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Potent, Sterile and Low-Protein Extracts of the Growth Hormone From the Anterior Hypophysis.*

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The following procedure has been used in obtaining extracts of the growth hormone of the anterior hypophysis for producing experimental gigantism in rats and provoking the growth of hypophysectomized puppies in the hands of Dr. Frederick L. Reichert.

2,000 gm. of beef anterior hypophysis. The anterior lobes of beef hypophyses had been stored for a period of between 18 and 24 months with solid CO_2 in an insulated container in a room held at —5.0° F., after having been ground in a Wiley laboratory mill.

12,000 cc. distilled water.

3,000 cc. 0.2/N Ba $(OH)_2$

Centrifugation: The alkaline mass was mixed by an electric household stirrer ("kitchen mechanic"); then centrifuged in a Sharples laboratory centrifuge. Large nozzles were used in the first centrifuging in order to remove the bulk of the insoluble material; the extract was then centrifuged at a rate of about 10 cc. per minute. The centrifuge was cooled with salt and ice at all times and the extract was kept surrounded with ice.

Neutralization: 0.2/N H_2SO_4 was added slowly with constant stirring by the same stirrer. Phenol red was used as indicator and the end point chosen was about pH 8.0, about 500 cc. being required. The Ba not already precipitated by H_2SO_4 was removed by a slight excess of Na_2SO_4.

Centrifugation: Sharples centrifuging was then repeated, finishing as before at a rate of 10 cc. per minute.

Filtration: A standard 14 cm. Seitz asbestos filter was used. A pressure of about 10 lbs. per square inch was applied by the use of a tire pump. Time required for filtration of 4 liters was about 45 minutes; towards the end, the rate of flow through the filter had decreased to half the original rate. Precautions were taken to maintain sterility of the solution after filtration. The extract was stored in 25 cc. lots in rubber-capped bottles and kept frozen until needed for injection.

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Yield: Approximately 15 liters.

Temperature during preparation: It is important to note that the temperature of the extract was never allowed to rise above 10° C. at any time during the preparation.

Bacterial content: Culture of the extract proved it to be sterile. (Dr. Reichert.)

Total solids: About 1.1%.

Color: Very pale, cloudy pink, presumably due to hemoglobin, almost clear in 100 cc. bottles; fluorescent.

Potency: Tested in normal adult female rats (weighing 250-270 gm.) the extract gave what we consider to be indubitable proof of the presence of the growth hormone; it created an increase in body weight of about 35 gm. in 20 days when administered in a daily intraperitoneal dose of 1/8 cc. and a growth of 55 gm. when given in 1 cc. daily doses.

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Effect of Morphine Sulphate by Mouth on Oxygen Consumption in Normal Humans.

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A preliminary report by M. E. Stark[1] has shown that morphine sulphate, presumably administered subcutaneously, significantly decreases oxygen consumption in normal humans, and, as might be expected, in a manner roughly proportional to the dose (0.37 mgm. to 0.48 mgm. per kg. weight). In studying the effects of the commonly used barbiturates on oxygen consumption, observation was also made of the action of morphine sulphate on some of the subjects. The drug was administered by mouth in doses from 0.1 mgm. to 0.5 mgm. per kg. weight. The technique was essentially the same as that employed by Miss Stark, except that the experiments did not run as long. The Sanborn apparatus and averages were used.

The results are shown in Table I. Where a change occurred in respiratory rate, it was usually slightly diminished. On the other hand, within the first hour after giving the drug, the pulse rate in-

[1] Stark, M. E., *Analgesia and Anesth.*, 1929, viii, 307.