932; Tainter and Cutting, 1934; Furth and Rapport, 1934]. The increased creatine and creatinine excretion found in these experiments would probably not be considered significant when calculated as total nitrogen.

SUMMARY.

The feeding of 25 mg. of 2:4-dinitrophenol per rat daily caused an increased excretion of calcium in the faeces similar to that produced by feeding desiccated thyroid. A marked creatinuria and an increased excretion of creatinine was also observed.

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Note added September 15th, 1935. After this paper had gone to press a paper by Lieben and Asriel [1935] came to the author's notice reporting an increased excretion of creatine in rabbits after the subcutaneous injection of dinitrophenol. Also Robbins [1935] recently reported that the administration of dinitrophenol to patients had no effect on their calcium and phosphorus excretion.