XLIII. STUDIES ON CALCIUM METABOLISM.

I. THE ACTION OF THE PARATHYROID HORMONE ON THE CALCIUM CONTENT OF THE SERUM AND ON THE ABSORPTION AND EXCRETION OF CALCIUM.

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THE numerous references to the low calcium content of the serum in *tetania parathyreopriva*, and more particularly the recent work of Collip [1925] on the extraction and action of the parathyroid hormone, make it clear that one action, at any rate, of the parathyroid glands is to control the concentration of calcium in the blood. So much may be taken as definitely established, but of the mode in which this control is exercised little is known. Clearly the parathyroids may affect the absorption or excretion of calcium, or may exert some controlling action on the equilibria between the different forms of combination in which calcium exists in the blood and between the concentrations in the blood and in the tissues.

In view of the ease with which the low serum calcium of *tetania parathyreopriva* can be raised by administration of calcium salts, it appears unlikely that the parathyroids act by controlling calcium absorption. Some doubt exists, too, as to whether they have any effect on the excretion of calcium. Thus Kishi [1924] found that after parathyroidectomy there was increased urinary, but unchanged faecal, excretion of calcium; MacCallum and Voegtlin [1909] found an increased faecal excretion, and Greenwald and Gross [1925, 1] found the total excretion to be decreased. These last authors, too [1926], state that long-continued administration of parathyroid to normal dogs brings about an increased calcium excretion.

The third possibility is one accepted by Greenwald and Gross [1925, 2], who have suggested that the parathyroid hormone is, or is necessary for the preparation of, the substance which keeps in solution the large excess of $Ca_3(PO_4)_2$ which Holt, La Mer and Chown [1925] have shown to be present in the blood. The excess of calcium excreted during long-continued administra-

tion of parathyroid they believe to be derived, ultimately, at any rate, from the bones.

In the work recorded in this paper we have aimed at obtaining some direct evidence as to the mode in which the parathyroid hormone exerts its action. The method of measuring the total excretion over a long period, first without and later with administration of parathyroid, is open to the objection that the differences usually observed are so slight as to be not far removed from the experimental error. They are not, moreover, easy of interpretation, for the faecal excretion is composed both of unabsorbed residues and of calcium re-excreted into the gut.

The effect of haemorrhage on the serum calcium.

Since many of our projected experiments necessitated the frequent drawing of blood samples and consequently involved considerable haemorrhage, it seemed advisable to test first the effect of haemorrhage itself on the calcium content of the serum.

Clark [1920], working with rabbits, found that extensive haemorrhage was followed by a considerable fall in the serum calcium, amounting to as much as 12 %.

We intended to use cats in our own experiments and therefore repeated Clark's work with these animals. To our surprise we found that, although the animal was bled freely at intervals of half an hour, there was no reduction in the serum calcium even at death. Numerous repetitions gave identical results; even with a loss of 60 % of the estimated total blood volume and a correspondingly large loss of haemoglobin, the serum calcium was never decreased by an amount greater than the experimental error of the method employed (that of Kramer and Tisdall [1921]). The results of these experiments are given in Table I.

The uniformity with which this effect was obtained determined us to repeat the experiments with the same species as was employed by Clark. In every case the results entirely confirmed Clark's observations.

There appears, therefore, to exist a definite species difference. The experimental conditions are identical in each series, the same anaesthetic being used, blood being taken at similar intervals and in similar amounts (with respect to the total blood volume), and the calcium estimations being carried out by the same method. We can offer no explanation of the difference, but it would seem to be in some obscure way connected with the susceptibility of the species to the action of the parathyroid hormone, for Greenwald and Gross [1926] have reported that, while cats, rabbits and rats are all more resistant than are dogs, rabbits and rats are even more resistant than are cats.

Table I. *The effect of haemorrhage on the serum calcium in the cat.*

No.	Weight of cat g.	Time min.	% haemo-globin *	Corpuscle volume % (haematocrit)	Mg. Ca per 100 cc. serum	Total haemor-rhage cc.	% re-duction in serum Ca	% re-duction in haemo-globin
I	2800	0	66	35	9·90	—	—	—
		30	55	32	9·84	—	—	—
		60	53	29	9·87	—	—	—
		90	50	24	9·90	47	Nil	15
II	3000	0	65	31	10·3	—	—	—
		40	—	—	10·25	—	—	—
		80	—	—	10·3	—	—	—
		120	50	25	10·3	—	—	—
		150	—	—	10·4	—	—	—
		180	40	21	10·3	81	Nil	37·4
III	3000	0	70	—	9·5	—	—	—
		60	—	—	9·5	—	—	—
		120	—	—	9·5	—	—	—
		180	47	—	9·5	57	Nil	33
IV	2980	0	66	44	9·90	—	—	—
		40	—	—	9·90	—	—	—
		80	—	—	9·90	—	—	—
		120	—	—	9·90	—	—	—
		150	50	30	9·90	80	Nil	24
V	3000	0	67	—	11·0	—	—	—
		60	—	—	10·95	—	—	—
		120	—	—	10·95	—	—	—
		180	40	—	11·0	90	Nil	40
VI	3020	0	—	—	9·25	—	—	—
		60	—	—	9·25	—	—	—
		120	—	—	9·25	—	—	—
		180	—	—	9·25	89	Nil	—

Table II. *The effect of haemorrhage on the serum calcium in rabbits.*

No.	Weight of rabbit g.	Time min.	% haemo-globin *	Corpuscle volume % (haematocrit)	Mg. Ca per 100 cc. serum	Total haemor-rhage cc.	% re-duction in serum Ca	% re-duction in haemo-globin
I	1500	0	62	30	12·90	—	—	—
		60	36	23	10·40	—	20	42
II	1000	0	53	32	11·20	—	—	—
		60	44	25	10·40	—	—	—
		100	38	23	9·50	—	14·4	28·5
III	2000	0	60	—	13·7	—	—	—
		30	52	—	13·2	—	—	—
		60	48	—	12·5	—	—	—
		90	40	—	12·4	44	8·6	33·3
IV	1950	0	60	27	11·5	—	—	—
		60	50	—	11·0	—	—	—
		120	20	15	10·0	33	10	66
V	1750	0	50	27	16·4	—	—	—
		75	40	21	13·6	30	17	20
VI	1690	0	60	28	11·9	—	—	—
		30	—	—	10·9	—	—	—
		90	—	—	10·5	—	—	—
		150	44	19	10·3	48	13·4	26·6

* Haemoglobin estimated colorimetrically against human standard.

The effect of parathyroid hormone on the blood serum of cats.

The parathyroid hormone used in our earlier experiments was kindly presented to us by Dr McNee, who had obtained it from Dr Collip; later we employed the commercial preparation "Parathormone Lilly," extracted by Collip's method. Table III shows the effect of subcutaneous injections of these preparations on cats.

Table III. *Effect of Collip's parathyroid extract, given subcutaneously, on the serum calcium in cats.*

(0·5 cc. (10 units) parathyroid extract given after withdrawal of first blood sample.)

		Mg. Ca per 100 cc. serum		
	Weight			
Exp.	g.	Before injection	15 hr.	Increase
I	2110	10·2	12·4	2·2
II	2440	9·7	11·9	2·2
III	2800	9·8	12·8	3·0
IV	3360	11·0	14·2	3·2
V	2800	10·4	14·1	3·7

10 units extract = amount necessary to produce in 15 hours a rise of 5 mg. per 100 cc. in a 2 kg. dog; therefore the extract is approximately half as active in cats as in dogs.

In every case it will be noted the serum calcium rose to an extent which, though less than would, presumably, have been observed in dogs, was well beyond the limits of experimental error and was undoubtedly real. The effect of intravenous injection of the hormone was next investigated. The cat was anaesthetised by means of paraldehyde and ether and a sample of blood having been withdrawn from the carotid, parathyroid hormone was given intravenously and thereafter blood was drawn at frequent intervals.

Table IV.

A. *Effect of intravenous injection of "Parathormone" in cats anaesthetised with paraldehyde and ether.*

	Weight of cat	Mg. Ca per 100 cc. serum	"Parathormone" injected	Mg. Ca per 100 cc. serum		
Exp.	g.	before injection	cc.	30 min.	120 min.	160 min.
I	2210	10·1	0·5	10·9	11·8	11·1
II	2900	10·0	0·5	11·6	12·0	10·4
III	3300	10·4	1·0	10·9	12·0	11·6

B. *Effect of intravenous injection of "Parathormone" in decerebrate and pithed cats.*

	Weight of cat	Mg. Ca per 100 cc. serum	"Parathormone" injected	Mg. Ca per 100 cc. serum	
Exp.	g.	before injection	cc.	45 min.	90 min.
I	3400	10·30	0·6	10·70	11·65
II	2890	9·6	0·6	11·70	12·00

The results of these experiments (Table IV) show that under these conditions the hormone exerts its action much more rapidly than when given hypodermically, the maximum rise in the serum calcium being attained in about 2 hours. The magnitude of the rise, however, is not greatly increased.

In Table IV (B) we also give the results of experiments in which parathyroid hormone was given intravenously to cats after decerebration which included removal of the pituitary gland, the spinal cord having also been destroyed. The rise in the serum calcium was of the same magnitude as that in anaesthetised animals (anaesthesia, as is shown *inter alia* by the results of the haemorrhage experiments, is without appreciable effect on the serum calcium). It is evident, therefore, that the effect of the parathyroid hormone is not exerted through the central nervous system.

We may remark at this point that *in vitro* we have observed a rapid inactivation of the hormone following its exposure to air (Table V).

Table V. *Effect of subcutaneous injection of Collip's parathyroid extract, which had been exposed to the air and kept at 37° for 24 hours, on the serum calcium in cats.*

Exp.	Weight of cat g.	Mg. Ca per 100 cc. serum before injection	Parathyroid extract injected (cc.)	Mg. Ca per 100 cc. serum 15 hr.
I	1980	9·5	0·5	9·5
II	2350	10·5	0·5	10·5

It is perhaps significant that the intravenous injection of sodium bicarbonate produces an effect exactly opposite to that of parathyroid. There is a rapid fall in the serum calcium reaching its maximum in some two hours, and thereafter returning to normal. Further, the two effects can be made to counterbalance each other exactly. As is shown in Table VI the simultaneous injection of sodium bicarbonate and of "Parathormone" (shown by control

Table VI.

A. *The effect of intravenous injection of sodium bicarbonate on the serum calcium in cats.*

Exp.	Weight of cat g.	Mg. Ca per 100 cc. serum before injection	$NaHCO_3$ injected g.	Mg. Ca per 100 cc. serum 30 min.	75 min.	100 min.
I	2110	10·8	0·8	10·0	9·4	8·8
II	2750	8·9	1·0	7·4	7·4	7·5

B. *The effect of simultaneous intravenous injection of $NaHCO_3$ and "Parathormone" on the serum calcium in cats.*

Exp.	Weight of cat g.	Mg. Ca per 100 cc. serum before injection	"Parathormone" (cc.) and $NaHCO_3$ (g.) injected	Mg. Ca per 100 cc. serum 30 min.	75 min.	100 min.
I	2750	8·8	0·7 cc. 1·0 g.	8·75	8·85	8·8
II	1870	10·2	0·7 cc. 1·0 g.	10·0	10·2	10·0

experiments to be active) leaves the serum calcium unaltered. Stewart and Haldane [1924] had previously found that oral administration of sodium bicarbonate caused a lowering of the human serum calcium (a result which we have confirmed), whereas oral administration of calcium chloride or of ammonium chloride, both of which produce an acidosis, caused a rise in the serum calcium.

These facts inevitably suggested the possibility that the action of "Parathormone" might be in some way connected with the neutrality-regulating mechanism. This idea, however, appears to have been partly disposed of by the recent work of Cantarow, Caven and Gordon [1926], who find that "Parathormone" is without effect on the CO_2-combining power of the blood.

Incidentally we may remark that oral administration of sodium bicarbonate affords two means of effecting a lowering of the blood calcium. By producing a decreased acidity of the intestinal contents it militates against the absorption of calcium; by bringing about an increased absorption of sodium, and hence an alkalosis, it may cause a direct lowering of the blood calcium. Following intravenous injection of sodium bicarbonate, the fall in the blood calcium can only be due to the alkalosis.

The effect of "Parathormone" on the absorption of calcium.

The direct measurement of the absorption of calcium is beset with many difficulties. Not only is the faecal output a combination of unabsorbed residues and of re-excreted calcium, the relative amounts of each being unknown, but the amount retained—which is all that can be measured—is obtained by difference from estimations of the total intake and total output, and is never more than a small fraction of either. The margin of error in such experiments is therefore considerable. Nor is it desirable to take the urinary excretion as an index of the amount of calcium absorption since it varies very considerably from day to day even under apparently standard conditions. The method of Bergeim [1926] undoubtedly gives a possible means of estimating absorption, but was published only after our experiments were under way. Indeed, excellent though it appears to be for many purposes, we believe it to be less satisfactory for our particular problem than the method we have employed. Briefly, our method consists in the complete removal of the alimentary canal distal to the oesophagus, *i.e.* the whole of that portion from which absorption may conceivably take place. If, under these conditions, the parathyroid hormone is able to exert its full effect on the serum calcium, it must mobilise calcium from some internal source or, possibly, act by controlling the rate of excretion. On the other hand, its inability to raise the serum calcium would indicate that normally the hormone draws on an external supply.

The technique of the experiments was as follows. Paraldehyde and ether were used to produce anaesthesia. The abdomen having been opened, the duodeno-jejunal junction was identified and the gut severed between two

ligatures at this point. The rectum was then drawn up out of the pelvis and cut across between ligatures close to the anus. The superior and inferior mesenteric arteries were ligated and the small and large intestine removed entirely. The duodenum was separated from the head of the pancreas, branches of the pancreatico-duodenal arteries being ligated. A series of ligatures were tied along the lesser and greater curvatures of the stomach from the pylorus to the cardia occluding the gastric vessels in the lesser omentum, and in the anterior layer of the greater omentum. The lower end of the oesophagus was ligated and cut across. The pylorus and first part of the duodenum were carefully separated from the portal vein, superior pancreatico-duodenal, splenic, and hepatic arteries, and the stomach and duodenum were removed by severing the omenta along the curvatures of the stomach. In this way the alimentary canal distal to the oesophagus was dispensed with, leaving the liver, spleen, and pancreas *in situ*, receiving an almost intact blood supply, that portion only of the portal circulation arising in the gut having been interfered with. The integrity of the arterial supply and venous return to these organs was in every case proved by the presence of arterial pulsation and by inspection of the venous flow. Where exclusion of liver, spleen and pancreas from the circulation was desired, the procedure was similar to that employed for evisceration alone, but it was unnecessary to separate the duodenum from the head of the pancreas. The hepatic artery and portal vein were ligated and cut in the free border of the lesser omentum, and the spleen and pancreas were removed entirely, after ligation of their respective arteries and veins.

At intervals blood was withdrawn through a cannula in the carotid artery, and injections were made through a cannula inserted in the external jugular vein. The animals were kept on artificial respiration and, despite the extensive surgical interference, their condition remained satisfactory throughout the experimental period. It was necessary, as a preliminary to the actual experiments, to ensure that in them the only variable condition was the presence or absence of added parathyroid hormone, since there were two other factors which might conceivably have caused an alteration in the calcium content of the blood. Firstly, it had to be shown that the operative shock was without effect in this direction. Secondly, in removing the whole of the alimentary canal, we had not only prevented absorption of calcium, but had also cut off one of the possible excretory routes. Control experiments in which evisceration was performed but no parathyroid hormone given showed that, over a period of three hours, the serum calcium remained absolutely unchanged. Whether the two factors are individually without effect, or whether their effects cancel one another was not determined, but was, for our purpose, a matter of no importance.

The results of a number of experiments on the effect of parathyroid hormone on the serum calcium of eviscerated cats are given in Table VII.

It will be noted that in every case the hormone produced a rise in the serum calcium, and comparison of these results with those given in Table IV shows

that the magnitude of the rise is as great in eviscerated as in normal animals. It seems fair, then, to conclude that the parathyroid hormone exerts its influence on the calcium content of the blood without drawing on external sources of calcium, *i.e.* without stimulating calcium absorption. Further, the liver, spleen and pancreas, have no special function as internal sources of calcium (though of course they may be used as reserves in the same way as other tissues) nor do they appear to influence the action of the hormone in any way.

Table VII.

A. *The effect of intravenous injection of "Parathormone" on the serum calcium of eviscerated cats.*

Exp.	Weight of cat g.	Mg. Ca per 100 cc. serum before injection	"Parathormone" injected (cc.)	Mg. Ca per 100 cc. serum	
				45 min.	90 min.
I	2760	9·0	0·7	9·8	11·2
II	2600	9·4	,,	11·2	11·8
III	3500	8·4	,,	11·4	10·2
IV	2990	9·6	,,	10·6	10·8
V	3330	10·0	,,	11·6	11·2
VI	3290	8·9	,,	10·0	10·8

B. *The effect of evisceration and the removal of the liver, spleen and pancreas on the serum calcium in cats.*

Exp.	Weight of cat g.	Mg. Ca per 100 cc. serum before removal		Mg. Ca per 100 cc. serum	
				45 min.	90 min.
I	2860	9·80	Intestine, liver,	9·80	9·80
II	3950	10·50	spleen and pan-	10·45	10·45
III	3800	9·90	creas removed	9·85	9·90

C. *The effect of "Parathormone" on the serum calcium of cats in which the liver, spleen, pancreas, intestine and stomach have been removed.*

Exp.	Weight of cat g.	Mg. Ca per 100 cc. serum before injection	"Parathormone" injected (cc.)	Mg. Ca per 100 cc. serum	
				45 min.	90 min.
I	4050	9·80	0·6	11·00	11·35
II	2980	10·30	0·6	10·70	11·65
III	4010	9·90	0·6	10·60	11·10

D. *The effect of intravenous injection of sodium bicarbonate on the serum calcium in cats in which the liver, spleen, pancreas, stomach and intestine have been removed.*

Exp.	Weight of cat g.	Mg. Ca per 100 cc. serum before injection	$NaHCO_3$ injected g.	Mg. Ca per 100 cc. serum	
				45 min.	90 min.
I	3950	10·50	1	8·80	8·80
II	3100	9·50	1	8·30	7·90

The effect of the parathyroid hormone on the excretion of calcium.

If the parathyroid hormone acts by controlling the rate of calcium excretion, then it can only produce an increase in the serum calcium by diminishing the excretion; any increase in the excretion can only be a secondary effect due to the increased calcium concentration in the blood, the excess calcium being derived from the tissues (since increased absorption has been ruled out).

Calcium is undoubtedly excreted by the kidneys, but we desired to assure ourselves definitely that, as stated in the literature, it is also excreted through the epithelium of the large intestine. Further, if such intestinal excretion took place, we needed quantitative data. As has previously been stated, the calcium content of the large intestine represents both the unabsorbed residue and any excreted calcium; to estimate the latter directly it is necessary to exclude the former. To secure this end we adopted the following method.

The cat was anaesthetised by paraldehyde and ether, and placed on artificial respiration throughout the experimental period. Cannulae were inserted into the carotid artery and the external jugular vein. The abdomen having been opened, the small intestine was cut across between two ligatures close to the ileo-caecal valve. The large intestine was brought out and the pelvic colon cut across at its junction with the rectum, all bleeding points being ligated. A large bore cannula was inserted into the lip of the caecum. The isolated loop of large intestine was thoroughly washed out with warm distilled water through the cannula, the removal of solid and semi-solid material and mucous being aided by gently squeezing the bowel with the fingers. Washing was continued until a clear sample was obtained, and this was reserved for analysis. After every washing a final clear sample was collected separately and analysed. Only those experiments were considered in which this sample contained merely traces of calcium and so indicated that the washing had been complete.

Table VIII. *The excretion of calcium by the large intestine and by the kidney in the cat.*

Exp.	Weight of cat g.			Mg. Ca in intestinal washings	Mg. Ca in urine	Mg. Ca per 100 cc. serum		
						At start of exp.	10 min. after injection	At close of exp.
I	2900	1st 3 hours	... ...	0·68	0·21	—	—.	—
		2nd 3 hours	... ...	0·66	0·14	—	—	—
II	2540	1st 3 hours	... ...	0·88	0·17	—	—	—
		2nd 3 hours	... ...	0·90	0·19	—	—	—
III	3010	1st 3 hours	... ...	0·33	0·22	—	—	—
		5 cc. 10 % CaCl₂ injected intravenously	...	—	—	10·2	22·0	15·0
		2nd 3 hours	... ...	4·27	0·25	—	—	—
IV	2700	1st 3 hours	... ...	0·68	0·16	—	—	—
		5 cc. 10 % CaCl₂ injected intravenously	...	—	—	—	—	—-
		2nd 3 hours	... ...	14·0	0·15	—	—	—
V	2890	1st 3 hours	... ...	0·30	0·21	—	—	—
		5 cc. 10 % CaCl₂ injected intravenously	...	—	—	9·0	21·0	10·0
		2nd 3 hours	... ...	9·60	0·23	—	—	—

After this preliminary washing the isolated loop of intestine was replaced within the abdomen, with the two ends slightly protruding, so that any contents would not escape. The washing was repeated after three and after six hours. In a number of experiments a cannula was inserted into the bladder and samples of urine collected (with washing) at the same time as the intestine was irrigated. Table VIII, giving the results of experiments of this type, shows

that calcium was excreted into the gut and that this excretion occurred at a fairly constant rate. Further, it was usually greater than the urinary excretion for the same period.

It remained possible that the calcium found in the large intestine did not constitute a true excretion, but had merely been secreted along with the mucus. To test this point, we allowed a preliminary control period of three hours, then injected calcium salts intravenously, and again washed out the gut after a further three hours. After the injection of calcium salt the excretion of calcium into the intestine was enormously increased although there was no corresponding increase in the mucous secretion. The urine did not show any comparable increase in calcium content (Table VIII). It seems, then, that not only is calcium excreted by way of the large intestine, but that this is the main excretory route.

The effect of the parathyroid hormone was tested by a method exactly similar to that described above, an injection of the hormone being given at the end of a three-hour control period. That the hormone was active was shown in each case by the withdrawal of blood samples at intervals and estimation of the serum calcium. The results, set forth in Table IX, showed the hormone to have little or no effect on the intestinal excretion of calcium.

Table IX. *The effect of intravenous injection of "Parathormone" on the excretion of calcium by the large intestine in cats.*

Exp.	Weight of cat g.	Mg. Ca in large intestine after 1st 3 hr. period	Mg. Ca per 100 cc. serum before injection	"Parathormone" injected cc.	Mg. Ca per 100 cc. serum 60 min. after injection	Mg. Ca in large intestine after 2nd 3 hr. period	Mg. Ca per 100 cc. serum 3 hr. after injection
I	2790	0·74	10·6	2	11·4	2·0	12·0
II	2400	0·70	9·9	2	11·1	0·72	11·5
III	2300	0·64	9·8	2	11·0	0·80	11·7
IV	3050	0·60	10·0	2	11·2	0·60	11·8

In only one experiment was a definite increase obtained, but, on the other hand, in no case was there a decrease. The latter, as has been pointed out, is the important finding, and indicates clearly that the parathyroid hormone does not control the rate of excretion of calcium by way of the large intestine. The fact that little or no increase in the calcium excretion was observed in these experiments is not surprising in view of the small rise in the serum calcium produced by the parathyroid hormone compared with that following the injection of calcium salts.

Indeed when one considers the amount of calcium excreted during the control period, it seems hardly possible that the parathyroid hormone could produce the observed rise in the serum calcium by diminishing this amount. The average of a number of experiments shows that the total excretion of calcium in the control period of three hours is 0·8 mg., the greatest excretion observed being just over 1·0 mg. Now in all our experiments the serum calcium

has been at least 1·0 mg. per 100 cc. higher at the end of the three-hour period than at the beginning, and the net rise has usually been greater than this. The smallest cat used in these experiments weighed 2500 g., which means an approximate blood volume of 200 cc. and therefore at least 100 cc. of serum. Hence an increase of 1 mg. per 100 cc. in the serum calcium involves the mobilisation of 1·0 mg. calcium at least, an amount greater than the total excretion during the period in which this rise has taken place. Obviously then, control of the rate of excretion cannot be the main mode of action of the parathyroid hormone. When one adds the experimental finding that the intestinal excretion, which accounts for much more than half of the total, is not diminished at all, it follows that the hormone does not act by controlling the rate of calcium excretion.

<div align="center">DISCUSSION.</div>

It is evident that the increase in the serum calcium following the administration of parathyroid extract is due to a withdrawal of calcium from the body tissues, for we have shown that the increase takes place without any diminution in the excretion, and without the possibility of any absorption from the alimentary canal. ·

Of the total serum calcium only about 60 % is readily diffusible through a collodion membrane [Cushny, 1920]. This readily diffusible fraction is apparently identical with Vines' "active calcium" [1924] which is precipitated by one equivalent of ammonium oxalate. Measurements of the ionic calcium [Neuhausen and Marshall, 1922] show that only 10–20 % of the total exists as ions. It is reasonable to suppose that the calcium mobilised by the parathyroid is readily diffusible, and that, therefore, the administration of parathyroid will be followed by a rise in the ratio of diffusible to total calcium in the serum. That such is actually the case has been stated by Vines [1924], and we have confirmed his statement [Stewart and Percival, 1927]. Several workers have shown that parathyroidectomy produces a relatively greater fall in the diffusible than in the non-diffusible calcium [Salvesen and Linder, 1924; Trendelenburg and Goebel, 1921; Moritz, 1925]. Hence it seems that on the amount of parathyroid hormone depends primarily the concentration of the readily diffusible calcium. This conclusion is to some extent in agreement with the suggestion of Greenwald and Gross [1925, 2] that the parathyroid hormone is, or is necessary for the production of, a substance (which Greenwald [1926] considers to resemble citric acid) capable of retaining in solution the excess $Ca_3(PO_4)_2$ which Holt, La Mer and Chown [1925] have shown to be present in blood.

The calcium drawn into the blood when parathyroid is administered must come from the soft tissues or from the bones—or from both. Using whole animals, it seems impossible to gain any direct information as to which alternative is taken, except by long-continued administration of the hormone, with, perhaps, actual tissue analysis. In short experiments the quantities

involved are hopelessly small. Even in long ones the large number of analyses of intake and output, with the relatively small differences on which to found conclusions as to the quantity of calcium retained or lost, render the results of doubtful value. Greenwald [1926], however, in an experiment of this kind claims that the amount of calcium lost during the period in which parathyroid was administered could only have come from the bones. He points out, however, that the blood may have drawn its extra calcium from the soft tissues which thus made up their supply at the expense of the bones. The rapidity with which parathyroid produces a rise in the blood calcium suggests, indeed, that the soft tissues form the primary source of supply.

We are at present engaged in a series of experiments by means of which we hope to gain some further information as to the immediate source of the calcium mobilised by the parathyroid hormone.

SUMMARY.

1. Extensive haemorrhage causes a lowering of the serum calcium in rabbits but not in cats. Rabbits are less susceptible to the action of parathyroid extract than are cats.

2. Parathyroid extract ("Parathormone") raised the serum calcium of cats when injected subcutaneously, and more rapidly when injected intravenously. The action is prevented by the simultaneous injection of sodium bicarbonate.

3. Since the parathyroid hormone can exert its full action on the serum calcium even after complete removal of the alimentary canal, it is concluded that the action does not consist in controlling the rate of calcium absorption.

4. The large intestine provides the main excretory route for calcium.

5. Following administration of parathyroid, and while the serum calcium is high, there is no diminution in the excretion of calcium. The parathyroids, therefore, do not act by controlling the rate of excretion.

6. The liver, spleen, pancreas, pituitary, thyroid, and central nervous system appear to have no special function in connection with the effect of parathyroid on the blood calcium.

7. It is considered that the parathyroid hormone controls the distribution of calcium between the blood and the tissues by regulating the proportion of the total serum calcium which is readily diffusible.

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