A Simplified Method for the Preparation of Corticotropin. (20351)

ROBERT J. BARTHOLOMEW.* (Introduced by E. B. Astwood.)

From the Ziskind Research Laboratories, New England Center Hospital, and Department of Medicine, Tufts College Medical School, Boston, Mass.

Previous work in this laboratory has shown that the corticotropic activity of dried pituitary gland can be satisfactorily extracted by glacial acetic acid and that a considerable concentration of the activity can be obtained by adsorption from dilute acetic acid onto cellulose(1) or oxycellulose(2), followed by elution with dilute hydrochloric acid. The specificity of oxycellulose suggested that, through its use, material of satisfactory purity for clinical application might be obtained from very crude extracts, such as those made with 80% acetic acid which has been found to dissolve about half of the dried pituitary tissue.

This present note reports a simplified method for obtaining, from either whole pituitaries of sheep or anterior lobes of pigs, a good yield of a highly active preparation. The actual working time is very small but, as described, is spread over 4 days.

Methods. In a representative experiment 20 g of dried powdered whole pituitary of sheep were stirred to a smooth paste with 90 ml of water, then 360 ml of glacial acetic acid were stirred in to make an even suspension. The mixture was then heated on a steam bath with frequent stirring until the temperature of the mixture reached 70°C. This required about one hour and at the end of this time the mixture had a translucent gelatinous appearance. The mixture was then allowed to cool, and diluted with water to 6 litres in a tall cylinder. Ten g of Hyflo Super Cel were added and the whole stirred mechanically for 10 minutes, and then allowed to stand overnight. The supernatant was then siphoned from the sediment of tissue fragments (which was covered by a compact layer of Super Cel) and transferred to another tall cylinder. Slightly more than 5.6 litres of opalescent supernatant were obtained and filtration was unnecessary. The protein concentration, as estimated by the biuret method(3), was 1.7 mg per ml, representing 51% of the weight of dry gland. To the supernatant was added 9.6 g of oxycellulose (Tennessee Eastman, Lot No. C 1060, 11.0% COOH, 0.13% N) and the mixture stirred mechanically for 24 hours, and then allowed to stand overnight. Before use the oxycellulose was washed on a coarse sintered glass filter with water, normal hydrochloric acid and deci-normal acetic acid. The amount used, 9.6 g, was quite arbitrarily chosen, being approximately equal to the weight of protein in the dilute acid extract. Most of the supernatant was siphoned off and discarded, then the remaining supernatant and the sediment of oxycellulose were transferred to a coarse sintered glass filter, filtered by suction and the residue washed on the filter with 2 or 3 small lots of deci-normal acetic acid. The corticotropin was then eluted from the oxycellulose by 4 washings, each 40 ml, of deci-normal hydrochloric acid, the first and second washings each being left in contact with the adsorbent for about an hour. The protein in the eluate as estimated by the biuret method was 0.90 mg per ml (or 144 mg in the total eluate), which represents 0.77% of the original powdered gland.

Assays were made on the dried pituitary powder and at various stages of the procedure by the ascorbic acid depletion method of Sayers *et al.*(4) as modified in this laboratory to a minimal effective dose method(1). The results in the particular preparation described and those in a similar preparation from anterior lobes of pig are shown in Table I. The values shown for the starting materials are in terms of actual weight of dried gland, the other values are in terms of protein indicated by the biuret estimations. Of the total 6 litres of extract only 5.6 litres (sheep) or 5.4 litres (pig) were used for the succeeding steps. The recoveries shown for the various fractions are not corrected for this.

* The Prince Henry Hospital, Sydney, Australia.

TABLE I. Yields in Corticotropic Activity in 2 Experiments Expressed as Minimal Dose to Induce a Fall in Adrenal Ascorbic Acid Concentration between 60 and 100 mg % in Hypophysectomized Rats.

Fraction	Sheep		Pig	
	Wt, g	Minimal effective dose, μg	Wt, g	Minimal effective dose, μg
Acetone-dried whole pituitary	20	10		
Acetone-dried anterior lobes			20	2.5
Extract in N. acetic acid	9.2	5	11.6	2.5
Supernatant after adsorption		>50		>50
Eluate in N/10 hydrochloric acid	0.144	0.1	0.320	0.05

The "direct adsorption method," as described here, was subsequently modified for use on fresh frozen sheep pituitaries. The frozen glands (water content approximately 80%) were rapidly broken into pieces of convenient size, and dropped into a macerator containing 320 ml of glacial acetic acid for each 100 g of frozen glands. The macerator was run at low speed until the lumps of frozen glands had sufficiently disintegrated, then at full speed until thorough maceration had been achieved (about 10 minutes). Heating, dilution and adsorption were then done essentially as in the original procedure, but it was found that centrifuging the dilute acid extract was preferable to sedimentation and that the addition of Super Cel was unnecessary and undesirable.

The method as used on acetone-dried pituitary was briefly described in September, 1951 (5), and was successfully applied by Sydnor and Sayers(6) to the extraction of corticotropin from blood.

Summary. A simple procedure is described whereby a good yield of highly active corticotropin can be obtained by direct adsorption from a crude pituitary extract. The method is equally suitable for pituitaries from sheep and from pigs.

1. Payne, R. W., Raben, M. S., and Astwood, E. B., *J. Biol. Chem.*, 1950, v187, 719.
2. Astwood, E. B., Raben, M. S., Payne, R. W., and Grady, A. B., *J.A.C.S.*, 1951, v73, 2969.
3. Gornall, A. G., Bardawill, C. J., and David, M. M., *J. Biol. Chem.*, 1948, v177, 751.
4. Sayers, M., Sayers, G., and Woodbury, L. A., *Endocrinology*, 1948, v42, 379.
5. Astwood, E. B., Rabin, M. S., and Payne, R. W., *Recent Progress in Hormone Research*, 1952, v7, 1.
6. Sydnor, K. L., and Sayers, G., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 432.

Received May 18, 1953. P.S.E.B.M., 1953, v83.

Mast Cell Resistance to Hormonal Influence.* (20352)

JAMES E. DEVITT, W. J. PIROZYNSKI, AND PETER B. SAMUELS.†
(Introduced by J. S. L. Browne.)
From the Department of Experimental Surgery, McGill University, Montreal, Canada.

Since their description by Ehrlich in 1879, the tissue mast cells have been a continual source of curiosity for histologists and physiologists. Over the years, these ubiquitous cells have been accused of some 30 different functions. Currently, evidence has been presented that these cells are controlled, or at least influenced, by endocrine glands, *viz.*: the pituitary-adrenal cortex(3,6,7) or pituitary-thyroid(1,2) systems. Only one of the papers (6) that have come to our attention records numerical counts to substantiate the author's opinion. Other workers have presented subjective impressions in lieu of statistically valid counts. The purpose of this study was to investigate tissue mast cell response to hormonal influence, utilizing numerical counts to indicate increased or decreased numbers of cells. The controversial field of tissue mast cell function will be avoided.

* Supported by a grant from the Cancer Research Society.

† U. S. Public Health Service Fellow.