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Assay of Adrenals for Lactogenic Hormone.*†

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Brownell, Lockwood and Hartman[1] postulated the existence of a specific lactation factor in the adrenals, "cortilactin," which they claimed was essential for normal lactation and separate from the life-sustaining factor. This material was separated from cortin by chilling out between 3 and −12° C. Spoor, Hartman and Brownell[2] precipitated out "cortilactin" isoelectrically from 86% alcohol at pH 5.8, following the method used by Riddle, *et al.*,[3] for extracting lactogenic hormone from pituitaries. The pigeon was used as an assay animal for this material, and an increase in crop gland weight served as a measure of its effect. They claimed that their best adrenal extracts were 1/10 as potent as a purified preparation of lactogenic hormone which contained 6 International Units (I.U.) per mg.

Inasmuch as this laboratory has been engaged in a study of the lactogenic hormone for a number of years, it was of interest to determine whether the adrenals contained true crop gland proliferating potency. The following materials were assayed: eschatin (Parke-Davis)‡ adrenal cortical extract (Upjohn),‡ whole macerated rabbit adrenals, and isoelectric precipitates of beef, hog and rabbit adrenals prepared by the Riddle, *et al.*,[3] method. The fresh beef and hog adrenals were secured from a local packing house. Only the beef adrenals were demedullated before extraction. All these materials were assayed by the highly sensitive intradermal "micro" pigeon test,[4, 5, 6] which enables one to measure quantitatively 1/160 I.U. of lactogenic

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1 Brownell, K. A., Lockwood, J. E., and Hartman, F. A., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 783.

2 Spoor, H. J., Hartman, F. A., and Brownell, K. A., *Am. J. Physiol.*, 1941, **134**, 12.

3 Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

‡ The two commercial adrenal cortical extracts were kindly furnished by the Parke-Davis and the Upjohn companies.

4 Lyons, W. R., and Page, E., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1049.

5 Bergman, A. J., Meites, J., and Turner, C. W., *Endocrinology*, 1940, **26**, 716.

6 Meites, J., Bergman, A. J., and Turner, C. W., *Endocrinology*, 1941, **28**, 707.

hormone. This "micro" assay technic, which is based upon an observed proliferation response in the crop gland rather than on an increase in crop gland weight, has made it possible to assay the minute amounts of lactogen present in the urine of women and men,[4, 7] goats,[8] and in the blood[9] and placentae[10] of rabbits.

It is obvious from the data (Table I) that the various adrenal preparations used in this experiment did not contain crop gland proliferating activity. The 3 positive responses which were obtained can be attributed to the occasional proliferations which are sometimes observed in untreated pigeons, presumably because they had recently been incubating eggs. In the case of the isoelectric precipitates of beef, hog and rabbit adrenals, injections were made only over one crop gland of each pigeon, leaving the other crop gland as a control. Regardless over which side the injections were made, the right crop glands weighed approximately 30-40% more on the average than the left crop glands. The same weight difference was found in 10 uninjected pigeons, indicating that no increase was obtained in crop gland weight.

It is difficult to account for the increase in crop gland weight which was obtained by Spoor, *et al.*,[2] with their "cortilactin," although it is possible that substances other than lactogen may produce increases in crop gland weight. In a thorough study involving 344 common

TABLE I.
Adrenal Substances Assayed for Lactogenic Activity in Common Pigeons.

Material	Amount of extract injected per bird	No. birds	Crop gland response
Eschatin (Parke-Davis)	10 dog units (.4 cc)	10	0 +, 10 —
Eschatin (Parke-Davis)	25 " " (1 ")	30	2 +, 28 —
Adrenal Cortical Extract (Upjohn)	50 " " (1 ")	10	0 +, 10 —
Whole Rabbit Adrenal	60 mg	10	0 +, 10 —
Whole Rabbit Adrenal	100 "	10	0 +, 10 —
Rabbit Adrenal Extract	equiv. 1.5 g fresh material	10	0 +, 10 —
Hog Adrenal Extract	equiv. 0.7 g fresh material	10	0 +, 10 —
Beef Cortical Adrenal Extract	equiv. 6.0 g fresh material	10	1 +, 9 —
Beef Cortical Adrenal Extract	equiv. 11.0 g fresh material	5	0 +, 5 —

[7] Meites, J., and Turner, C. W., *J. Clin. Endocrinology*, 1941, **1**, 918.

[8] Hurst, V., Meites, J., and Turner, C. W., *J. Dairy Sci.*, 1941, **24**, 499.

[9] Meites, J., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 190.

[10] Turner, C. W., and Meites, J., *Endocrinology*, 1941, **29**, 165.

pigeons, McShan[11] found that an increase in crop gland weight often did not indicate a positive lactogenic response, since such glands were visibly unproliferated. On the other hand, some crop glands which were visibly proliferated weighed no more than the crop glands of normal, uninjected pigeons. Since the proliferation of the crop gland mucosa, forming "crop milk", is a specific reaction to lactogenic hormone, it would seem that an assay method based upon such a response would be more accurate than one based on an increase in crop gland weight, which may not be specific for lactogenic hormone.

On the basis of the findings reported here, in which several types of adrenals were extracted by the same method used by Spoor, *et al.*, for "cortilactin", and despite the fact that a pigeon assay method at least 160 times as sensitive as that employed by the latter workers was used, it is concluded that the adrenals do not contain crop gland proliferating activity and therefore no lactogenic hormone.

Summary. Two commercial adrenal cortical extracts, whole untreated rabbit adrenals, and isoelectric precipitates of beef, hog and rabbit adrenals were assayed for lactogenic hormone by the sensitive intradermal "micro" pigeon crop gland technic. None of the adrenal compounds were found to contain lactogen.

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Synergism Between Thyrotropic and Growth Hormones of Pituitary. Body Weight Increase in Hypophysectomized Rat.*

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It has been shown that the body weight gain stimulated in rats by alkaline extracts of beef anterior piuitary could be increased by adding relatively small amounts of thyroxin.[1, 2] At these dose

[11] McShan, W. H., Ph.D. Thesis, 1936, University of Mo.

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[1] Smith, P. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1252.

[2] Evans, H. M., Simpson, M. E., and Pencharz, R. J., *Endocrinology*, 1939, **25**, 175.