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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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. *Hydrocortisone* (Hydrocortone Acetate, Merck; Saline suspension, 25 mg/ml) was administered at 6 mg/100 g, I.P. on first day, in Exp. A and B; at 3 and 6 mg/100 g, S. C. on subsequent days in Exp. A and B respectively. Antibiotics (Combiotic, Pfizer; Crystalline Penicillin G. Procaine, 200,000 U and Crystalline Dihydrostreptomycine, 250 mg/ml of saline suspension) were administered (0.2 ml/100 g, S.C.) on alternate days throughout treatment. Animals were killed by decapitation at progressively later intervals from first injection of hydrocortisone. *Adenohypophyses* were collected, pooled and processed, as previously described,¶ for ACTH assay by *in vitro* technic of Saffran and Schally(2). The results, computed and tested for validity according to Bliss' methods for factorial analysis and analysis of variance(3) 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 internal (*within* assays) and external (*between* assays) variances of combined estimates (unweighted logarithmic means) by computing their standard errors from the averages of crude and unweighted $S\bar{m}'s$(3). *Adrenal glands* were dissected free of fat and weighed to the nearest 0.05 mg. To dissociate growth from treatment effects, results were expressed as percentages of mean control values for each time interval.

Results. An immediate, slight (14%) and short-lived (2 hr) depletion‡ of *pituitary ACTH* (Table I, Fig. 1A) was followed by rise to a peak of 133% of initial level, reached after 4 hours and tapering off slightly in the next 20 hours. A steep, though somewhat irregular, decrease in concentration, bearing no significant relationship to dosage, was observed thereafter. Less than 10% of the original concentration was found after 24 days of the 6 mg regimen. No ACTH could be detected, within limits of sensitivity of the assay (less than 1.5 mu/mg of pituitary, when sample was assayed at 4 times the usual concentration) after 55 days of hydrocortisone administration at the 3 mg dosage level. This regimen (3 mg/100 g, daily) induced an exponential reduction of the *adrenal weight* to hypophysectomy level (10-14 mg, corresponding to 22-34% of controls)§ within 32-55 days. Hydrocortisone administration also resulted, from onset, in complete arrest of

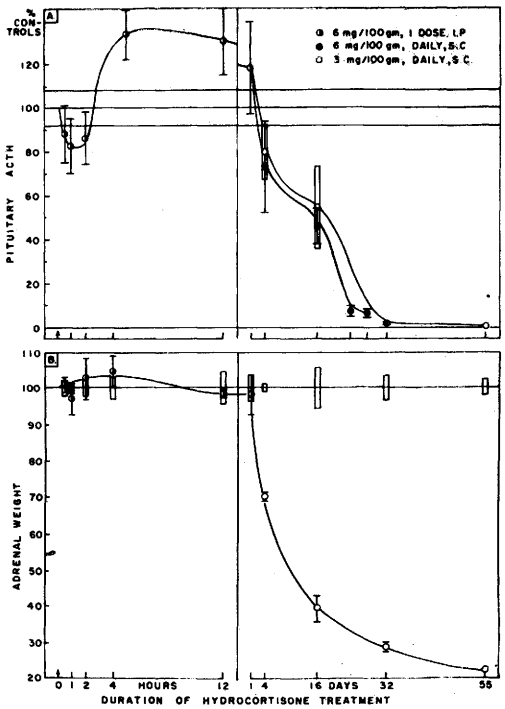

FIG. 1. Effect of hydrocortisone on pituitary ACTH concentration (A) and adrenal wt (B). Black & white and monochrome dots, in A, respectively correspond to means of 2 assays, with *S.E.*'s computed from avg of crude and unweighted $S\bar{m}$'s, and to single assays, with *S.E.*'s derived from internal *Sm*'s. Control baseline was obtained from mean of 13 assays. Dots and their respective control baselines, in B, correspond, for all but 55-day time ordinate (1 experimental value), to means of 4-6 determinations.

¶ Fortier, C., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 628.

‡ Though none of the values corresponding to first 3 time ordinates differs significantly from the control mean, their grouping is suggestive of a true fall.

§ Nearly identical values were recorded by Harris and Jacobsohn(4) 42-45 days after hypophysectomy in male rats.

TABLE I. Effect of Hydrocortisone on Pituitary ACTH Concentration.

Interval	ACTH/mg fresh tissue (mu)	95% confidence limits (mu)	Index of precision (λ)†
	Exp. A§		
0			
Mean of 7 assays ± *S.E.**	84.0 ± 7.9		.120 ±.015
30 min.	65.3	38.9–144.4	.115
1 hr	55.7	43.5– 73.1	.054
2	62.3	45.9– 89.4	.066
4	105.7	63.8–282.8	.113
12	95.2	63.2–167.9	.084
1 day	76.0	55.6–114.1	.068
4	66.9	45.7–107.7	.083
16	46.2	12.4–139.0	.175
32	1.6	1.0– 2.2	.065
55‡	0 (<1.5)		
	Exp. B‖		
0			
Mean of 6 assays ± *S.E.**	65.2 ± 4.7		.100 ±.020
30 min.	65.4	40.0–127.6	.108
1 hr	67.4	53.7– 87.9	.038
2	65.8	38.6–146.0	.103
4	91.9	76.7–113.0	.034
12	93.3	67.4–145.5	.065
1 day	100.3	85.3–121.1	.031
4	48.2	22.3–117.3	.144
16	30.2	17.4– 44.1	.082
24‡	4.7	1.1– 8.4	.107
28‡	4.2	1.3– 7.8	.077

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

† Stand. dev. (s) of response (Y) divided by slope (b) of assay: $\lambda = s/b$ (3).

‡ Sample obtained from 1 donor, instead of usual 3.

§ Hydrocortisone acetate, 6 mg/100 g, I.P. on first day; 3 mg/100 g, S.C. on subsequent days.

‖ Hydrocortisone acetate, 6 mg/100 g, I.P. on first day, S.C. on subsequent days.

growth which is admittedly related to the protein catabolic effect of glucocorticoids(5,6). The few animals which survived beyond the first 30 days of treatment evidenced marked cachexia.

Discussion. The foregoing observations on pituitary ACTH-depleting effect of chronic hydrocortisone administration are in general agreement with previously reported results of cortisone administration. From direct comparisons of the adrenal ascorbic acid-discharging activity of pituitaries from intact and cortisone-treated (100 mg, daily) dogs, Farrell and Laqueur(7) reported that the ACTH content was depressed to 54, 3 and 2% of the normal, after 14, 44 and 71 days of treatment. Using similar procedure in rats, Michailova (8) observed a reduction to 61 and 55%, after 19 and 24 days of cortisone (2.5 mg, daily) administration. More recently, Kitay *et al.*(9), who used a modification of Saffran and Schally's assay, found a 41% depletion, in the rat, after 7 days of treatment with cortisone (5 mg, daily). No previous observation is available on the immediate effects of steroid injection, displayed, in the present study, by a transient fall and a subsequent rise of the pituitary ACTH concentration.

Interpretation of the observed changes in pituitary ACTH concentration is largely dependent on information provided by indices of ACTH release. Thus, from adrenal ascorbic acid (AA) depletion noted one hour after cortisone injection in the rat(10), taken in conjunction with the reported blockade of the AA response to stress, from hydrocortisone administration 4 hours prior to its onset(11), it may be inferred that the initial post-injection fall of pituitary ACTH is related to ACTH-releasing effect of the injection *per se*, and its subsequent rise, to inhibitory effect of the steroid on further release. Sustained and complete blockade of release during the chronic phase of treatment is evidenced by the exponential decline of adrenal weight to hypophysectomy level. Were release and synthesis the only factors to be considered, total inhibition of synthesis could be inferred from failure of pituitary ACTH to rise under these conditions. Its very fall, however, points to involvement of a third factor: inactivation of the stored hormone. Whether the observed rate of ACTH disappearance (half-life of 5.2 ± 3.5 days, computed from regression of x (time) on y (log concentration)) corresponds to inactivation alone or in opposition to residual synthesis, cannot be ascertained from our data.

Summary. Pituitary ACTH and adrenal weight were concurrently determined, in the rat, during treatment with hydrocortisone (3 and 6 mg/100 g, daily). An immediate and slight depletion of pituitary ACTH was fol-

lowed by rise to a peak of 133% of the initial level, reached after 4 hours and tapering off in the next 20 hours. A sharp decrease was observed thereafter. Less than 10% of the original concentration was found after 24 days of the 6 mg regimen. No ACTH could be detected after 55 days, at the 3 mg dosage level. This regimen (3 mg) induced an exponential reduction of the adrenal weight to hypophysectomy level within 32-55 days. From available indices of ACTH release under comparable conditions, it is inferred that the initial post-injection fall of pituitary ACTH is related to the ACTH-releasing effect of the injection *per se,* and its subsequent rise, to the inhibitory effect of the steroid on further release. Sustained and complete blockade of release during the chronic phase of the treatment is suggested by the rate of adrenal weight loss. The concomitant disappearance of pituitary ACTH is imputed to inactivation of the stored hormone in the absolute or relative absence of synthesis.

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Lipid Composition of Human Serum Lipoprotein Fraction with Density Greater than 1.210.* (24508)

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On ultracentrifugation of samples of untreated human serum, Turner *et al.*(1) found that the fraction of high density at the bottom of the tube contained phospholipid and neutral fat in concentrations approximating those of whole serum, but little cholesterol. Subsequently, Turner *et al.*(2) estimated that approximately 65% of the serum phospholipid was present in a lipoprotein containing no free cholesterol. Hillyard, Entenman, Feinberg and Chaikoff(3) reported that on ultracentrifugation of human serum, the density of which had been raised to 1.220 by addition of potassium bromide, a fraction sedimented which contained an average of 13% of the total serum phospholipid and small quantities of cholesterol. Using more vigorous centrifugation of serum with density raised to 1.21, Havel, Eder and Bragdon(4) sedimented a fraction which contained lipid phosphorus but practically no cholesterol. They estimated that such a lipoprotein, which varied little in concentration between healthy subjects and patients with hyperlipemia, could account for no more than 15% of the total serum phospholipid. The lipid (ethanol-acetone soluble) phosphorus, which was not dialyzable against 0.15 M sodium chloride, was precipitable with serum proteins by zinc hydroxide, soluble in water, insoluble in petroleum ether, and migrated in starch electrophoresis largely with the a_1 globulin-albumin fraction. Similarities of this phosphorus fraction and a serum phos-

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