AN EXTRACT OBTAINED FROM THE EXTERNAL BOVINE PARATHYROID GLANDS CAPABLE OF INDUCING HYPERCALCEMIA IN NORMAL AND THYREO-PARATHYROPRIVIC DOGS.

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Vassale (1) in 1905 claimed to have prepared an active parathyroid extract, but failed to describe his process of extraction. There is no available evidence supporting his contentions. Berkeley and Beebe (2) (1909) recovered a nucleoprotein from the bovine parathyroid glands which completely relieved acute parathyroid tetany in dogs. Their process consisted in shaking freshly ground gland tissue at room temperature for 2 hours with 6 to 8 volumes of physiological salt solution to which had been added 2 drops of 10 per cent sodium hydroxide. The resulting product was filtered and preserved with chloroform. This preparation was claimed to be effective orally but better subcutaneously or intraperitoneally. It was, however, not very stable, being readily destroyed by heat and cold and slowly by digestive ferments.

Hanson (3) in a series of papers (1923–24) described a parathyroid preparation which he called "hydrochloric X." It was made by boiling fresh parathyroid glands with 0.1 N hydrochloric acid. He succeeded in relieving parathyroid tetany in dogs for varying periods of time. The same author (4) showed recently that this extract induced a serum calcium increase in thyreo-parathyroprivic dogs. Berman (5) reported the preparation of a crystalline substance by means of acid-alcohol extraction of bovine parathyroid glands which possessed the property of inducing hypercalcemia. His report was very brief, and gave nothing in detail. Collip (6), a short time ago, described an active serum calcium-increasing extract of the parathyroids, obtained by boiling
them with hydrochloric acid. His preparation was active when administered to either normal or thyreoparathyroprivic dogs. In general the extracts made by the three foregoing investigators were similar.

Other attempts have been made to prepare active parathyroid extracts, notable among which were those of Massaglia, and MacCallum and Vogel (7). The preparation of the former was composed of a mixture of calcium salts and parathyroid substance, hence making difficult an estimation of its value. MacCallum and Vogel triturated bovine parathyroid glands with Ringer's solution and glycerol, and injected the resulting extracts intravenously in thyreoparathyroprivic dogs. They succeeded in at least temporarily or partially relieving tetany without modifying the blood calcium. They raised the question whether or not some other factor influenced tetany than just the one controlling the blood calcium.

The present writers, hoping to prepare an active serum calcium-raising extract from the external bovine parathyroid glands which would serve for human application, made numerous preparations, and found that the above mentioned activity lay invariably in the acid extracts. The details of this investigation are recorded in the following pages.

EXPERIMENTAL.

I. Preliminary Remarks.

In the following experiments normal and thyreoparathyroprivic dogs were employed in testing the effect of parenterally administered parathyroid extracts on the serum calcium levels. All dogs were kept on a standard diet, fasted for about 16 hours before commencement of experiments, and in the cases where the observations were conducted over not more than 1 day, the animals received neither food nor water during that time. Both male and female, as well as young and old, dogs served as experimental animals.

The calcium analyses were performed according to the technique of Kramer and Tisdall (8). Over a long series of observations normal variations of serum calcium during all hours of the day lay well within 10 per cent in each dog. When the difference was greater, it could be ascribed to some good cause, such as extremely
warm weather or drinking much water. The variation in serum calcium in different dogs ranged from 10.0 to 13.0 mg. per 100 cc. of serum with the great bulk of values occupying a range of from 11.0 to 12.5 mg. Duplicate analyses were always made, and in most instances the 0.01 N potassium permanganate titrations were within 0.01 cc. of each other. Occasionally, 0.02 cc. differences were encountered, and when greater than the latter, the results were discarded. A difference of 0.01 cc. in titration is equivalent to 0.2 mg. of calcium per 100 cc. of serum. But one modification was made in the above method which was to permit a 1 hour contact between the blood serum and the ammonium oxalate instead of a half hour as directed. We could find no appreciable increase in the values obtained by permitting a contact of more than 1 hour.

II. General Comment.

Extracts were prepared from fresh moist and fresh acetone-desiccated bovine parathyroid glands by a number of methods. In each case the ultimate product represented the extractive of a large quantity of gland tissue. As a rule, the extracts were divided into two fractions by precipitation of proteins with neutralization and addition of acetone or alcohol.

The following groups of extracts were prepared and tested for serum calcium-raising properties by parenteral administration in dogs.

A. Inactive Extracts.
1. Extraction of pulped glands with neutral distilled water.
2. Extraction of pulped glands with 65 per cent alcohol.
3. Extraction of pulped glands with 0.1 per cent acetic acid in 40 per cent alcohol.
4. Extraction of pulped glands with 0.5 per cent sodium hydroxide in 65 per cent alcohol.
5. Extraction of pulped glands with 0.4 per cent sodium hydroxide in 40 per cent alcohol.
6. Extraction of pulped glands with 0.4 per cent sodium hydroxide.
7. Extraction of pulped glands with ether.
8. Extraction of pulped glands with acetone.

B. Active Extracts (made at room temperature).
1. Extraction of pulped glands, after ether and acetone treatment, with 0.5 per cent hydrochloric acid in 65 per cent alcohol.
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2. Extraction of pulped glands with 0.3 per cent hydrochloric acid in 40 per cent alcohol.
3. Extraction of pulped glands with 0.3 per cent hydrochloric acid.

C. Active Extracts (made by boiling).
1. Extraction of pulped glands by boiling with 0.1 N hydrochloric acid for 15 minutes to 2 hours.
2. Extraction of acetone-desiccated pulped glands by boiling with 0.2 to 0.5 per cent hydrochloric acid for 15 minutes to 2 hours.
3. Extraction of acetone-desiccated and chloroform-defatted pulped glands by boiling with 0.2 to 1.0 per cent hydrochloric acid for 15 minutes to 2 hours.

Activity is apparently absent from all types of extract save the acid-aqueous and acid-alcohol. Hence, the original preparation described by Hanson and the acid-alcohol extracts of Berman contain the calcium-controlling hormone of the parathyroids. The recent work of Collip substantiates these results. The positive findings of the present writers are recorded below.

III. Serum Calcium-Increasing Extracts of Fresh Moist Bovine Parathyroid Glands and Their Effects on Thyreoparathyroprivic and Normal Dogs.

A. Preparation in Acid Solutions at Room Temperature.

(1). Alcoholic Hydrochloric Acid Extract.—112 gm. of fresh frozen parathyroid glands were finely ground and extracted 16 hours at room temperature with 300 cc. of 0.3 per cent hydrochloric acid in 40 per cent alcohol. Precipitation of colloids was induced by neutralization to the isoelectric point and the addition of 1 volume of acetone. The preparation was then filtered, the clear filtrate concentrated in vacuo, and later dried on a hot air bath at 50-60°C. Yield 1.8 gm. 0.5 gm. of this extract was dissolved in 8 cc. of physiological salt solution and injected subcutaneously in normal dog No. 43.

(2). Aqueous Hydrochloric Acid Extract.—140 gm. of fresh parathyroid glands were finely ground and extracted 16 hours at room temperature with 300 cc. of 0.3 per cent hydrochloric acid. The preparation from this point was treated identically as in (1) above. Yield 3.5 gm. 1 gm. of this product was dissolved in 8 cc. of physiological salt solution, and injected subcutaneously in normal dog No. 53.

(3). Which Tissue Constituent Is the Source of the Activity? 60 gm. of fresh parathyroid glands were carefully trimmed of extraneous fat, and the clean pulp finely ground. Yield 23 gm.

(a). The clean finely ground pulp was extracted for 16 hours at room
temperature with 100 cc. of ether to which had been added 3 drops of concentrated hydrochloric acid. The supernatant ether was then decanted, the residue was washed with two small portions of fresh ether, the ether washings were united with the original solvent, and the total was evaporated to dryness on a hot air bath at 50–60°C. Yield 3.0 gm. This extract was emulsified with about 10 cc. of water, and injected subcutaneously in normal dog No. 57.

(b) After the ether extraction, the residue was extracted for 4 hours at room temperature with 100 cc. of acetone. Then the acetone was decanted, the residue washed with two small portions of fresh acetone, the acetone washings were united with the original acetone extract, and the total was evaporated in vacuo to remove all of the acetone. Yield 10 cc. of aqueous solution. This was then injected subcutaneously in normal dog No. 58.

c) After ether and acetone extraction the undissolved gland residue was digested for 16 hours at room temperature with 100 cc. of 0.5 per cent hydrochloric acid in 65 per cent alcohol, neutralized, filtered, and the filtrate concentrated to a volume of 5 cc. in vacuo. This product was injected subcutaneously in normal dog No. 59.

The results of the foregoing observations are described graphically in Chart 1.

B. Preparation in Acid Solutions by Boiling.

(1) 30 gm. of fresh parathyroid glands were finely ground, boiled 15 minutes with 500 cc. of 0.1 N hydrochloric acid, cooled, freed from fat by skimming, and filtered. The pale yellow, hazy filtrate was bottled and preserved in the ice chest. 5 cc. of this filtrate were injected intramuscularly in thyreoparathyroprivic dog No. 77.

(2) Identical preparation with B (1) above save for boiling 2 hours instead of 15 minutes. (Hanson's hydrochloric X.) 5 cc. of this filtrate were administered intramuscularly in thyreoparathyroprivic dog No. 80.

The results of these two experiments are recorded in Chart 2.

IV. Serum Calcium-Increasing Extracts of Fresh Acetone-Desiccated Parathyroid Glands and Their Effect on Normal Dogs.

A. Comparison of Hot and Cold Acid Extracts.

(1) Extraction with Acid at Room Temperature.—5 gm. of acetone-desiccated fresh pulped glands were extracted for 4 hours at room temperature with 100 cc. of 0.2 per cent hydrochloric acid, filtered, excess protein was removed by adding strong sodium hydroxide to the isoelectric point, filtered again, and the clear filtrate concentrated to 10 cc. in vacuo. To the somewhat turbid concentrate, 2 cc. of 0.1 N sodium hydroxide were added to aid solution. All of the product was injected subcutaneously in normal dog
No. 69. (1 gm. of acetone-desiccated tissue is equivalent to approximately 7 gm. of fresh moist glands.)

**Chart 1.** Injections made at time designated by arrow. Maximum serum calcium increase in Dog 43, 10.7 per cent.

- 53, 29.6
- 59, 16.0

**Chart 2.** Injections made at time designated by arrow. Maximum serum calcium increase in Dog 77, 7-24 hrs. 26.7 per cent.

- 80, 8
- 40.1

(2). *Extraction with Acid by Boiling.*—5 gm. of acetone-desiccated glands (same lot as used in (1) above) were boiled for 15 minutes with 100 cc. of 0.2 per cent hydrochloric acid, cooled, filtered, and the extract was completed exactly as in A (1) above. Final volume was 14 cc., all of which was administered subcutaneously in normal dog No. 70.
The results of the above two experiments are recorded in Chart 3.

**CHART 3.** Injections made at time indicated by arrow.

Maximum serum calcium increase in Dog 69, 18-24 hrs. 27.9 per cent.

" " " " 70, 18-24 " 97 " "

Last serum calcium analysis, 12 mg. at 168th hr.

**B. Comparison of Similar Extracts Obtained by Boiling Acetone-Desiccated, and Acetone-Desiccated Chloroform-Defatted Tissues with Dilute Hydrochloric Acid.**

(Acetone-desiccated glands lose about 40 per cent of their weight by continuous chloroform extraction.)

(1) 1 gm. of acetone-desiccated glands was boiled with 20 cc. of 0.2 per cent hydrochloric acid for 15 minutes, cooled, and filtered. The excess protein was removed from the filtrate by the addition of strong sodium
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hydroxide to the isoelectric point and subsequent filtration. The resulting filtrate was evaporated to dryness in a vacuum autoclave, and the desiccated product dissolved in 10 cc. of sterile water. 5 cc. of this solution were injected subcutaneously in normal dog No. 74.

(2). 1 gm. of acetone-desiccated chloroform-defatted glands (same lot of glands as above) was boiled for 15 minutes with 20 cc. of 0.2 per cent hydrochloric acid, cooled, and filtered. The preparation was completed identically as in B(1) above, and 5 cc. of the resulting solution were given subcutaneously in normal dog No. 75.

The results of these observations are recorded in Chart 4.

![Chart 4](http://www.jbc.org/)

**CHART 4.** Injections made at time designated by arrow. Maximum serum calcium increase in Dog 74, 6-9 hrs. 21.5 per cent. " " " " " 75, 18 " 94.4 " "

**V. Effect of Purification of the Parathyroid Extracts on Their Potency.**

10 gm. of finely ground fresh acetone-desiccated and chloroform-defatted parathyroid glands were boiled with 250 cc. of 0.1 \( n \) hydrochloric acid for 30 minutes, and then cooled to room temperature. 10 cc. of the filtrate were obtained for testing the potency of the crude product. 2.5 cc. portions of this filtrate were injected intramuscularly in normal dogs Nos. 83 and 84.

To 220 cc. of the unfiltered crude product, strong sodium hydroxide was added to the point of maximum precipitation, and 1175 cc. of 95 per cent alcohol were added to further coagulate proteins. This mixture (approximately 80 per cent alcohol) was permitted to stand 16 hours, and then filtered.
The clear filtrate was concentrated in vacuo to remove all of the alcohol. It was then made up to the original volume of 220 cc. with distilled water and preserved with chloretone. 2.5 cc. portions of this preparation were administered intramuscularly to normal dogs Nos. 85 and 86.

These experiments are recorded in detail in Chart 5.

![Chart 5](image)

Chart 5. Injections made at time designated by arrow. Maximum serum calcium increase in Dog 83, 7 hrs. 8.0 per cent.

- " " " " " " 84, 7-24 " 33.33 " "
- " " " " " " 85, 7 " 12.0 " "
- " " " " " " 86, 7 " 20.0 " "

![Chart 6](image)

Chart 6. Injections made at time designated by arrow. Maximum serum calcium increase in Dog 79, 7 hrs. 28.8 per cent.

- " " " " " " 82, 7 " 7.2 " "

VI. Effect of the Age of the Extract on its Potency.

A preparation was made 16 months ago according to the method of Hanson wherein 30 gm. of freshly frozen and trimmed
Extract from Bovine Parathyroid Glands

Parathyroid glands were boiled for 2 hours with 500 cc. of 0.1 N hydrochloric acid, cooled, freed from fat by skimming, filtered, and preserved in the ice chest. Before employing this material for testing it was neutralized to the point of maximum precipitation with strong sodium hydroxide, and further coagulated by the addition of 1 volume of 95 per cent alcohol. This mixture was then filtered, and the clear filtrate concentrated on the steam bath to one-tenth of its original volume. The resulting slightly turbid amber-colored solution was preserved with chloretone. 5 cc. portions of this product were injected intramuscularly into thyreoparathyroprivic dogs Nos. 79 and 82 with the results tabulated in Chart 6.

Discussion.

In Section III A and Chart 1 are presented evidence indicating that the bovine parathyroid glands contain an acid-extractable hormone capable of inducing hypercalcemia in dogs. It further shows that the active substance is probably obtainable from the lipid-free constituents of the glands, and is rendered soluble in both alcoholic- and aqueous-acid media although alcohol is in no wise essential.

Boiling the finely ground glands with dilute hydrochloric acid is as good if not a better means of extracting the hormone than digestion at room temperature as is demonstrated by Section III B and Chart 2. The hormone is active in increasing the serum calcium of thyreoparathyroprivic as well as of normal dogs. In fact, we have evidence indicative of greater responsiveness of the former to the action of the hormone. The active substance must be quite stable when it is not affected by boiling with acid.

Acetone-desiccated fresh parathyroid glands are as serviceable as the fresh moist gland for the preparation of extracts as is shown in Section IVA and Chart 3. In this section, the evidence suggests that boiling with acid is preferable to extraction at room temperature. Dog 70, although its serum calcium was increased practically 100 per cent, showed no symptoms attributable to the change. Furthermore, large doses are shown to exert their influence over a relatively long period as is here demonstrated, the serum calcium decreasing slowly over a week's time.

The contents of Section IV B and Chart 4 corroborate the obser-
vations recorded in Chart 1 wherein it is suggested that the lipoid constituents of the glands are not the source of the hormone. In these two experiments, it is shown that lipoid-free tissue yields a very potent extract by boiling with dilute hydrochloric acid. The difference in response between Dogs 74 and 75 to these two extracts may be due to the fact that the acetone and chloroform, desiccated and defatted, material contains more (about 40 per cent), potentially active tissue per gm. of weight than does the acetone, desiccated and defatted.

In Section V and Chart 1 is evidence indicating that most of the protein bulk may be removed without appreciably affecting the potency of the extract. It is possible that some may be lost by precipitation of the colloids for the hypercalcemia test is not sufficiently accurate to detect small losses. The results also show the variation in response of different dogs to the same dose of the hormone. Unrecorded data show this variation to be quite conspicuous, and that in general, dogs which respond well at one time, do so at other times and that dogs which respond poorly, also do so consistently. Certain observations point to the fact that young dogs are most responsive, yet there is considerable variability among them. There is also a suggestion that response depends to some degree, at least, on readily available calcium, for when relatively small amounts of calcium lactate are given orally at the time of injection of the hormone, there is a rapid increase in the serum calcium.

The parathyroid hormone is very stable even when preserved in 0.1 \( N \) hydrochloric acid for a period of over a year as is shown by the data in Section VI and Chart 6. In this case, a preparation, made according to the method of Hanson, was preserved for 16 months in the ice chest, and was then still very active. The difference in response between Dogs 79 and 82 shows that the variation existing in normal dogs is shared by thyreoparathyroid-privic dogs. In some unrecorded data, operated dogs have shown variations in response at different times. There is some indication that hypoparathyroid dogs with a moderate degree of hypocalcemia respond most readily to injections of the hormone. Some dogs in tetany have been relieved of their symptoms without showing any serum calcium increase, substantiating MacCallum and Vogel's observations, and other dogs or the same dogs at other
times have shown a very rapid increase in their serum calcium simultaneous with relief from symptoms.

The observations with boiled hydrochloric acid extracts corroborate the results published originally by Hanson and later by Collip. The results with acid-alcohol extracts verify the work of Berman. The relative stability of the hormone to treatment with boiling acid and long preservation in the ice chest serve as contradictory evidence against the claims of Berkeley and Beebe.

CONCLUSIONS.

1. A hormone is obtainable from fresh bovine parathyroid glands by aqueous or alcoholic hydrochloric acid extraction which when given parenterally to dogs possesses the property of relieving tetany and inducing hypercalcemia.

2. Boiling the glands with dilute hydrochloric acid is preferable to extraction at room temperature.

3. The lipid-free portion of the glands is the potentially active tissue.

4. Very little, if any, potency is lost in the removal of proteins by neutralization to the isoelectric point and addition of alcohol to a concentration of 80 per cent.

5. The hormone is relatively stable as judged by the vigorous treatment it withstands in the course of its preparation, and its retention of activity during 16 months preservation in the ice chest.

6. Definite conclusions are in some cases not justified because of the limited accuracy of the hypercalcemia test.

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