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EXTRACTION AND PURIFICATION OF BEEF
PARATHYROID PRINCIPLE USING
COUNTERCURRENT
DISTRIBUTION

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INTRODUCTION

The function of the parathyroid glands has long been an enigma. A major obstacle to the elucidation of parathyroid activity is the extraction of a pure gland principle. Numerous attempts have been made to reduce the gland to a single homogenous compound containing all the activity of the gland itself. Little progress has been made since Collip first produced his hot HCl extract and showed that it was complete replacement therapy following parathyroidectomy (1).

Recently, the problem has been subjected to countercurrent distribution techniques by Craig and Rasmussen (2), following a modification of the extraction procedure of Auerbach (3).

This work is an attempt to verify the published results of Craig and Rasmussen (2).

METHODS AND MATERIALS

Beef parathyroid glands were obtained from a local packing plant and were frozen immediately following removal from the animals. A total of eight-hundred eighty-five grams of fresh gland tissue was used.

This was ground in a meat grinder after being allowed to thaw and then was mixed in small portions with acetone in a Waring Blender until a relatively thick brei was obtained. The larger strands of tissue were removed and rerun in the Blender. The acetone had been redistilled over potassium permanganate and potassium carbonate.

The acetone was removed in vacuo and the gland mince was then mixed with chloroform (reagent grade) and allowed to stand with frequent stirring for one-half hour. It was then filtered under suction. The residue was again mixed with chloroform and allowed to stand overnight in the cold and again filtered. The residue was washed on the filter paper with chloroform after each filtration. The residue was dried in vacuo and yielded 81.2 grams of dry powder.

Forty grams of this powder was then treated by a modification of the method of Craig and Rasmussen(2). The powder was stirred with 10 ml/gm of 70% phenol for one hour and the residue was removed by centrifugation. Phenol extraction

of the residue was repeated, allowed to stand for twenty minutes, and centrifuged. To the combined supernate (600 milliliters) was added three liters of 1:1 acetone:acetic acid and 15 milliliters of 1 Molar NaCl and the whole was allowed to stand for one hour in the cold. Inactive precipitate was removed by filtration. To the filtrate was added an equal volume of peroxide free ether (3.3 liters) and the mixture was allowed to stand overnight in the cold. Following centrifugation, the supernate was decanted, the residue was washed twice with ether and dried in vacuo over anhydrous calcium chloride. This procedure yielded 1.78 grams of fine white powder (which was 4.5% of the original dried gland material). Thirty milligrams of this material was removed for assay in mice by the method of Shane(4). See Graph I.

The remaining 1.75 grams of powder was dissolved in 30% acetic acid. To this was added 35% NaCl to a final concentration of 7.5% and further inactive precipitate was removed by centrifugation. To the three-hundred milliliters of supernate was added 100% trichloroacetic acid to a final concentration of 7.5%, whereupon the active material precipitated and was centrifuged out.

In order to remove the trichloroacetic acid an ion exchange resin was employed. Amberlite IR45 was utilized (5 grams) in a column. It was made the acetate form by addition of saturated sodium acetate until the column removed no more acetate. Readings of pH served to indicate the completeness of uptake of acetate.

The active material was suspended in 0.1 Molar acetic acid and this colloidal suspension was then added to the resin column and the main body of fluid collected in a beaker (42 ml).

The column was then washed with 0.1 Molar acetic acid in 1-2 milliliter aliquots which were collected for detection of protein on the Beckman Ultraviolet Spectrophotometer at 277 millimicrons.

Essentially all the protein was obtained in the first twelve samples. The pooled aliquots made approximately 60 milliliters, to which was added a saturated (40%) solution of NaCl to a final concentration of 1%. This mixture was entered in the first six tubes of the countercurrent distribution machine, and distributed for 103 transfers in a solvent system of 6% acetic acid: 1% NaCl versus 1:1 butanol:propanol.

Tubes number 30 to 95 were removed, the upper phase drawn off and allowed to settle overnight. The upper phase was analyzed on the Beckman Spectrophotometer at 277 millimicrons and the peak absorption band was identified at tube number 51 (Figure 1).

The large volume of protein in the samples tended to make some unevenness in volume of the upper phase, so the volume of each upper phase was measured and multiplied by the optical density of the sample on the Beckman to give an estimate of the absolute amount of protein available. This is shown in Graph 2. The upper phases were pooled and evaporated down to a small volume which was added to the pooled lower phase to make a total of 90 milliliters of aqueous solution. This solution contained all the active fraction.

Before lyophilization of the final product, removal of the NaCl was attempted by the

following procedure. Amberlite IR400 resin in the chloride form was converted to the hydroxyl form and then to the bicarbonate form by use of saturated NaCl and saturated sodium bicarbonate, respectively. Strong cation exchange IRC50 in the Hydrogen form was made with hydrochloric acid.

These two resins were mixed in equal proportions in a column. The saline solution of the parathyroid extract was put through the column to remove the NaCl. The column was washed with distilled water until the reading of samples on the Beckman at 277 millimicrons was 0.025 optical density. Thus, virtually all the protein which was present came off. The volume of the effluent was 150 milliliters.

Lyophilization was carried out in the centrifugal freeze dry apparatus over phosphorous pentoxide, (which removed water and organic acid). A special dry ice trap was designed by Dr. A.R. McIntyre and Mr. L. Laughlin of this department in order to remove any remaining alcohol. Evacuation was maintained at between 50 and 150 microns of mercury pressure. The final product was obtained in small vials as a fine white powder.

RESULTS

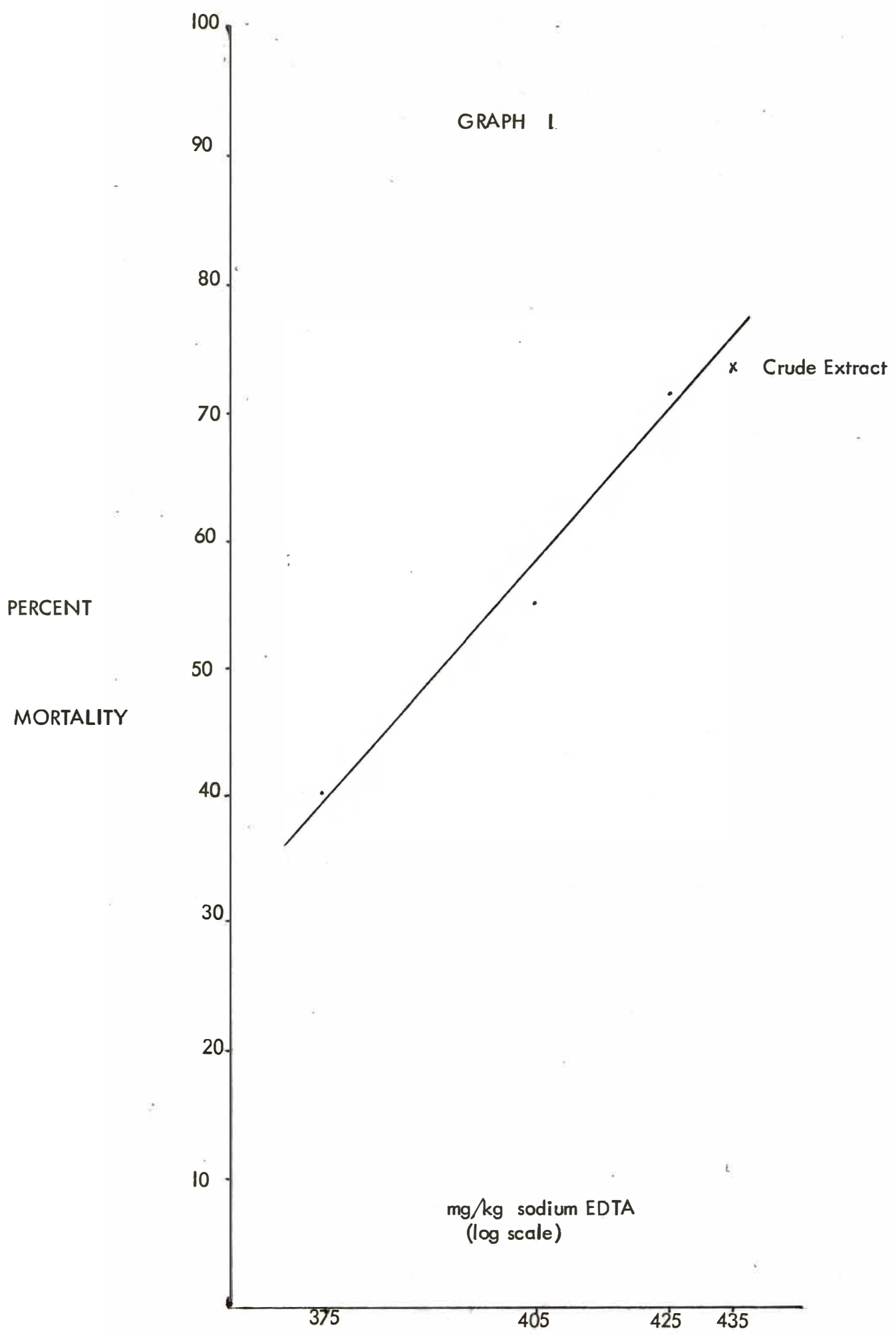
Thirty milligrams of dried gland powder were removed following extraction by modification of the method of Auerbach (3). This was estimated to have an activity of 150 units per milligram.

This material was assayed against standard Parathormone (Lilly). The unknown extract was injected into mice in an estimated concentration of 1 unit per gram of body weight after the method of Shane (4). Standard Parathormone was injected at a dose of 2 units per gram. As can be seen from Graph I, the experimental group responded in a manner similar to the controls, indicating that approximately 2 units per gram had been given. This meant that at this stage in the extraction procedure the extract was indeed quite potent.

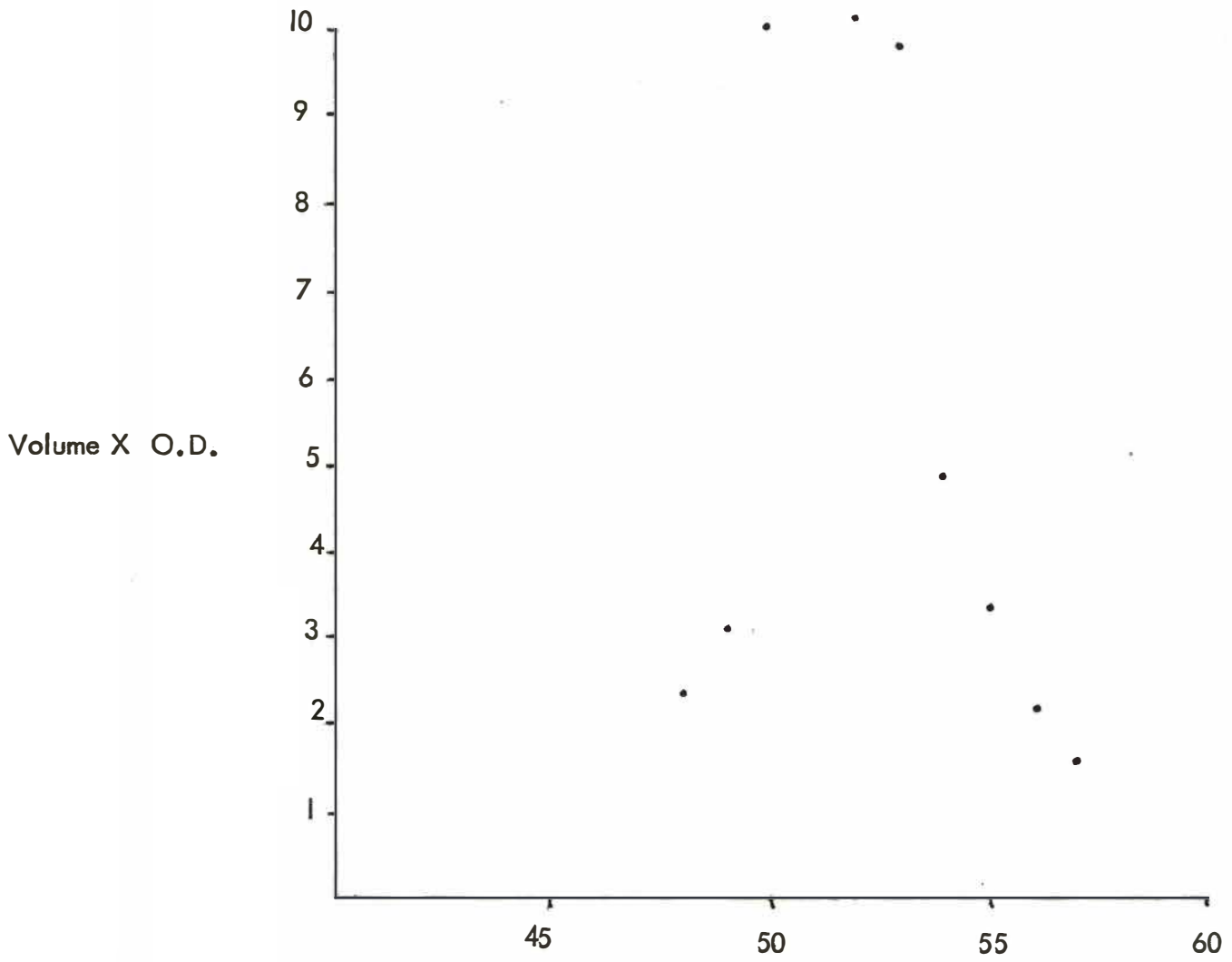
FIGURE I

<u>TUBE</u>	<u>VOLUME</u>	<u>OPTICAL DENSITY</u>	<u>PRODUCT</u>
48	11.6 cc	0.199	2.31
49	11.7	0.259	3.03
50	10.7	0.935	10.00
51	10.2	1.036	10.57
52	9.8	1.031	10.10
53	14.8	0.659	9.75
54	8.9	0.536	4.77
55	8.2	0.391	3.21
56	7.6	0.284	2.16
57	6.6	0.246	1.62

GRAPH I.



GRAPH 2
PARATHYROID EXTRACT



DISCUSSION

The foregoing experiments indicate that the method of Rasmussen and Craig is indeed an advance over previous methods of extraction. Midway in the procedure the extract was very active, possibly more so than they had believed. The final product awaits assay by the very precise method of Munson (5).

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