CLXXXIII. THE EFFECT OF PARATHYROID HORMONE UPON THE SERUM CALCIUM AND CALCIUM EXCRETION OF NORMAL AND ADRENALECTOMIZED RATS.

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It has been shown by Pugsley [1932] that administration of parathyroid hormone to rats causes a marked increase in urinary calcium excretion. With daily injections, the peak of the excretion is reached on the 4th or 6th day; after this time a gradual decrease in the calcium excretion to the control level occurs. Morgan *et al.* [1934] claim that the gradual failure of response to parathyroid hormone is associated only with a low-calcium diet and low Ca : P ratio; such a diet was used in the above work. That diet is an important factor in the response obtained to parathyroid hormone has been shown by the work of Allardyce [1931] on dogs, and of Shelling *et al.* [1933] on rats.

Schour & Rogoff [1936] have reported that the changes in the teeth following adrenalectomy in the rat are similar to those observed after administration of parathyroid hormone. They suggest a possible interrelationship between the parathyroid glands and the adrenals. It has been shown by Rubin & Krick [1933] that the loss of certain inorganic ions including calcium contributes to the development of the symptoms which follow adrenalectomy in the rat, and that administration of these ions will prevent the onset of the symptoms. The various factors involved in adrenal insufficiency in the rat have been reviewed recently by Cleghorn *et al.* [1936] and are outside the scope of this paper.

A study has been made of the effect of parathyroid hormone upon the serum calcium and calcium excretion of normal and adrenalectomized rats maintained on a supplement of 1 % sodium chloride. Data are also included on the effect of parathyroid hormone on the calcium excretion and calcium balance of rats on an adequate calcium diet and a normal Ca : P ratio.

Methods.

The rats were kept in Hopkins's metabolism cages and the urine and faeces collected separately. The collections were made over a 2-day period and the results are expressed in mg. Ca/rat/day. The analytical methods used in handling the excreta were similar to those described by Pugsley [1932]. The diet had the following composition: 31.1 % cornmeal, 31.1 % wheat flour, 2.1 % wheat germ, 31.1 % Purina, 2.1 % brewer's yeast, 2.2 % cod liver oil and 0.3 % sodium chloride. It contained 0.544 % Ca and 0.508 % P.

The rats were adrenalectomized under ether anaesthesia. 1 % NaCl was given as drinking fluid 10 days before the operation and continued throughout the experimental study. No difficulty was encountered in keeping the rats alive

with this treatment. Adult male rats 5–6 months old were used in this part of the work. Parathormone (Eli Lilly Company¹) was used in all the experiments. The dosage is expressed in Collip units.

The effect of parathyroid hormone upon the excretion of calcium in the urine of normal and adrenalectomized rats.

The effect of 1% NaCl upon the excretion of Ca in the urine of normal and adrenalectomized rats is shown in Fig. 1. The data for this chart are based on the average excretion of 10 rats in each group. The rats in the upper half of

Fig. 1. Upper chart: The effect of 1% NaCl as drinking fluid upon the excretion of Ca in the urine of normal and adrenalectomized rats. Adrenalectomized on the 8th day. Lower chart: The effect of 5 units of parathyroid hormone daily upon the excretion of Ca in the urine of normal rats receiving water as drinking fluid. Injections begun on the 8th day and continued throughout experiment.

the chart were placed on the NaCl 8 days before the operation and the urinary excretion of Ca followed. It is seen that the excretion is approximately 5–6 times that of the control rats receiving water (see first 8 days in lower part of chart). No significant difference in the Ca excretion in the urine is shown after adrenalectomy. The effect of 5 units of parathyroid hormone per day upon the excretion of Ca in the urine of normal rats is shown in the lower part of the chart. Injections were started on the 8th day and continued throughout the experiment. The maximum excretion of calcium occurred on the 4th day of injection; after this the excretion gradually returned to the control level.

Fig. 2 shows the effect of 5 units of parathyroid hormone daily upon the Ca excretion of normal rats (upper half of chart) receiving 1% NaCl as drinking fluid. The rats were adrenalectomized on the 8th day and injections started on the 12th day. It is seen that the response is markedly increased (when compared with the normal rats shown in Fig. 1) and the adrenalectomized rats gave a greater response than the normal rats on 1% NaCl. The additional effect on the Ca excretion cannot be attributed wholly to the NaCl, since over the 24-day period of injection the adrenalectomized rats excreted 22.0 mg. Ca and the normals 17.7 mg. Ca in the urine. This difference seems to indicate that adrenalectomized rats are more sensitive to the parathyroid hormone (Fig. 2). Over the

¹ The authors are indebted to the Eli Lilly Company, Indianapolis, for supplying the parathormone for these experiments.

same period, normal rats receiving 5 units of parathyroid hormone per day and with water as drinking fluid excreted 6.4 mg. Ca, while the adrenalectomized rats on NaCl but no parathyroid hormone excreted 5.5 mg. Ca (Fig. 1). Thus it is seen that the substitution of 1% NaCl for water renders rats more sensitive to the parathyroid hormone, and adrenalectomy appears to enhance this effect. In a similar experiment, in which adrenalectomized rats received 10 units of

Fig. 2. Upper chart: The effect of 5 units of parathyroid hormone daily upon the excretion of Ca in the urine of normal rats receiving 1% NaCl as drinking fluid. Note increased response as compared with Fig. 1. Lower chart: The effect of 5 units of parathyroid hormone daily upon the excretion of Ca in the urine of adrenalectomized rats receiving 1% NaCl drinking fluid. Rats adrenalectomized on 8th day and injections begun on 12th day. Note greater response of adrenalectomized rats.

parathyroid hormone daily and 1% NaCl as drinking fluid, they excreted 37.7 mg. Ca in the urine over a period of 24 days. The maximum excretion occurred on the 4th day and amounted to 7.6 mg. Ca. This is a larger excretion of Ca in the urine than was ever obtained by the administration of 40 units of parathyroid hormone daily to normal rats with water as drinking fluid.

The effect of parathyroid hormone upon the serum calcium of normal and adrenalectomized rats.

This experiment was carried out similarly to the one described above. The hormone was injected daily and the blood obtained 12 hours after the injection by cutting the carotid artery and jugular vein and allowing the blood to flow into a centrifuge-tube. Ca was determined in the serum by the method of Clark & Collip [1925].

Table I, col. 2, shows the effect of 20 units of parathyroid hormone daily on the serum Ca of normal rats receiving water. It is seen that the maximum on the 4th day of injection is 14.2 mg./100 ml. Col. 3 shows the response of adrenalectomized rats receiving 1% NaCl. The maximum here is 17.6 mg./100 ml. on the 3rd day of injection and 17.0 mg./100 ml. on the 4th day. The figures in the brackets refer to the number of determinations, and the average serum Ca of the group is taken. These rats, on the 3rd and 4th days of injection, showed symptoms of overdosage somewhat similar to those manifested in the dog. All of them showed hyperaemic areas in the stomach and caecum. In two cases there was

Table I. *The effect of daily injections of parathyroid hormone upon the serum calcium of normal and adrenalectomized rats.*

Expressed in mg./100 ml.				
Days	Normal 20 units	Adrenalecto- mized + 1% NaCl 20 units	Normal 1% NaCl 5 units	Adrenalecto- mized + 1% NaCl 5 units
Control	10.3 (40)	10.6 (9)	10.8 (4)	10.6 (4)
1	11.3 (3)	12.2 (2)	—	—
2	13.5 (3)	14.2 (3)	12.2 (4)	12.7 (4)
3	13.9 (3)	17.6 (5)	12.4 (4)	13.2 (4)
4	14.2 (4)	17.0 (5)	12.4 (4)	13.2 (4)

marked congestion of the alimentary tract and the presence of blood in stomach and intestines was noted. The condition of the stomach and intestines resembled that described by Collip [1926] as occurring in the dog when lethal amounts of parathyroid hormone were given. Normal rats receiving water tolerate 20 units of parathyroid hormone daily without any ill effects, and it is not until the dosage is raised to 40 units per day that ill effects occur, but even then the haemorrhagic condition of the stomach and intestines was not seen. Cols. 4 and 5 show the effect of 5 units of parathyroid hormone daily upon the serum Ca of normal and adrenalectomized rats receiving 1% NaCl as drinking fluid. The serum Ca response is slightly greater in the adrenalectomized rats.

The effect of continued injections of parathyroid hormone upon the calcium excretion and calcium balance of normal rats.

The rats used in this experiment consisted of a litter of eight. They were weaned at 24 days of age, placed in the metabolism cages and the Ca excretion and balance followed for 104 days. 4 rats in the group were kept as controls and

Fig. 3. The effect of continued injections of parathyroid hormone upon Ca excretion and Ca balance of young rats on an adequate Ca diet. Amount of hormone injected daily indicated by values below arrows. Continuous line = injected rats; broken line = control rats.

the other 4 were injected continuously with increasing amounts of the hormone as indicated in Fig. 3. Apart from the increase in urinary Ca which occurred during the first injections of the hormone, no essential difference can be detected between

the two groups of rats. The Ca balance curves of the control rats (broken line) and the injected rats (solid line) follow each other very closely. The increase in urinary Ca due to the injections of the hormone does not alter the marked positive balance of these growing rats, since an increased excretion of 1.0–1.5 mg. Ca is insignificant in calculating a positive balance of 40–45 mg. No increased excretion of Ca in the faeces was found with this dosage of the hormone. An interesting relationship between the growth curves and the Ca balance curves is shown in this experiment. The rats have the greater positive balance of Ca during the period of rapid growth and as the growth curve flattens out the Ca balance becomes less positive. The decrease in the Ca balance is brought about by an increased excretion of Ca in the faeces.

These results show that rats become refractory to a given dose of parathyroid hormone when placed on an adequate Ca diet with a normal Ca : P ratio.

DISCUSSION.

The substitution of 1% NaCl for water as drinking fluid and the resulting increased Ca excretion is possibly connected with the diuresis which takes place. This increased excretion of Ca is similar to that obtained by the administration of ammonium chloride to rats [Pugsley & Anderson, 1934]. The enhancement of the response to parathyroid hormone of adrenalectomized rats is of interest in view of the results of Schour & Rogoff [1936]. Our experiments indicate that Ca is more readily mobilized from the bones after adrenalectomy.

The results shown in Fig. 3 support the previous findings of Pugsley [1932] that daily injections of parathyroid hormone in the rat lead to an increased urinary excretion of Ca which gradually decreases to the control level in spite of continued injections. They are not in line with the suggestion of Morgan *et al.* [1934] that the gradual failure of response was due to the low-Ca diet which was used. These rats became refractory to the hormone, showing the same positive balance as the control rats. A low-Ca diet may be a contributing factor to the gradual decrease in response to continued injections of parathyroid hormone, since the rats used in these experiments on the high-Ca diet did not become resistant to the hormone as soon as the rats reported on the low-Ca diet. However, other factors discussed by Thomson & Collip [1932] play a role in the gradual decrease in response to continued injections of parathyroid hormone.

SUMMARY.

The substitution of 1% NaCl for water as drinking fluid renders rats more sensitive to parathyroid hormone. Adrenalectomized rats maintained on 1% NaCl show a greater increase in serum Ca and Ca excretion after injection of parathyroid hormone than normal rats under the same conditions. A haemorrhagic condition of the stomach and intestine was produced in adrenalectomized rats by the daily administration of 20 units of parathyroid hormone.

The Ca excretion and Ca balance of young rats on an adequate-Ca diet was followed. With continued injections of parathyroid hormone, these rats showed the typical increase in Ca excretion in the urine, which gradually decreased to the normal level.

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REFERENCES.

- Allardyce (1931). *Amer. J. Physiol.* **98**, 417.
Clark & Collip (1925). *J. biol. Chem.* **63**, 461.
Cleghorn, Cleghorn, Forester & McVicar (1936). *J. Physiol.* **86**, 229.
Collip (1926). *The Harvey Lectures*.
Morgan, Kimmel, Thomas & Samischi (1934). *J. biol. Chem.* **106**, 531.
Pugsley (1932). *J. Physiol.* **76**, 315.
—— & Anderson (1934). *Biochem. J.* **28**, 754.
Rubin & Krick (1933). *Proc. Soc. Exp. Biol., N.Y.*, **31**, 228.
Schour & Rogoff (1936). *Science*, **83**, 267.
Shelling, Ascher & Jackson (1933). *Johns Hopk. Hosp. Bull.* **53**, 458.
Thomson & Collip (1932). *Physiol. Rev.* **12**, 309.