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gain in intact or adrenalectomized rats; it has weak physiological activity favorable for the adrenalectomized rat (unpublished).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.

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

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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-

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TABLE I. Quantitative Urinary Steroids.

Patient	Porter-Silber chromogens	Tetrahydro S	17-ketosteroids*	Dehydroepiandrosterone†
	mg/24 hr			
V.G.	89	13	134	36
J.B.	50	15	152	75
V.F.	72	44	70	29
R.S.	61	11‡	57	19
G.T.	20	7	23	3
T.R.	6	.4	130	47
G.H.§	.6	.3	107	34
J.H.	5	.7	4.5	.6
M.S.	5	.3	44	17
Normal ♂	7	.9	12	

* Modification of Callow's method.
† Paper chromatography and micro Zimmerman.
‡ Excretion of dihydro S—3 mg/24 hr.
§ 15-mo-old boy.

tion of one of the probable intermediates in synthesis of hydrocortisone. Since one of our patients with adrenocortical carcinoma excreted 60 mg of Porter-Silber chromogens daily and was without clinical evidence of Cushing's syndrome we searched for tetrahydro S in his urine and subsequently in the urine of 8 other patients with adrenocortical carcinoma. This resulted in the finding of increased urinary excretion of tetrahydro S in 5 patients and the presumptive identification of pregnan-17α, 21-diol-3, 20-dione (dihydro S) in one patient.

Methods. Most urines examined had been stored at −4°C up to 2 years. Aliquots of 24-hour urines were hydrolyzed with beef liver beta glucuronidase (Ketodase®) at pH 5.0 for 5 days. They were then continuously extracted with ether at pH 1 for 48 hours, and for 24 hours at a concentration of sulfuric acid of 1 normal. After washing with 1N sodium hydroxide and water, aliquots of the crude material in ethanol were chromatogrammed in the Bush B_5 system, a toluene-ethylene glycol system, or in the E_4 system of Eberlein and Bongiovanni(4). This last proved most useful as blue tetrazolium-reacting steroids more polar than tetrahydro S have low Rf's. Tetrahydro S moved 19 to 23 cm in 16 hours at room temperature. The steroids were eluted with cold methanol and determined quantitatively with blue tetrazolium(5). Recovery experiments with tetrahydro S from paper averaged between 85 and 110% Tetrahydro S was identified by its mobility in the 3 solvent systems, its reaction with blue tetrazolium and phenylhydrazine and infra-red spectroscopy.† Dihydro S was presumptively identified in urine of R. S. by its mobility in 2 solvent systems, and comparison of its acetate with authentic dihydro S acetate in the E_1 system(4) and its reaction with blue tetrazolium and phenylhydrazine.

Results. In Table I, data are presented comparing 24-hour excretion of tetrahydro S in 9 patients with proven adrenocortical carcinoma with excretion of Porter-Silber chromogens, 17-ketosteroids, and dehydroepiandrosterone. Whenever excretion of Porter-Silber chromogen was elevated, tetrahydro S was likewise increased. However, when 17-ketosteroids alone were elevated, excretion of tetrahydro S was low.

Discussion. Of the steps leading to synthesis of hydrocortisone, 11-beta hydroxylation is generally considered the final one. Excretion of tetrahydro S undoubtedly closely corresponds to production of Compound S. Thus, these results suggest that when an adrenocortical carcinoma produces excessive amounts of hydrocortisone, 11-beta hydroxylation of Compound S may be the rate limiting step in the series of reactions leading to synthesis of hydrocortisone. Whether this is peculiar to adrenocortical carcinoma or is a general consequence of the high rate of production of hydrocortisone is unanswered by these data. Evidence to support the first alternative is the demonstration that a patient with an adrenocortical adenoma excreted only small amounts of tetrahydro S in spite of an excretion of tetrahydro E and tetrahydro F as great as in patients with adrenal carcinoma and Cushing's syndrome(1). It is apparent, of course, that in the 4 patients without elevated urinary Porter-Silber chromogens, only small amounts of tetrahydro S were detected.

Hydroxylation at C_{17} was not similarly impeded as no alpha-ketols less polar than dihydro S were seen in any of the chromatograms. This would exclude the presence of

† We wish to thank Dr. Thomas F. Gallagher of Sloan-Kettering Institute for the infra-red spectrometry.

4-pregnen-21-ol-3, 20-dione (tetrahydrol DOC) in amounts greater than 500 μg per 24 hours. The inconsistency between high excretion of Porter-Silber chromogens and absence of Cushing's syndrome in patient R.S. could not be attributed to excretion of tetrahydro S and dihydro S as this patient also excreted amounts of tetrahydro E and tetrahydro F which were well above normal. The occurrence of dihydro S was unexpected. To our knowledge, this compound has not previously been found in urine. As it has been shown that reduction of the C_3-keto group is the rate-limiting step in metabolism of the 3-keto, Δ4 group(6), no dihydro S should be excreted. It was noted in only one patient, R.S., who had extensive hepatic metastases at the time. Whether or not this affected the further metabolism of dihydro S is conjectural.

Summary. Tetrahydro S was excreted in amounts ranging from 7 to 44 mg daily in 5 patients with adrenocortical carcinoma exhibiting excessive production of Porter-Silber chromogens. Dihydro S was presumptively identified in the urine of one of these patients. The amount of tetrahydro S in 4 patients with adrenocortical carcinoma with normal levels of Porter-Silber chromogens was likewise normal.

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Immunological Tolerance to Male Skin Isografts in Female Mice.* (24325)

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Eichwald and Silmser(1) reported among certain members of inbred strains of mice a histo-incompatibility determined by the sex. This observation has been confirmed by Prehn and Main(2) and Short and Sobey(3) for some strains of mice but not for others. In these studies, isografts from male to female mice of either the A or C_{57} BL strain are often unsuccessful. When skin isotransplants are made from female to male, male to male or female to female, skin grafts are regularly successful and resemble autografts. The basis for rejection of male skin isograft by the female of the same inbred strain has been interpreted by Eichwald *et al.*(4) and Snell(5) as the function of an immune response due to a histocompatability gene located on the Y-chromosome. According to this theory the female (XX), which lacks the donor's isoantigen originating from a gene residing on the Y-chromosome, is capable of an immune response and rejects the skin isotransplant. However, other possible explanations for failure of male skin isotransplants to survive on females have been considered. For example, endocrinological influences, by mechanisms not yet understood, might account for the results observed(4). Reasoning that rejection of male skin by females of the same strain is an immunological phenomenon based on the same principles as those responsible for skin homograft rejection, it seemed possible that the effects of the so-called Y-antigen might be

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