Pituitary ACTH and Plasma Free Corticosteroids Following Bilateral Adrenalectomy in the Rat.*† (24506)

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From a study of changes in pituitary ACTH, plasma free corticosteroids and adrenal weight induced in the rat by a surgical procedure (splenectomy), it was concluded (unpublished) that the initial postoperative depletion of pituitary ACTH, and its subsequent rise, reflected opposite effects of stress and corticosteroids on release of the hormone. The present report on the effect of hypocorticoidism on ACTH release and synthesis was based on concurrent determinations of pituitary ACTH and plasma free corticosteroids, following bilateral adrenalectomy in the rat.

Materials and methods. Male Sprague-Dawley rats (175-225 g, initial B.W.) were acclimatized for 2 weeks to laboratory conditions (constant lighting and temperature (25 ± 1°C); Purina Fox-Chow and water *ad lib.*) and distributed into equal numbers of experimental and control groups of 3 subjects of uniform weight. *Bilateral adrenalectomy* was performed by lumbar approach, under ether anesthesia. The animals were maintained postoperatively on 0.9% NaCl drinking solution, and killed by decapitation at progressively later intervals from onset of anesthesia. They were closely examined postmortem for presence of ectopic or regenerated adrenal tissue, and discarded in the few instances when such tissue was detected. The *adenohypophyses* were collected, pooled and processed, as previously described,¶ for ACTH assay by *in vitro* technic of Saffran and Schally(1). The results, computed and tested for validity according to Bliss' methods for factorial analysis and analysis of variance (2), were expressed in milliunits (mu) of ACTH/mg of fresh tissue (with confidence limits (antilog $C^2M \pm CtSm$) for odds of 19 in 20), and in percentage of mean control level. Equal emphasis was placed on the internal (*within* assays) and external (*between* assays) variances of the combined estimates (unweighted logarithmic means) by computing their standard errors from the averages of crude and unweighted $S\bar{m}$'s. In a separate experiment performed under strictly identical conditions, *arterio-venous blood* from neck vessels was collected into heparinized beakers for plasma free corticosteroid determination by modification(3) of Silber's fluorometric method(4). The results, expressed in corticosterone equivalents, as well as in percentage of the control baseline, were corrected for fluorescence of unknown origin, observed to persist in blood of adrenalectomized(4,5) and hypophysectomized(5) rats, by subtracting 5 (a value corresponding to this fluorescent residue) from the original estimates.

Results. Pituitary ACTH response to bilateral adrenalectomy (Fig. 1) was poly-

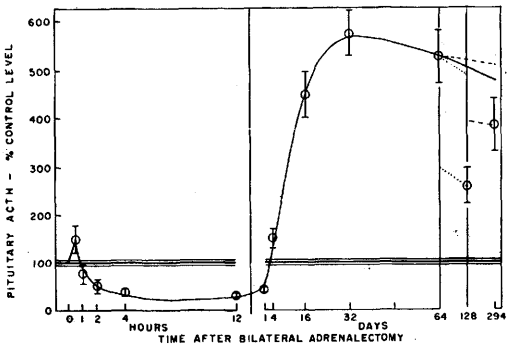

FIG. 1. Effect of bilateral adrenalectomy on pituitary ACTH concentration. Dots correspond to means of 3 assays for the first time ordinate, of 2 for the others; control baseline, to mean of 18 assays. In this as in following figure, stand. errors are respectively indicated by T-shaped bars for experimental, and parallel lines for control, values.

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TABLE I. Effect of Bilateral Adrenalectomy on Pituitary ACTH Concentration.

Interval	ACTH/mg fresh tissue (mu)	95% confidence limits (mu)	Index of precision (λ)†
0			
Mean of 18 assays $\pm S.E.$*	40.6 ± 1.7		.105 ±.009
30 min.	89.8	28.3–282.2	.168
	40.0	25.2– 63.6	.091
	62.4	33.3–218.2	.143
	60.7 ± 12.9		
1 hr	39.8	13.6–122.4	.169
	23.6	7.9– 47.9	.148
	30.7 ± 7.8		
2	15.7	4.3– 26.9	.125
	28.3	11.9– 53.0	.132
	21.1 ± 5.7		
4	13.8	6.0– 21.6	.090
	19.4	12.3– 27.9	.060
	16.4 ± 2.6		
12	11.6	4.1– 19.1	.102
	13.7	2.3– 27.2	.171
	12.6 ± 1.9		
1 day	15.8	10.6– 21.2	.057
	19.4	3.0– 38.8	.174
	17.5 ± 2.4		
4	54.9	30.6–148.7	.131
	69.2	45.3–126.5	.094
	61.7 ± 8.6		
16	173.5	119.5–272.4	.082
	192.0	64.5–865.8	.182
	182.4 ± 19.9		
32	226.4	153.2–399.3	.082
	241.2	151.3–512.7	.104
	233.3 ± 20.8		
64	197.6	118.4–305.6	.094
	234.0	160.7–392.5	.081
	214.8 ± 21.9		
128	97.4	52.3–152.3	.099
	112.5	68.4–227.5	.110
	104.7 ± 13.6		
294	142.8	78.1–305.7	.125
	172.6	101.6–668.2	.132
	157.0 ± 22.5		

* Stand. errors ($S.E.$'s) of combined estimates (unweighted logarithmic means) were computed from averages of their crude and unweighted $S\bar{m}$'s.

† Stand. dev. (s) of response (Y) divided by slope (b) of assay: $\lambda = s/b$ (2).

phasic: transient rise within first 30 minutes, followed by fall to 40% of control level after 4 hours, slower rate of decline and stabilization at low level for the next 20 hours, subsequent rise, to a crest of 575% on 32nd day, and slow regression thereafter to lower, though still markedly elevated, values. The mean angle of decline from the 64th day was computed from values recorded after 128 and 294 days. An identical pattern characterized the 2 series of determinations carried out independently (Table I).

Free corticosteroids (Fig. 2) disappeared from the plasma within 4 hours. The residual fluorescence, inherent to the method(3,4) and corresponding to 5 γ of corticosterone/100 ml of plasma (uncorrected scale), was remarkably constant thereafter, as shown by standard error of 0.1, computed from all determinations in chronic groups.

Discussion. A slight increase of pituitary ACTH concentration, following bilateral adrenalectomy in the rat, was recently reported by Kitay *et al.*(6) on the basis of assays at a single postoperative interval (7 days), by modification of Saffran and Schally's technic. Previous and more detailed studies were based on Sayers' assay(7). From single point determinations, in which the potency of the unknown in terms of adrenal ascorbic acid-depleting activity was computed directly from a standard log dose-response chart, Gemzell *et al.*(8) reported that bilateral adrenalectomy induced a progressive fall of pituitary ACTH to a minimum of 30% of the preoperative level after 24 hours. This was followed by a rise to 178 and 191% of the control values, after 7 and 21 days respectively. For lack of determinations before the first hour, the initial rise in

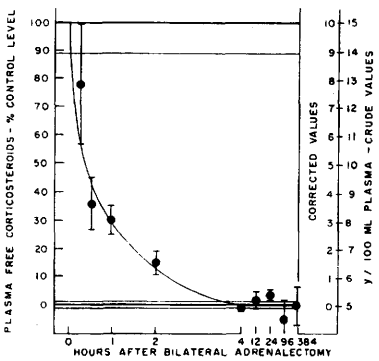

FIG. 2. Disappearance of plasma free corticosteroids, following bilateral adrenalectomy. Dots and control baseline respectively correspond to means of 4 and 42 determinations.

concentration, in the present study at the 30-minute postoperative interval, was not detected. Percentagewise, the results of Gemzell *et al.* are in fair agreement with ours, with respect to the subsequent 24-hour depletion, but differ markedly thereafter. In view of the utilization by this group of a uniform aliquot (0.01 mg of dry pituitary) throughout their series, the comparatively low values recorded at 7- and 21-day p.o. intervals may be attributed to improper adjustment of the unknown to the useful range (rectilinear portion of log dose-response slope) of Sayers' assay.‡ The same may hold, to a lesser extent, with regard to the low post-adrenalectomy values (114, 214 and 254% after 7, 14 and 18 days respectively) reported by Sydnor and Sayers (9) on the basis of 5-point assays, in which samples from adrenalectomized animals were uniformly determined at half the concentration used for controls.

The amplitude of the observed changes in pituitary ACTH concentration provides clear evidence of a dissociation between rates of ACTH release and synthesis. A constant level of pituitary ACTH admittedly corresponding to a steady state, defined by equal rates of synthesis and release, increasing and decreasing levels will be respectively indicative of a predominance of synthesis over release and *vice versa*. Given similar trends of storage and release, the trend of synthesis would be readily available through algebraic summation. The interpretation of our data is facilitated, in this regard, by recent observations of Brodish and Long(10). Using a cross-circulation technic, in which the adrenal ascorbic acid (AA) response of the hypophysectomized recipient to 20 ml of circulated blood from the donor, indicated the latter's relative blood ACTH level(11), these investigators reported that bilateral adrenalectomy resulted, within 15 minutes, in a significant rise of blood ACTH which gradually subsided until the 12th hour, when minimal values were recorded. A secondary rise to supernormal values was observed thereafter. It levelled off, after 2 weeks, until the end of observation period (4 weeks). Notwithstanding the limitations of the cross circulation technic,§ it is noteworthy that similar time relationships characterize succession of events initiated by adrenalectomy at blood and pituitary levels. In both instances, the initial phase of the reaction involves immediate rise followed by a fall to minimum level after 12 hours, the secondary phase, a subsequent rise to supernormal levels. It would thus appear that, following a transient burst of synthesis, reflected by concomitant initial peaks of pituitary and blood ACTH, a predominant, though gradually receding, acceleration of rate of release accounts for pituitary ACTH depletion, a greater and sustained acceleration of synthesis, for its secondary rise.

A comparison of pituitary ACTH responses to bilateral adrenalectomy and to splenectomy, 2 forms of surgical trauma respectively characterized by rapid disappearance and equally rapid rise of blood corticosteroids, illustrates the latter's involvement in adrenocorticotrophic regulation. No initial rise in concentration was observed after splenectomy. A fall of lesser amplitude (40, as compared to 70% after adrenalectomy) was followed by a rise to a transient peak, sharply contrasting in magnitude and duration with the upward phase of the response to adrenalectomy. From available indices of ACTH release, it appears furthermore that, whereas the fall can be ascribed, in both instances, to a predominantly accelerated rate of release, the secondary rise is related to depressed release after splenectomy, and to predominant synthesis after adrenalectomy. It may be inferred therefrom that 1) the ACTH-releasing effect of stress, common to the 2 experimental conditions, is markedly influenced by the level of circulating adrenal cortical hormones, and that 2) withdrawal of these hormones enhances both release and synthesis of

‡ This range reportedly extends from 0.15 to 2.5 mu of ACTH/100 g, B.W.(8), as compared to 3-300 mu/100 mg adrenal, for Saffran and Schally's assay(2).

§ Adrenals of recipient animal being already stimulated (17% AA depletion) by the ACTH released as a result of cannulation of donor(10,11), this technic provides an unusually narrow margin of sensitivity to additional stimulation.

ACTH, but has a predominant effect on the latter. The eventual arrest and reversal of this reaction may be tentatively related to the intervention of a complementary feed-back mechanism of a more sluggish type, as suggested by Gemzell and Heijkenskjöld's observation of a delayed and cumulative depressing effect of exogenous ACTH on the synthesis of the hormone in the adrenalectomized animal(12).

Summary. Pituitary ACTH and plasma free corticosteroids were concurrently determined following bilateral adrenalectomy in the rat. Free corticosteroids disappeared from the plasma within 4 hours. Pituitary ACTH rose to 150% of the control level within the first 30 minutes, fell to 40% after 4 hours, to 30% after 12, stabilized at this level for the next 12 hours, rose from then on to supernormal levels, up to a crest of 575% on the 32nd day, and regressed, thereafter, to lower, though still markedly elevated, values. From a correlation of these findings with available data on blood ACTH levels under comparable conditions, it is inferred that, following a transient burst of synthesis reflected by the initial peak, a predominant, though gradually receding, acceleration of the rate of release accounts for the pituitary ACTH depletion, a greater and sustained acceleration of synthesis, for its subsequent rise. It is further suggested that the ACTH-releasing effect of stress is markedly influenced by the level of circulating adrenal cortical hormones, and that withdrawal of these hormones enhances both release and synthesis of ACTH, but has a predominant effect on the latter.

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Effect of Hydrocortisone on Pituitary ACTH and Adrenal Weight in the Rat.*† (24507)

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From previous study of changes in pituitary ACTH concentration induced, in the rat, by bilateral adrenalectomy(1), it was inferred that withdrawal of the adrenal cortical hormones enhances both release and synthesis of ACTH, but has a predominant effect on synthesis. As a correlate, the present report on effect of hypercorticoidism on adrenocorticotrophic activity was based on concurrent determinations of pituitary ACTH and adrenal weight during hydrocortisone administration in the rat.

Materials and methods. Male Sprague-Dawley rats (175-225 g, initial B.W.) were acclimatized for 2 weeks to constant lighting

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