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Cushing's Syndrome V. Chemical Inhibition of 11β-Hydroxylating Enzyme Systems in Hyperplastic and Neoplastic Adrenal Glands.* (25793)

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The clinical distinction between adrenal hyperplasia and adrenal tumor as the cause for Cushing's syndrome has been based in part upon tests which inhibit ACTH secretion (1), and also upon response of the adrenal glands to stimulation with exogenous ACTH (2-4). In general, adrenal tumors are neither suppressed by exogenous steroids nor stimulated with ACTH, whereas hyperplastic glands usually hyper-respond to stimulation with ACTH and are suppressed by administration of exogenous corticosteroids. However, these tests do not permit absolute distinction between the two pathological states (5-7). Su 4885, (2-methyl-1, 2-bis-(3-pyridyl)-1-propanone),‡ when administered in proper dosage to the human subject, selectively causes inhibition of 11β-hydroxylation within the adrenal cortex. This substance has been extensively studied in the normal individual and in patients with adrenal and pituitary dysfunction(7,8). Differences in response to Su 4885 have been noted to occur between patients with adrenal tumors and those with bilateral hyperplasia(8). The purpose of this investigation has been to define the action of Su 4885 upon the biosynthetic mechanisms within the adrenal gland in Cushing's syndrome when due to either bilateral adrenal hyperplasia or neoplasia.

Methods and materials. In all, 7 patients with documented Cushing's syndrome were studied. Two patients were shown to have adrenal carcinoma and 5 to have bilateral adrenal hyperplasia. The urinary ketogenic steroids were determined by a modification of the Norymberski technic(9). The methods for extraction, separation and identification of the various corticosteroid metabolites have been reported(10). In brief, urinary steroids after extraction were separated by chromatography on $Bush_1$ and $Bush_5$ systems. Eluted steroid fractions were quantitated by a modified Porter-Silber reaction. The tetrahydro S§ metabolite was identified by melting point determinations and infrared spectra, together with similarities in Rf values to a standard on several different chromatographic systems. Twenty-four-hour urine collections were obtained for several days before administration of the Su 4885 compound and for several days thereafter. 750 mg of Su 4885 were administered every 4 hours for 6 doses except in the case of St. who received a total of 200 mg in this period of time.

Results. Adrenal hyperplasia. Administration of Su 4885 over a one day period to 5 patients with bilateral adrenal hyperplasia and Cushing's syndrome resulted in a marked increase in urinary ketogenic steroids (Table I). In 4 of these 5 patients, maximal rise occurred the day following administration of Su 4885. In the fifth patient, Gu., maximal increase was observed on day of administration of the compound.

The results of identification and quantitation of the various hydrocortisone and com-

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§ 3α, 17α,21-triol, pregnan-20-one.

TABLE I. Effect of Su 4885 on Urinary Ketogenic Steroid Excretion in Patients with Cushing's Syndrome.

	Patient	Sex	Age	Ketogenic steroids (mg/24 hr) Day 1	Day 2*	Day 3	Day 4
Adrenal hyperplasia	Ma.	♀	61	34	29	66	
	Ho.	♀	40	31	44	101	
	Co.	♂	51	28	39	65	
	Gu.†	♀	52	46	61	41	
	Gs.	♀	38	22	49	85	
" carcinoma	Fl.	♂	33	40	39	25	28
	St.	♂	1½	12	5	7	12

* Su 4885 administered in divided doses on Day 2.
† Unilateral adrenalectomy performed 6 yr previously.

TABLE II. Effect of Su 4885 on Individual Urinary C-21 Steroids in Patients with Cushing's Syndrome.

		Tetrahydro F, tetrahydro E & allotetrahydro F	Tetrahydro S	Dihydro S	Total
Adrenal hyperplasia (patient Ho.)*	Before Su 4885	27.2	1.1	0	28.3
	After "	2.6	27.5	12.7	42.8
Adrenal carcinoma (patient Fl.)	Before "	31.5	1.6	0	33.1
	After "	5.8	12.3	0	18.1

* This study upon patient Ho. was performed prior to that reported in Table 1.

pound S metabolites in a representative patient are shown in Table II. Tetrahydro F‖, tetrahydro E¶ and allotetrahydro F** fell from a control level of 27.2 mg/day to 2.6 mg/day, whereas values for urinary tetrahydro S rose from 1.1 to 27.5 mg/day. These changes reflect inhibition by Su 4885 of 11β hydroxylation within the hyperplastic adrenal gland. A compound tentatively identified as dihydro S‡‡, on the basis of running rates similar to the authentic compound in several chromatographic systems, appeared in the urine as a result of Su 4885 administration to patient Ho.

Adrenal carcinoma. Administration of Su 4885 to 2 patients with adrenal carcinoma resulted in an actual decrease in excretion of urinary ketogenic steroids (Table I). Analysis of specific urinary steroids in one patient showed a decrease in tetrahydro F, tetrahydro E and allotetrahydro F levels from 31.5 mg/day to 5.8 mg/day following administration of the compound, whereas the level of tetrahydro S rose from 1.6 to 12.3 mg/day. However, total amount of identifiable steroid metabolites in the urine fell from 33.1 to 18.1 mg/day (Table II).

Discussion. In a patient with Cushing's syndrome due to bilateral adrenal hyperplasia, as in the normal individual, administration of adequate amounts of Su 4885 results in a definite increase in urinary ketogenic steroids. This increase is caused by a marked rise in tetrahydro S excretion and not by an increased excretion of hydrocortisone metabolites. In fact, the latter group of steroids decreases after Su 4885 administration. Su 4885 apparently causes a decrease in amount of circulating hydrocortisone, thereby diminishing the usual inhibiting influence of hydrocortisone upon the pituitary gland. The pituitary consequently secretes excessive amounts of ACTH, which in turn stimulate the adrenal cortex. However, as the 11β-hydroxylating enzyme systems are inhibited, the adrenal glands secrete large amounts of compound S rather than hydrocortisone. The metabolites of compound S are identified as ketogenic steroids in the urine.

Following administration of Su 4885, the metabolites of the hydrocortisone in the urine of patients with adrenal carcinoma decrease

‖ 3α,11β,17α, 21-tetrol, pregnan-20-one.
¶ 3α,17α,21-triol, pregnane-11, 20-dione.
**3α,11β,17α,21-tetrol, allopregnan-20-one
‡‡ 17α,21-diol, pregnane-3, 20-dione.

in a manner similar to the response of patients with adrenal hyperplasia and normal individuals. Therefore, it seems clear that Su 4885 inhibits 11β-hydroxylation in adrenal carcinoma as well as in non-neoplastic glands. The difference in response as regards total urinary ketogenic steroids apparently resides in the failure of the neoplastic glands to respond to stimulation with the endogenous ACTH released after Su 4885 administration. Patients with carcinoma of the adrenal usually do not show rises in plasma or urinary steroid levels when ACTH is administered, nor do their urinary steroids diminish following suppression of ACTH with exogenous steroids.

The response to Su 4885 may prove useful as a clinical test for differentiating adrenal carcinoma from hyperplasia(8) but more data must be amassed before generalizations can be made. Analogs of this compound may also assume a role in the chemotherapy of adrenal carcinoma.

Summary. Administration of Su 4885 resulted in inhibition of 11β-hydroxylation within the adrenal cortex in 7 patients with Cushing's syndrome. In 5 patients with adrenal hyperplasia, this resulted in a rise in values of urinary ketogenic steroids whereas in the 2 patients who had adrenal carcinoma no such rise was observed. This difference in the 2 groups of patients is ascribed to the state of adrenal responsiveness to endogenous ACTH.

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In vitro Passive Transfer of Atopic Hypersensitivity. (25794)

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During experiments on allergic histamine release from leukocytes of atopic subjects(1-3), it was shown that histamine release varies in relation to antigen concentration(1). To study the effect of antibody concentration, attempts were made *in vitro* passively to sensitize leukocytes from non-allergic individuals. That this might be done successfully seemed reasonable in view of the fact that normal skin is regularly sensitized by allergic sensitizing antibody. This is the basis of the Prausnitz-Küstner reaction. Also, Humphrey and Jacques(4) demonstrated that normal rabbit platelets will release histamine in presence of homologous rabbit antibody and antigen.

Methods. Heparinized whole blood was obtained from normal skin test negative individuals. Plasma containing sensitizing antibody was procured from subjects highly allergic to ragweed pollen (3-4 plus reaction to skin test with 1/40 ml ragweed extract containing 100 protein N units per ml). The capacity of ragweed antigen to produce histamine release in the whole blood of the donor subjects is shown in Table I. In some experiments donor plasma was diluted with 0.15 M NaCl prior to mixing with normal whole blood. In other experiments, plasma was col-