CLII. THE EFFECT OF THYREOTROPIC HORMONE AND OF DESICCATED THYROID UPON CREATINE AND CREATININE EXCRETION.

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CRAMER AND KRAUSE [1913] observed a creatinuria in both men and dogs as a result of feeding thyroid tissue. Gross and Steenbock [1921] found that feeding sheep thyroid to a pig on a nitrogen-free diet led to an increase in the creatine excretion. Patients with Graves' disease excrete excessive amounts of creatine in the urine. It has been suggested by Shorr *et al.* [1933] that the thyroid hormone is involved in the breakdown and building up of phosphocreatine. Eimer [1931] reported a significant increase in the total creatinine excretion of healthy subjects when fed desiccated thyroid. Recently, Shire and Zwarenstein [1933] have shown that the administration of anterior pituitary extracts causes an increased excretion of creatinine in normal rabbits but not in castrated animals.

Methods.

Adult rats were placed on the following diet 3 weeks before the metabolism studies were started: wheat flour 1000 g., corn meal 1000 g., oatmeal 1000 g., linseed meal 300 g., wheat germ 82 g., brewer's yeast 182 g., cod-liver oil 180 ml. We have found the diet to be an important factor in studying creatine metabolism. The addition of meat or fish meal to the diet gives relatively high and irregular values for the creatine excretion. The rats were kept in Hopkins's metabolism cages and the urine and faeces collected separately. The urine was collected over a period of 48 hours, and the samples were made up to a volume of 20 ml.; the creatinine was estimated on a convenient aliquot by the method of Folin [1914].

The creatine was calculated by difference after autoclaving the urine in the presence of picric acid, and it is expressed here in terms of creatinine. The oxygen consumption was measured in a multiple chamber respiration apparatus, similar to that described by Benedict [1930-31]. The metabolic rate is expressed as the percentage increase in oxygen consumption over that determined in the pre-experimental period. Since fasting alone has an effect upon the creatine and creatinine excretion, the metabolic rates could not be taken under fasting conditions; however, on a series of several hundred rats we have found that the oxygen consumption, measured under feeding conditions at approximately the same hour each day, tends to be remarkably constant (unpublished data). Hypophysectomy was performed on the rats by the parapharyngeal approach of Selye [Collip *et al.*, 1933]. Two weeks were allowed after the operation before the creatinine studies were started.

The thyreotropic extract which was used showed on assay 100 units per ml. [Anderson and Collip, 1933]. One unit represents the smallest amount of the hormone which, when given twice daily to an hypophysectomised rat, will produce a 20 % increase in oxygen consumption within 96 hours.

Results.

Fig. 1 shows the effect of feeding desiccated thyroid upon the metabolic rate and the creatine and creatinine excretion of normal rats. The values in this chart represent the average of 6 rats. After a control period of 6 days, the rats were each given 2 grains of desiccated thyroid daily in their food. A very striking increase in creatine output occurred, which reached a peak on the twelfth day of thyroid feeding. It then commenced to drop off gradually, in

Fig. 1. The effect of feeding 2 grains of desiccated thyroid daily upon the metabolic rate and the creatine and creatinine excretion of adult rats.

spite of a rising metabolic rate. The creatinine excretion was increased slightly during the first 2 weeks of thyroid administration. After 24 days of thyroid feeding the animals were in a poor condition due to the extreme hyperthyroidism, and 3 rats of the series died before the experiment was finished. The drop in the metabolic rate which occurred in the sick rats lowered the average rate for the group.

In another series of experiments, a group of 4 rats was given thyreotropic hormone. The effect upon the metabolic rate and the creatine and creatinine excretion is shown in Fig. 2. Fifty units of thyreotropic hormone were injected intraperitoneally twice daily for a period of 32 days. A rise in the creatine excretion occurred immediately after beginning the injections, and the excretion then gradually fell to the normal level. The metabolic rate rose to a level of + 35 %, and then gradually fell, finally below the pre-experimental level.

Fig. 3 shows the effect of the thyreotropic hormone upon the metabolic rate and the creatine and creatinine excretion of hypophysectomised rats. A group

of 4 animals was studied. The thyreotropic hormone in doses of 50 units twice daily was started on the tenth day. It is seen that the creatine excretion reached a peak by the fourth day of injection and then began to decline while the metabolic rate was still rising. This suggests that the relationship between the two is not as direct as one might conclude from Fig. 2. The amount of creatinine

Fig. 2.

Fig. 3.

Fig. 2. The effect of 50 units of thyreotropic hormone upon the metabolic rate and the creatine and creatinine excretion of adult male rats.

Fig. 3. The effect of 50 units of thyreotropic hormone upon the metabolic rate and the creatine and creatinine excretion of hypophysectomised rats.

excreted remained unchanged throughout the experiment and was essentially the same as that of normal rats. This differs from the findings of Braier [1931], who reported a 35–40 % decrease in the creatinine excretion of hypophysectomised dogs.

The thyreotropic hormone produced a rise in the creatine output of the dog. This is shown in Table I. It will be noted that a diuresis occurred simultaneously with the creatinuria.

Table I. *Creatine and creatinine excretion of a dog treated with thyreotropic hormone. Wt. 26.6 kg.*

	Day	Urine volume ml. per day	Creatinine g. per day	Creatine as creatinine g. per day
Control period	2	867	1.103	0.357
	4	725	0.668	0.195
	6	1135	1.003	0.334
Experimental period. Injections started on 6th day; 500 units thyreotropic hormone twice daily, intravenously	8	1250	0.817	0.807
	10	1335	0.861	0.795
	12	1675	0.885	1.273
	14	1070	0.718	0.968
	16	1375	0.891	0.663
	18	1425	0.789	0.479
	20	1550	0.790	0.603
	22	1375	0.715	0.524
Recovery period. Injections stopped on 22nd day	24	1360	0.743	0.262
	26	1050	0.550	0.088
	28	930	0.756	0.055
	30	1000	0.515	0.110

The effect of small doses of thyreotropic hormone on creatine excretion has been studied. In Table II it is seen that 6 units of thyreotropic hormone are

Table II. *The effect of graded doses of thyreotropic hormone upon the creatine and creatinine excretion of normal rats.*

	Day	Group I (average of 4 rats)			Group II (average of 4 rats)			Group III (average of 4 rats)		
		Crea- tinine mg.	Crea- tine mg.	Meta- bolic rate	Crea- tinine mg.	Crea- tine mg.	Meta- bolic rate	Crea- tinine mg.	Crea- tine mg.	Meta- bolic rate
Control period	2	3.65	1.16	—	2.80	0.58	—	3.08	0.69	—
	4	3.33	1.09	—	3.22	0.64	—	2.96	0.36	—
	6	3.28	1.04	Normal	2.84	0.79	Normal	2.74	0.56	Normal
Injections started on 6th day		Dose: 50 units b.i.d.			Dose: 12 units b.i.d.			Dose: 6 units b.i.d.		
Experimental period	8	3.03	2.17	—	2.94	0.62	—	2.64	0.50	—
	10	3.31	3.71	—	2.81	0.74	—	2.64	0.57	—
	12	3.24	2.78	+34.8 %	2.82	1.20	—	2.73	1.06	—
	14	3.44	2.30	—	3.08	1.20	+17.3 %	2.93	1.27	+28.7 %
	16	3.32	2.41	—	3.03	0.53	—	3.15	0.28	—
	18	3.40	2.06	+28.1 %	3.09	0.44	+16.7 %	3.17	0.34	+19.1 %

The thyreotropic hormone in doses as low as 6 units twice a day causes an increase in creatine excretion in the normal rat. The creatine and creatinine are expressed in mg. per 100 g. body weight per day. The creatine is expressed in terms of creatinine. The "metabolic rate" is the percentage increase in oxygen consumption over that determined in the control period.

sufficient to cause a significant rise in the creatine excretion and metabolic rate of normal rats. In the hypophysectomised rat, 1 unit causes a more striking increase in the creatine output than is produced after 6 units in the normal rat (Table III).

Table III. *The effect of thyreotropic hormone upon creatine and creatinine excretion of hypophysectomised rats.*

	Day	Group I (average of 4 rats)			Group II (average of 2 rats)			Group III (average of 2 rats)		
		Crea- tinine mg.	Crea- tine mg.	Meta- bolic rate	Crea- tinine mg.	Crea- tine mg.	Meta- bolic rate	Crea- tinine mg.	Crea- tine mg.	Meta- bolic rate
Control period	2	3.34	0.77	—	2.85	0.77	—	2.65	0.95	—
	4	3.02	0.90	—	2.79	0.17	—	3.14	0.28	—
	6	2.90	0.83	Normal*	2.81	0.15	Normal*	2.77	0.18	Normal*
Injections started on 6th day		Dose: 50 units b.i.d.			Dose: 2 units b.i.d.			Dose: 1 unit b.i.d.		
Experimental period	8	3.10	2.12	—	2.95	0.14	—	3.00	0.12	—
	10	3.28	3.38	+31.5 %	2.94	2.42	+26.4 %	2.74	2.52	+20.9 %
	12	3.24	3.08	—	2.56	2.24	—	3.08	2.92	—
	14	3.18	2.62	+37.8 %	2.95	1.26	+11.1 %	2.90	2.62	+ 5.0 %
	16	3.14	2.30	—	2.82	0.80	—	2.83	1.07	—
	18	2.90	2.09	+26.3 %	2.78	0.10	+21.0 %	2.74	0.26	+10.9 %

The hypophysectomised rat is exceedingly sensitive to the thyreotropic hormone. It is seen here that one unit of the hormone twice a day causes a significant rise in the creatine excretion.

* Normal for hypophysectomised rats.

Since fairly small amounts of the thyreotropic hormone will produce a significant increase in creatine excretion in the normal rat, this may give us a satisfactory method of assaying the hormone. The simplicity of the test would

be a distinct advantage over our present method of measuring the increase in metabolic rate in the hypophysectomised rat.

Purified extracts of the growth hormone and of the adrenotropic hormone have been injected into normal rats and the creatine and creatinine excretion followed over a period of 18 days. The effect of thyreotropic extract which had been inactivated by boiling for 2 hours at p_H 5 was also studied. The average daily output of creatine on the eighth day of the growth extract was 0.71 mg. per 100 g., of the adrenotropic 0.55 mg., and of the boiled thyreotropic 0.38 mg., while the controls receiving active thyreotropic hormone excreted 2.88 mg. of creatine on the eighth day of injection.

A substance which inhibits the action of thyreotropic hormone in raising the metabolic rate has been reported by Collip and Anderson [1934; Anderson and Collip, 1934]. Creatine and creatinine excretion has been studied on a group of normal rats receiving the antithyreotropic extract, together with the thyreotropic hormone. The results are given in Table IV. A fall in metabolic rate

Table IV. *The effect of antithyreotropic and thyreotropic hormones upon the creatinine and creatine excretion of normal rats.*

	Day	Group I (average of 4 rats)			Group II (average of 4 rats)		
		Creatinine mg.	Creatine mg.	Metabolic rate	Creatinine mg.	Creatine mg.	Metabolic rate
Control period	2	2.81	0.72	—	2.92	0.60	Normal
	4	2.94	0.51	—	2.88	0.57	—
	6	3.00	0.82	—	2.86	0.50	—
	8	2.74	0.59	—	3.02	0.48	—
	10	2.86	0.68	Normal	2.90	0.60	Normal
Injections started on 10th day		Dose: thyreotropic 25 units b.i.d.			Dose: thyreotropic 25 units b.i.d.; antithyreotropic extract 1 ml. b.i.d.		
Experimental period	12	2.50	0.82	—	2.98	0.81	—
	14	2.95	1.05	+ 16.7 %	3.01	1.54	—
	16	2.90	1.22	—	3.12	1.21	- 11.6 %
	18	3.10	1.74	—	3.14	1.15	—
	20	2.74	1.05	+ 37.4 %	2.58	1.07	- 6.0 %
	22	2.67	1.06	—	3.10	1.12	—
	24	2.70	0.82	—	3.00	0.89	—
	26	2.88	0.42	+ 20.1 %	3.18	0.66	- 12.9 %
	28	2.66	0.76	—	2.88	0.51	—
	30	2.64	0.40	—	3.24	0.51	- 20.1 %
	32	3.10	0.30	—	3.18	0.57	—
	34	2.80	0.20	+ 2.0 %	3.12	0.53	—

occurred in the group of animals receiving the antithyreotropic substance together with the thyreotropic hormone (Group II); however, the creatine excretion in this group was practically the same as in the group receiving thyreotropic hormone only (Group I).

SUMMARY.

1. The feeding of desiccated thyroid to normal rats caused a rise in the creatine excretion, which reached its peak on the tenth day and then gradually decreased while the metabolic rate was still rising.

2. The thyreotropic hormone caused a temporary increase in creatine excretion both in the normal and the hypophysectomised rat.

3. A significant increase in creatine excretion was produced with 6 units of thyreotropic hormone in the normal rat and with one unit in the hypophysectomised rat.

4. The thyreotropic hormone caused an increase in the creatine excretion of the dog.

5. Purified extracts of the growth hormone and of the adrenotropic hormone had no effect on the creatine excretion of normal rats. Thyreotropic extract inactivated by boiling also gave negative results.

6. Antithyreotropic substance, which inhibited the rise in metabolic rate by thyreotropic hormone, did not prevent an increase in creatine excretion.

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