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Extraction of Gonad Stimulating Substances of Anterior Lobe of the Hypophysis.

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It has been shown by Fevold, *et al.*[1, 2] that the gonad stimulating principles of the anterior lobe of the pituitary gland can be extracted almost quantitatively from powdered glands by means of 50% aqueous pyridine. On account of the difficulty in evaporation of the pyridine extract and the molesting odor, other methods of extracting have been used in the following experiments.

It was found that a solution of 6% normal butyl alcohol, or a 3% solution of amyl alcohol in water would also extract the active principles in a very efficient manner. The extract can very easily be concentrated by vacuum distillation at low temperature (below 40°C.) so it offers advantages over aqueous pyridine in this respect.

A comparison of the efficiency of the 2 solvents, 50% aqueous pyridine and 6% aqueous butanol, is given below. The procedure used was that given by Fevold, *et al.*, and was the same in both cases. One hundred grams of the dried acetone extracted pituitary gland from horses were used. The material was extracted each time for 12 hours with 700, 600, 600, 600 cc. of the respective solvent—50% pyridine or 6% butyl alcohol solution.

Each extract was then purified as follows: After evaporation *in vacuo* at a temperature below 40° to a volume of 500 cc., 4½ volumes of acetone were added. The precipitate thus obtained was emulsified in 1,500 cc. of water and 750 cc. of acetone were added. The insoluble material was re-extracted with 33% acetone for a second time. The combined 33% acetone solution was evaporated *in vacuo*. The residue was emulsified in 500 cc. water, and 70 cc. of saturated alcoholic benzoic acid were added. The material brought down with the precipitate of benzoic acid was washed with acetone to remove the benzoic acid, then extracted twice with 150 cc. of water; the aqueous extract was mixed with the filtrate from the benzoic acid and precipitated with 4½ volumes of acetone. The

[1] Fevold, H. L., Hisaw, F. L., and Leonard, S. L., *Am. J. Physiol.*, 1931, **97**, 293.

[2] Fevold, H. L., Hisaw, F. L., Hellbaum, A., and Hertz, R., *Am. J. Physiol.*, 1933, **104**, 710.

precipitate was purified by dissolving it in 100 cc. of water. The addition of 50 cc. of acetone removed some impurities. The bulk of the active principle was then precipitated with 150 cc. of acetone. The small quantity of active material that remained in solution was precipitated with an excess of acetone, purified by fractional precipitation with acetone and dissolved together with the main fraction in 100 cc. of water.

The extracts were tested on immature female rats 22 days of age. They were injected twice daily, ¼ cc. per injection for 3 days, and the animals were autopsied on the morning of the sixth day. The results are given in Table I.

TABLE I.

Biological Assay of Preparations.
Ovarian Weights*

Amount injected cc. per rat	Ext. No. 609 mg.	Ext. No. 611 mg.
0.1	135	186
0.05	89	109
0.025	53	56
0.01	23	24

* The weights given are the average weight of the ovaries of two animals.
Ext. 609 = 50% aqueous pyridine extraction.
Ext. 611 = 6% " butanol "

The results show that both extracts have approximately a potency of 100 units per cc.,[2] the butanol extract being perhaps slightly more active. However, this is within the limits of variation.

Butyl alcohol itself is not a solvent for the hormone; neither is pure water a satisfactory medium of extraction. The possibility that a change of surface tension might play a rôle was considered. A 6% butyl alcohol solution in Traube's stalagmometer gave 261 drops; a 3% commercial amyl alcohol solution 225 drops, as compared with 100 drops of water. These figures are the highest obtainable with any of the lower alcohols in solutions of not more than 10%. Incidentally, an extraction carried out with a 3% amyl alcohol solution had approximately the same potency as the butanol extract.

The extracts can be filtered through Berkefeld medium filters and Chamberland L-3 filters without noticeable loss of potency.

Studies on the blood pressure in dogs showed that the pyridine extract retains a definite amount of the pressor principle of the pituitary, whereas its content in the butyl alcohol extract is insignificant.