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 postoperative	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 postoperative	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.

14087 P

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.

TABLE I.

Adrenal Cholesterol Values of Normal and Adrenotropic Hormone Treated Male Rats, Each 24 Days Old.

Type of rat*	No. of animals	Cholesterol concentration in adrenal glands: Mg/100 mg fresh adrenal tissue	P value	Mg/100 g body wt	P value
Control, injected with water	22	3.5 ± 0.18†		1.12 ± 0.06	
3 hr after inj. of 2 mg A.C.T.‡	16	1.6 ± 0.08	<0.01§	0.57 ± 0.03	<0.01
6 hr after inj. of 2 mg A.C.T.	4	2.0 ± 0.12	<0.01	0.73 ± 0.06	0.02
9 hr after inj. of 2 mg A.C.T.	4	2.3 ± 0.18	0.02	0.83 ± 0.09	0.05
24 hr after inj. of 2 mg A.C.T.	5	3.6 ± 0.07	0.8	1.37 ± 0.08	0.05
Inj. thrice daily 3 days, total 2 mg A.C.T.	4	4.4 ± 0.11	0.05	1.68 ± 0.11	<0.01
Inj. thrice daily 3 days, total 5 mg A.C.T.	5	5.0 ± 0.4	<0.01	2.52 ± 0.25	<0.01
3 hr after inj. 1.0 pressor unit pitressin (Parke-Davis)	9	3.0 ± 0.13	0.1	0.92 ± 0.08	0.05

*All animals received food and water *ad libitum*.

†Means and standard errors. Standard error $= \sqrt{\frac{\text{sum of squares of deviations from mean,}}{n\,(n-1)}}$

where n = number of observations.

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

§P values as compared to control groups.

are here reported were obtained from two sources, from the Biochemical Laboratory of the Connecticut Agricultural Experiment Station, through the courtesy of Dr. Rebecca Hubbell, and from The Breeding and Laboratory Institute, Brooklyn. Twenty-four-day-old male animals were used. Only healthy, growing rats are suitable for the studies of adrenal cholesterol changes inasmuch as it has been found that inanition or infection will markedly deplete the adrenals of cholesterol. Similar observations on the effect of infection on the adrenal cholesterol level have been recently reported by Sperry and Stoyanoff.[2] Injections were made intraperitoneally, generally in a volume of 0.25 ml for each injection. At the appropriate time, the rats were killed with illuminating gas, the adrenals carefully dissected out and weighed. Total cholesterol determinations were conducted on either single or paired adrenal glands of each rat by the method described by Sperry.[3]

In view of the fact that the purified adrenotropic hormone preparations exhibited slight pressor (cat)† and antidiuretic (rat) activity,[4] the action of posterior pituitary pressor substance (Parke-Davis) on adrenal cholesterol levels was examined. One milligram of purified adrenotropic hormone was found to contain an amount of pressor and antidiuretic activity equivalent to 0.6 unit of Parke-Davis Pitressin.

The cholesterol data which have been obtained are presented in Table I. The standard errors of each series are also included in the table, together with the P values, which have been calculated by the method of Fisher[5] for small series in order to determine which differences among the several groups of experimental animals are significant.

The data indicate a definite lowering of the adrenal cholesterol level 3 hours after injection of adrenotropic hormone. Subsequently, the adrenal cholesterol concentration rises and returns to normal within 24 hours following hormone administration. Moreover, the repeated injection of the hormone over a period

† Grateful acknowledgment is made to Dr. L. S. Goodman of the Department of Pharmacology for the pressor assay in the cat.

2 Sperry, W. M., and Stoyanoff, V. A., *J. Nutrition*, 1938, **9**, 131.

3 Sperry, W. M., *Am. J. Clin. Path.*, Tech. Suppl., 1938, **2**, 91.

4 Gilman, A., and Goodman, L. S., *J. Physiol.*, 1937, **90**, 113.

5 Fisher, R. A., *Statistical Methods for Research Workers*, 1932, 4th Ed., Oliver and Boyd, London.

of 3 days raises the adrenal cholesterol concentration above that of control animals. This is particularly striking with a total dose of 5 mg of the adrenotropic preparation. It is to be noted that the increases in cholesterol concentration are greater when calculated on the basis of animal body weight. This is due to the fact that adrenal hypertrophy is occurring under the influence of the hormone, and cholesterol is being deposited in the new adrenal tissue in a concentration at least as great as that present in the adrenal gland before stimulation.

The results in Table I suggest that the adrenotropic hormone is concerned with the regulation of metabolic changes in the adrenal gland involving cholesterol. In view of the fact that the active principles of the adrenal cortex are also steroids, it is possible that variations in the cholesterol content of the adrenal also reflect changes in concentration of steroid hormones. This suggestion finds support in evidence of increased function of the adrenal cortex following adrenotropic hormone administration, as measured by liver glycogen changes[6] and in the report[7] that adrenotropic hormone increases the resistance of hypophysectomized and normal rats to cold, starvation and anoxia.

It may be added that the cholesterol concentration of the adrenals of 45-day-old hypophysectomized rats has been found to be elevated in comparison to the cholesterol levels in the adrenals of unoperated animals of the same age. Cholesterol data similar to those which are reported have also been obtained by employing 2 mg of an adrenotropic hormone fraction prepared from whole sheep pituitary glands by a procedure essentially that described by Li, Simpson and Evans.[7]

Summary. Pituitary adrenotropic hormone produces a distinct lowering of the adrenal cholesterol level 3 hours after injection of 2 mg into 24-day-old male rats; repeated injections over a period of 3 days results in an increase in adrenal cholesterol concentration above that of control animals.

[6] Grattan, J. F., and Jensen, H., *J. Biol. Chem.*, 1940, **135**, 511.

[7] Li, C. H., Simpson, M. E., and Evans, H. M., *Science*, 1942, **96**, 450.

14088 P

Excretion of Keto Acids and Hydroxyphenyl Compounds in Pernicious Anemia.

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It has been found in this laboratory that the urinary excretion values for keto acids of patients with untreated pernicious anemia in relapse show a marked decrease when liver extract is administered by intramuscular injection. Since there are several indications that tyrosine metabolism may be disturbed in pernicious anemia, it was of interest to measure the urinary hydroxyphenyl compounds in this disease in order to determine whether or not their excretion values could be correlated with those of the keto acids. Previous experiments by Becher[1] have shown a rise in blood phenols in pernicious anemia and, in contrast, Volterra[2] observed a low excretion of volatile phenols in the urine in untreated pernicious anemia and a greatly increased excretion of these compounds after liver therapy.

Twenty-four-hour urine samples were collected in acid and analyzed for keto acids by

[1] Becher, E., Litzner, S., and Täglich, W., *Z. klin. Med.*, 1926, **104**, 195.

[2] Volterra, A., *Schweiz. med. Wochschn.*, 1939, **69**, 627.