CORTICOTROPINS (ACTH)

X. Biological Investigations on α-Corticotropin

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Plates 29 to 30

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The isolation and structure of α-corticotropin, a polypeptide of 39 amino acid residues, derived from sheep pituitary glands and possessing an adrenocorticotropic (ACTH) activity of 150 I.U. per mg., have been reported from this laboratory (1–3). The present paper is concerned with various biological properties of the peptide hormone.

A. Stimulation of the Adrenal Glands in Normal and Hypophysectomized Rats

Experiments with Normal Rats.—The increment of adrenal weight in 21-day-old normal male rats induced by the administration of ACTH preparations has been suggested by Moon (4) as a criterion for the assay of ACTH potency. Because various substances and conditions will produce adrenal hypertrophy in the intact animal, this criterion is not specific, and hence, as has been discussed elsewhere (5), this type of assay does not furnish a reliable index of degree of biological potency. It is, however, useful for detecting activity in a preparation, since adrenocorticotropically active preparations should be capable of increasing the adrenal weight in normal animals.

The hormone peptide was suspended in peanut oil and 5 per cent beeswax according to the procedure described by Bruce, Parkes, and Perry (6). Male rats, 28 days of age, of the Long-Evans strain were injected intraperitoneally with 0.1 ml. of the hormone suspension once daily for 4 days, and the animals were autopsied 24 hours after the last injection. As Table I reveals,[1] a daily dose of 15 μg of α-corticotropin for 4 days produced an increase in

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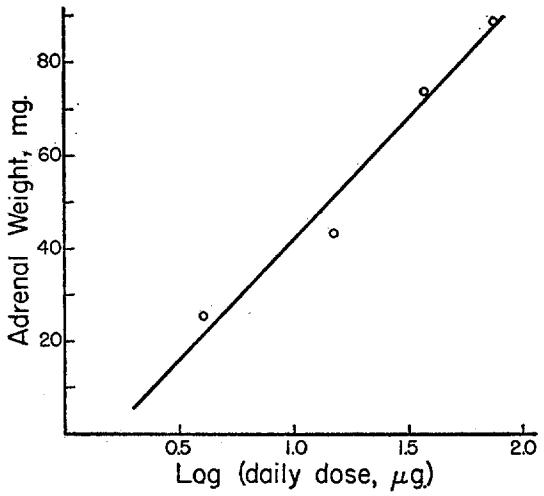
[1] It may also be noted that the gain in body weight during the 4 day injection period decreases as the dosage of α-corticotropin increases. This growth-inhibiting effect of α-corticotropin will be discussed on page 343.

adrenal weight of nearly 100 per cent, and an 80 per cent reduction of thymus weight. It is of interest to note that a dose of 4 μg. of the peptide hormone administered daily over a 4 day period did not result in any significant change in the adrenal weight, whereas the same daily dose produced marked reduction of thymus weight. The observation that the involution of



TEXT-FIG. 1. Increment of the adrenal weight as the function of α-corticotropin dosage in immature male rats.

TABLE I

Effect of α-Corticotropin on the Adrenal Weight of Immature Male Rats

Daily dose	No. of rats*	Body weight		Adrenals	Thymus
		Onset	Autopsy		
μg.		gm.	gm.	mg.	mg.
0	34	64 ± 3‡	86 ± 2	23.5 ± 0.8	240.9 ± 13.7
4.0	17	67 ± 2	83 ± 3	25.1 ± 1.3	134.1 ± 14.3
15.0	11	66 ± 4	75 ± 3	43.1 ± 1.7	42.2 ± 3.9
37.5	38	66 ± 3	72 ± 2	73.8 ± 2.9	33.0 ± 1.5
75.0	12	60 ± 3	64 ± 4	88.9 ± 6.4	24.4 ± 2.4

* 28 day old male rats.

‡ Mean ± standard error.

the thymus gland is a more sensitive indicator of ACTH activity than is the increment of adrenal weight in normal rats is in accord with earlier findings (7, 8).

It can been seen in Text-fig. 1 that the weight of adrenal glands induced by α-corticotropin is a function of the daily dosage of the hormone employed:

$$A = 51.3 \log D - 9.4 \qquad (1)$$

in which A is the adrenal weight in mg. and D the daily dose in micrograms; this assay procedure has an index of precision (λ) of 0.31.

Experiments with Hypophysectomized Rats (with H. D. Moon and A. Lostroh).—Once it had been shown by Smith (9) that atrophy of the adrenal cortex follows hypophysectomy of the rat, and that pituitary implants will restore the adrenal cortex, a method was available for measuring the ACTH activity of pituitary extracts. The obvious advantage in using hypophysectomized animals for the bioassay of ACTH preparations is that, through the removal of the pituitary gland, all so called non-specific effects are eliminated; consequently, any effects that are noted can be ascribed wholly to the action of the injected material. As early as 1933, Collip *et al.* (10), proposed an assay for adrenal-stimulating substances using the hypophysectomized rat. Estimates of potency were to be based on a comparison made between the weight

TABLE II

Effect of α-Corticotropin on Adrenal and Thymus Weights of Hypophysectomized Male Rats

Daily dose	No. of animals*	Body weight at autopsy	Adrenal	Thymus
μg.		*gm.*	*mg.*	*mg.*
0	21	117 ± 2‡	11.2 ± 0.2	264 ± 7
1.5	4	121 ± 6	15.5 ± 0.7	248 ± 13
3.8	9	123 ± 4	20.8 ± 0.6	110 ± 10
7.5	8	120 ± 4	23.9 ± 1.1	82 ± 7
15.0	11	138 ± 5	29.5 ± 1.4	117 ± 16
22.5	11	139 ± 6	31.3 ± 1.3	78 ± 10

* Operated on at 40 days of age; intraperitoneal injections (0.1 ml.), begun 4 days later, were administered once daily for 4 days; and autopsy was performed 24 hours after the last injection. The hormone was suspended in beeswax-peanut oil.

‡ Mean ± standard error.

of one adrenal removed prior to treatment and the weight of the remaining adrenal subjected to the influence of the ACTH preparation. Later, other investigators (5) employed hypophysectomized rats for the standardization of ACTH preparations on the basis of either partial repair of the atrophied adrenals, or of maintenance of normal adrenal weight by injections initiated immediately after hypophysectomy.

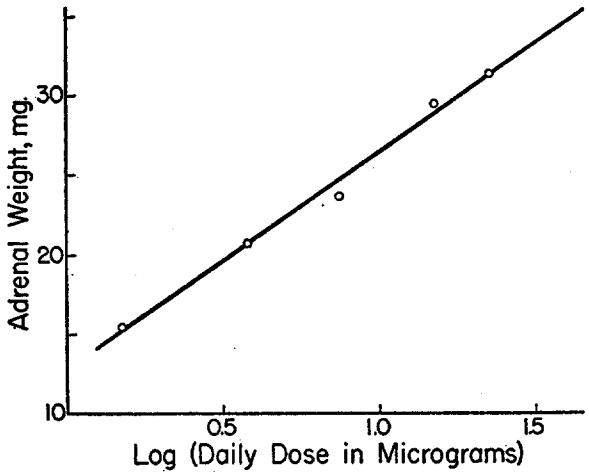
In the present experiments, male rats of the Long-Evans strain, hypophysectomized at 40 days of age, were injected intraperitoneally with α-corticotropin in beeswax-peanut oil suspension (0.1 ml.), once daily for 4 days, beginning 4 days after hypophysectomy. Twenty-four hours after the last injection, the animals were autopsied; the adrenal and thymus glands were dissected and weighed, and the adrenals were fixed in formalin or Zenker-formol. Paraffin sections, 7 μ thick, were prepared and stained with hematoxylin and eosin. Frozen sections, 10 μ thick, were stained with oil-red O and hematoxylin.

Table II shows the response of the adrenals to graded doses of α-corticotropin. It can be seen that a daily dose of 7.5 μg. in 4 days gave a 100 per cent

increment of adrenal weight over the control; a straight line relationship exists between the weight of the adrenal glands and the logarithmic function of the daily dose as shown in Text-fig. 2. By the method of least squares, this relationship may be expressed by the following equation:

$$A = 13.7 \log D + 12.8 \quad (2)$$

in which A is the adrenal weight in milligrams and D the daily dose in micrograms. The index of precision (λ) was calculated to be 0.26. Thus, according to this equation, a dose of 5 μg. of α-corticotropin administered once daily over a 4 day period would cause an increase in the adrenal weight of hypophysectomized male rats (40 days old at operation and 4 days postoperatively) of from 11.2 to 22.4 mg. As would be expected, the thymus glands of the rats treated with α-corticotropin were smaller than those of the controls. It is of interest to note that the reduction of thymus weight is more marked in normal rats (Table I) than in hypophysectomized animals (Table II).



TEXT-FIG. 2. Increment of the adrenal weight as the function of α-corticotropin dosage in hypophysectomized male rats.

The microscopic changes in the adrenal glands that follow the administration of α-corticotropin can be seen in Figs. 1 through 8. The adrenal cortices of the hypophysectomized controls were composed of small cells with small nuclei. The junction between the zona glomerulosa and zona fasciculata was devoid of lipide, but small amounts of lipide were present in the remainder of the cortex. The cells of the adrenal cortices of the hypophysectomized rats injected with α-corticotropin were larger than those of the controls. Cortical hyperemia was manifested by dilatation of the sinusoids. With the lower dosages, lipide appeared in the cells of the junctional zone; in addition, the large intracytoplasmic lipide-globules characteristic of the fasciculata and

reticularis of the hypophysectomized animal were replaced by fine droplets. At higher dose levels the cells of the adrenal cortex increased progressively in size, and there was some decrease in the staining reaction for lipide. These histological patterns are similar to those previously described by other investigators (11) for ACTH-stimulated adrenal glands of hypophysectomized rats.

B. Effect on the Level of Circulating Eosinophils in Hypophysectomized Rats

(with G. F. Hungerford and W. O. Reinhardt)

The level of circulating eosinophils falls within a few hours after stimulation of the adrenal glands or after the administration of adrenal cortical steroids. This phenomenon has been employed as a basis for the assay of adrenal steroids in adrenalectomized mice (12) and of ACTH in normal mice (13). An eosinopenia has also been reported following the administration of ACTH preparations to hypophysectomized rats (14). However, good dose-response curves were not obtained with these earlier ACTH preparations when they were assayed on the basis of eosinopenic response. Hence, it was of interest to investigate the effect of α-corticotropin in this regard. It will be seen that assays performed on the basis of the eosinopenic response of hypophysectomized rats to α-corticotropin exhibit satisfactory dose-response relationships below the 10 μg. dose level.

Eosinophil counts were made daily for the first 5 days after operation on hypophysectomized male rats of the Long-Evans strain, weighing 200 to 350 gm. If the hypophysectomized animals were not used for corticotropin assay within 2 days after hypophysectomy, it was found necessary to stimulate the adrenal glands with a single injection of a potent ACTH preparation 24 hours before the assays were performed in order to insure responsiveness to the effects of the α-corticotropin.

All blood counts were made from tail vein blood. The Spiers-Meyer diluent (15) was used for the direct eosinophil counts. A dilution of 1:20 was made and the counts were performed on a Fuchs-Rosenthal hemocytometer 0.2 mm. deep, two chambers being counted for each sample. An eosinophil count was taken prior to injection of the test substance, and another was taken 4 hours later; the change between the two counts was calculated as a percentage of the initial value. If an initial count was lower than 100 eosinophils per mm^3 of blood, the animal was not used. Total white blood counts were made using a 1.0 per cent acetic acid diluent. A 1:20 dilution was made and counts were performed on a Levy hemocytometer. Blood smears were made and stained with Giemsa; between 100 to 200 cells were counted. From these values, the total number of lymphocytes (mononuclear leucocytes) was calculated. The α-corticotropin samples were dissolved in a 0.9 per cent saline solution, and a crystalline suspension of hydrocortisone acetate (Merck) was used for comparative assay. Control animals received a 0.9 per sent saline solution alone. All injections were given intraperitoneally.

Table III presents the percentage of change in eosinophil counts after the administration of graded doses of two preparations of α-corticotropin.[2] Al-

[2] Preliminary experiments indicate that α-corticotropin does not exhibit an eosinopenic effect in hypophysectomized-adrenalectomized rats.

though higher doses were given, the response was found to be maximal at the 10 μg. dose level. The dose-response curve could be plotted as a straight line. It is also interesting to note that α-corticotropin at a dose level of 2 μg. is as effective in drepressing the number of circulating eosinophils as 100 μg. of hydrocortisone in crystalline suspension.

When the effect of α-corticotropin on the number of circulating lymphocytes was investigated at one dose level (100 μg.), the total number of blood

TABLE III

Effects of α-Corticotropin and Hydrocortisone on Blood Eosinophils of Hypophysectomized Rats

Test substance	Dose	No. of rats	Change in eosinophil levels
	μg.		*per cent*
α-Corticotropin (CC4H′-D)	10	8	−89 ± 5*
	7	6	−85 ± 3
	5	9	−76 ± 3
	3	9	−51 ± 10
	1	10	−23 ± 8
Saline	0.5 cc.	19	−2 ± 5
α-Corticotropin (CC8H′-2)	5.0	12	−70 ± 6
	3.5	13	−54 ± 13
	2.0	19	−41 ± 10
	0.5	6	−25 ± 10
Saline	0.5 cc.	22	8 ± 7
Hydrocortisone	1000	10	−95 ± 2
	250	9	−82 ± 8
	100	10	−39 ± 13

* Mean ± standard error.

lymphocytes was found to be significantly decreased (−44 ± 5 per cent in 9 rats as compared with +38 ± 16 per cent in 5 rats of the saline control group).

C. Effect on the Muscle Glycogen of Hypophysectomized Rats

(with P. Fønss-Bech)

In earlier investigations, no effect with respect to maintenance of muscle glycogen in hypophysectomized animals was found to be exercised by ACTH concentrates (16, 17). However, it will be seen that α-corticotropin is highly active in this respect in fasted hypophysectomized animals.

Male rats of the Long-Evans strain, weighing 280 to 300 gm., were hypophysectomized 2 weeks prior to the experiment. Some were adrenalectomized in addition, 5 days prior to the

experiment; the doubly operated animals were maintained with daily injections of either 0.5 mg. of hydrocortisone acetate or 1.0 mg. of desoxycorticosterone acetate per animal. All animals were fasted for 24 hours before autopsy, and the α-corticotropin, as a beeswax-peanut oil suspension, was administered intraperitoneally 16 hours prior to sacrifice. Analytical procedures were similar to those described by Russell and Wilhelmi (18).

TABLE IV

Effect of α-Corticotropin on Muscle Glycogen of Hypophysectomized Male Rats Fasted for 24 Hours

Dose*	Muscle glycogen	
	Without hydrocortisone	With hydrocortisone‡
μg.	*mg. per cent*	*mg. per cent*
0	278 ± 9.7 (5)§	414 ± 19.9 (5)
1.5	307 ± 14.4 (5)	464 ± 11.6 (4)
7.5	370 ± 17.0 (5)	559 ± 29.6 (4)
37.5	405 ± 11.1 (5)	588 ± 34.1 (4)

* Dose per 100 gm. body weight.

‡ 0.1 mg. hydrocortisone acetate injected daily for 7 days prior to sacrifice.

§ Mean ± standard error (No. of rats).

TABLE V

Effect of α-Corticotropin on Muscle Glycogen of Hypophysectomized-Adrenalectomized Rats Fasted for 24 Hours

Dose*	Muscle glycogen	
	Hydrocortisone‡	Desoxycorticosterone§
μg.	*mg. per cent*	*mg. per cent*
0	453 ± 29.3 (4)‖	683 ± 18.8 (9)
50	426 ± 41.7 (5)	625 ± 17.0 (9)

* Dose per 100 gm. body weight.

‡ Daily doses of 0.5 mg. hydrocortisone acetate begun on the day of adrenalectomy and administered for 5 days prior to sacrifice.

§ Daily doses of 1.0 mg. desoxycorticosterone acetate begun on the day of adrenalectomy and administered for 5 days prior to sacrifice.

‖ Mean ± standard error (No. of rats).

Inspection of the data, presented in Table IV, obtained with hypophysectomized rats shows that without hydrocortisone, α-corticotropin in a single dose of 7.5 μg. per 100 gm. body weight prevents significantly a decrease of muscle glycogen. When the animals were pretreated with 0.5 mg. of hydrocortisone daily for 7 days before sacrifice, the dose of α-corticotropin required for an increment of the muscle glycogen is much smaller; namely 1.5 μg. This may be taken to mean that the presence of hydrocortisone enhances the myoglycostatic effect of α-corticotropin. It may be noted further that, with

or without pretreatment with hydrocortisone, the level of muscle glycogen is elevated in proportion to the increase in the dosage of α-corticotropin. In rats that were adrenalectomized as well, the peptide hormone was without effect (Table V). It is of interest to note that the steroid hormones (hydrocortisone and desoxycorticosterone) used for maintenance of the latter animals, themselves caused an elevation of the muscle glycogen levels of both hypophysectomized and hypophysectomized-adrenalectomized rats (Tables IV and V). Earlier studies have shown that corticosterone and cortisone, as well as crude cortical extract, are capable of preventing the depletion of muscle glycogen in rats after hypophysectomy (16, 19, 20).

TABLE VI

Antagonism between α-Corticotropin and Somatotropin as Evidenced by Tibia Test

Group*	Total dose	Width of uncalcified cartilage plate	Adrenals	Thymus
	μg.	*μ*	*mg.*	*mg.*
Control	0	163	7.9	164
Somatotropin	60	244	8.3	176
α-Corticotropin‡	100	148	11.8	87
Somatotropin + α-Corticotropin‡	60 + 100	251	12.4	109
α-Corticotropin§	100	116	19.3	44
Somatotropin + α-Corticotropin§	60 + 100	126	20.9	44

* 10 animals per group.

‡ Administered intraperitoneally in aqueous solution.

§ Administered subcutaneously in peanut oil–beeswax suspension.

D. Effect on Growth in Hypophysectomized and Hypophysectomized-Gonadectomized Rats

The early observation of Moon (21) on the retardation of the somatic growth of young castrated male rats by crude ACTH preparations has been confirmed by various investigators (22) using partially purified ACTH concentrates. It is now known that α-corticotropin is a potent growth inhibitor, as evidenced by its antagonistic action to the growth-promoting activity of somatotropin (growth hormone) in hypophysectomized and hypophysectomized-gonadectomized rats.

Effect on the Tibia (with I. I. Geschwind).—

Hypophysectomized female rats of the Long-Evans strain (26 to 28 days of age at operation), were injected 12 to 13 days postoperatively, once daily for 4 days. Twenty-four hours after the final injection, the animals were autopsied, the tibias excised, and the uncalcified portion of the proximal epiphyseal cartilage of the tibia was stained with silver nitrate as previously described (23). In some experiments, the α-corticotropin was mixed with somatotropin (isolated from anterior lobes of bovine pituitaries by the published method (24)) in

aqueous solution which was then administered intraperitoneally, and in others, the peptide hormone alone was injected subcutaneously in beeswax-peanut oil suspension.

The results are summarized in Table VI; these experiments have demonstrated that in aqueous solution, very high doses of α-corticotropin, either alone or in conjunction with growth hormone, had little effect on the width of the tibial cartilage, despite the fact that significant involution of the thymus did occur. When administered in a delaying medium, namely, beeswax-peanut oil, α-corticotropin causes a very marked thymic involution, a greater increase in adrenal weight, a highly significant decrease in the control cartilage width, and an almost complete inhibition of the response to growth hormone.

Effect on Body Growth (with T. Hayashida).—

TABLE VII

Effect of α-Corticotropin, Somatotropin and Their Combination on Adrenal, Thymus, Spleen, and Body Weights of Hypophysectomized-Ovariectomized Rats

Experiment*	Daily dose	Body weight			Adrenal	Thymus	Spleen
		Onset	Autopsy	Changes			
	mg.	*gm.*	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*
Control	0	135.0	134.0	−1.0	8.3	208	501
Somatotropin	0.300	136.0	160.3	+24.3	13.4	418	652
α-Corticotropin	0.018	141.0	129.5	−11.5	28.7	Nil	251
Somatotropin + α-Corticotropin	0.300 + 0.018	140.0	139.1	−0.9	29.9	Nil	450

* Female rats (45 to 50 days old) hypophysectomized 2 days after ovariectomy; injections begun 5 days after hypophysectomy and continued daily for 10 days; 7 animals in each group.

Female rats of the Long-Evans strain were ovariectomized at the age of 48 days and hypophysectomized 2 days later; subcutaneous daily injections of α-corticotropin (18 μg.) and growth hormone (300 μg.) were begun 5 days after hypophysectomy and were continued for 10 days. The animals were maintained on a standard enriched diet[3] in dry powder form,

[3] *Diet composition:* *per cent*

Ground whole wheat	67.5
Casein, tech	15.0
Skim milk powder	7.5
Sodium chloride	0.75
Calcium carbonate	1.5
Melted fat (hydrogenated vegetable oil)	6.75
Fish oil (sardilene) 750 units vitamin A and 300 units vitamin D per gm.)	1.0

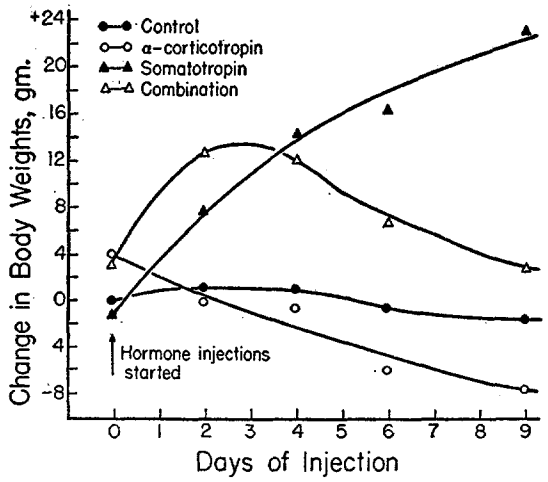
Approximate composition: *per cent*

Protein	24.5
CHO	54.9
Fat	8.9

Sufficient KI solution is added to furnish 0.9 μg. of iodine per gm. of diet.

given *ad libitum*. They were weighed every 2nd day, and autopsy was performed 24 hours after the last injection. Growth hormone was administered subcutaneously in aqueous solution and α-corticotropin was given in the form of a 5 per cent beeswax-peanut oil suspension.

It can be seen from Table VII and Text-fig. 3 that the animals given α-corticotropin alone lost 11 gm. of body weight in 10 days, whereas the controls lost 1 gm. during the same period. The animals treated with somatotropin alone gained at the rate of about 2.5 gm. per day. When α-corticotropin was administered together with somatotropin, the growth-promoting effect of the latter was almost completely neutralized by a daily dose of 18 μg. of the



TEXT-FIG. 3. The influence of α-corticotropin on body weight of hypophysectomized-ovariectomized rats. Daily dosages: α-corticotropin, 0.018 mg.; somatotropin, 0.300 mg. The body weight of the rats in all experimental groups at beginning of injections is in the range of 137 to 141 gm.; 7 rats per group.

former. In each of the groups, treated with α-corticotropin or with α-corticotropin plus somatotropin, complete involution of the thymus gland was noted. It is of interest that not only is marked involution (50 per cent) of the spleen[4] produced by the administration of α-corticotropin at 18 μg. per day, but also the usual stimulating effect exercised by growth hormone on the spleen is counteracted by the simultaneous injection of α-corticotrophin.

E. Melanocyte-Stimulating Activity of α-Corticotropin

(with I. I. Geschwind)

In previous publications from this laboratory (25, 26) it has been demonstrated that most of the melanocyte-stimulating activity found in crude

[4] The histological changes produced in the spleen and adrenal glands by the combined effect of somatotropin and α-corticotropin will be reported elsewhere.

corticotropin concentrates can be separated from the adrenal-stimulating activity by chromatographic and electrophoretic methods. For example, in one of the steps in the procedure for the isolation of α-corticotropin (2) these two activities are resolved in the main during chromatography on the XE-97 cation exchange column, where the activity associated with the melanocyte-stimulating hormone (MSH; intermedin) passes through the column unretarded. Hence, there seems little question that MSH is not identical with corticotropin. It was also pointed out, however, that it had not been found possible to separate completely the melanocyte-stimulating activity from the corticotropic activity in ACTH preparations. Moreover, the association between these two activities has persisted in the pure corticotropin preparations obtained by means of the countercurrent distribution procedure. A

TABLE VIII

Melanocyte-Stimulating Activity of α-Corticotropin Preparations

Preparation	No. of assays*	Activity
		units/gm.
8H′2	4	0.24×10^8
10H′B_2-D	1	1.01×10^8
21H′B_2	1	0.80×10^8
34H′B_2	1	0.64×10^8
42H′B_2-D	3	0.28×10^8
52H′B_2-D	7	0.40×10^8
55H′B_2-D	1	0.80×10^8

* Each assay was performed on 2 to 4 pieces of frog skin at 2 different dose levels.

number of such preparations have been extensively assayed by the method of Shizume *et al.* (27), with isolated frog skin used as the substrate. The results of these assays, presented in Table VIII, indicate that activities ranging from 0.25 to 1.0×10^8 units per gram[5] were to be found in all preparations tested, with an average activity of 0.60×10^8 units/gm. The variable activity observed is, in all probability, due to a partial inactivation which occurs during the countercurrent distribution process.

It has also been reported (25, 26) that brief treatment of crude corticotropin preparations with alkali at elevated temperatures effects a potentiation of their melanocyte-stimulating activity at the same time as a pronounced loss of their adrenal ascorbic acid–depleting activity. To determine the potentiating effect on α-corticotropin of treatment with alkali, the hormone was heated for 5 to 10 minutes in a boiling water bath, in a solution which was 0.1 N with respect to NaOH. Multiple assays with preparations 42H′B_2-D and 52H′B_2-D demonstrated a threefold potentiation of activity to have taken place.

[5] The unit is that defined by Shizume *et al.* (27).

α-Corticotropin is derived from sheep pituitaries; the ovine melanocyte-stimulating hormone has not as yet been isolated and little is known regarding the effect of alkaline heat treatment on the intermediate lobe hormone derived from this species. Moreover, no generalizations from one species to another can be made since porcine MSH is not potentiated by alkali whereas the bovine hormone is (28). Consequently, in the absence of any data on sheep MSH, the activity of α-corticotropin can only be compared with the sole MSH that has been thoroughly studied; *i.e.*, the porcine hormone. The specific activity of the latter hormone (29) is 100 or more times more active as a melanocyte stimulator than is α-corticotropin. Nevertheless, the high and comparatively constant melanocyte-stimulating activity present in α-corticotropin preparations reinforces the probability that it is an inherent activity of this hormone. The probable basis for this effect, in terms of chemical structure, has recently been presented (29).

F. Fat-Mobilizing (Adipokinetic) Activity of α-Corticotropin

(with I. I. Geschwind and M. Sideman)

It has been known for about 20 years that injection of extracts of the anterior pituitary gland causes an increase in liver lipides, by mobilizing lipide from the body depots (30). Early experiments on the effects of injection of crude corticotropin preparations have been summarized by Levin and Farber (31), who were of the opinion that growth hormone, and not corticotropin, was the pituitary factor involved. Recently, Rosenberg (32) demonstrated that oxycellulose-purified corticotropin concentrates possessed marked adipokinetic activity. However, since the effect was discernible in the adrenalectomized animal (maintained on steroid therapy), confirming a finding first reported by Payne (33), Rosenberg suggested that the activity might reside in a contaminating adipokinin, rather than being an extra-adrenal function of the corticotropin molecule.

The adipokinetic activity of a number of hormones has been determined over a period of several years in this laboratory. All results suggest that adipokinetic activity[6] is shared by a number of different hormones, and in each case responds to chemical or enzymatic modification in the same way as the characteristic activity of the hormone in question, suggesting that there exist many different "adipokinins." Herein is reported the data concerned with the adipokinetic activity of α-corticotropin.

Adipokinetic activity was measured according to the procedure of Campbell (34). Virgin female mice (Swiss white) weighing 17 to 20 gm. were fasted for 7 hours. All preparations

[6] Adipokinetic activity refers to the ability to increase the amount of fat in the liver within a short period of time. How this is accomplished—by mobilization of fat from depots, by decreased oxidation or by increased lipogenesis—is not taken into account, nor is the chemical composition of the fat.

(in beeswax–peanut oil suspension) were administered intraperitoneally at the beginning of the period of fast and the animals were sacrificed 7 hours later. Their livers were then removed, washed, and freeze-dried. Liver lipides were determined by a modification (32) of the procedure of Folch *et al.* (35).

The results obtained with a typical corticotropin preparation are presented in Table IX. It may be seen that as little as 0.5 μg. elicits a highly significant increase in liver lipide during a 7 hour fast. Furthermore, it is evident that a positive correlation exists between the dose of hormone administered and the amount of lipide in the liver. By way of comparison, it has been found that 8 to 16 times as much of growth hormone is required to produce a similar response.

In order to find out whether or not the adipokinetic effect of corticotropin can still be observed in the absence of the adrenals, a series of experiments was performed on animals

TABLE IX

Adipokinetic Effect of α-Corticotropin in Fasted Mice

Dose	No. of animals	Amount of fat in fat-free dry liver*
μg.		*per cent*
0	7	27.6 ± 2.5‡
0.5	7	39.9 ± 2.7
2.5	6	52.0 ± 2.5
10.0	7	58.4 ± 4.4

* $\frac{\text{Weight of total lipide}}{\text{Weight of dry liver} - \text{weight of total lipide}} \times 100.$

‡ Mean ± standard error.

adrenalectomized 3 days prior to the experiment. Some of these animals received no maintenance therapy with sterioids, while others were maintained with daily subcutaneous injections of 0.1 to 0.2 mg. of hydrocortisone acetate. All operated animals were fed a diet of bread and milk, and saline solution. The results of experiments employing such animals confirmed (Table X) the findings of Payne (33), Levin and Farber (31), and Rosenberg (32) that corticotropin preparations show no adipokinetic effect in adrenalectomized animals not maintained with cortical hormones. On the other hand, maintenance of such animals with adrenal steroids permitted the development of an adipokinetic effect. In no case, however, was the response as great in the maintained adrenalectomized animal as it was in the normal animal.

Thus, the adipokinetic effects demonstrated by more crude preparations have also been observed in pure α-corticotropin, a hormone whose structure has been established (3). However, since there would still seem to be some question as to whether the adipokinetic activity is inherent in the corticotropin molecule, a series of experiments was performed with corticotropin preparations that had been submitted to peptic and chymotryptic hydrolysis. It has been previously demonstrated (3, 36) that limited digestion with pepsin alters very little the adrenal-stimulating function of corticotropin. On

TABLE X

Effect of Adrenalectomy on Adipokinetic Action of α-Corticotropin in Fasted Mice

Experiment	Animals*	Dose	No. of animals	Amount of fat in fat-free dry liver
		μg.		*per cent*
	N	0.0	12	34.5 ± 2.6‡
	N	0.5	8	45.0 ± 2.8
I	A	0.5	4	36.1 ± 5.0
	A§	0.0	12	34.1 ± 2.9
	A§	0.5	5	40.7 ± 5.8
	N	0.0	7	33.7 ± 2.6
	N	1.5	8	57.4 ± 4.6
II	A	1.5	4	32.1 ± 2.3
	A§	0.0	6	33.8 ± 1.3
	A§	1.5	6	50.1 ± 1.3

* N, normal; A, adrenalectomized.

‡ Mean ± standard error.

§ Maintained with 0.1 to 0.2 mg. of an aqueous suspension of hydrocortisone acetate per day for 4 days.

TABLE XI

Effect of Limited Peptic and Chymotryptic Digestion on Adipokinetic Activity of α-Corticotropin

Experiment	Treatment	Dose	No. of animals	Amount of fat in fat-free dry liver
		μg.		*per cent*
	None	0	7	38.2 ± 1.8
	"	20	6	71.5 ± 5.8
I	Pepsin*	20	5	47.7 ± 2.7
	"	50	6	68.0 ± 2.7
	None	0	4	30.0 ± 1.9
	"	50	6	74.8 ± 6.2
II	Chymotrypsin‡	20	6	34.5 ± 3.3
	"	50	5	37.4 ± 4.6

* Hydrolyzed with pepsin (ratio of enzyme/substrate by weight = 1:300) for 2 hours at 25°C.

‡ Hydrolyzed with chymotrypsin (ratio of enzyme/substrate by weight = 1:300) for 3 hours at 25°C.

the other hand, even limited chymotryptic digestion results in an extensive loss of such activity. The results presented in Table XI demonstrate that limited digestion with pepsin alters the adipokinetic activity of corticotropin only slightly—proportionately to the small decrease in the adrenal ascorbic acid–

depleting activity. On the other hand, chymotryptic digestion abolishes almost entirely both the adipokinetic and ascorbic acid–depleting activities of corticotropin.

It is interesting to compare these results with unpublished data on the effect of enzymatic digestion on the adipokinetic activity of growth hormone preparations. When conditions of digestion identical with those reported above are employed, it has been found that an almost complete loss of adipokinetic activity occurs following treatment with pepsin, whereas chymotryptic digestion leaves this activity intact. In the case of this hormone, it has been previously established (37) that peptic digestion causes complete loss of growth-promoting activity, whereas limited chymotryptic digestion, such as that employed here, has very little effect on the functions of the hormone as a growth stimulator. Thus, the adipokinetic activities in α-corticotropin and in growth hormone respond in an opposing fashion to treatment with these two enzymes; this furnishes a further illustration of the observation, noted above, that when a hormone is subjected to chemical treatment, the adipokinetic activity varies in accordance with the major activity associated with that particular hormone.

The entire body of these results support the contention that an adipokinetic function is an inherent activity of corticotropin. The hormone apparently serves a dual purpose by first stimulating the adrenal to secrete steroids, which then serve as "permissive" agents to potentiate the extra-adrenal adipokinetic effect of the hormone.

G. Effect on the Accessory Sex Glands of Castrated Hypophysectomized Rats

(with A. Lostroh)

Davidson and Moon (38) were the first to report that crude ACTH preparations stimulated the release of androgen(s) from the adrenal glands of immature Long-Evans male rats, as evidenced by the growth of the sex accessories of castrated animals. Under these same conditions, no stimulation was observed in castrated-adrenalectomized animals (39), demonstrating that the effect was not an extra-adrenal one but was mediated through the adrenal glands. Although Nelson confirmed these findings (40), the negative reports found in the later literature suggest that this stimulation may not be produced by more purified ACTH preparations (41). Recently, we have reinvestigated this problem in hypophysectomized-castrated male rats of the Long-Evans strain and have found that ACTH is the only pituitary hormone that is directly responsible for the androgen production (42). Inasmuch as the ACTH preparation used in the earlier investigation could be further purified, it remained to be determined whether or not similar results could be demonstrated with pure α-corticotropin.

Male rats of the Long-Evans strain were castrated at 43 days of age and hypophysectomized at 44 days. Some of the animals were also adrenalectomized; these animals were maintained on 1 per cent NaCl solution. For the triple operation, the testes and the right adrenal were removed at 43 days, and the left adrenal was removed on the 46th day. Twenty-four hours after the final operation, a regimen of single daily subcutaneous injections of α-corticotropin in a 5 per cent beeswax–95 per cent peanut oil medium was initiated. On the day following the tenth injection, the animals were anesthetized with nembutal; the adrenals, ventral prostate, and seminal vesicles were removed and weighed. The sex accessories were immediately fixed in neutral formalin, and were subsequently sectioned by the paraffin method, and stained with hematoxylin-eosin.

TABLE XII

Effect of α-Corticotropin on the Weights of Sex Accessories of Hypophysectomized-Castrated and Hypophysectomized-Castrated-Adrenalectomized Male Rats

Animals*	Treatment	Daily dose	No. of rats	Body weights		Adrenals	Ventral prostate	Seminal vesicles
				Onset	Autopsy			
		μg.		*gm.*	*gm.*	*mg.*	*mg.*	*mg.*
H-C	Saline	0	6	122	120	8.8 ± 0.3‡	11.6 ± 1.2	12.3 ± 1.7
"	α-Corticotropin	15.0	3	119	102	27.6 ± 4.7	14.8 ± 4.7	19.5 ± 5.4
"	"	22.5	8	114	99	53.2 ± 5.0	15.8 ± 1.8	13.9 ± 0.9
H-C-A	DOCA§	100.0	4	111	114	0	12.3 ± 1.6	11.5 ± 0.7
"	α-Corticotropin	22.5	4	112	101	0	9.2 ± 2.0	11.9 ± 0.6
"	α-Corticotropin + DOCA	22.5 + 100.0	6	106	113	0	11.0 ± 1.1	11.9 ± 0.5

* All animals castrated at 43 days, hypophysectomized at 44 days and sacrificed at 55 days. H, hypophysectomized; C, castrated; A, adrenalectomized.

‡ Mean ± standard error.

§ Desoxycorticosterone acetate injected as a sesame oil solution.

The results summarized in Table XII demonstrate that α-corticotropin given at a level of 22.5 μg. per day can effect a slight increase in the weight of the ventral prostate of the hypophysectomized-castrated male rat in the presence of the adrenal glands, but not in their absence. No consistent change was observed in the weight of seminal vesicles. While the weight increase in the ventral prostate of the hormone-treated group was not significantly greater than that of the saline-treated control ($0.1 > p > 0.05$), a definite histological improvement accompanying this increase was apparent in all the animals injected with 22.5 μg. of α-corticotropin. The degree of stimulation evidenced by the height of the glandular epithelium is illustrated in the photomicrograph shown in Fig. 9. A daily dose of 15 μg., which produced adrenal weights comparable to those of non-hypophysectomized animals, induced no evidence of androgen secretion.

H. Evidence for the Secretion of Progestogens by α-Corticotropin-Stimulated Adrenal Glands

(with W. R. Lyons)

It was reported earlier from this laboratory (43) that highly purified preparations of ACTH are capable of causing deciduoma formation in hypophysectomized-oophorectomized rats. This was taken to indicate that ACTH-stimulated adrenals secrete progestogens which are in turn responsible for the formation of deciduomata. These observations have now been confirmed with the pure peptide hormone, α-corticotropin.

Female rats of the Long-Evans strain were hypophysectomized and oophorectomized at 30 days of age; daily injections of α-corticotropin suspended in 5 per cent beeswax in peanut oil were begun on the day of operation and continued for 7 days. On the 3rd day, the uteri of all the animals were threaded and necropsy was performed on the day following the final injection. Uteri that did not show discrete gross tumors at the thread sites were studied histologically and graded on the basis of stromal cell hypertrophy.

TABLE XIII

Effect of α-Corticotropin on Deciduoma Formation in Hypophysectomized-Oophorectomized Rats

Experiment	No. of rats	Daily dose	Adrenals	Deciduoma		Open vaginae
				Gross	Histological	
		μg.	mg.			
Control	27	0.0	8.6	0	0	0
I	4	7.5	21.1	0	2	1
	4	22.5	32.1	0	3	2
	4	75.0	68.3	4	4	2
II	3	75.0	96.0	3	3	0

A summary of the results can be seen in Table XIII. Two preparations of α-corticotropin were employed; at a daily dose level of 75 μg. both induced small but positive deciduomata in the gross. Groups that received daily doses of 7.5 μg. and 22.5 μg. showed only slight uterine swelling around the thread sites; but endometrial sections showed beginning deciduomal reactions in the form of enlarged stromal cells in some of these animals.

Some of the rats receiving one of the α-corticotropin preparations showed open vaginae at necropsy, although they had been oophorectomized on the 30th day. There was no assurance that this was an effect of an adrenocortical estrogen, since the vaginae were studied only after 7 days of treatment. At that time the epithelium was lightly mucified, but not cornified.

In summary it may be said that an adrenocortical progestogen may be

detected in rats in which a hyperadrenocortical state has been induced with α-corticotropin. The development of deciduomata under the influence of a progestogen seems all the more remarkable because at the same time other corticoids known to be inhibitory to progestogen (*e.g.*, cortisone) are also formed in and secreted by the abnormally enlarged and overstimulated adrenals.

I. Corticotropin as a Galactopoietic Hormone

(with W. R. Lyons)

In the reviews by Folley and his colleagues (44–46) and by Mayer and Klein (47) the importance of the adrenocortical hormones in lactation has been stressed. The need in lactation for one or another of the adrenal steroids in the absence of the pituitary or the adrenal, and for ACTH in the absence of the pituitary, has clearly placed ACTH by the side of lactogenic hormone as a galactopoietic hormone—a substance that increases the milk yield of an animal already lactating (48, 49).

In our first experiments on replacement therapy in hypophysectomized rats an abundant milk secretion was induced by giving a crude lactogenic preparation containing enough ACTH to maintain the adrenal cortices and enough somatotropin to permit body weight increase in the test rats (50). However, even though the glands were filled with milk and active suckling was observed, starvation of the young still occurred, presumably because there was insufficient oxytocin to permit milk ejection. Until recently, pure ACTH has not been available in large enough quantities for testing its galactopoietic potency, and it has been more practicable to use the adrenocortical steroids (51–55). The availability of α-corticotropin has now permitted us to establish that this hormone is as important as lactogenic hormone in milk secretion.

Long-Evans rats bred for the first time at 2½ to 3 months of age were hypophysectomized 11 days after the detection of sperm; and, following delivery, were injected daily subcutaneously with different levels of α-corticotropin in beeswax and peanut oil. A potent sheep lactogenic hormone preparation (56) containing approximately 30 I.U. per mg., no detectable ACTH or thyrotropin, and not more than 0.5 per cent somatotropin activity was administered in daily subcutaneous injections at the 5 mg. level, alone and in combination with α-corticotropin. Injections were continued for the first 10 days after delivery; and necropsies were performed on the 11th day. The weight of the left adrenal was ascertained at necropsy, and mammary glands were fixed and studied after staining in alum-carmine and clearing in methyl salicylate.

The test animal used in this experiment was usually found to secrete a small amount of milk for a day or 2 after delivery, apparently because of lactogenic substance released from the placenta before parturition (53). But by the 11th postpartum day the glands of 3 of the uninjected hypophysectomized controls were found to be regressing and devoid of milk in the gross (see Table XIV), as

were the glands of the rats that received only α-corticotropin. The rats injected with lactogenic hormone alone manifested partial maintenance of their lobular structure, but secreted less than the small amount of milk seen on the day of parturition in uninjected animals. All the animals given 5 mg. of lactogenic hormone daily plus 25, 50, or 100 γ of α-corticotropin showed well-developed glands filled with milk. Only the 100 γ dose of α-corticotropin stimulated the adrenals to the degree observed in normal rats at the end of pregnancy. In order to quantitate the efficiency of lactogenic hormone and ACTH as galactopoietic agents, it will be necessary to test them in a complete nursing program with the addition of somatotropin, oxytocin, and possibly thyrotropin.

TABLE XIV

Galactopoietic Effect of α-Corticotropin in Combination with Lactogenic Hormone in Rats

No. of rats	α-Corticotropin	Lactogenic hormone	Left adrenal	Milk secretion
	μg.	*mg.*	*mg.*	
3	25	0	14.6	−
3	25	5	19.0	+
3	50	5	16.6	+
3	100	5	45.3	+
3	0	5	13.1	(±)*
3	0	0	10.7	−
3‡	0	0	40.2	+

* Slight residual secretion in a few scattered small lobules.

‡ Normal rat on 1st postpartum day.

DISCUSSION

The various methods that have been advanced for the estimation of adrenal-stimulating activity are generally derived from the manifestations of either direct or indirect effects of ACTH on normal or hypophysectomized animals. The direct effects are primary; that is, they follow directly from the hormone administration without the intervention of adrenal cortical steroids. They include depletion of adrenal ascorbic acid, hypertrophy of the adrenals, and repair of the histological changes in the adrenals produced by hypophysectomy. The indirect effects are those that result from the formation and release of the adrenal cortical steroids following the administration of ACTH; they include eosinopenia, lymphopenia, involution of thymus and spleen, and inhibition of body growth. All of these effects have now been shown to be properties of α-corticotropin. Moreover, the peptide hormone is capable of maintaining muscle glycogen in hypophysectomized rats and of mobilizing fat into the liver of fasted mice.

In both normal and hypophysectomized rats, the increment of adrenal weight

produced by α-corticotropin (administered in a beeswax–peanut oil suspension) is directly proportional to the logarithmic function of the daily dose as shown in Equations 1 and 2. When the hormone peptide was injected as an aqueous solution for a period as brief as 4 days, the adrenal-stimulating activity was found to be greatly reduced (Table VI). As would be expected, the adrenals of hypopsectomized rats respond with greater sensitivity, and a higher degree of precision can be obtained with them than with those of normal animals. It may be seen in Table II, that within 4 days a daily dose of approximately 1 I.U. of α-corticotropin will elicit an increase of 100 per cent in the adrenal weight of hypopsectomized rats. This increase may be employed as a routine procedure for the assay of corticotropin preparations.

The observations that have been made to the effect that in the ovariectomized-hypophysectomized female rat α-corticotropin induces deciduoma formation whereas in the castrated-hypophysectomized male rat it stimulates the growth of the ventral prostate as evidenced by the histological changes illustrate the difficulties that may be encountered in assaying the anterior pituitary hormones through their effects on their respective target organs. Earlier investigations, and the concepts stemming from them, require more careful interpretation in the light of presently accruing knowledge concerning the ability of the adrenal cortex and gonads to secrete a considerable array of steroid compounds, some of which may be estrogenic, progestogenic, or androgenic. Furthermore, the fact that various steroids elaborated in the same organ or different organs may operate together antagonistically or synergistically renders extremely difficult the quantitation of such end results as interruption of the estrous cycle, vaginal cornification or mucification, progestational proliferation or deciduoma formation in the uterus, various stages of mammary growth, and many other manifestations. A good example of this difficulty is furnished by the careful quantitative work on the synergistic and antagonistic action of steroids done by Hisaw and Velardo (57, 58) in the course of their studies on deciduoma formation. Hence, it is not surprising that these investigators (59) have subsequently demonstrated that ACTH inhibits progesterone-induced deciduoma formation, a finding entirely in disagreement with our data reported herein and also those previously published (43). This discrepancy was described by Hisaw and Velardo themselves (59) as probably only apparent and not real, since different degrees of adrenal stimulation were involved, theirs being "moderate" and ours "grossly exaggerated," at least in cases positive for deciduoma formation. It is likely that in our experiments the adrenals under the stimulation of α-corticotropin secreted progestogen in amounts that overrode any inhibiting effects on the part of the cortisone-like steroids that were concomitantly being secreted in amounts sufficient to obliterate the thymus.

It is now beyond any doubt that the melanocyte-stimulating activity represents one of the intrinsic biological properties of the α-corticotropin molecule.

When the activity of α-corticotropin is compared with that of the pure MSH peptide isolated from the posterior lobes of pituitary glands (29, 60), it may be estimated that the former is about 1/100th as potent as the latter. Preparations of corticotropin A from pig glands have also been shown to possess melanocyte-stimulating activity (61, 62). As a structural consideration, it is of interest to note that a portion of the amino acid sequence in the MSH molecule (29, 63) is closely related to a sequence found in all corticotropin preparations (3, 62, 64), as follows:

Corticotropins: Ser.|Tyr.|Ser.|Met.Glu.His.Phe.Arg.Try.Gly.|Lys.|Pro.|Val. . . .

β-MSH: H-Asp.Glu.Gly.Pro.|Tyr.|Lys.|Met.Glu.His.Phe.Arg.Try.Gly.|Ser.|Pro.|Pro.Lys.Asp.OH

It is very probable that the melanocyte-stimulating activity of corticotropins can be attributed to the presence of this amino acid sequence in the hormone

TABLE XV

Erythropoietic Activity of α-Corticotropin in Hypophysectomized Rats

Daily dose	No. of rats	Body weight	Hematocrit determination	Total red cell volume/100 gm. B.W.	Adrenal	Thymus
μg.		*gm.*	*per cent*	*ml.*	*mg.*	*mg.*
0	6	75 ± 1.2*	27.7 ± 1.2	1.43 ± .02	6.0 ± 0.3	81 ± 5
5	6	77 ± 1.0	30.6 ± 1.3	1.52 ± .04	8.5 ± 1.0	82 ± 7
10	5	75 ± 1.0	37.2 ± 1.0	1.88 ± .04	11.5 ± 0.9	<50
25	6	76 ± 1.0	48.4 ± 2.1	2.18 ± .04	25.3 ± 3.0	Depleted
50	6	72 ± 1.0	43.5 ± 1.3	2.11 ± .03	35.0 ± 2.0	Depleted

* Mean ± standard error.

molecule. The final proof of this assumption must await the synthesis of this peptide.

Melanocyte stimulation is not the only function of α-corticotropin in the absence of adrenal glands. Recent studies of Menkin (65) have shown that the hormone peptide exerts the direct local effect of repressing the increased capillary permeability of an inflammed area in adrenalectomized rats. Moreover, the adipokinetic activity of α-corticotropin herein reported can be discerned in adrenalectomized animals maintained with corticoid therapy. This points up a misconception that has been prevalent, arising from the name originally coined for the major biological activity of the substance; *i.e.*, adrenocorticotropic hormone. It has been assumed for a long time that any ACTH preparation which can elicit a biological function in adrenalectomized animals is contaminated with some active component(s) other than the ACTH itself. However, the observations discussed above clearly demonstrate that α-corticotropin, which is known to be pure (1, 2) and whose chemical structure has been elu-

cidated (3), can exercise physiological functions without the participation of the adrenal gland. Similar confusion arising from terminology has recently been discussed in connection with pituitary growth hormone (66).

In addition to the various biological functions that can be exercised by α-corticotropin, as reported herein, it has been demonstrated that the hormone is very potent as an erythropoietic factor,[7] according to assay by the Fe^{59}-labelled cell dilution method in hypophysectomized rats (67). As can be seen in Table XV, a daily dose of 25 μg. of α-corticotropin administered to the hypophysectomized animals is capable of elevating the total red cell volume from 1.43 ml. per 100 gm. of body weight, to 2.18 ml., a level identical with that found in normal rats (68). It may be further noted that the erythropoietic activity of the hormone increases with the dosage, reaching a maximum at a daily dose of 25 μg. There appears to be some indication of an inhibition of this increment in red cell volume at the higher dose levels. Whether or not the adrenal cortex is essential for the erythropoietic activity of α-corticotropin remains to be investigated.

The same animals that were used for the determination of the erythropoietic activity (Table XV) were also tested for the calorigenic response to the graded doses of α-corticotropin.[8] Whereas the hypohysectomized control animals had a standard metabolic rate of 19 cal./m.^{2} hr., after 12 days of injection all animals receiving daily doses of α-corticotropin of 10 μg. or more showed a significant elevation in this rate. Thus at a daily dose of 10 μg., the rate was 24.4; at 25 μg., 31.1; and at 50 μg., 36.9 cal./m.^{2} hr. The corresponding rate for normal animals was found to be 41.8.

Finally, a few words may be said about the biological activity of the hormone in human subjects. Two groups of investigators (69, 70) have carried out metabolic investigations with α-corticotropin in man; both groups have shown that the hormone peptide is active in stimulating human adrenals. Forsham *et al.* (69), reported that when α-corticotropin was assayed in man on the basis of steroidogenesis elicited by intravenous injections, an activity equivalent to 150 I.U. per mg. of the peptide was obtained.

SUMMARY

Purified α-corticotropin has been reported to exercise the following biological effects: (*a*) stimulation of the adrenal glands in normal and hypophysectomized rats, (*b*) production of blood eosinopenia in hypophysectomized rats, (*c*) maintenance of muscle glycogen in hypophysectomized rats, (*d*) inhibition of growth-promoting activity of somatotropin, (*e*) stimulation of melanocytes in the skin of frogs, (*f*) mobilization of fat into the liver of fasted mice, (*g*) stimulation

[7] It is a pleasure to acknowledge, with thanks, the work of Drs. M. E. Simpson and A. N. Contopoulos on the assay of the erythropoietic activity of α-corticotropin.

[8] We are indebted to Dr. E. Evans for these determinations. Full details will be published elsewhere (Evans, E., Simpson, M. E., and Contopoulos, A. N., *Endocrinology*, in press).

of the accessory sex glands of castrated-hypophysectomized male rats, (*h*) induction of deciduoma formation in hypophysectomized-oophorectomized rats, and (*i*) elevation of the total red cell volume in hypophysectomized rats. α-Corticotropin has also been shown for the first time to act in synergism with lactogenic hormone as an essential galactopoietic hormone.

The ability of α-corticotropin to elicit biological responses in the absence of the adrenal cortex is discussed.

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EXPLANATION OF PLATES

PLATE 29

FIG. 1. Hypophysectomized control. Hematoxylin and eosin. × 16

FIG. 2. Hypophysectomized control. The junctional, lipide-free zone between the zona glomerulosa and zona fasciculata is composed of atrophic cells which have scanty cytoplasm and pyknotic nuclei as compared with the cells on either side. Hematoxylin and eosin. × 110.

FIG. 3. Hypophysectomized rat treated with 5 μg. of α-corticotropin. Hematoxylin and eosin. × 16.

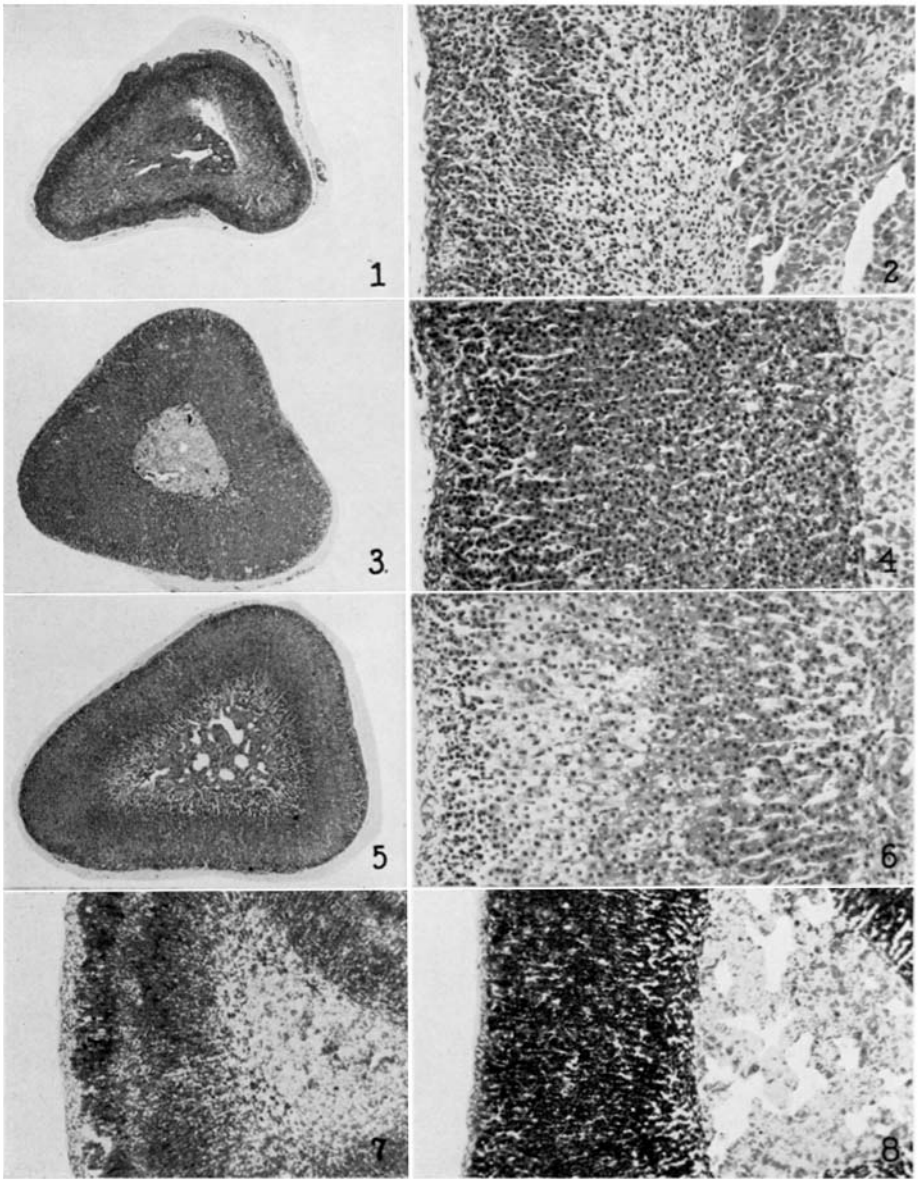
FIG. 4. Higher magnification of Fig. 3. The cortical cells of all zones exhibit nuclear and cytoplasmic restitution. Some of the sinusoids are dilated. × 110.

FIG. 5. Hypophysectomized rat treated with 30 μg. of α-corticotropin. Hematoxylin and eosin. × 16.

FIG. 6. Higher magnification of Fig. 5. The adrenal cortical cells are larger than those of rats treated with 5 μg. of α-corticotropin. The sinusoids, likewise, are dilated to a greater degree; × 110.

FIG. 7. Hypophysectomized control. The lipide-free zone between the zona glomerulosa and zona fasciculata appears as a distinct pale area. Oil–red O and hematoxylin. × 49.

FIG. 8. Hypophysectomized rat treated with 5 μg. of α-corticotropin. Lipide has been deposited in the junctional zone. The entire cortex shows an increased amount of lipide; × 49.



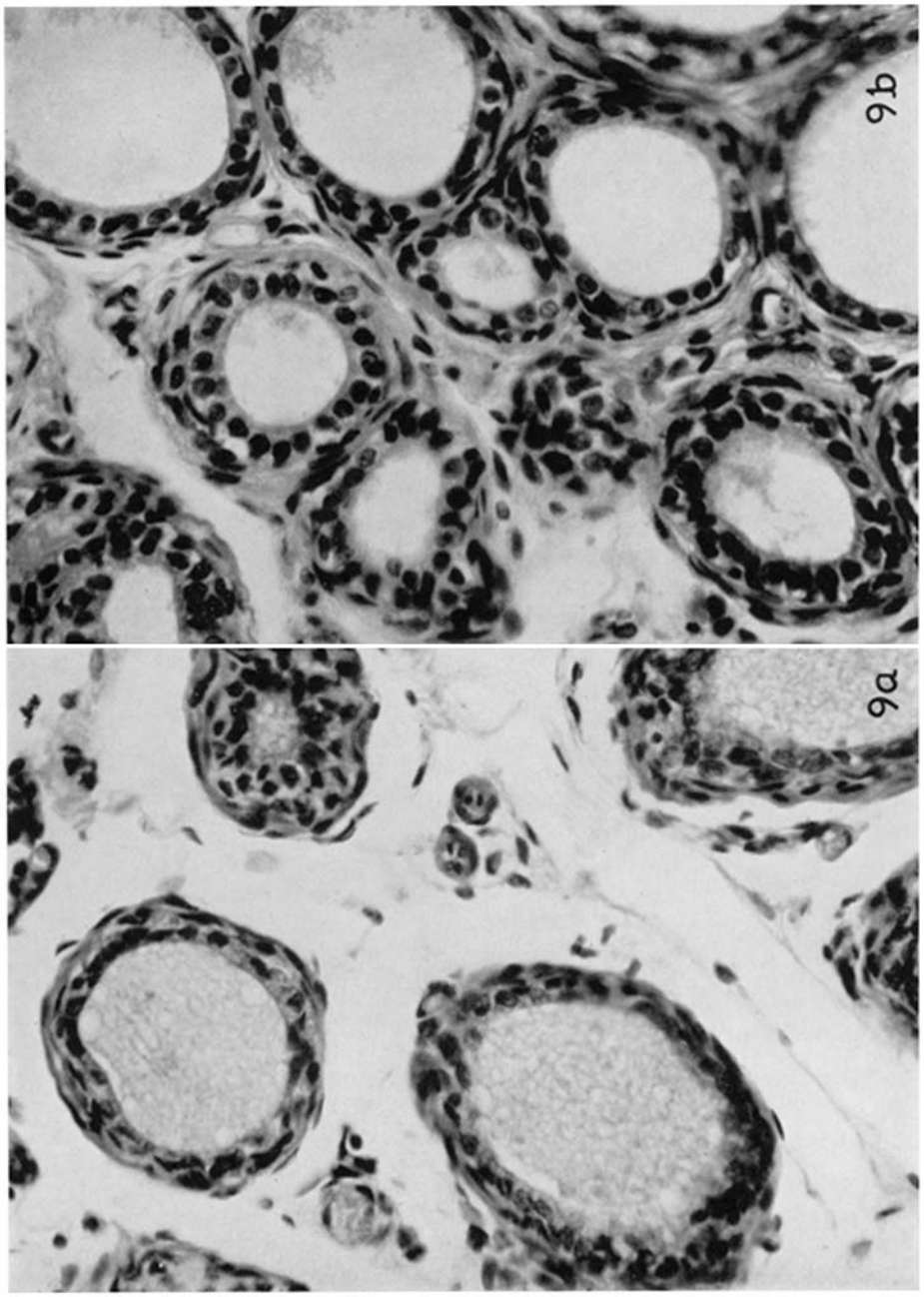
(Li *et al.*: Corticotropins. X)

PLATE 30

FIGS. 9 a and 9 b. Ventral prostate of the Long-Evans rat, operated at 44 days of age and sacrificed at 55 days. Hematoxylin and eosin. × 560.

FIG. 9 a. Hypophysectomized-castrated male rat injected with saline; weight of prostate, 11.2 mg.

FIG. 9 b. Hypophysectomized-castrated male rat injected with a daily dose of 22.5 μg. of α-corticotropin for 10 days; weight of prostate, 15.2 mg.



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