CCXLVIII. THE EFFECT OF PARATHYROID HORMONE AND OF TUBERCULOSIS ON THE SERUM AND TISSUE CALCIUM OF GUINEA-PIGS

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EPISODES in the treatment of human tetany led Linder *et al.* [1930-31] to the conclusion that infection plays a large part in the success or failure of parathyroid extracts in raising the serum calcium and relieving the symptoms. When infection was present these extracts failed, and a similar effect of infection on the action of insulin is well established [Graham, 1924-25]. The experiments reported here were undertaken to study this and to investigate the effect of the parathyroid hormone on the distribution of calcium in the bone, brain and liver of normal guinea-pigs and of guinea-pigs infected with tubercle bacilli. An experimental clinical investigation was begun at the same time; the results showed that the nature of the infection was most important in influencing the response to injections of parathyroid hormone, typhoid fever decreasing it but pulmonary tuberculosis in its active stages increasing it [Linder, 1935].

EXPERIMENTAL.

Young guinea-pigs weighing 150-200 g. were used. They were fed on bran, pollard and the outer leaves of cabbage. Four groups were studied; a control group, a group given injections of "Parathormone" (Eli Lilly and Co.), a group infected with tubercle bacilli by injection of a culture into the groin and a group similarly infected and treated with parathormone a month later when the infection was generalised. The dosage was 15 units the first day, 25 the second, 35 the third, 50 the fourth and the animal was killed the fifth day. The animals were not fed that morning and were killed by cutting the throat. The carotid blood was collected and the serum used for calcium determination. The humeri were removed, one being used for histology and the other being cut in two longitudinally, digested in pancreatic extract and 2% sodium carbonate to remove the marrow and bleached in dilute alkaline hydrogen peroxide. Absorption of the finer trabeculae was studied with a hand lens and graded by independent observers.

The livers of the parathormone-treated animals sometimes contained small pale areas which were removed for section; the cells showed degenerative changes but only in one instance was there histological evidence of calcification, and the calcium content of the remainder of this liver was not exceptional. The liver and brain, which were already drained of blood were weighed, mashed with a sharp knife, dried in an oven and ashed in small silica basins; the ashing was commenced over a low gas flame so that very little smoking occurred and was completed in an electric furnace at a dull red heat which was maintained for about 10 min. The ash contained a small amount of carbon. It was extracted

with about 2 ml. 50 % hydrochloric acid on a water-bath for 15 min.; the acid was transferred to a graduated centrifuge-tube and the extraction was repeated with successive portions of dilute acid until the centrifuge-tubes contained 6 ml. in the case of the brains and 9 ml. in that of the livers. The contents of the tubes were mixed by inversion and the traces of carbon removed by centrifuging. 5 ml. of the brain extract and two 4 ml. samples of the liver extract were transferred to centrifuge-tubes for calcium determination. 1 ml. of saturated ammonium oxalate was added and then ammonia until the mixture was alkaline to methyl red; it was then reacidified with acetic acid and any phosphate precipitate redissolved. The tubes were capped and placed in boiling water until a definite cloud of calcium oxalate was seen. After standing for 24 hours the analysis was completed by the technique of Clark and Collip [1925].

Results.

The results of the individual observations are given in Fig. 1, in which the mean value for each category is indicated by a horizontal line. These means of small samples have been compared by Fisher's [1930] method of the calculation

Fig. 1. Individual observations on the calcium content of serum, brain and liver of young fed guinea-pigs. Effect of parathyroid extract and of tuberculosis.

of *t*. The corresponding value of *P* was found from his Table of *t*. For the means to differ significantly *P* must be 0.05 or less. The means and the comparisons between them are given in Tables I-III.

Sera (Table I). The mean value of the serum calcium of the control guinea-pigs was 11.2 mg./100 ml. Bodansky *et al.* [1930] found a mean of 10.5 mg. After parathormone the mean was 12.0 mg. which just fails to be significantly greater than the mean of the controls; the value of *t* required for significance is 2.028

Table I. *Means of the series of serum calcium determinations and their statistical comparison.*

Series	Observations in series	Mean mg./100 ml.	Standard deviation	Mean deviation of mean	Compared with series	Difference of means mg./100 ml.	<i>t</i>	<i>P</i>
Control	29	11.18	1.01	0.187	—	—	—	—
Parathormone	8	12.01	1.13	0.400	Control	0.83	2.01	0.05 +
Tubercle	6	10.87	1.01	0.412	Control	0.31	0.69	Very large
Tubercle and parathormone	8	12.84	1.16	0.412	Control	1.66	4.03	0.01
Tubercle	—	—	—	—	Tubercle and parathormone	1.97	3.30	0.01
Parathormone* (fasted)	3	15.23	1.51	0.872	Control	4.05	6.36	0.01

* A series of animals given a single large dose in the fasting state.

and that obtained 2.01. A longer series would probably show a small real difference. A few observations made after a single dose of 50 units in fasting animals killed 24 hours after the injection gave a mean of 15 mg.; this greater and significant increase confirms observations of Bodansky *et al.* [1930]. Infection with tuberculosis did not alter the mean serum calcium, but parathormone acting on a tuberculous animal produced an increase which was significant in comparison with the means of the controls and of the tuberculous animals.

Bone. The bones showed definite absorption of the fine trabeculae in all the parathormone-treated animals. Tuberculous infection produced no change in the bones by itself, and did not appear to alter the extent of the absorption caused by parathormone. The histology did not show any specific tuberculous lesion in the bones.

Brain (Table II). The mean calcium content of the brains in the control series was 6.7 mg. per 100 g. fresh brain. The mean of the series treated with

Table II. *Means of series of brain calcium determination and their statistical comparison.*

mg. Ca per 100 g. fresh brain.								
Series	Observations in series	Mean	Standard deviation	Mean deviation of mean	Compared with series	Difference of means	<i>t</i>	<i>P</i>
Control	27	6.68	2.12	0.407	—	—	—	—
Parathormone	13	6.70	1.67	0.464	Control	0.02	—	—
Tubercle	7	5.47	0.843	0.318	Control	1.21	1.47	0.1 +
Tubercle and parathormone	7	7.11	1.71	0.645	Control	0.43	0.49	Very large
Tubercle	—	—	—	—	Tubercle and parathormone	1.64	2.29	0.05 -

parathormone was identical. The mean of the brains of the untreated tuberculous animals was lower, but the difference was not large enough to be significant; after parathormone these brains showed a significantly larger amount of calcium, but the increase was not sufficiently large to be real when compared with the normal controls. Parathormone therefore has no effect on the brain calcium under the conditions of these experiments with the possible exception of a small effect in the tuberculous animal.

Liver (Table III). The mean liver calcium in the control series was 4.2 mg. per 100 g. fresh tissue. This was definitely increased by parathormone and by tuberculous infection; the latter caused the greater increase. Tuberculosis increased the rise due to parathormone to a significant extent, but parathormone

Table III. *Means of series of liver calcium determinations and their statistical comparison.*

mg. Ca per 100 g. fresh liver.								
Series	Observations in series	Mean	Standard deviation	Mean deviation of mean	Compared with series	Difference of means	<i>t</i>	<i>P</i>
Control	23	4.17	1.025	0.213	—	—	—	—
Parathormone	7	5.21	0.852	0.321	Control	1.04	2.44	0.02
Tubercle	6	5.81	0.727	0.297	Control	1.64	3.69	0.01
Tubercle and parathormone	4	6.47	0.768	0.383	Control	2.30	4.25	0.01
Tubercle	—	—	—	—	Tubercle and parathormone	0.66	1.38	0.2
Parathormone	—	—	—	—	Tubercle and parathormone	1.26	2.43	0.05 -

did not increase the rise due to tuberculosis alone. Tubercle therefore seems the more potent agent. A solitary observation of 18.5 mg. was obtained after parathormone and this has not been included in the above data as it seemed likely that some other factor such as calcification must be operative.

DISCUSSION.

Bodansky *et al.* [1930] found a mean increase of 2.4 mg. in the serum calcium of young guinea-pigs from repeated doses of parathormone such as those given in the present experiments. Repeated doses of 10 units were without effect on the serum although slight absorption of bone was detected. Cohn and Stohr [1933] produced no effect on serum or bone with repeated doses of 1-5 units. Our results with the larger dosage showed but a questionable increase. The serum determinations in the tuberculous animals support the clinical observation that parathyroid hormone gives a greater response in an animal with an active tuberculous focus. The human cases were with one exception mild and the patients well nourished, but the guinea-pigs when exposed to the combined effects of tubercle and parathormone constantly lost weight, whereas they did so only occasionally and to a less extent when these agents were acting separately; so far as the animals were concerned it might have been that a starvation factor was operative in increasing the response as in the case of the fasting animal with the large single dose.

It is well known that the calcium content of the tissues is very different from one kind of animal to another, and that in the same kind of animal there is much greater individual variation than in the serum calcium. In any series, by one worker using one kind of animal under the same conditions, the highest figure is seldom less than three times the lowest. This applies to the present series and makes it difficult to demonstrate small changes caused by such an agent as parathormone. It is probable that in the tissue cells the calcium is present in protein-bound, diffusible and ionised components as it is in the serum. As it is believed that the ionised form is the most important in influencing cell activity, it is possible that variations in the amount of this fraction are buffered

by the presence of the protein-bound fraction, itself inactive and subject to considerable variation in amount.

In the following remarks the calcium figures quoted from the literature have been reduced to mg. per 100 g. of fresh tissue; to effect this the figures for the dry weight have been divided by 4.

Rona and Heubner [1919; Heubner and Rona, 1923] found that cat brain contained 8–17 mg. of calcium, and that this amount was not influenced by calcium therapy. Hess *et al.* [1932] found 25–65 mg. in rat brain and they quoted dog and human brain as containing about 5 mg. and rabbit brain 125 mg. In rats with rickets the brain calcium was greatly reduced, but neither parathyroidectomy nor the injection of parathyroid extract influenced it. Underhill and Jaleski [1933] reported that 2–8 mg. was the titre in dogs and that parathyroidectomy had no effect. Eaves [1931] gave 4–6 mg. as the limits in four normal human brains but found pathological brains containing 8 mg. or more. The range of the present series is from 4.3 to 11.1 mg. with more observations in the lower half of the field, and no effect of parathormone was shown. Except for the changes reported in rickets by Hess the brain calcium seems resistant to external influences.

Heubner and Rona [1923] found 4–11 mg. of calcium in the liver of cats, and Dennis and Corley [1925] 7–11 mg. in rabbits; both failed to influence it by giving calcium salts. Underhill and Jaleski [1933] reported 1–3.5 mg. in dogs without change after parathyroidectomy. Barral and Barral [1928] found 0.3–6.8 mg. in rabbits. Ramage *et al.* [1933] found 6–40 mg. in the livers of sick children. Burns and Elliott [1935] found 0.5–2.5 mg. in the livers of guinea-pigs which had been bled and 3–5 mg. in the livers of those which had not; they found that pneumonia increased the liver calcium of rats from under 3 to as much as 10 mg. The observations presented here give the range of liver calcium in young guinea-pigs as 2.5–7.5 mg. per 100 g. fresh liver with a mean of 4.2 mg. and show that the liver calcium was increased by tuberculous infection and by parathormone. Both increases were small, but the two agents acted together to give a greater effect than either alone. Tuberculosis appeared to be the more potent.

The liver calcium appears more labile than the brain calcium but conditions under which the liver discharges calcium have not been found. The liver is probably not a calcium store of great quantitative importance, and unlike bone it seems to take up calcium under the influence of parathormone. This suggests that it may have a part to play in the finer regulation of the serum calcium, which would entail its taking up calcium when the serum calcium tends to rise.

The observations have added a few facts about the influence of tuberculous infection on parathyroid action, but the clinical results referred to at the commencement of this paper show that tuberculous infection was an unfortunate one to have chosen for the investigation of the general action of infections on the response to parathyroid hormone.

SUMMARY.

1. Injection of repeated large doses of parathyroid extract into a series of young guinea-pigs produced a mean increase of the serum calcium which was not quite large enough to be statistically significant.
2. Similar treatment of guinea-pigs which were infected with tubercle bacilli produced a larger mean response which was significant.
3. No difference could be detected in the degree of decalcification of bone caused by the parathormone in the two classes of animal.

4. Parathyroid extract had no effect on the calcium content of the brains of healthy guinea-pigs. It possibly increased the calcium in the brains of tuberculous animals.

5. The liver calcium was increased by parathyroid extract and by tuberculous infection. Tuberculous infection increased the response to parathyroid.

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