CCLI. SOME EXTRACTS OF PARATHYROID GLANDS CONTAINING AN ANTI-GROWTH FACTOR. I.

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PREVIOUS work on the parathyroid gland [Collip, 1925; Collip and Clark, 1925; Hjort, Robison and Tendick, 1925] has shown the presence of a serum-calcium raising factor. From experiments on animals injected with a preparation of this gland one of us [Thompson, 1931] was led to suspect the presence of an anti-growth factor. It seemed possible that this activity might be due to the serum-calcium raising factor itself or to the presence of a specific anti-growth factor as an impurity in the extract. A series of extracts was therefore prepared with the intention of eliminating the Collip factor as far as possible. Collip and Clark [1925] have shown that the latter is precipitated at p_H 4.8. All extracts, however prepared, were adjusted to this p_H and allowed to stand until no further precipitation took place.

The preliminary extracts were prepared by the following methods.

- (1) Aqueous extraction.
- (2) Extraction with 95 % alcohol.
- (3) Extraction with 80 % alcohol containing 5 % by weight of sulphuric acid, afterwards neutralising with sodium hydroxide to p_H 4.8, filtering off the sodium sulphate and concentrating *in vacuo*.
- (4) Collip's method, heating with dilute hydrochloric acid, making alkaline to p_H 8 to 9, precipitating inert matter by bringing to p_H 5.6, acidifying to p_H 4.8 and allowing to precipitate.
- (5) Method (4), but previously defatting the glands with acetone.

Acid alcohol extraction.

The results of animal tests with these fractions encouraged us to adopt method (3) as a basis for further investigations. Berman [1926] used a similar method for preparing an extract containing the Collip factor, but the acid alcohol extract was neutralised to litmus, thus retaining the Collip factor in solution. Incidentally it appears that Berman's extracts must have contained sodium sulphate, the alcohol concentration not being sufficiently high to remove it.

Method (3) was therefore elaborated for use with larger quantities of material. 100 g. of fresh parathyroid glands, freed from adherent fat and connective tissue and finely minced, were shaken continuously for 48 hours with 1 litre of alcohol (85 % by volume) containing 5 % by volume of sulphuric acid. The mixture of acid and alcohol must be made slowly and the temperature kept low, otherwise sodium ethyl sulphate is formed which appears in the final product. This compound has been found to have no growth-retarding action on rats.

The gland residue was then strained off, and the liquid was neutralised to methyl red by the gradual addition of 50 % sodium hydroxide solution, keeping the mixture cool. Alcohol was then added so as to give a final concentration of 75 % by volume in the mixture. This ensures the almost complete precipitation of the sodium sulphate, on cooling to about 5°. After keeping at this temperature overnight the sodium sulphate and other precipitated material were removed by filtration through a Büchner funnel. The filtrate was concentrated under reduced pressure at about 15–20 mm. until all the alcohol was removed. The volume of the concentrated liquid was adjusted so that 1 cc. was equivalent to 2 g. of parathyroid gland, and the p_H was adjusted

Fig. 1.

to 4.8. The extract was then allowed to stand at a temperature of about 5° until no further precipitation occurred, when it was filtered through a Seitz filter. The extract was used in this condition for animal tests without the addition of a preservative. For a description of the method of testing and for the physiological evidence for the anti-growth factor see Robinson and Thompson [1932].

A number of extracts prepared by this method have given definite evidence of the presence of an anti-growth factor. Fig. 1 shows the effect of such an extract on the growth of rats. Six rats were used as controls and six were given daily injections of 2 cc. of the extract, other conditions being the same.

Defatting by acetone. The large amount of fat in parathyroid glands prevents the efficient penetration of the gland material by the cold acid alcohol. Previous treatment of the minced glands with cold acetone appeared to give a more complete extraction. The fat so obtained appeared to possess a slight activity, as the following experiment shows.

200 g. of the fat extracted by acetone were dissolved in 750 cc. of light petroleum and shaken for 24 hours with 1 litre of 70 % by volume alcohol containing 10 % by volume of sulphuric acid. After separation the light petroleum layer was washed with 70 % alcohol and the combined acid alcohol extracts were treated as described above. The final concentration of the solution was, however, adjusted so that 1 cc. was equivalent to approximately 25 g. of gland instead of 2 g. The effect of this extract on rat-growth is shown in Fig. 2. The difference in the average increases in weight over 4 weeks is insignificant. Nevertheless if the last two weeks only be considered the growth of the injected rats was practically stationary during this period while the average increase of the controls was 13 g.

Fig. 2.

Defatting with benzene. In order to avoid this loss of active material benzene was added to the minced gland with the acid alcohol and the mixture was shaken together. This was an improvement on previous acetone extraction in removing the fat and allowing better extraction of the gland material.

400 g. of parathyroid glands were minced and shaken for 48 hours with

700 cc. of benzene and a cooled mixture of 300 cc. of sulphuric acid, 1800 cc. of water and 900 cc. of 95 % alcohol. The acid alcohol was separated and treated as in the first method.

Removal of fat by heat. The minced glands were mixed with alcohol (30 % by vol.) containing 5 % by vol. of sulphuric acid and heated to 70° for a few minutes until the fat was completely melted and rose to the surface. After cooling, the cake of fat was removed and the acid alcohol and gland material were shaken together for 48 hours. The liquid was filtered, neutralised with sodium hydroxide solution, and alcohol added to 75 % by vol. concentration as in the first method. The extract obtained was found to be active (see Fig. 3, extract No. 601). The fat obtained in this method was dissolved in benzene and extracted with acid alcohol. The results confirmed the previous finding that the fat contains some of the anti-growth factor.

Fig. 3.

Salting-out. Extracts prepared by the above methods have been salted out with sodium sulphate at p_H 4.8 with a view to removing the Collip factor more completely. This has been repeated a number of times and in all cases in which it has been done an increase in the anti-growth potency has been observed. The usual procedure was to saturate the solution at p_H 4.8 with sodium sulphate at room temperature and filter off the precipitate formed, which was usually small in amount. The precipitate was redissolved in a smaller volume of water at p_H 4.8 and again salted out in the same way. The combined filtrates were treated with alcohol to a concentration of 75 % by volume and the sodium sulphate was filtered off after crystallising at 5° overnight. The alcohol was removed from the filtrate under reduced pressure and the concentrate was made up to the original volume with water.

The results on growth of such an extract (40 SO) are shown in Fig. 4.

The total average increase in the injected rats is only half the increase obtained with the extract before salting-out (40). Table I. Tests on a solution of the salted-out precipitate have failed to show any activity.

Alkaline extraction. 1200 g. of minced parathyroid gland were heated to 40–45° with 2400 cc. of 2 % sodium hydroxide solution and kept at that temperature for a few minutes, then at 37° for 24 hours, toluene being added as a preservative. After separation of the fat the liquid was neutralised to p_H 7 and centrifuged. The clear liquid was treated with alcohol until the concentration was 75 % by volume, the sodium sulphate was removed in the usual way, and the filtrate concentrated as usual. This extract appeared to have little or no activity.

Treatment with activated charcoal. 300 cc. of an active extract (601) were shaken with 6 g. of activated charcoal (Darco) for half an hour and filtered. The filtrate (601 C) still contained the anti-growth factor in undiminished amount (Fig. 3).

Fig. 4.

Treatment with hydrogen peroxide. 1 cc. of 30 % hydrogen peroxide was added to 300 cc. of an active extract (40 SO) and the mixture kept overnight at 22°. The excess of hydrogen peroxide was removed by the addition of manganese dioxide which was filtered off when the hydrogen peroxide was completely decomposed. This treatment appears in some cases to destroy the whole of the anti-growth activity [Robinson and Thompson, 1932] but the destruction is not always complete; for example, treatment with hydrogen peroxide reduced the potency of extract (40 SO) and the extract so obtained (40 SO.HP) appeared to have about the same potency as the original extract (40) before the salting-out process had increased its activity (see Fig. 4).

Chemical properties of the extracts. Extracts prepared by the acid alcohol method contain about 3 % of total solids, of which about 1.5 % are ash. The nitrogen content is about 0.2 %. Picric acid gives a slight precipitate, and phosphotungstic acid a heavy one. They give marked xanthoproteic and Millon's reactions which are reduced in intensity by the salting-out treatment and by treatment with activated charcoal. Ehrlich's reaction for tryptophan is also positive, but is very faint when applied to the extract after treatment with hydrogen peroxide. The extract of the fat was very low in solids and gave none of these reactions.

SUMMARY.

1. Extracts have been prepared from parathyroid glands containing a factor which retards the normal growth of rats.
2. Extraction with acid alcohol gives active extracts.
3. Alkaline extraction gives little or no activity.
4. Removal of the fat gives better extraction, but the fat may contain some of the activity.
5. Salting-out increases the potency of active extracts, the salted-out material having no activity.
6. Treatment with activated charcoal does not affect the potency.
7. Treatment with hydrogen peroxide may partially or completely destroy the activity.

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