strated an enzyme system localized in the microsomal fraction of liver cells, which oxidizes hexobarbital to keto-hexobarbital. The investigation of the activity of this enzyme system in these strains is now underway.

Summary. 1. Significant differences in sleeping time have been demonstrated between various strains of mice, inbred and non-inbred, when injected with 125 mg per kilo body weight of hexobarbital. 2. Three different levels of genetic homozygosity were represented in the various strains tested. The most closely inbred strains (A/L, DBA/2, A/He, BALB/c, C57L, C57BL/6, C57BR/cd, C3HfB, BRSUNT, SWR) showed the least variation, the less closely inbred strain (CFW) showed more variation, and the non-inbred Swiss strain showed the most variation.

1. Russell, E. S., Newfeld, E. F., and Higgins, C. T., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 761.

2. Law, L. W., Morrow, A. G., and Greenspan, E. M., *J. National Cancer Inst.*, 1952, v12, 909.

3. Tuba, J., *Cancer Res.*, 1952, v12, 113.

4. Wragg, L. E., and Spiers, R. S., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 680.

5. McLaren, A., and Michie, D., *Nature,* 1954, v173, 686.

6. Baten, W. D., 1938, Elementary Mathematical Statistics, New York, John Wiley & Sons, Inc.

7. Quinn, G. P., Axelrod, J., and Brodie, B. B., *Fed. Proc.*, 1954, v13, 395.

8. Cooper, J., and Brodie, B. B., *J. Pharm. Exper. Therap.*, 1954, v110, 12.

Received August 26, 1955. P.S.E.B.M., 1955, v90.

Blood ACTH Levels Following Subcutaneous Injections.* (22040)

E. A. LAZO-WASEM AND S. W. HIER. (Introduced by Claire E. Graham.)

Wilson Laboratories, Chicago, Ill.

The adrenocorticotropic hormone (ACTH) is known to disappear rapidly from the circulating blood. Van Dyke *et al.*(1) have estimated the average life of the ACTH molecule in the circulation to be approximately 17 minutes, and half-life values of about 5 minutes have been estimated for ACTH administered intravenously to intact rats(2,3). The half-life of endogenous ACTH in the adrenalectomized rat has been reported to be approximately one minute(4). A rapid rate of disappearance of ACTH from the circulation is also indicated by clinical studies. Intravenous infusion of ACTH over a prolonged period brings about a much greater adrenal response than the single injection of a comparable dose of the hormone(5). It becomes of interest, therefore, to establish the rate at which exogenous ACTH disappears from the circulation following a single extravascular administration.

In these studies a large dose of ACTH was administered subcutaneously to hypophysectomized rats and blood ACTH levels were estimated at various intervals after treatment.

Method. Male hypophysectomized rats were obtained from the Hormone Assay Laboratories, Chicago, and used within a 24-hour post-operative interval. An ACTH standard dose response curve was prepared for this study based on accumulated data representing

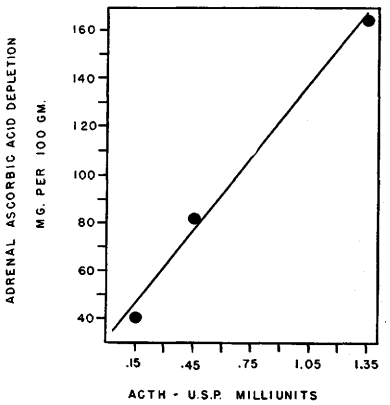

FIG. 1. ACTH standard dose-response curve. Each point represents the mean response of 25 hypophysectomized rats 1 hr after intrav. injection with U.S.P. ACTH Reference Standard.

* The authors acknowledge the helpful assistance of Mr. William Watson and Miss Joan Michalski.

TABLE I. Depletion of Adrenal Ascorbic Acid in Recipient Rats following Injection of 4 ml of Blood from Donor Rats Previously Injected with 10 U.S.P. Units of ACTH.

Min. after subcut. inj. of donor rats	No. of rats	Adrenal ascorbic acid depletion (mg/100 g) of recipient rats 1 hr after intrav. inj.	Blood ACTH of donor rats (mU/4 ml)*
1	6	77	.44
2	6	90	.56
4	4	134	1.00
8	5	119	.88
10	3	106	.76
16	6	123	.92
20	3	43	.12
32	3	69	.36
40	3	58	.32
80	3	10	.00

* Obtained by interpolation of column 3 using Fig. 1.

25 rats at each point injected intravenously with U.S.P. ACTH Reference Standard (Fig. 1). Measurements of blood ACTH were made by the technic of Sayers and Burke(6). Hypophysectomized rats, 250 g, were injected subcutaneously with 10 U.S.P. ACTH units in 0.5 ml water. At various time intervals following treatment 4 ml of blood from a previously injected rat were collected from the abdominal aorta into a heparinized syringe. The blood was immediately injected into the jugular vein of a recipient 100 g hypophysectomized rat. The donor rat was then discarded. Individual rats were used for each blood collection. Adrenal ascorbic acid of recipient animals was determined 1 hour after the transfusion. These values were subtracted from uninjected control values to estimate adrenal ascorbic acid depletion. Blood ACTH values were obtained by interpolating the depletion values on Fig. 1.

Results. Table I presents the ascorbic acid depletion values found in recipient rats receiving 4 ml of blood from donor animals.

The concentration of ACTH in each ml of rat blood (obtained from Table I) is shown graphically in Fig. 2. Detectable ACTH activity is found in the blood within one minute after subcutaneous injection.

The blood ACTH level rises rapidly and reaches a peak concentration between 4 and 8 minutes after injection. After 20 to 40 minutes the blood ACTH is almost back to zero and 80 minutes after injection no detectable activity is found.

In a separate experiment, adrenals were removed from the donor rats at the time that blood sampling took place. The adrenal ascorbic acid depletion was determined and is shown plotted with blood ACTH levels in Fig. 3. Significant depletion of adrenal ascorbic acid in the donor rats does not occur until about 28 minutes after the peak level of ACTH in the blood is reached.

Discussion. The present data indicate that exogenous ACTH, even when administered subcutaneously in a large dose rapidly disappears from the blood. Endogenous ACTH, too, disappears quickly from the blood after ether anesthesia(6) and after scald(7).

Pincus *et al.*(8) showed that up to 70% of added ACTH activity is lost within 1 hour from blood. Since such *in vitro* inactivation may be prevented by previously heating the blood(8), it is likely that at least some of the disappearance of ACTH from blood *in vivo* may be due to enzymatic inactivation.

The time relationship between ACTH administration and adrenal ascorbic acid depletion has been the subject of many studies (9,10), but this is believed the first investigation where the actual ACTH concentration in the blood was correlated with its corresponding adrenal ascorbic acid depletion. This is significant since it shows that despite an unphysiologically high ACTH blood concentration a 28-minute lag in the adrenal response still occurs.

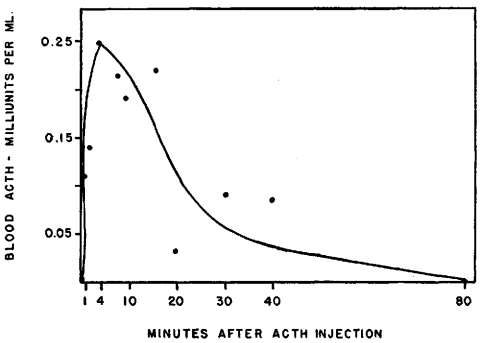

FIG. 2. Blood levels of ACTH in hypophysectomized rats after a single subcut. injection of 10 U.S.P. ACTH units. Each point represents mean response of 3 to 6 animals.

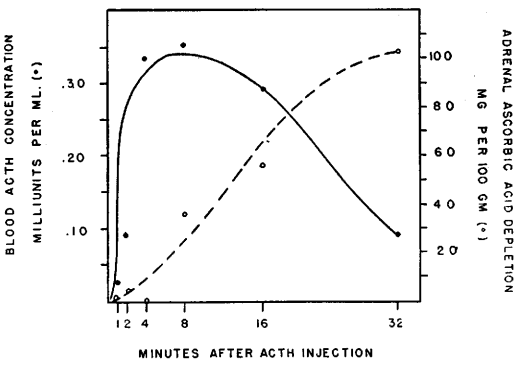

FIG. 3. Simultaneous measurement of blood ACTH concentration and adrenal ascorbic acid in hypophysectomized rats treated subcut. with 10 U.S.P. ACTH units. Each point represents mean response of 3 animals.

The highest blood concentration of ACTH observed in this study (Fig. 3) was 0.35 milliunit per ml blood. The blood volume of a rat is estimated to be 6 ml per 100 g(11,12). In the 250 g rats used in these experiments a total of only 6.1 milliunits, therefore, appears to be present in the circulation at the time of the highest blood concentration out of 10,000 milliunits injected. This indicates massive inactivation of the hormone at probably both the site of injection and after it enters the circulation.

Summary. The rate of disappearance of exogenous ACTH from the blood in the hypophysectomized rat has been determined. Following subcutaneous injection of 10 U.S.P. ACTH units detectable blood ACTH activity was found within 1 minute. A peak concentration of 0.25 to 0.35 milliunit per ml blood took place 4 to 8 minutes after injection. No detectable activity was found after 80 minutes. Significant adrenal ascorbic acid depletion did not occur until about 28 minutes after the peak concentration of ACTH in the blood was reached.

1. Van Dyke, D. C., Simpson, M. E., Li, C. H., and Evans, H. M., *Am. J. Physiol.*, 1950, v163, 297.

2. Greenspan, F. S., Li, C. H., and Evans, H. M., *Endocrinol.*, 1950, v46, 261.

3. Gemzell, C. A., Van Dyke, D. C., Tobias, C. A., and Evans, H. M., *ibid.*, 1951, v49, 325.

4. Sydnor, K. L., and Sayers, G., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 729.

5. Renold, A. E., Forsham, P. H., Maisterrena, J., and Thorn, G. W., *New England J. Med.*, 1951, v244, 796.

6. Sayers, G., and Burke, R., *Fed. Proc.*, 1955, v14, 131.

7. Sayers, G., *J. Clin. Endocrinol.*, 1955, v15, 754.

8. Pincus, G., Hechter, O., and Hopkins, T., *ibid*, 1952, v12, 920.

9. Sayers, G., Sayers, M. A., Fry, E. G., White, A., and Long, C. N. H., *Yale J. Biol. Med.*, 1944, v16, 361.

10. Sayers, G., and Sayers, M. A., *Ann. N. Y. Aacd. Sci.*, 1949, v50, 522.

11. Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1933, v99, 457.

12. Metcoff, J., and Favour, C. B., *Am. J. Physiol.*, 1944, v141, 695.

Received August 29, 1955. P.S.E.B.M., 1955, v90.