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Biological Half-Life of Endogenous ACTH.* (20476)

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The relatively high concentration of adrenocorticotrophic hormone (ACTH) in the blood of the adrenalectomized rat(1,2) makes it possible to determine the biological half-life of endogenous hormone in this animal.

Methods. Male rats from the Holtzman-Rolfsmeyer farm weighing 200 to 300 g were acclimatized for 2 weeks in the laboratory (constant lighting and constant temperature, 76 ± 2°F) before use in the experiments.

Two separate experiments were performed to determine the rate of disappearance of ACTH from the blood of adrenalectomized rats following hypophysectomy. One week (Exp. 1) or 2 weeks (Exp. 2) after bilateral adrenalectomy,† the animals were anesthetized with ether and either sham-hypophysectomized (pituitary exposed, but not removed) or hypophysectomized. The sham-hypophysectomized animals served as zero-time controls. All rats were decapitated‡ and blood was drained from the trunk into 4 volumes of glacial acetic acid. Blood samples were collected exactly one minute after sham-hypophysectomy, and 1, 2, or 3 minutes after hypophysectomy. The blood-glacial acetic acid mixtures from individual rats of each group were pooled and processed by the oxycellulose§ method(3).

Exp. 3 was designed to determine if the rapid disappearance of ACTH from the blood of adrenalectomized rats one minute after hypophysectomy could be attributed to inactivation in blood itself. Two weeks after bilateral adrenalectomy, 76 rats were exposed to ether for 2 minutes, then bled from the abdominal aorta for a period of one minute. One-third of each blood sample was added immediately to 4 volumes of glacial acetic acid; the remaining two-thirds were quickly transferred to a centrifuge tube, incubated for one minute at 37°C, and then immediately added to 4 volumes of glacial acetic acid. The non-incubated (control) and the incubated blood-glacial acetic acid mixtures were pooled separately and processed by the oxycellulose method.

The pH of the HCl-eluate of each pool of processed blood was adjusted to 4 immediately before assay for ACTH in male hypophysectomized rats by the adrenal ascorbic acid-depletion method(4). The estimate of the concentration of ACTH in blood is based on the assumption that the oxycellulose method recovers 100% of the hormone. Studies on ACTH added to hypophysectomized rat blood indicate that 96 ± 6% of the hormone is recovered(5).

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† Adrenalectomized animals were given 0.9% sodium chloride solution to drink.

‡ Decapitated in order to prevent the adenohypophysis of the sham-hypophysectomized rat from contributing to blood ACTH during bleeding.

§ Samples of oxidized cellulose (10-12% COOH) were generously supplied by the Research Laboratories, Tennessee Eastman Co.

TABLE I. Blood ACTH.

Exp. No.	Test substance	Dose (ml per 100 g test rat)	Ascorbic acid depletion (mg/100 g adrenal) Individual responses*	Avg	Estimate of conc., M ± S.E.M., mu† per 100 ml
1	U.S.P. ACTH Std.	1.0 mu	112, 80, 103, 145, 117	111	
		.25	43, 57, 34, 27, 50	42	
	Adrenx rat blood, 1' after sham-hypox	6.0 ml	50, 122, 82, 109	104	
		3.0	84, 30, 61	58	11.8 ± 2.5
		1.5	27, 24, 27, 52	33	
	Adrenx rat blood, 1' after hypox	12.0 ml	111, 93, 109, 87, 88	98	
		6.0	46, 55, 40, 63	54	6.0 ± 1.3
		3.0	15, 13, 82	37	
	Adrenx rat blood, 2' after hypox	12.0 ml	74, 76, 35	86	
		6.0	31, 45, 20, 36, 30	32	3.8 ± .9
		3.0	5, 3, −28, 5, 10	−1	
	Adrenx rat blood, 3' after hypox	12.0 ml	65, 48, 118, −16	77	
		6.0	48, 23, 35, 40	36	2.8 ± 1.2
		3.0	42, −25, 16, −36, 36	7	
2	U.S.P. ACTH Std.	1.0 mu	188, 157, 160, 137, 145, 170	160	
		.5	102, 80, 125, 107, 69, 69	92	
		.25	63, 37, −4, 87, 77, 86	58	
	Adrenx rat blood, 1' after sham-hypox	6.0 ml	124, 112, 134, 142, 107	124	
		3.0	54, 97, 50, 71, 95, 82	75	11.0 ± 1.7
		1.5	2, 98, 44, 77, 18, 42	47	
	Adrenx rat blood, 1' after hypox	12.0 ml	90, 105, 118, 145, 58, 75	98	
		6.0	45, 76, 61, 56	60	3.8 ± .6
		3.0	27, 17, −34, 36, −2	9	
	Adrenx rat blood, 3' after hypox	24.0 ml	55, 108, 121, 123, 119	105	
		12.0	34, 65, 38, 68, 18, 11	40	2.0 ± .6
		6.0	9, 65, 31, −7	25	
3	U.S.P. ACTH Std.	1.0 mu	140, 122, 171, 102, 132, 122, 136	131	
		.5	95, 64, 89, 80, 129, 127, 128	102	
		.25	49, 56, 65, 111, 105, 71	76	
	Adrenx rat blood, (control)	6.0 ml	105, 103, 222, 165, 128	145	
		3.0	44, 121, 138, 40, 55, 52, 80	80	13.7 ± 3.0
		1.5	83, 106, 50, 55, 32, 61, 20	58	
	Adrenx rat blood (incubated 1' at 37°C)	12.0 ml	150, 124, 196, 178, 169	163	
		6.0	153, 116, 135, 138, 146, 85	129	13.6 ± 2.7
		3.0	33, 93, 81, 128, 85	84	

* Difference in ascorbic acid concentration in right adrenal removed prior to injection of test substance and concentration in left adrenal removed 1 hr after injection.

† One mu equals one-thousandth of a U.S.P. unit of ACTH.

Results. The individual and average responses (adrenal ascorbic acid depletion) to the test material and to the Standard ACTH assayed on the same day, as well as the estimates of blood-ACTH concentrations, are presented in Table I. The data indicate that approximately 75% of the biological activity disappears from the blood within 3 minutes after hypophysectomy. Estimates of biological half-life were obtained by plotting the logarithm of the concentration of blood-ACTH against time, and by calculating the lines of best fit by the method of least squares (Fig. 1). The estimates for the 2 experiments, 0.95 and 1.25 minutes, are in good agreement.

The results of Exp. 3 indicate there is no appreciable loss of biological activity when adrenalectomized rat blood is incubated *in vitro* for one minute at 37°C. The estimate of the blood-ACTH concentration of the control (non-incubated) blood is not significantly different from that of the incubated blood. It appears that the rapid disappearance of

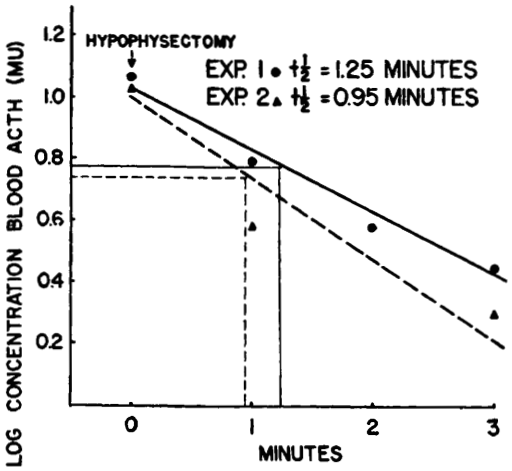

FIG. 1. Rate of disappearance of ACTH from blood of adrenalectomized rats following hypophysectomy (zero-time). See text for explanation.

ACTH from the blood of adrenalectomized rats one minute after hypophysectomy cannot be attributed to destruction of ACTH in blood itself.

Discussion. The 2 estimates of biological half-life of endogenously produced ACTH, 0.95 and 1.25 minutes, are in good agreement. The difference in the 2 estimates may be explained on the basis of the error of the bioassay method for the analysis of ACTH rather than on incomplete or inconsistent recovery of the hormone from blood by the oxycellulose method. Actually, incompleteness of recovery would have little, whereas inconsistency of recovery would have a significant, influence upon the estimation of the half-life of ACTH. The results of 7 separate experiments on recovery of Standard ACTH added to hypophysectomized rat blood indicate that the oxycellulose method recovers 96 ± 6% of the hormone(5). An analysis of the results of 14 separate experiments indicates that the mean concentration of ACTH in blood of acutely exsanguinated, adrenalectomized rats with intact pituitaries is 12.8 ± 0.6 mu per 100 ml. The small standard errors of these estimates provide strong evidence for consistency of recovery of endogenous ACTH by the oxycellulose method.

No previous reports have appeared in the literature regarding the biological half-life of endogenous ACTH. Van Dyke *et al.*(6) have calculated the rate of disappearance of endogenous ACTH and have estimated the ". . . average life of the ACTH hormone molecule in the circulation of the rat at physiological levels" to be approximately 17 minutes. This estimate was based on adrenal weight changes in parabiotic rats, a bioassay technic which is quantitatively inadequate to measure ACTH. The estimate of the half-life of ACTH in the present study has been made, necessarily, in adrenalectomized rats. It is not technically feasible to determine the half-life in rats with intact adrenal glands. The concentration of ACTH in blood of the intact rat is too low, even under conditions of stress, to make a quantitative analysis of the type presented in this report. If the adrenals "utilize" ACTH, and on this point there are no definitive data, one would expect the half-life to be even shorter in the intact than in the adrenalectomized animal.

Endogenously produced ACTH appears to have a shorter half-life than exogenous ACTH. Values of 5.5 minutes(7) and 5 minutes(1) have been reported for sheep ACTH administered to intact rats. Sheep ACTH apparently has the same half-life in adrenalectomized rats(1). Calculation of the data presented by Richards and Sayers(8) indicate that the half-life of rat ACTH administered to intact rats is approximately 2 minutes, a value in close agreement with the estimates for endogenous ACTH presented in this study. The reason for the difference between the half-life of injected sheep and injected rat ACTH is not apparent. It is reasonable to assume that a study of endogenously secreted ACTH has more physiological significance.

The mechanisms concerned with the rapid disappearance of ACTH from the circulation are unknown. Inactivation in blood itself cannot be a major factor. Experiments on renal excretion of administered ACTH in rat(8) and man(9) indicate that no appreciable fraction of the hormone is excreted in biologically active form. However, no quantitative data are available on renal excretion of endogenous ACTH. ACTH is rapidly inactivated when incubated with slices or homogenates of various tissues(10). *In vitro* studies of this nature are difficult to interpret in

physiological terms, since disintegrated cells exhibit an abnormally high degree of proteolytic activity. For example, intact pituitaries incubated for one hour at 37°C show no loss of ACTH activity, whereas crushed pituitaries lose practically all activity within a few minutes after cell injury(11).

Characterization of the dynamics of the pituitary-adrenocortical system would add appreciably to our understanding of the mechanisms which influence the rate of elaboration of ACTH and the cortical steroids. It is now definitely established that ACTH release from the adenohypophysis is accelerated within a remarkably short time after application of a stressful stimulus. The indirect studies of Gray and Munson(12) suggest that acceleration occurs within 10 seconds after application of a stress. Recent direct analyses of blood-ACTH in this laboratory(5) indicate that the blood titer of ACTH is significantly increased within 2 minutes after subjecting animals to stressful stimuli. The present study establishes the fact that ACTH, following its release from the pituitary, has an extremely brief sojourn in the blood.‖ It appears reasonable to conclude that the dynamics of ACTH release and disappearance are characterized by time factors which do not contribute an appreciable degree of inertia to the pituitary-adrenocortical system.

No precise observations have been made on the time-lag between increased rate of release of ACTH and of increased rate of secretion of corticosteroids by the adrenal. The concentration of total 17-hydroxycorticosteroids(13) and of 17-hydroxycorticosterone(14) in blood is significantly increased 15 minutes after a single intravenous injection of ACTH in man. Experiments are in progress in animals to determine with accuracy the time which elapses between accelerated ACTH release and accelerated adrenocorticosteroid secretion.

Another important time-relationship, and the one which may contribute the greatest degree of inertia to the pituitary-adrenocortical system, is the period which elapses between the reduction of the blood-ACTH titer to pre-stress levels and the subsidence of the accelerated secretory activity of the adrenal cortex. Only meagre information is available on this aspect of the problem. Sweat *et al.* (14) found 17-hydroxycorticosterone concentration in blood to remain abnormally high for 4 hours after a single intravenous injection of 15 units of ACTH in man; apparently the secretory activity of the adrenal cortex is accelerated for a relatively long period after a brief stimulus with ACTH. More direct observations have been made by Farrell and Sweat(15) who determined steroid output by the adrenal after hypophysectomy; steroid output at one and 2 hours after hypophysectomy was 50 and 20%, respectively, of preoperative levels.

Finally, the rate of utilization, degradation and excretion of blood adrenocorticosteroids must be determined in order to gain a complete characterization of the dynamics and, in turn, of the regulatory mechanisms of the pituitary-adrenocortical system.

Summary. The biological half-life of endogenous ACTH in blood of the adrenalectomized rat is approximately one minute (0.95 and 1.25 minutes for 2 experiments). No appreciable loss of biological activity occurs when adrenalectomized rat blood is incubated *in vitro* for one minute at 37°C. The rapid disappearance of ACTH from the blood of the adrenalectomized rat one minute after removal of the adenohypophysis cannot be attributed to inactivation of the hormone by blood itself.

‖ It is to be noted that the concentration of ACTH in blood of adrenalectomized animal is greater than that in blood of intact animal. The discussion assumes that rate of disappearance of ACTH from the circulation is the same at all blood concentrations. The validity of the assumption can only be tested when a method is available for determining rate of disappearance of endogenous ACTH at low concentrations.

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Chemical and Biological Studies on *1,2*-Dihydro-*s*-triazines. IV. Activity in Pteroylglutamic Acid-*Streptococcus faecaelis* No. 8043 Systems. (20477)

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Preliminary communications from these laboratories reported the synthesis(1,2) of an hitherto undescribed class of compounds, the *1,2*-dihydro-*s*-triazines which exhibit significant anti-malarial*(1-3), anti-tumor(3), and anti-vitamin(1,4) activity. Details of the synthesis, chemical characterization and identification of these compounds are recorded elsewhere(2,5). During the course of these studies, certain derivatives of this series also have been synthesized independently by others in connection with studies on the identification of the active metabolite of paludrine(6,7).

The biological activity of the symmetrical dihydrotriazines was first observed in these laboratories early in 1950 when the parent-compound of the series (D-19 • HCl) was studied in routine screening assays† for microbiological activity. Since that time, more than 250 samples of 71 derivatives of this series have been studied. The purpose of the present communication is to report the activity of these compounds in *Streptococcus faecalis* No. 8043‡-pteroylglutamic acid (PGA) assay systems.

Methods. PGA assays(8) using *Streptococcus faecalis* were done in Difco PGA Assay Medium or the casein hydrolysate medium prepared as described by Tepley and Elvehjem(9). The basal medium was diluted (usually 1:1) with glass-distilled water to yield uniformly reproducible optical densities of 4.0-6.0 x 10 from minimal inocula with the addition of PGA and no growth in the absence of PGA. Media were handled in chemically clean glassware, dispensed in 10 ml volumes in 22 x 175 mm optically standardized tubes, closed with loose fitting aluminum caps and autoclaved 5 minutes at 15 lb pressure. Crystalline PGA, other metabolites and the various dihydrotriazines were prepared in appropriate concentrations in sterile glass-distilled water and added aseptically to the cooled sterile medium to the desired final concentration. Assays were incubated at 37°C for 24 hours. Optical density was read di-

* We are indebted to J. H. Williams and his colleagues of the Lederle Laboratories, Pearl River, N. Y. for the anti-malarial assays.

† The routine screening program, which comprises several standard microbiological assay systems as well as studies with a number of miscellaneous bacteria and fungi was organized in 1947 as one part of a broad program in the chemotherapy of cancer initiated by Sidney Farber.

‡ A. T. C. C. No. 8043.