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Preparation of Pituitary Adrenotropic Hormone.*

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This is a preliminary description of a relatively simple procedure devised for the isolation of purified and highly active preparations of adrenotropic hormone from whole hog pituitary glands. The extraction and fractionation procedures consist of the following steps: (1) Extraction and isolation of a fraction designated as crude prolactin by the procedure originally developed by Lyons¹ and adopted by White, Bonsnes and Long² as the first step for the subsequent preparation of crystalline prolactin. This "crude prolactin" contains adrenotropic hormone activity. (2) Solution of the crude prolactin preparation in 2% concentration at pH 9.0 and removal of precipitates which form by careful adjustment of pH to 8.0, 6.6 and 5.4. (3) Supernatant obtained in Step 2, after removal of pH 5.4 insoluble material (prolactin), is freed from traces of prolactin by the addition of 7 ml of saturated $(\text{NH}_4)_2\text{SO}_4$ to each 100 ml of solution. The precipitate is separated and discarded. (4) Acetone is added to the supernatant to a concentration of 80%. The adrenotropic fraction precipitates. (5) This precipitate is dissolved in water (volume used being two-thirds of that employed in Step 2) and the solution mixed with one-half its volume of concentrated NH_4OH . The ammoniacal solution is allowed to stand at room

temperature for 7 hours. (6) Acetone is then added to above solution to a concentration of 90%. (7) The precipitate is dissolved in one-third volume of water used in Step 2, and dialyzed to remove salt. (8) The adrenotropic fraction is precipitated by lowering of pH to 4.7. The precipitate may be dried by washing 3 times with acetone at the centrifuge and finally drying *in vacuo* over concentrated sulfuric acid. The yield of dry, purified adrenotropic fraction has been of the order of 500 mg from each 1000 g of whole hog pituitary glands.

It may be noted that the yield of the crude prolactin fraction is strikingly higher, approximately 3 to 4 times, from hog pituitary glands than from a similar weight of beef pituitary tissue.

The adrenotropic preparations have been assayed by two methods: (1) restitution of atrophied adrenals in 45-day-old, hypophysectomized, male rats, 10 days post-operative; and (2) maintenance of adrenal size in 45-day-old, male hypophysectomized rats. Using the first method of assay, a total of 5 mg of adrenotropic hormone preparation, given 3 times daily for 3 days, resulted in restoration of the adrenal size to that found in unoperated control animals of the same age. The maintenance method of assay showed that 0.05 mg of adrenotropic hormone preparation, given daily for 14 days beginning at the first day postoperative, was adequate for maintaining normal adrenal size. The adrenotropic preparations thus have a potency of the order of that of the preparation recently described by Li, Simpson and Evans.³ The assay data are summarized in Table I.

From preliminary data available at the present time, the purified adrenotropic hormone preparations give no definite evidence of

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¹ Lyons, W. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1936-37, **35**, 645; in *Cold Spring Harbor Symposia on Quantitative Biology*, Cold Spring Harbor, 1937, **5**, 198.

² White, A., Bonsnes, R. W., and Long, C. N. H., *J. Biol. Chem.*, 1942, **143**, 447.

³ Li, C. H., Simpson, M. E., and Evans, H. M., *Science*, 1942, **96**, 450.

TABLE I.
Assay of Adrenotropic Hormone Preparation in 45-day-old Hypophysectomized Male Rats.

Type of rat	No. of animals	Avg change in body wt,* g	Avg paired adrenal wt, mg	Avg paired testes wt, mg
Intact, normal	9		24.2 (19.3-30.0)†	2,266 (1,890-2,660)†
Hypophysectomized, uninj. control	13	—15	12.1 (9.0-15.8)	480 (234-700)
Hypophysectomized, inj. 3 times daily 3 days, total dose 5 mg A.C.T.,‡ beginning 10 days post-operative	9	—15	20.0 (16.5-29.9)	676 (390-1,130)
Hypophysectomized, inj. daily 14 days with 0.1 mg A.C.T. per day, beginning first day postoperative	12	—12	23.5 (20.0-26.3)	372 (154-573)
Hypophysectomized, inj. daily 14 days with 0.05 mg A.C.T. per day, beginning first day post-operative	9	— 7	20.8 (17.5-26.5)	285 (119-636)

*Between day of operation and time of autopsy.

†Figures in parentheses indicate range of values observed.

‡A.C.T. = adrenotropic hormone preparation.

the presence of the following types of pituitary activities: lactogenic, growth-promoting, gonadotropic, or thyrotropic. Assays for posterior pituitary principles indicate that one milligram of the adrenotropic preparation has a pressor and antidiuretic activity equivalent to approximately 0.6 unit of Pitressin (Parke-

Davis).⁴ The purified adrenotropic hormone fraction is homogeneous in the Tiselius apparatus, at four different hydrogen ion concentrations, and in the ultracentrifuge.

⁴ Sayers, G., Sayers, M. A., White, A., and Long, C. N. H., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 200.

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Effect of Pituitary Adrenotropic Hormone on Cholesterol Content of Rat Adrenal Glands.*

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In the course of studies of the physiological effects of adrenotropic hormone, striking decreases in adrenal cholesterol content were found to occur in normal 24-day-old male rats within 3 hours following administration of

2 mg of the hormone preparation described in the preceding paper.¹ Subsequently, the adrenal cholesterol concentration returns to normal within 24 hours after hormone injection. Continued injection of the adrenotropic hormone for 3 days raises the cholesterol content of the adrenals to values above those observed in adrenals of normal, uninjected rats.

The rats on which adrenal cholesterol data

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¹ Sayers, G., White, A., and Long, C. N. H., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 199.