27°C and 26°C for 9 minutes. The colored liquid is then transferred to a fused glass absorption cell and read in the photelometer with an orange filter at exactly 10 minutes. The value is read from a calibration curve obtained from the analyses of standard cholesterol solutions in chloroform made exactly in the same manner. The curve plotted on semi-logarithmic paper is a straight line.

The accuracy and duplicability of the method is attested to by the findings in Table I. Added cholesterol was recovered with the same degree of accuracy.

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Effect of Adrenocorticotropic Hormone in 4-Day-Old Rats.*

HENRY D. MOON. (Introduced by H. M. Evans.)

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Adrenocorticotropic hormone (A-C-T) has been assayed in this laboratory using the 21-23-day-old male rat.[1] This test requires relatively large amounts of A-C-T. For this reason the response of 4-day-old rats to A-C-T has been studied with the hope of finding them to be more sensitive.

On the day of birth, rats were grouped into litters of 8, containing both males and females. Beginning on the 4th day postpartum, the rats in each litter were injected intraperitoneally with 0.1 ml of A-C-T preparations (previously assayed in 21-23-day-old rats)

TABLE I.
Effect of A-C-T on 4-day-old Rats.

No. of rats	Dose units	Avg body wt		Avg wt of adrenals	Avg wt of thymus
		Init. grams	Final grams	mg	mg
16	0.05	9.5	12	3.7	10.5
16	0.10	9.6	14.2	4.0	9.5
16	0.15	9.5	11.5	4.3	5.1
16	0.25	9.0	11.2	4.4	5.3
16	0.50	—	10	6.1	2.5
40 controls		9.8	14.0	2.4	20.9

* Aided by grants from the Board of Research of the University of California and Rockefeller Foundation of New York City. Assistance was rendered by the Federal Works Progress Administration, Project O.P. 65-1-08-62 Unit A-5.

[1] Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 649.

daily for 3 days. The litter was then sacrificed 24 hours after the last injection, the adrenals and thymuses dissected out, fixed, and then weighed. The adrenals were weighed together and the thymuses were weighed separately unless the atrophy was nearly complete. In some cases only 4 of the littermates were injected and 4 kept as controls. Table I shows the effects of A-C-T on adrenals and thymuses of 4-day-old rats.

Mammotropic† and growth‡ preparations as well as inactivated A-C-T preparations were given to the 4-day-old rats. Thymic atrophy was not produced by these preparations.

TABLE II.
Effect of Mammotropic and Growth on 4-day-old Rats.

No. of rats	Extract	Total dose units	Avg adrenal wt, mg	Avg thymus wt, mg
16	Mammotropin	502	2.6	19.7
16	Growth hormone	5§	2.6	24.6
16	" "	10	2.9	28.2

2 Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 645.

§ 1 unit of Growth Hormone: the daily amount required to cause plateaued female rats to gain 2 g per day over a period of 20 days.

Celloidin sections were cut of the adrenals and thymuses, and stained with hematoxylin and eosin. Microscopic examination revealed that the adrenals of the rats injected with 0.05 units showed definite evidences of stimulation.|| When compared to the controls, the cells throughout the adrenal cortex were larger; there was an increase in the number of mitoses; there was vascular congestion in the cords of cells in the zona fasciculata; there was an increase in the amount of fat vacuoles. These changes were more marked in the adrenals of rats injected with larger amounts of A-C-T.

The atrophic thymuses of rats treated with A-C-T showed a loss of the lymphocytes. There appeared to be a *relative* increase in number of epithelial cells, although there was no evidence of actual hyperplasia of the epithelial elements of the thymus.

It is to be noted that even at the lowest level of A-C-T administered (0.05 units) there was gravimetric (Table I) as well as histologic evidence of stimulation of the adrenal cortex. Thus 0.05 unit will cause a 50% increase in the adrenal weights of 4-day-old rats,

† Prepared and kindly supplied by Dr. W. R. Lyons of this laboratory.

‡ Prepared and kindly supplied by Mr. Donald Meamber of this laboratory.

|| With more active A-C-T preparations, this response was obtained with as little as 0.2 mg of total solids. These preparations were obtained by repeated solution and precipitation from acid acetone solutions and isoelectric precipitations from aqueous solutions as described in the method first published (*Cf.* Ref. 1).

whereas it requires one unit to cause a 50% increase in the adrenal weights of 21-day-old rats. We believe that 4-day-old rats afford a more satisfactory method of assay for A-C-T because of greater sensitivity.

Summary. The response of 4-day-old rats to various anterior pituitary extracts has been described. It was noted that 4-day-old rats responded with adrenal hypertrophy and thymic atrophy to much smaller amounts of A-C-T than 21-23-day-old rats. Mammotropic and growth preparations did not cause adrenal hypertrophy and thymic atrophy. It is concluded that the 4-day-old rats will be a more sensitive test animal for A-C-T preparations.

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Effect of Adrenocorticotropic Hormone on the Thymus of Rats.*

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It was reported in earlier papers that the administration of adrenocorticotropic hormone (A-C-T) to immature rats resulted in adrenal hypertrophy[1, 2] and thymic atrophy.[2, 3] It has also been noted that large amounts of A-C-T will also cause a depression of somatic growth, whereas the viscera continue to grow. These findings have been studied considerably more in detail in this laboratory.†

A-C-T, prepared and assayed by the method previously published,[1] was given at various levels to 21-23-day-old normal male rats in 3 daily injections. The rats were autopsied 24 hours after the last injection. It was noted that the thymic atrophy was proportional to the amount of A-C-T administered as seen in Table I.

Atrophy of the thymus following the administration of the gonadotropic hormone of pregnant mare serum was reported by Evans and

* Aided by grants from the Board of Research of the University of California and Rockefeller Foundation of New York City. Assistance was rendered by the Federal Works Progress Administration, Project O.P. 65-1-08-62 Unit A-5.

1 Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 649.

2 Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 34.

3 Evans, H. M., Moon, H. D., Simpson, M. E., and Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 419

† Results on the inhibition of growth to be published later.