# Recovery of Growth Hormone in Purification of Corticotropin. (19136)

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Current procedures for the extraction and purification of pituitary growth hormone have given consideration to the reported ready destructibility of the active principle(1), consistent with estimations of a large molecular size and protein character(2,3). Sensitivity to heat(1,2,4,5) and to acid(2) have been noted and most procedures have been performed in alkaline or near-neutral media (1-3,6,7), and sometimes in the cold(1,3). It is perhaps surprising, therefore, to find that the method recently reported by Astwood, Raben, Payne, and Grady(8) for the purification of the highly acid-resistant corticotropin leaves in the final residual solution an abundance of active growth hormone.

Initial extraction of acetone-dried pig anterior pituitary powder with glacial acetic acid at 70°C was followed by removal of an acetone precipitate containing thyrotropin, gonadotropins, and some luteotropin; crude corticotropin was then obtained by precipitation with ether(9). The crude corticotropin, with a potency of about 2 u/mg, was dissolved with warming in 0.1 N acetic acid and stirred with 8% its weight of powdered oxidized cellulose (10.4% COOH) for 20 hours at room temperature. The oxycellulose removed 2% of the solids of the pituitary extract and 90% of the corticotropic activity. The remaining acetic acid fraction, containing about 12% of the solids of the original acetone-dried pituitary powder and 98% of the solids of the crude corticotropin, was found to be rich in growth hormone. Retreatment of this fraction with a 20% weight of oxidized cellulose removed almost all the residual corticotropin without detectable loss of growth hormone. When tested in hypophysectomized Sprague-Dawley male rats,† weighing 80 to 100 g, by the weight gain produced with daily intraperitoneal injections, this fraction appeared to be roughly 20% as active as the purified growth hormone preparation of Wilhelmi (Table I). Adjustment of the pH of the acetic acid fraction to 5.5 in the presence of 0.3 N potassium acetate caused 78% of the material to precipitate from a 2.5% solution. The precipitate (No. 4, Table I) was about as active as the whole acetic acid fraction. Similarly, precipitation of 70% of this fraction at pH 3.5 did not effect a separation of growth activity from the major portion of the solids (No. 3, Table I). Purification was achieved, however, with a modification of Wilhelmi's procedure(3), by removing the precipitate formed at pH 8.5, and adding 95% ethanol to the supernatant to 47.5 or 60% concentration. The alcohol precipitate containing 10.5 to 13% of the solids was approximately as potent as the purified preparation used for reference (No. 6, Table I).

Growth hormone had a relatively low affinity for oxycellulose and the cellulose appeared to be a poor fractionating agent for growth hormone when employed in the manner described. Comparing the adsorption of

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† Some of the animals used were hypophysectomized by the Charles River Breeding Laboratory, Boston.

TABLE I. Growth Effect as Tested by Daily Intraperitoneal Injections in Hypophysectomized Rats.

| No. | Substance tested | No. of rats | Test period, days | Daily dose, μg | Avg wt gain per day, g |
|---|---|---|---|---|---|
| 1 | Acetic acid fraction | 5 | 9 | 100 | .9 |
| | | 3 | 11 | 250 | 1.3 |
| 2 | a. 16.5% of (1) eluted from oxycellulose | 3 | 11 | 200 | 1.6 |
| | b. Unabsorbed portion | 5 | 10 | 100 | 1 |
| | | 5 | 4 | 500 | 2.3 |
| 3 | pH 3.5 ppt.—70% of (1) | 2 | 6 | 250 | 1.5 |
| 4 | pH 5.5 ppt.—78% of (1) | 5 | 10 | 250–2d.<br>100–8d. | 1.1 |
| 5 | pH 8.5 ppt.—57% of (1) | 7 | 13 | 100 | .3 |
| | | 7 | 7 | 250 | .4 |
| 6 | Ethanol ppt. of pH 8.5 soluble—10.5% of (1) | 11 | 10 | 30 | 1.1 |
| 7 | Wilhelmi growth hormone | 3 | 10 | 25 | 1.1 |
| | | 4 | 10 | 30 | .7 |

an electrophoretically homogeneous growth hormone preparation(3), kindly provided by A. E. Wilhelmi, with that of purified corticotropin, it was found that the amount adsorbed on 1 g of oxycellulose (10.4% COOH) from a 0.1 N acetic acid solution, and eluted with 0.1 N hydrochloric acid, was approximately 6 mg of the growth hormone, and 340 mg of corticotropin(10). Treatment of the acetic fraction with a 12.5-fold weight of 19% carboxyl oxycellulose yielded only 16.5% of the material in the eluted fraction. The growth activity, furthermore, appeared to be about equally concentrated in the eluted and the unadsorbed fractions (No. 2, Table I).

Studies on the stability of growth hormone have led Li to conclude that growth hormone is more stable in alkali than acid(11). This is in contrast to earlier work which had indicated to Evans, Meyer, and Simpson that the hormone was more susceptible to alkali(1), and to Collip that growth hormone was most stable at pH 2(4). The preparations described here survived a glacial acetic acid extraction at 70°C and solution in 0.1 N acetic acid for 2 days at room temperature and for 5 to 6 weeks at 10°C.

Kamm and coworkers reported that the posterior lobe principles could be extracted with glacial acetic acid at 25°C but that the extraction was facilitated by the presence of 2% water(12). Evans, Meyer, and Simpson found the growth hormone to be extractable at 20°C with 95 to 98% acetic acid, but not with glacial acetic acid(1). It has recently been shown that the presence of as little as 0.5% water improves the efficiency of extraction of corticotropin with acetic acid at room temperature(13). The glacial acetic acid used for the extraction of corticotropin (14,9,8), and as used in the present work, was standard reagent grade acid, stated to contain about 0.5% water.

*Summary.* Extraction of acetone-dried hog anterior pituitary glands with glacial acetic acid at 70°C and removal of corticotropin with oxycellulose provides a method for the preparation of corticotropin and growth hormone in a single procedure.

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