group were significantly lower than in both control groups.

In both experiments the ratios of comb weight to body weight were significantly lower in experimental than in control groups. Such findings are interpreted as evidence that retardation of comb growth in animals receiving cord emulsion exceeded retardation in body growth. This ratio is of particular interest in comparing the group receiving liver-in-adjuvant and the spinal cord group. Although body weights of these groups did not differ significantly from each other, this ratio did reveal statistically significant differences.

In Exp. 2, testicular weights and ratios of testicular to body weight were significantly lower in the experimental than in control groups. There were no statistically significant differences in control groups.

Discussion and summary. Injection of homologous spinal cord in Freund's adjuvant into 7-day-old cockerels retards body growth with disproportionate retardation of comb and testicular growth. Preliminary microscopic examination of combs revealed decreased mucoid matrix of the intermediate layer of the dermis in experimental animals, suggesting a hormonal mechanism(1). Freund and co-workers(2) reported production of aspermatogenesis in guinea pigs by injection of homologous testicular tissue and adjuvant. In studying the effects of other tissues, these investigators found mild testicular damage and aspermatogenesis after injecting homologous *CNS* tissue and adjuvant into guinea pigs. The effects of these injections on secondary sex characteristics were not studied. They believed that, although CNS and testes shared a common antigen, the testicular injury was secondary to physio-pathologic changes associated with meningo-encephalitis (loss of body weight and other symptoms). In our chickens the incidence of encephalomyelitis was very low. Although body weights were retarded in these animals and in animals receiving liver-in-adjuvant, ratio of comb weight to body weight was lower only in the experimental group, indicating a disproportionate retardation of comb growth. It appears that some factor more specific than decreased body weight and changes associated with encephalomyelitis is operative. Whether it is a common antigen shared by CNS tissue and testes or whether it is inhibition of hypothalamic-hypophyseal function is purely speculative.

1. Hardesty, Mary, *Am. J. Anat.*, 1931, v47, 277.
2. Freund, J., Lipton, M. M., Thompson, G. E., *J. Exp. Med.*, 1953, v97, 711.

Received June 17, 1958. P.S.E.B.M., 1958, v99.

Effects of 11-Oxy Corticoids on Renal Excretion of Water and Electrolytes in Force-Fed Intact Rats.* (24323)

W. J. EVERSOLE AND B. E. ROMERO
Dept. of Biology, University of New Mexico, Albuquerque

Hydrocortisone, prednisolone, or prednisone were considered equally effective in augmenting urine volume and renal excretion of Na and K in water-loaded adrenalectomized rats (1). Recently, Houlihan and Eversole (unpublished) found that these and other 11-oxy steroids augmented urine volume and potassium excretion in force-fed adrenalectomized rats. Our experiments were conducted to determine (1) whether presence of adrenals would modify renal responses to 11-oxy corticoids and (2) relative effectiveness of corticoids in inducing adrenal atrophy.

Methods. Eleven groups of Long-Evans male rats (approx. 230 g) were used. The control group received no hormone while the

* The 11-oxy steroids were supplied by Dr. C. W. Winter, Merck Inst. for Therapeutic Res. The work was aided by N.I.H. grant. Final draft was written by W. J. Eversole, Guggenheim Fellow, Div. Cancer Res., Perugia, Italy. The authors thank Dr. Lucio Severi for use of facilities.

others were treated with one of the following corticoids: cortisone, prednisone, cortisone acetate, hydrocortisone, prednisolone, hydrocortisone acetate, 9α-fluorohydrocortisone, corticosterone, 11-dehydrocorticosterone, and $\Delta^{1,4}$-pregnadiene-17, 20, 21-triol-3, 11-dione (Δ^1 U). Degree of adrenal atrophy, daily urine volume, daily excretion of Na and K, terminal blood serum levels of Na and K, and body weight changes were criteria in evaluating hormonal effects. The rats were kept in wire-bottomed cages in air-conditioned quarters (76-78°F) and tube-fed a warmed, semiliquid diet by French catheter and veterinary syringe. The diet, patterned after Ingle and Oberle(2), consisted of: 120 g Cellu flour, 40 g Osborne-Mendel salt mixture, 100 g brewer's yeast powder, 10 g wheat germ oil, 10 g cod liver oil, 200 g corn oil, 160 g casein, 200 g corn starch, 190 g dextrose, 200 g sucrose, .1 g Vit. K, and water to make 2 l. Amount of food administered was gradually increased from 5 to 20 ml/day over a 6-day period. During preliminary feeding, rats were allowed access to Purina Chow and water, but on day 7 and through 11-day treatment they received 10 ml food in morning, 12 ml water at noon, and 10 ml of food again in afternoon. Each rat received 1 mg hormone/day: .4 in morning and afternoon and .2 mg at noon. Steroids were dissolved in alcohol, suspended in Merck's Aqueous Vehicle No. 1, and injected subcutaneously. Animals were in individual metabolism cages which separated urine from feces. Body weights and urine volumes were recorded daily and evaporative loss was assumed constant. On alternate days excretion of total Na and K was determined. Terminally, after 18 hr fast, blood collections were made from decapitated trunks, and only clear serum was analyzed for Na and K. At sacrifice, the adrenals were excised, dissected free of adhering fat, and weighed. Standard error was calculated by use of formula recommended by Fisher(3): $\text{S.E.} = \pm\sqrt{\frac{\Sigma d^2}{n(n-1)}}$. Differences between means were considered significant if P values were .05 or less.

Results. Table I shows values obtained for urine volume, urinary Na and K, and serum Na and K. Seven of the 10 steroids aug-

TABLE I. Effects of Adrenal Steroids on Water and Electrolyte Balance in Force-Fed Intact Rats. (1 mg/day for 11 days.)

	Urine vol, ml/day	Urinary Na, meq/day	Urinary K, meq/day	Serum Na, meq/l	Serum K, meq/l
Intact control	9.8 ± .28* (9)† (81)‡	2.26 ± .05 (9) (45)	1.90 ± .04 (9) (45)	145 ± 1.89 (9) (9)	7.4 ± .39 (9) (9)
Cortisone	10.7 ± .24 (6) (54)	2.49 ± .07 (6) (30)	2.07 ± .05 (6) (30)	140 ± .88 (6) (6)	7.2 ± .10 (6) (6)
Prednisone	11.9 ± .20 (6) (54)	2.44 ± .06 (6) (30)	2.09 ± .06 (6) (30)	145 ± .98 (6) (6)	7.3 ± .40 (6) (6)
Cortisone acetate	11.4 ± .30 (6) (54)	2.40 ± .06 (6) (30)	2.13 ± .07 (6) (30)	142 ± .79 (6) (6)	7.2 ± .17 (6) (6)
Hydrocortisone	11.3 ± .32 (5) (45)	2.41 ± .07 (5) (25)	2.06 ± .05 (5) (25)	141 ± .84 (5) (5)	7.4 ± .22 (5) (5)
Prednisolone	13.0 ± .22 (6) (54)	2.47 ± .03 (6) (30)	2.14 ± .04 (6) (30)	145 ± 1.67 (6) (6)	7.9 ± .32 (6) (6)
Hydrocortisone acetate	12.1 ± .32 (6) (54)	2.52 ± .07 (6) (30)	2.19 ± .09 (6) (30)	142 ± 1.20 (6) (6)	7.6 ± .71 (6) (6)
9α-fluorohydrocortisone	11.1 ± .52 (6) (54)	2.27 ± .06 (6) (30)	2.03 ± .06 (6) (30)	162 ± 3.40 (5) (5)	5.2 ± .43 (5) (5)
Corticosterone	9.0 ± .32 (7) (63)	2.31 ± .06 (7) (35)	1.81 ± .05 (7) (35)	150 ± 2.36 (7) (7)	7.4 ± .20 (7) (7)
11-dehydrocorticosterone	10.2 ± .28 (6) (54)	2.34 ± .06 (6) (30)	2.02 ± .04 (6) (30)	143 ± 2.23 (6) (6)	7.3 ± .33 (6) (6)
Δ^1-U	9.8 ± .28 (6) (54)	2.35 ± .05 (6) (30)	1.86 ± .04 (6) (30)	149 ± 1.10 (6) (6)	7.0 ± .33 (6) (6)

* Mean ± S.E. † No. animals. ‡ No. cases (determinations).

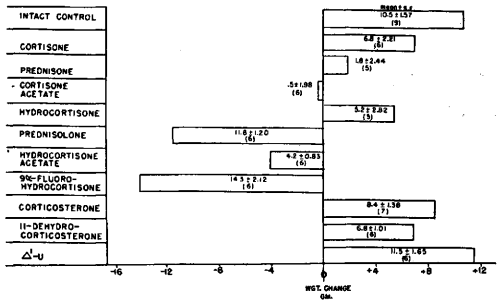

FIG. 1. Effects of adrenal steroids on body wt in force-fed intact rats. (1 mg/day for 11 days.)

mented significantly the volume of urine excreted, with prednisolone most effective; corticosterone, 11-dehydrocorticosterone, or Δ^1 U were ineffective in this respect. Cortisone, hydrocortisone, their acetates and Δ^1 analogues, augmented urinary excretion of Na significantly while other steroids had little or no effect. The corticoids, with 2 exceptions, augmented significantly urinary excretion of K. The depression of urinary K after corticosterone or Δ^1 U was small and statistically insignificant. Cortisone and 9α-fluorohydrocortisone were the only steroids affecting serum electrolytes. Cortisone caused small but significant fall in serum sodium, whereas 9α-fluorohydrocortisone caused marked increase in serum Na and severe drop in serum K.

The steroids (Δ^1 U excepted) depressed body weight gain; prednisolone and 9α-fluorohydrocortisone were most effective in promoting body weight loss (Fig. 1).

Adrenal weights were depressed to different degrees depending upon the steroid employed (Table II). There was significant adrenal atrophy, either on an actual or relative weight basis, after treatment with hydrocortisone acetate or prednisolone. Treatment with 11-dehydrocorticosterone or Δ^1 U caused significant decreases in relative but not actual weights, whereas corticosterone significantly depressed actual but not relative weight. Further work, involving increased dosage and numbers, would be necessary to clarify these details; only major points will be discussed.

Discussion. These experiments demonstrated that small daily doses of cortisone or hydrocortisone and their acetates and Δ^1 analogues uniformly elicited increases in urinary excretion of water, sodium, and potassium in the intact force-fed rat. These steroidal effects were similar to those obtained in the adrenalectomized rat (unpublished) except they demonstrated more clearly that, in addition to stimulating water and K excretion, they augmented urinary excretion of Na. It appears that these effects are characteristic ones for these steroids in the rat regardless of whether the adrenals are absent or present (4,5).

Nine α-fluorohydrocortisone elicited moderate increase in urine volume, failed to influence significantly urinary Na or K, and elevated serum Na and depressed serum K. This latter finding, reported previously(6,7), further emphasizes the desoxycorticosterone-like effects of 9α-fluorohydrocortisone in promoting exchange of K for Na between extra- and intracellular fluids.

Corticosterone, 11 - dehydrocorticosterone, and Δ^1 U had no effects on renal excretion of water and electrolytes. The former 2 steroids are generally considered to have weak physiological activity and the latter has been shown recently to exert mildly beneficial effects in the adrenalectomized rat. It is likely that increasing the dosage would have led to observable effects(8).

The acetates and Δ^1 analogues were more effective than parent steroids in depressing body weight and such effects correlated well with urinary loss of water and electrolytes.

TABLE II. Effects of Adrenal Steroids on Adrenal Weight in Force-Fed Intact Male Rats.

Treatment	No. cases	Adrenal wt* Relative, mg/100 g BW	Adrenal wt* Actual, mg
None, controls	9	18.8 ± 1.2	48.8 ± 2.1
Cortisone	5	18.1 ± .4	45.2 ± .1
Prednisone	6	18.2 ± 1.8	43.2 ± 4.6
Cortisone acetate	6	17.2 ± .7	44.2 ± 1.7
Hydrocortisone	6	18.4 ± 1.2	46.1 ± 3
Prednisolone	6	14.0 ± .3	31.9 ± .1
Hydrocortisone acetate	6	13.8 ± .9	34.6 ± 2.5
9α-fluorohydrocortisone	5	16.9 ± 1.5	40.4 ± 3.6
Corticosterone	7	15.7 ± 1.2	38.9 ± 1.9
11-dehydrocorticosterone	6	11.7 ± 2.1	40.8 ± 3.9
Δ^1-U	6	12.9 ± .6	39.2 ± 5

* Mean ± S.E.

Although 9α-fluorohydrocortisone was more effective than the other steroids in depressing body weight, it was poorly effective in promoting urinary loss of water, Na, and K; reason for excessive body weight loss is unknown. Δ^1 U was the only steroid that promoted body weight gain in intact or adrenalectomized rats; it has weak physiological activity favorable for the adrenalectomized rat (unpublished).

Experiments dealing with effects of 11-oxy steroids on adrenal weight demonstrated that each compound caused some adrenal atrophy, but only hydrocortisone acetate and prednisolone were significantly effective on both actual and relative weight bases. These 2 steroids are then presumably the most effective ones of the 10 used in inhibiting endogenous production of ACTH and adrenal cortical hormones.

Summary. In the force-fed intact rat, treatment with small daily doses of cortisone or hydrocortisone or their acetates and Δ^1 analogues had slight, if any effects on serum Na and K but augmented urinary excretion of water (urine volume), Na and K, suppressed body weight gain, and caused reduction in adrenal weight. Hydrocortisone acetate and prednisolone were most effective of 10 steroids tested in causing adrenal atrophy. Treatment with 9α-fluorohydrocortisone resulted in marked increase in serum Na, severe decrease in serum potassium, marked loss in body weight, no change in urinary sodium, and mild increases in urinary volume and K. Corticosterone, 11-dehydrocorticosterone, or $\Delta^{1,4}$-pregnadiene-17, 20, 21-triol-3, 11-dione (Δ^1 U), in the doses employed, failed to affect water and electrolyte balance. These 3 compounds tended to suppress adrenal weight and, excepting Δ^1U, caused slight reduction in body weight gain. In general, renal responses were similar to those found previously in adrenalectomized rats.

1. Chart, J. J., Hetzel, N., Gaunt, R., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 73.

2. Ingle, D. J., Oberle, E. A., *Am. J. Physiol.*, 1946, v147, 222.

3. Fisher, R. A., *Statistical Methods for Research Workers*, 1950, Hafner Pub. Co., N. Y.

4. Sala, G., Leutcher, J. A., *Endocrinol.*, 1954, v55, 516.

5. Ingle, D. J., Prestud, M. C., Rice, K. L., *ibid.*, 1950, v46, 510.

6. Leith, W., Beck, J. C., *J. Clin. Endocrinol.*, 1957, v17, 280.

7. Swingle, W. W., Baker, C., Eisler, M., Le Brie, S. J., Brannick, L., *Endocrinol.*, 1955, v57, 220.

8. Ingle, D. J., Sheppard, R., Oberle, E. A., Kuizenga, M. H., *ibid.*, 1946, v39, 52.

Received June 24, 1958. P.S.E.B.M., 1958, v99.

Excretion of Pregnan-3α, 17α, 21-Triol-20-One (Tetrahydro S) by Patients with Adrenocortical Carcinoma.* (24324)

MORTIMER B. LIPSETT AND BARBARA DAMAST (Introduced by Roy Hertz)
Dept. of Health, P.H.S., National Cancer Inst., Endocrinology Branch, Bethesda, Md.

An increased excretion of Porter-Silber chromogens in urine has generally implied the presence of increased amounts of tetrahydrocortisone (tetrahydro E) and tetrahydrohydrocortisone (tetrahydro F), the reduced products of cortisone and hydrocortisone respectively. However, in 2 patients with adrenocortical carcinoma, tetrahydro S has been shown to comprise the major part of the urinary neutral reducing lipids and exceed excretion of tetrahydro E and tetrahydro F(1). In a patient with the hypertensive variant of congenital adrenal hyperplasia, tetrahydro S was the major component of the Porter-Silber chromogens(2). It was isolated in increased amount from urine of 2 patients with adrenocortical carcinoma(3). Since excretion of tetrahydro S probably accurately reflects secretion of 4-pregnen-17α, 21-diol-3, 20-dione (Compound S), determination of its urinary excretion provides data regarding the secre-

* We wish to thank Dr. Hildegarde Wilson of the Nat. Inst. of Arthritis and Metabolic Diseases for generous advice.