

## Preparation and Biological Assay of Adrenocorticotropic Hormone.

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Adrenocorticotropic extracts† were tested in normal 21-day-old male rats and in hypophysectomized rats. The amount of adrenocorticotropic hormone necessary to cause an increase of 50% over the adrenal weight of controls when injected into 21-day-old male rats in 3 doses over a period of 3 days was defined as one normal rat unit. A total dose of 20 mg. was usually found to be equal to one unit.

The injections of large amounts of beef brain (1000 mg.) produced no significant change in adrenal weights of normal 21-day-old male rats. Adrenocorticotropic preparations from beef anterior pituitaries showed the same activity as adrenocorticotropic preparations from whole sheep pituitaries, indicating that posterior lobe substances were not causing the hypertrophy of the adrenal cortex in the normal male rats.

Male rats between 40 and 50 days old were hypophysectomized,‡ and at least 30 days were allowed for atrophy of the adrenals before injections were begun. The completeness of the operation was checked by examination of the sella turcica under dissecting binocu-

TABLE I.  
The Effect of Adrenocorticotropic Extracts in Hypophysectomized Rats.

Extract	Hypophysectomized Rat	Total Dose of Adrenocorticotropic Hormone,		Wt. of 2 Adrenals, mg.	Increase Over Controls		Wt. of Thyroids, mg.	Body Growth, gm.
		units	Length of Treatment, days		mg.	%		
M 126	W 63	5.1	10	19.9	9.4	89.5	—	2
	W 44	5.1	10	23.8	12.3	117.1	—	2
	GH 37	5.1	10	21.0	10.5	100.0	—	—8
M 133	BH 8421	5.3	10	22.0	11.5	109.0	9.5	0
	BH 8406	5.8	10	22.8	12.3	117.1	12.5	2
	W 8383	5.8	10	24.5	14.0	133.2	9.0	—3
Controls (7 rats)	0.0	0	10.5	—	—	13.1	—	
				(6.0-13.5)			(8.0-17.2)	

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† Adrenocorticotropic extracts were prepared by the method described by Dr. William R. Lyons in the accompanying paper. The precipitate obtained at pH 6.5 was the corticotropic fraction.

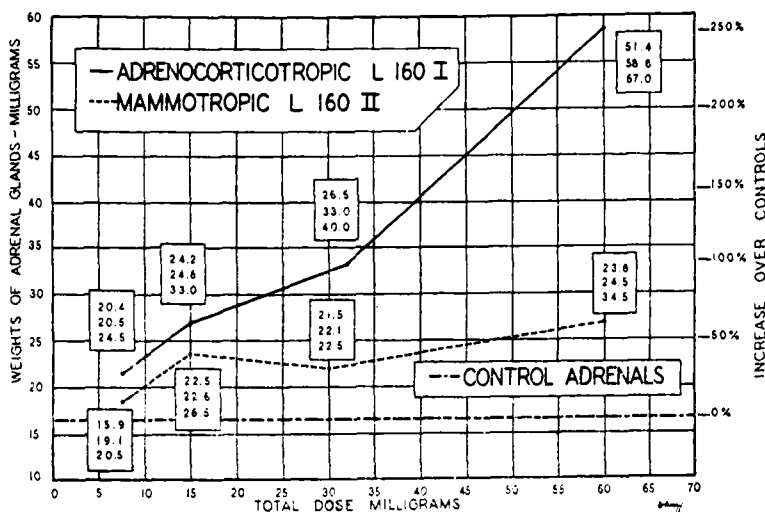
‡ The hypophysectomies were performed by Mr. L. L. Bennett.

lars. Table I shows the effect of adrenocorticotrophic extracts in hypophysectomized rats.

From the results obtained in hypophysectomized rats, it can be seen that the adrenocorticotrophic preparations contained no growth hormone at the level tested. The thyroids showed no histological evidence of stimulation.

Adrenocorticotrophic extracts when injected subcutaneously into squabs for 4 days showed no stimulation of the thyroids or of the testes. The crop glands were stimulated.

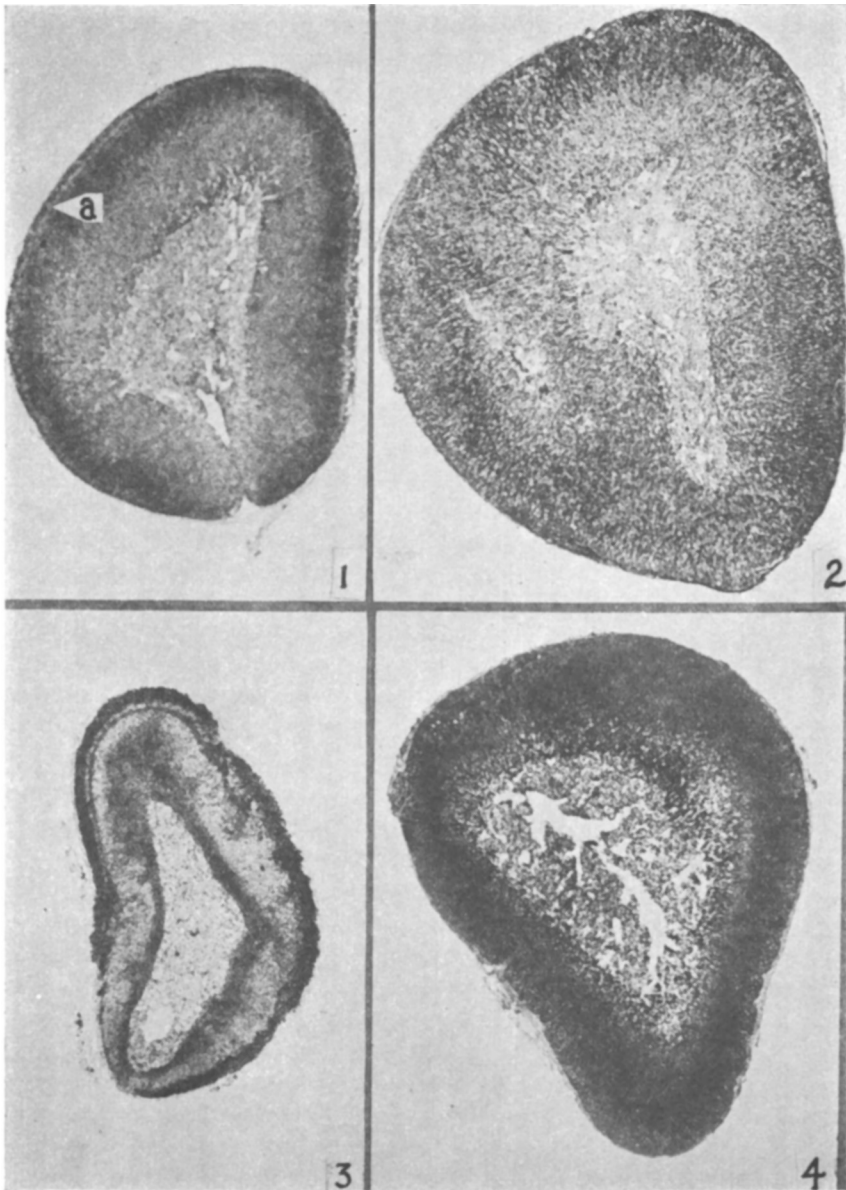
Adrenocorticotrophic and mammotropic fractions from the same extract were compared at various levels for their adrenocorticotrophic potencies in normal 21-day-old male rats. The results are given in Graph 1.



GRAPH 1.  
Effect of Adrenocorticotrophic and Mammothropic Fractions, Normal 21-Day-Old Male Rats.

The adrenal cortices of normal immature rats injected with adrenocorticotrophic hormones<sup>§</sup> (Fig. 2) differ markedly from the untreated controls (Fig. 1). There is an *increased* fat content throughout the whole adrenal cortex. In untreated controls there is an intermediate zone between the glomerulosa and the fasciculata in which there is very little or no fat. One of the earliest signs of stimulation is the presence of fat in the cells of this zone. In injected rats there is hypertrophy and hyperplasia of the cortical cells. The hyper-

<sup>§</sup> Work in progress indicates that the cortical hypertrophy obtained in immature rats with adrenocorticotrophic extracts is not affected by the removal of all thyroid tissue visible under dissecting microscopes.



## PLATE I.

Adrenals Stained with Sudan Black and Haematoxylin.

FIG. 1. Untreated normal 24-day-old male rat.  $\times 20$ . a. Fat-free zone between the zona glomerulosa and the zona fasciculata.

FIG. 2. Normal 24-day-old male rat injected with 2 units of adrenocorticotrophic hormone.  $\times 20$ .

FIG. 3. Untreated hypophysectomized rat.  $\times 20$ .

FIG. 4. Hypophysectomized rat injected with 5.8 units of adrenocorticotrophic hormone.  $\times 20$ .

plasia is limited to the cells of the zona glomerulosa and the outer zona fasciculata. There is a definite hyperemia proportional to the amount of adrenocorticotropic extract injected.

The adrenal cortices of hypophysectomized rats treated with adrenocorticotropic extracts (Fig. 4) show a restoration of the cytoplasm of the cortical cells. The nuclei of the cells are no longer pycnotic. There is a great increase in the fat content of the cortical cells.

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#### Studies on the Enumeration of Marine Anaërobic Bacteria.

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Considerable difficulty has been experienced in estimating the abundance of marine anaërobic bacteria although they have been demonstrated<sup>1</sup> in nearly all samples of water or mud examined. Most of the conventional procedures<sup>2</sup> such as the incubation of plates in anaërobic jars have failed to yield reproducible results and moreover, the use of such complicated, space- and time-consuming apparatus is entirely impracticable aboard a rolling boat at sea. The application of oval tubes as described by Anderson<sup>3</sup> for the enumeration of anaërobics has exceeded expectations.

Ordinary round glass tubes with sealed ends as used by Roux<sup>4</sup> and Burri<sup>5</sup> are satisfactory for the cultivation of anaërobics but the curvature of the glass makes it virtually impossible to count the colonies which develop, particularly when it is necessary to use a hand lens. The Kimball Glass Company fabricated special oval tubes for us with flat, parallel sides, thereby obviating this difficulty. The oval tubes are 6x14 mm. in cross-section and 380 mm. long with one end permanently sealed and the other end flared to facilitate the introduction of the medium. The tubes are sterilized in a pipet can. A test-tube containing 10 cc. of nutrient agar recently heated to nearly 100°C. to expel oxygen and cooled to 45° is inoculated

<sup>1</sup> ZoBell, C. E., and Anderson, D. Q., *Am. Assn. Petrol. Geol.*, 1936, **20**, 258.

<sup>2</sup> Hall, I. C., *J. Bact.*, 1929, **17**, 255.

<sup>3</sup> Anderson, D. Q., In press.

<sup>4</sup> Roux, E., *Ann. de l'Inst. Past.*, 1887, **1**, 49.

<sup>5</sup> Burri, R., *Centralbl. f. Bakt.*, Abt. 1, Orig., 1902, **8**, 533.