THE EFFECT OF A PARATHYROID HORMONE ON NORMAL ANIMALS.

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(Received for publication, December 26, 1924.)

INTRODUCTION.

In a previous communication (1) it was shown that tetany in parathyroidectomized dogs could be prevented or controlled by the administration of a special extract of the parathyroid glands of the ox. It was also shown that this extract exercised a direct control of the concentration of calcium in the blood and that coincident with the marked improvement in the clinical condition of parathyroidectomized dogs following the administration of the parathyroid hormone there was observed an elevation of the level of blood calcium. It was noted that the calcium content of the blood rose gradually, but very definitely, over a period of some hours, that a maximum point was finally reached, and that a return to lower levels of calcium concentration was gradually accomplished. In each experiment of this nature there was therefore a definite blood serum calcium curve somewhat indicative of the effectiveness of the preparation used in treatment.

It seemed to us that the final proof of the existence in potent form of the active principle of the parathyroid gland, in the extracts used in the early experiments, had been obtained by the successful replacement therapy controlled by blood calcium studies earlier reported. The next problem which was studied, therefore, was the effect of these potent extracts upon the normal animal. The results of a number of experiments in which our extract has been administered to normal animals are reported in this communication.
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Methods.

Normal dogs were used. The extract was administered by subcutaneous injection. Blood samples for analysis were taken at frequent intervals from a leg vein. Calcium was estimated in the blood serum by the Kramer-Tisdall method (2) with slight modifications. Phosphorus was estimated on a few samples by the Briggs method (3). Alkali reserve was determined on occasion by the Van Slyke method (4), using whole blood equilibrated with alveolar air. The extracts containing the hormone were made as previously reported.

Results.

The effect of single injections of the extract into normal dogs upon the blood serum calcium values is shown graphically in Chart 1. Blood serum calcium curves following a single injection of the extract into parathyroidectomized dogs are shown for comparison in Chart 2.

The effect of two injections of the extract into normal dogs is shown in Chart 3, while in Chart 4 the curves obtained for blood serum calcium following two injections of the extract into parathyroidectomized dogs are given.

The effect of successive injections of graded doses of the extract into six normal dogs is shown in Chart 5.

In Charts 6 to 12 are shown the effects of repeated doses of the extract at different intervals.

The results of the administration of the extract to normal animals by stomach tube are shown in Chart 13.

Discussion.

Effect of a Single Injection of the Extract into a Normal Dog.

The effect of a single injection of the extract into a normal dog is, in as far as the blood serum calcium curve may be used as an index, much the same as the effect of a single injection into a parathyroidectomized animal. There is a gradual rise in blood serum calcium values which reaches a maximum in from 5 to 9 hours and then follows a return to normal, the slope of the curve being almost the same as that of the earlier rising curve. By comparison, the effect in these experiments on blood serum cal-
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cium value of a single injection of the extract into parathyroidectomized dogs is more marked. The initial rise in calcium content of the serum was usually greater than was the case in normal animals, and the return to the original level was more prolonged. This observation was in keeping with our experience in treating parathyroidectomized dogs with the extract. In the

![Chart 1](http://www.jbc.org/)

**Chart 1.** Dog 57, ♀ 18 kilos, 20 cc extract = 10 glands.

- 58, ♀ 15 " 10 " " = 5 "
- 60, ♀ 11 " 2.5 " " = 1.25 "
- 61, ♀ 16.5 " 3 " " = 1.5 "
- 63, ♂ 16 " 5 " " = 2.5 "
- 64, ♂ 14 " 10 " " = 5 "
- 65, ♂ 22 " 10 " " = 5 "
- 73, ♀ 7.5 " 2.5 " " = 1 gland.
- 80, ♂ 12.5 " 2.5 " " = 1 "

majority of cases it has been found that a single daily injection is sufficient to ward off tetany. A few animals, as the protocols published in a previous communication will indicate, require treatment at less than 24 hours intervals. Two injections daily into such animals are not without danger, however, as overdosage phenomena may be encountered.
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Effect of Two Injections of the Extract into Normal and Parathyroidectomized Dogs.

The effect of two injections of the extract is largely dependent upon the time interval between the injections. In the normal dog, if the second injection is administered early on the rising calcium curve, the general character of the curve is very little altered.

![Graph showing calcium levels over time for different dogs with varying amounts of extract.]
CHART 3. Dog 72, 8 kilos, 2 cc. extract = 1 gland. Potency test.

"74, ♂ 10 " 2.5 " special extract = 4 glands.

"76, ♀ 12.5 " 2.5 " extract = 1 gland. Passed through Mandler filter.

"77, ♀ 18.5 " 1st injection 4 cc.; 2nd injection, 2.5 cc. Potency test.
Slightly higher values for blood serum calcium may result, and the return to normal is somewhat more prolonged. If the second injection is made at or near the peak of the curve following the single injection, a dip in the curve will most likely result before the second injection has begun to affect materially the level of blood serum calcium. Pyramiding or cumulative action may be most strikingly demonstrated by such treatment (Chart 4). Dog 51 showed typical symptoms of hypercalcemia during the day following the two injections and finally died at 6 p.m. of that day. Dog 50, on the other hand, made a complete recovery, and is still alive. This animal has frequently been allowed to develop tetany and has in every instance completely recovered from such attacks following a single injection of the extract.
Effect of Successive Injections of the Extract into Normal Dogs.

When several injections of the extract are made into normal dogs at intervals of a few hours the phenomenon of pyramiding is manifested in the blood serum calcium curve. The blood serum calcium under such circumstances rises to a very high level. If injections be discontinued the blood serum calcium values may gradually approach normal or death may intervene when hypercalcemia is still manifested. Even after injections have been discontinued and the blood serum calcium content has fallen to lower levels, death may still occur in the course of 24 to 48 hours, probably as the direct result of the condition of extreme hypercalcemia earlier existent. The results of six experiments carried out simultaneously on six normal dogs are shown in Chart 5. Each animal was injected at approximately 2 hour intervals with the same extract. The dosage used varied from 1 to 6 cc. of a preparation, 2 cc. of which were equivalent to one ox parathyroid gland. When symptoms became very pronounced injections were discontinued. Two dogs in this series died, the other four made a complete recovery. It would appear that blood serum calcium values above 15 mg. per 100 cc. are essential before definite symptoms of hypercalcemia (hormone over-dosage) are manifested. The animals gradually become weak. There is as a rule diarrhea and vomiting, and, in fatal cases, blood-stained fluid may be vomited or passed by the bowel. The condition of atonia in fatal cases becomes more marked, and complete collapse ultimately follows. The circulation becomes impaired, and in some instances it is almost impossible to get blood samples even when venesection is resorted to. It becomes increasingly difficult to secure serum from blood specimens, and blood taken at death has on occasion been centrifuged for an hour without any appreciable separation of the cells from serum taking place. Accurate viscosity measurements have not been made in these experiments, but by rough methods it is evident that there is at least 100 per cent increase in blood viscosity in the terminal state. There is, however, no great change in hemoglobin values. 5 to 15 per cent increases in hemoglobin have been noted, which, in view of the frequent bleedings, would indicate a definite decrease in plasma volume, but yet nothing like sufficient decrease to account for the profound change noted in
the character of the blood. The blood chemistry in hypercal-
cemia due to parathyroid hormone overdosage promises to be a
most interesting study. In this communication we are only
able to report a few results on this subject. The problem is
being studied in a systematic manner, however, and a detailed
report will be made of the findings at a later date.

Relationship Between Size of Dose of the Extract and the
Physiological Effect.

The physiological effect of the extract administrations would
seem to be more dependent upon the time interval between in-
jections than upon the size of dose, provided always that a known
potent preparation of the extract is used and something more than
the minimal effective dose is administered. The blood serum
calcium curves obtained on six animals above referred to and
shown graphically in Chart 5 illustrate this phenomenon ex-
ceptionally well. The dosage used in this series of experiments
varied 600 per cent, and it is interesting to note that the two
cases which ended fatally in this instance received 2 and 6 cc.
doses, respectively, while complete recovery occurred in the four
animals which received 1, 3, 4, and 5 cc. doses, respectively.
The general character of the blood serum calcium curves is the
same in all instances. Chart 11, which illustrates the blood
serum calcium curve obtained in our first experiment of this
nature, shows that the effect of a single massive dose (18 cc. = 9
ox glands) is very little different from the effect of a much smaller
dose (see Chart 1). The decrease in blood calcium obtained in
this animal some 3 hours following the second injection is of
interest. It is suggestive of a latent period and in this instance
this would correspond with the passing off of the effect of the
first dose. A third injection resulted in pyramiding and a great
prolongation of the period of hypercalcemia. No ill effects were
noted in this animal.

The extract has been administered to a number of human
subjects, and blood serum calcium values have been increased
thereby. The minimal effective dose in the human individual is
probably about one-third to one-half an ox gland. Definite
rises in blood calcium have been obtained with 1 cc. doses (= 0.5
gland), while no effect has been obtained with single 0.5 cc.
doses of the same preparation on the same subject.
CHART 5. Dog 61, 18.5 kilos, 1 cc. = 0.5 gland injected approximately every 2 hours. 11 injections in all.

62, ♀ 16.5 " 2 " = 1 " " " " 2 " " 9 " " " "
63, ♂ 10 " 3 " = 1.5 glands " " " " 2 " " 10 " " " "
64, ♂ 14 " 4 " = 2 " " " " 2 " " 9 " " " "
65, ♂ 22 " 5 " = 2.5 " " " " 2 " " 11 " " " "
66, ♀ 16.5 " 7 " " " = 3 " " " " 2 " " 9 " " " "

Calcium

Hrs.

Mg
Dep
1000
Chart 6. Dog 77, 9 18.5 kilos, 6 cc. extract = 2 glands injected at intervals indicated by arrows on the chart.
Chart 7. Dog 74, 10 kilos, 6 c.c. extract = 2 glands injected as indicated by arrows on the chart.

The broken line indicates phosphorus; the solid line calcium values.
CHART 8. Dog 75, 9.10.5 kilos, 6 cc. extract = 2 glands injected as indicated by arrows on the chart.
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Effect of Varying the Interval between Successive Injections of the Extract into Normal Animals.

The effect of varying the interval between successive injections of the extract into normal animals is shown in Charts 6 to 12, inclusive. Dog 77 (Chart 6) received 6 cc. of extract (= 2 ox glands) in each of six injections spaced over 24 hours. The blood calcium rose steadily to 20.5 mg. per 100 cc. during this period, injections were then discontinued, and the animal made a complete recovery. Dog 74 (Chart 7) received the same dosage as Dog 77 administered five times over 24 hours. This animal died. There was a premortal fall in blood serum calcium in this instance followed by a subsequent rise. Phosphorus was determined on blood serum samples in this animal and the sharp rise in the phosphorus curve coincident with the preterminal fall in calcium is suggestive of a protective response on the part of the organism to combat the extreme hypercalcemia. Phosphates have been shown to lower blood calcium (5), and a mobilization of phosphorus may well be a natural protective response in hypercalcemia.

Dog 75 (Chart 8) received five injections, each equivalent to two ox glands, during 38 hours. No ill effects were noted, and pyramiding is not so pronounced as in the case of Dogs 77 and 74. A condition of mild hypercalcemia was, however, maintained over a period of many hours. Should this hormone have a clinical use it is just this sort of result, mild but continued hypercalcemia, which should be aimed at in the treatment.

The experiment on Dog 78 (Chart 9) is very instructive. This animal was injected with 6 cc. of extract (= 2 glands) on three occasions during 18 hours. It will be noted that the effect of the first injection had almost passed off when the second injection was made at 11½ hours. The third injection was made, however, while the effect of the second was at or about its maximum. The result was marked pyramiding and a greatly prolonged period of hypercalcemia. Dog 79 (Chart 10) represents a somewhat similar experiment. The result here is suggestive of cumulative action and is indicative of the fact that pyramiding may be obtained in normal animals even with two injections per day. 2 days later when this animal had an almost normal blood serum calcium value (11.6 mg.), 2 cc. injections (= 0.66 of an ox
Chart 9. Dog 78, ♀ 18 kilos, 6 cc. extract = 2 glands injected as indicated by arrows on the chart.
CHART 10. Dog 79, ♂ 31.5 kilos, 6 cc. extract = 2 glands injected as indicated by arrows on the chart.
gland) were made every 20 minutes for 13 hours. At the end of 8 hours the animal had diarrhea, was vomiting, but was otherwise normal. The blood calcium curve it will be noted is very little different from a number of others (Chart 5). There is evidently a limit to the rate at which calcium can be mobilized even though injections be made as frequently as was the case in this experiment. At the end of 13 hours injections were discontinued. The animal showed signs of general weakness during the next 2 days, but it was anticipated that a recovery might be made. Death, however, supervened. It was absolutely impossible to secure serum from the blood specimen taken shortly after death, so an analysis for calcium could not be made. Phosphorus was determined on whole blood, and the result was 13 mg. per 100 cc. The values for phosphorus shown on the chart represent analyses on serum, the final value of 13, being on whole blood, has been omitted from the graph. This same blood sample had a urea nitrogen of 111 mg., a non-protein nitrogen of 120 mg., creatinine 3.5 mg., and creatine 6 mg. per 100 cc., and a chlorine calculated as sodium chloride of 0.330 per cent.

**Blood Chemistry at Death Following Hypercalcemia.**

As yet we have been able to make only a few studies of the variations in various blood constituents which are induced by parathyroid hormone overdosage resulting in death. This part of the problem promises to be full of matters of great interest and it is now being studied in a systematic manner. In addition to the calcium curve in parathyroid hormone overdosage it is now proposed to determine the curve for halogen, sodium, potassium, magnesium, urea, non-protein nitrogen, total nitrogen, and osmotic pressure, as determined by the cryoscopic method.

The following data relating to the blood chemistry in fatal hypercalcemia have already been obtained.

1. Halogens.—Five cases only have been studied. In each instance there has been observed a gradual fall in defibrinated blood halogen which was most pronounced in the terminal state.

2. Urea Nitrogen.—The urea nitrogen has risen in each experiment and has reached a very high level at death.

3. Non-Protein Nitrogen.—This has paralleled somewhat urea nitrogen.

4. Cryoscopic Measurements.—In four cases studied there has been a
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depression in the A of 0.09-0.14°C at the terminal state as compared with controls.

6. Blood Sugar.—This has been found to be within the normal range.

6. Viscosity.—An enormous increase in viscosity has always been observed.

7. Protein Content.—No exact measurements of protein content have been made, but it is a significant fact that the usual Folin-Wu tungstic acid precipitation technique applied to defibrinated blood, taken terminally has given at times highly colored filtrates which could be avoided by the use of double amounts of 10 per cent sodium tungstate and 2/3 N sulfuric acid.

8. Alkali Reserve.—This has not been followed to a great extent as yet, but it would appear that there is little or no change in this value early in the condition but that a marked drop occurs terminally.

The following results have been obtained in four cases.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Control.</th>
<th>Terminal.</th>
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<tbody>
<tr>
<td>Dog 90.</td>
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<tr>
<td>Halogen</td>
<td>315</td>
<td>286</td>
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<tr>
<td>Urea nitrogen</td>
<td>16</td>
<td>75</td>
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<tr>
<td>Non-protein nitrogen</td>
<td>43</td>
<td>162</td>
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<tr>
<td>Δ</td>
<td>0.61°C.</td>
<td>0.75°C.</td>
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<tr>
<td>Dog 86.</td>
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<td></td>
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<tr>
<td>Halogen</td>
<td>320</td>
<td>270</td>
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<tr>
<td>Urea nitrogen</td>
<td>14</td>
<td>63</td>
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<tr>
<td>Non-protein nitrogen</td>
<td>41</td>
<td>166</td>
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<tr>
<td>Δ</td>
<td>0.60°C.</td>
<td>0.71°C.</td>
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<tr>
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<td>257</td>
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<tr>
<td>Urea nitrogen</td>
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<td>72</td>
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<tr>
<td>Non-protein nitrogen</td>
<td>41</td>
<td>219</td>
</tr>
<tr>
<td>Δ</td>
<td>0.63°C.</td>
<td>0.74°C.</td>
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<tr>
<td>Dog 89.</td>
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<td></td>
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<tr>
<td>Halogen</td>
<td>335</td>
<td>273</td>
</tr>
<tr>
<td>Urea nitrogen</td>
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<td>72</td>
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<tr>
<td>Non-protein nitrogen</td>
<td>26</td>
<td>184</td>
</tr>
<tr>
<td>Δ</td>
<td>0.59°C.</td>
<td>0.72°C.</td>
</tr>
</tbody>
</table>
The enormous increases in non-protein nitrogen and urea nitrogen occur only many hours after hypercalcemia has been established and are to be regarded as preterminal phenomena. The decrease in halogen is a matter of great interest because it may be by slight changes in the chlorine content of the blood that a diuretic action and a decrease in edema has been manifested in the human individual treated with the extract.

**Blood Serum Phosphorus during Parathyroid Hormone Overdosage.**

As yet only a few determinations of blood serum constituents other than calcium have been determined in experimental hypercalcemia. The results which have so far been obtained on phosphorus values for blood serum during parathyroid hormone overdosage would indicate that there is very little change during the early hours of hypercalcemia. If the condition of high blood serum calcium is maintained, however, and a fatal issue finally results, the blood serum phosphorus rises markedly. The cases of Dogs 74 (Chart 7) and 79 (Chart 12) have already been referred to. The blood serum of Dogs 62 and 66 (Chart 5) was
Chart 12. Dog 79, \(\sigma\) 31.5 kilos, 2 cc. = 0.66 gland injected every 20 minutes for 13 hours.
analyzed for phosphorus and in each case a terminal rise was noted. In the case of Dog 62

At 10 hours, blood serum calcium was 15.9 mg. and phosphorus 5.6 mg.

At death, calcium was 17.2 mg. and phosphorus 8.3 mg.

In the case of Dog 66

The control calcium was 10.3 mg. and phosphorus 4.9 mg.

At 4 hours, calcium was 13.4 mg. and phosphorus 5.5 mg.

At death, calcium was 16.5 mg. and phosphorus 9.9 mg.

These experiments indicate that the phosphorus content of the blood serum tends to rise during hypercalcemia, but more especially in the later stages of this condition.

**Effect of the Extract Administered to Normal Dogs by Stomach.**

In view of the fact that definitely positive results had previously been obtained both in parathyroidectomized dogs and in man by the oral administration of the extract, it was somewhat surprising to find that it is practically impossible to produce overdosage effects in normal dogs by the intermittent administration of the extract by stomach tube. Chart 13 is illustrative of three experiments of this type. The explanation of such results was readily obtained, however, when known potent preparations
were submitted to the action of pepsin and trypsin. In all instances where this has been done, there has been almost complete loss of potency of the preparations so treated. The effectiveness of oral administration, therefore, must lie in rapid absorption and so circumvention of enzyme action. Subcutaneous administration of the extract is in our opinion the most effective and also the most readily controllable.

**Standardization of the Extract.**

Physiological standardization of the extract would seem to be possible. It is too early as yet to attempt to define a unit. The influence of the weight of the animal on the blood serum calcium curve has yet to be definitely determined. The minimal effective dose on a dog of definite weight would seem to be something to strive to determine. In our experience known potent preparations (that is tested on parathyroidectomized dogs in tetany) are capable of causing an elevation in the level of blood calcium in the normal dog.

A few dogs, which might in a sense be termed refractory, have been encountered in which little appreciable change in blood serum calcium content has been manifested following a single injection. Such animals have invariably shown hypercalcemia when the injections of the extract have been continued. Successive administrations of small doses at intervals of 2 to 4 hours are, therefore, recommended in potency testing. Several blood serum calcium determinations should then be made, and error thus reduced to a minimum.

It is felt that all parathyroid hormone preparations should be tested physiologically before using. Up to the present, the dosage has been expressed in terms of the average ox gland. In other words the fresh glands have been counted out and extracted. The gram basis will be used in the future, however.

**SUMMARY.**

1. The effect of the subcutaneous and oral administrations of a parathyroid hormone to normal dogs is reported.

2. It has been found that a potent extract, administered by subcutaneous injection, causes an elevation in the level of blood
serum calcium. The change so produced in calcium values follows a typical curve. These curves have been contrasted with similar curves obtained on parathyroidectomized dogs.

3. The effect of successive injections of a potent extract into normal dogs has been studied. A condition of profound hypercalcemia has thereby been produced. Such a condition when long maintained may result in death.

4. The effect of varying the dosage and varying the time of successive administrations has been studied.

5. The blood in fatal cases of hypercalcemia shows very definite changes of both a physical and chemical nature.

It is a pleasure to acknowledge the financial assistance afforded to the carrying out of this work by a grant from the Carnegie Foundation.

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