LXXXVIII. THE EFFECTS OF PARATHYROID FEEDING ON CALCIUM AND CREATINE METABOLISM.

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It has been known for some years that removal of the parathyroid glands results in a condition of tetany. A large number of observations on the nervous and metabolic changes in tetania parathyreopriva have been recorded, and from these experiments conclusions have been drawn as to the probable function of these glands and two main hypotheses advanced to explain the effect of their removal. The first theory associates the nervous changes with a deficiency of calcium, and the second attributes them to the production of a toxic agent, possibly guanidine, which is closely related to creatine.

The experiments of MacCallum and Voegtlin [1909] and MacCallum and Vogel [1913] have indicated that the parathyroids are associated with the retention of calcium. Korenchevsky [1922] finds that no change in the calcium content of bone results on removal of the parathyroids. Vines [1921, 1922] has applied the use of parathyroid extracts to the treatment of certain diseases which he associates with a diminution in the ionic calcium of the blood, and by feeding parathyroid he was able to raise the ionised calcium to the normal value.

Collip, Clark and Scott [1925] report that parathyroid extracts are of great benefit in removing the tetany of parathyroidectomised dogs and that administration of potent extracts to normal dogs results in hypercalcaemia.

Paton and Findlay [1916] have indicated that the symptoms of parathyroidectomy are due to the liberation of methylguanidine. Frank, Stern and Nothman [1921] suggest that the toxic agent is dimethylguanidine. Both these substances are closely related to creatine and creatinine. An increase in the excretion of creatine after parathyroidectomy has been found by Greenwald [1911] and a decrease in creatinine by Burns [1916] and by Greenwald. Henderson [1918] has shown that tetania parathyreopriva is associated with an increase in muscle creatine and a decrease in the total and free guanidine of muscle. Hammett [1921, 1] has demonstrated a direct relationship between the parathyroids and creatine metabolism. He found that the addition of parathyroid tissue to muscle extracts retarded the formation of creatinine which normally occurs during incubation.

The present investigation was started with the object of ascertaining the effect of parathyroid feeding and injections on the creatine metabolism of rats, as measured by muscle creatine and urinary creatine and creatinine. It was later extended, and a further series of experiments was carried out to determine the action of parathyroid feeding on calcium metabolism with reference to bone calcium.

The thyroid and parathyroid glands of several rats were examined for histological changes. The results of this part of the work will be reported later.

In every experiment the rats were weighed twice a week so that variations in weight and growth could be followed, and in all experiments except the injection series the food was weighed out and the residues collected daily and weighed in order to determine the effect of parathyroid feeding on appetite.

Methods.

Feeding. Adult and young rats of both sexes were used for these experiments. They were fed on a diet of bread, butter and water. The desiccated parathyroid, in doses of 0·02 g. or 0·1 g., was mixed with a little bread and water and given to the rats before their main meal. No further food was given until all the parathyroid had been eaten. The rats were fed at approximately the same time each afternoon and the food removed early next morning. The parathyroid was administered daily for periods of 2 or 4 weeks.

Injections. The parathyroid extract was injected daily for 4 weeks in doses of 0·5 cc. (= 0·00085 g. desiccated gland).

In another series of experiments calcium lactate (0·21 g.) was given daily with and without parathyroid.

Estimations. Muscle Creatine. The total creatine and creatinine of muscle was estimated as creatinine by the method of Janney and Blatherwick [1915]. The right hind limb was freed from muscle, as far as possible without injury to the blood vessels to avoid errors due to adherent blood. The muscle was well minced and a portion (3·5 g.) used for the creatine estimation; the other portion was used to determine the water content.

Urinary Creatine and Creatinine. This series of experiments was carried out on four rats which were kept in small metabolism cages. In the experiments on the first three rats, 48-hourly samples of urine were collected for a period of 4 weeks. During the first 2 weeks the rats were fed on bread and butter alone, and during the second 2 weeks they received 0·02 g. parathyroid daily in addition. In the case of the fourth rat 24-hourly samples of urine were collected. The rat received bread and butter for the first 8 days, then 0·02 g. parathyroid was added daily to the diet for 11 days. The urine was collected in a flask containing a trace of thymol, and was made up, together with the washings of the floor of the cage, to constant volume as recommended by Burns and Orr [1914]. Estimations were done on 24-hourly samples of urine + thymol to determine if any change in creatine-creatinine content takes

place if urine is kept for a day before estimating. The results showed that no appreciable change occurs.

The creatine and creatinine were estimated by the micro-method of Folin [1914] using a standard of 0·2 mg. creatinine per cc. which was made up fresh for each estimation. Picric acid, purified and tested by the method of Folin and Doisy [1916] was used throughout the experiments. In order to correct any personal errors in reading the colorimeter, curves were plotted similar to those reported by Mellanby [1908] and by Hunter and Campbell [1916]. The urine was tested throughout the experiments for acetone bodies, but none was found.

Bone Calcium. The bones used in every case were the right fibula, tibia and femur. The fibula and tibia were separated from the femur and all three carefully freed from muscle, cartilage, etc. After drying at 107° for 16–17 hours to determine moisture content, the calcium was estimated in the dried bone by Aron's [1908] method.

Results.

Appetite and Weight. Observations were made on 118 rats but no definite relationship between appetite and weight was recorded. There appeared to be a slight increase in the appetite of the rats which received parathyroid; this effect of parathyroid feeding has also been observed by Kojima [1917]. In the young rats the variations in weight between the controls and those receiving parathyroid were too small for it to be concluded that parathyroid feeding has any effect on growth, confirming the results of Cameron and Carmichael [1921].

Muscle Creatine. The results for the average muscle creatine values for the different series are given in Table I. In the results for the adult rats (series 1–6) the only marked difference found is in series 4, and this was due to one of the rats giving an abnormally high value (0·546), so that it may be concluded that parathyroid feeding has no effect on muscle creatine.

Table I.

Series	No. of rats	g. creatine per 100 g. moist muscle	g. creatine per 100 g. dry muscle	g. moisture per 100 g. muscle	Diet daily g.	Mode and duration of administration
Adult Rats.						
1	21	0·478	1·923	75·2	*N*	—
2	18	0·483	1·877	74·4	0·02 *P*	Orally—2 weeks
3	8	0·476	1·993	76·0	0·02 *P*	,, 4 ,,
4	3	0·507	2·022	74·8	0·1 *P*	,, 4 ,,
5	6	0·481	1·843	73·3	0·21 Ca lactate	,, 2 ,,
6	7	0·475	1·879	74·6	0·21 Ca lactate 0·02 *P*	,, 2 ,,
Young Rats.						
7	4	0·396	1·496	73·4	*N*	,, 2 ,,
	4	0·419	1·633	74·4	0·02 *P*	
8	4	0·459	1·836	74·9	*N*	,, 2 ,,
	4	0·468	1·832	74·5	0·02 *P*	

N = Normal diet. *P* = Parathyroid.

The results for the young rats confirm those of previous observers that there is less creatine in the muscle of young rats than in that of adult rats. In both series a slightly higher creatine value for moist muscle was found in the rats which received parathyroid.

Urinary Creatine and Creatinine. The results of the urine estimations are given in Table II. The results are expressed as the average for the period of time mentioned. The normal average creatinine coefficient of rats 1, 3 and 4 is 13·5. Rat 2 is omitted from the average as it is apparently an exception showing a very high creatinine coefficient (19·5) which is associated with an abnormally high creatinine excretion in proportion to its weight. The normal average creatine of rat's muscle is given in Table I as 0·478 g. per 100 g. moist muscle. These results, taken in conjunction with those of Myers and Fine [1913], suggest that there is a relationship between the creatinine elimination and the percentage of muscle creatine of a given species, and the values obtained for the rat place it between the rabbit and man.

Table II. *Urine estimations.*

Adult Rats.

Average values only are given.

Rat	Creatinine per 2 days mg.	Creatine per 2 days mg.	Creatinine coefficient	Diet g.	Duration of experiment (days)
1 (♂)	12·9	2·8	11·4	*N*	14
	14·35	2·7	12·8	0·02 *P*	14
Difference	+1·45	−0·1	+1·4		
2 (♂)	16·54	1·9	19·5	*N*	14
	14·8	5·8	17·9	0·02 *P*	14
Difference	−1·74	+3·9	−1·6		
3 (♂)	15·33	2·37	14·5	*N*	14
	15·03	2·61	13·1	0·02 *P*	14
Difference	−0·30	+0·24	−1·4		
	Creatinine per day	Creatine per day			
4 (♀)	7·86	1·8	14·6	*N*	8
	7·6	2·2	13·9	0·02 *P*	11
Difference	−0·26	+0·4	−0·7		

N = Normal diet. *P* = Parathyroid.

Creatine in varying quantities is found to be present normally in rat's urine [Folin and Morris, 1913].

An increase in the average creatine excretion is shown by rats 2, 3 and 4. This increase is associated with a decrease in creatinine excretion, and in rats 3 and 4 might be due to the alteration in the creatine-creatinine ratio, and not to a direct effect on creatine metabolism, since these rats show practically no alteration in the amount of total creatinine excreted. Rat 2, on the other hand, shows a marked increase in the amount of total creatinine excreted and therefore suggests that parathyroid feeding not only alters the creatine-creatinine ratio in the urine, but also stimulates the production of creatine.

The results of the first experiment apparently contradict this effect. During the first 12 days on normal diet the creatinine excretions of this rat varied from 11·7–12·8 mg. per day. During the 2 days previous to the commencement of parathyroid feeding the creatinine excretion rose to 15·5 mg.—higher than the average value when receiving parathyroid. The apparent contradiction of these results, then, may be due to the fact that the value of creatinine excretion in rat 1 rose suddenly during the normal period and might have remained at this high level regardless of addition of parathyroid to the diet.

Bone Calcium. Table III shows the average values for the calcium content of the bone of rats on normal diet and of those receiving parathyroid. In the case of the adult rats the bone calcium content is very similar for all three series, whilst the very slightly higher average value found in the case of the young rats fed on parathyroid as compared with the controls does not warrant the conclusion that parathyroid feeding has any definite effect.

Table III.

Adult Rats.

Series	No. of rats	g. Ca per 100 g. moist bone	g. Ca per 100 g. dry bone	g. moisture per 100 g. bone	Diet g.	Mode and duration of administration
1	7	16·2	23·3	30·6	*N*	None
2	7	15·9	22·2	28·9	0·02 *P*	Orally—4 weeks
3	3	15·5	22·7	31·3	0·1 *P*	„ 4 „

Young Rats.

4	3	11·8	19·8	39·9	*N*	Orally—4 weeks
	3	12·3	20·8	40·2	0·02 *P*	
5	5	9·71	17·3	45·9	*N*	Orally—4 weeks
	5	10·07	18·4	44·9	0·02 *P*	
6	3	9·17	17·2	45·9	*N*	Injected—4 weeks
	3	9·46	18·4	48·4	0·0008 *P*	

N = Normal diet. *P* = Parathyroid.

General Discussion.

The output of guanidine after parathyroidectomy, and the close chemical relationship between creatine and guanidine, suggest that the parathyroid may be an important factor in creatine metabolism. The experiments of Hammett [1921, 2] indicate that parathyroid extract inhibits the conversion of creatine into creatinine in incubated muscle. If muscle creatine is the origin of urinary creatinine, as is believed by some observers, it would be expected, since parathyroid extract inhibits the conversion of creatine to creatinine in muscle, that feeding with parathyroid would cause a decrease in the elimination of the latter associated with an increase in that of the former. The results reported here support this view.

The negative effect of parathyroid feeding on muscle creatine itself may be partly due to the fact that muscle is normally practically saturated with

creatine, and even if this substance is fed, only a trace is taken up by muscles of adult animals.

Experiments [Hammett, 1921, 2] which have been carried out on rats normally used for experimental purposes and those which are "stock" and only come into such human contact as is incident to cleaning, feeding, etc., showed that there was a marked difference in the response of the two groups to removal of the parathyroids. The majority of the animals which survived belonged to the "experimental group" and Hammett suggested that their survival was due to an inherently smaller proportion of tetany-producing substances. The rats used in the present work were not equally tractable; some were much more excitable than others and this difference in excitability, if considered with the results of Hammett, and those of Pekelharing [1911] on creatine and muscle tone, may explain variations found in muscle creatine. Where a high state of neuro-muscular tension existed a higher creatine content of muscle would be found.

Summary.

1. Administration of extracts of parathyroid has no effect on the weight of adult animals or on the growth of young animals.

2. There appears to be an increase in the appetite of rats fed with parathyroid.

3. Parathyroid feeding has no effect on creatine metabolism as measured by muscle creatine.

4. Parathyroid feeding causes an alteration in the ratio of creatinine to creatine excreted, resulting in the elimination of more creatine and less creatinine.

5. Parathyroid feeding and injections have no effect on the deposition of calcium in bone.

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