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Edward G. Biglieri, ... , Paul E. Slaton Jr., Peter H. Forsham

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IN VIVO AND IN VITRO STUDIES OF ADRENAL SECRETIONS IN CUSHING'S SYNDROME AND PRIMARY ALDOSTERONISM *

By EDWARD G. BIGLIERI, SATOSHI HANE, PAUL E. SLATON, JR., AND
PETER H. FORSHAM WITH THE TECHNICAL ASSISTANCE OF MARY ANNE
HERRON AND SHIRO HORITA

(From the Metabolic Research Unit and the Department of Medicine, University of California
School of Medicine, San Francisco, Calif.)

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Abnormalities in aldosterone secretion are unusual in Cushing's syndrome even in the presence of hypokalemic alkalosis (1). Similarly, increased cortisol excretion is not seen in primary aldosteronism (2-7). Little information regarding corticosterone secretion is available in either condition. Increased excretion of corticosterone metabolites has been suggested in several cases of primary aldosteronism (8, 9). The concentration of aldosterone and frequently of corticosterone is increased in primary aldosteronism tumor tissue (10-13). Cortisol content in adrenal tissue of patients with Cushing's syndrome is not increased (10, 12, 14), nor does the rate of production *in vitro* increase, although total production by the entire hyperplastic gland or adenoma is greater than in normal tissue (15).

We measured secretory rates of cortisol,¹ corticosterone, and aldosterone in normal subjects, patients with Cushing's syndrome, and patients with primary aldosteronism. In addition, urinary aldosterone was quantitated in seventeen patients with Cushing's syndrome and in two patients who had been on continuous steroid therapy for more than 9 years. The steroid content of the adrenal tumor and the contiguous gland was measured in nine patients with primary aldosteronism. Similar measurements were made in three hyperplastic glands and three adenomas from patients with Cushing's syndrome. Steroid con-

centration of the adrenal vein was determined in two patients with Cushing's syndrome and six with primary aldosteronism.

METHODS

Cushing's syndrome. The 17 patients with Cushing's syndrome included 3 with adrenocortical carcinomas, 4 with adrenocortical adenomas, and 10 with hyperplasia, two of them associated with pituitary tumors. The diagnoses were established by elevated basal Porter-Silber chromogens, clinical symptoms, and subsequent surgical operation. Hypertension was present in 14 of the 17 patients. Slight hypokalemic alkalosis was observed in but a single patient with adrenocortical carcinoma.

Primary aldosteronism (12 patients: 7 males, 5 females). All patients with primary aldosteronism had hypokalemia, hypertension, hypervolemia, increased excretion of urinary aldosterone, and unilateral adenomas were found in eleven. Normal adrenal glands were found in a single patient, K.L., in whom only secretory rates were measured.

Secretory rates of cortisol, corticosterone, and aldosterone. Measurements were made while patients were receiving constant metabolic diets containing more than 110 mEq of sodium. The radioisotopes ² cortisol-4-C¹⁴ (SA 42.4 μ c per mg), corticosterone-4-C¹⁴ (SA 44.2 μ c per mg), and 7-H³-d-aldosterone (SA 20 μ c per μ g) were purified by paper chromatography and kept in 100% ethanol until use. Secretory rates of cortisol were determined by a modification of the techniques of Cope and Black (16), Peterson (17), and Flood and co-workers (18); corticosterone was measured by the technique of Peterson (19) and aldosterone by modified techniques of Peterson (17), and Flood and co-workers (18). One μ c (0.022 mg) of cortisol-4-C¹⁴, 1 μ c (0.022 mg) of corticosterone-4-C¹⁴, and 3 μ c (0.15 μ g) of 7-H³-d-aldosterone were added to 20 ml isotonic saline and simultaneously injected intravenously between 8 and 10 a.m. A 24-hour urine specimen was collected. Inulin, or creatinine clearances, or both were normal or high. More than 90% of the in-

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¹ Names of compounds listed: cortisol = 17-hydroxycorticosterone, corticosterone = 4-pregnene-11 β ,21-diol-3,20-dione, aldosterone = 11 β ,21-dihydroxy-18-formyl-4-pregnene-3,20-dione, desoxycorticosterone = 21-hydroxy-4-pregnene-3,20-dione, and 11-desoxycortisol = 17 α ,21-dihydroxy-4-pregnene-3,20-dione.

² Radioactive isotopes were gifts of the Endocrinology Study Section, National Institutes of Health, Bethesda, Md.

jected dose was excreted in the first 24 hours, and total injected radioactivity was used in calculating the secretory rate. The specific activity of a metabolite was determined as follows.

a. *Cortisol and corticosterone.* A 50- to 100-ml sample was washed with 2 vol ethyl acetate before hydrolysis with Ketodase (β -glucuronidase) at pH 4.8 at 45° C for 18 hours. After incubation, tetrahydrocorticosterone² was extracted from the urine by two treatments with 4 vol carbon tetrachloride. The same urine was then treated twice with 2 vol methylene dichloride to extract tetrahydrocortisol and tetrahydrocortisone. The extracts were washed with 5% Na₂CO₃, then 0.1 N HCl, were dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness. Each extract was chromatographed on Whatman no. 2 filter paper for 6 hours in an iso-octane: tertiary butanol: water (50:50:50 v/v) system. Chromatograms were then scanned for radioactivity with a Vanguard Autoscanner. The radioactive areas were eluted and rechromatographed on Whatman no. 2 paper in a toluene: methanol: water (125:50:50 v/v) system, 3½ hours for tetrahydrocorticosterone and 24 hours for tetrahydrocortisol and tetrahydrocortisone. The metabolites were then identified by scanning for radioactivity and were eluted. Specific activity was determined by chemical quantitation with the alkaline blue tetrazolium reaction and by C¹⁴ activity in a liquid scintillation spectrometer. The specific activities of tetrahydrocortisol and tetrahydrocortisone were virtually identical.

b. *Aldosterone.* The specific activity of the 3-oxo conjugate was used to determine the secretory rate of aldosterone. A 100-ml sample was hydrolyzed at pH 1 for 24 hours, extracted with dichloromethane, and dried. After the extract was chromatographed in a cyclohexane: benzene: methanol: water (50:100:100:40 v/v) system for 16 hours, the aldosterone was acetylated with C¹⁴-acetic anhydride for quantitation, and chromatographed as aldosterone diacetate in 3 different systems by the method described by Kliman and Peterson (20).

Tissue and adrenal vein content. The adrenal tissue was immediately frozen after removal until extraction could be done. One-half to 1 g adrenal tumor or adrenal gland, and 1 to 2 ml adrenal venous plasma were prepared as previously described (21), the extracts were acetylated with tritiated acetic anhydride, chromatographed in 3 different systems, and analyzed for steroid content by the double isotope derivative technique using the C¹⁴-acetates of cortisol, corticosterone, aldosterone, desoxycorticosterone, and 11-desoxycortisol to monitor losses.

The acetylated steroids were extracted with dichloromethane, dried, and chromatographed in a cyclohexane: benzene: methanol: water (100:25:100:10 v/v) system for 8 hours, which provided separation of cortisol acetate, desoxycorticosterone acetate, and an area containing aldosterone diacetate, corticosterone acetate, and 11-desoxycortisol acetate. Cortisol acetate was eluted and chromatographed in a cyclohexane: dioxane: methanol: water (100:75:50:25 v/v) system for 24 hours. After elution with methanol, cortisol acetate was dried, oxidized to

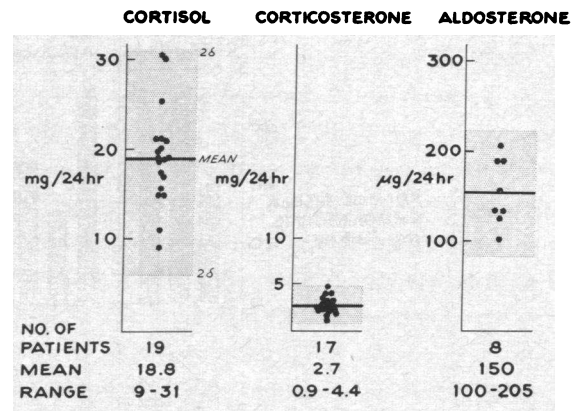


FIG. 1. SECRETORY RATES IN NORMAL SUBJECTS. Stippled areas indicate 2 SD.

cortisone acetate, and chromatographed in a carbon tetrachloride: methanol: water (100:100:25 v/v) system for 16 hours before counting for tritium and C¹⁴ in a liquid scintillation spectrometer. Desoxycorticosterone acetate was chromatographed in a cyclohexane: dioxane: methanol: water (100:25:100:10 v/v) system for 10 hours, eluted, dried, and reduced with sodium borohydride. Final chromatography was accomplished in a cyclohexane: nitromethane: methanol (100:50:50 v/v) system for 24 hours before counting. Separation of corticosterone acetate, aldosterone diacetate, and 11-desoxycortisol acetate was achieved in the cyclohexane: dioxane: methanol: water (100:75:50:25 v/v) system in 16 hours. Aldosterone diacetate and corticosterone acetate were oxidized with chromium trioxide and then chromatographed in cyclohexane: benzene: methanol: water (100:50:100:25 v/v) for 12 hours before final elution and counting. 11-Desoxycortisol acetate was reduced with sodium borohydride before final chromatography in a cyclohexane: benzene: methanol: water (100:50:100:25 v/v) system for 10 hours.

Urinary steroids. Urinary aldosterone was measured by the double isotope derivative method of Kliman and

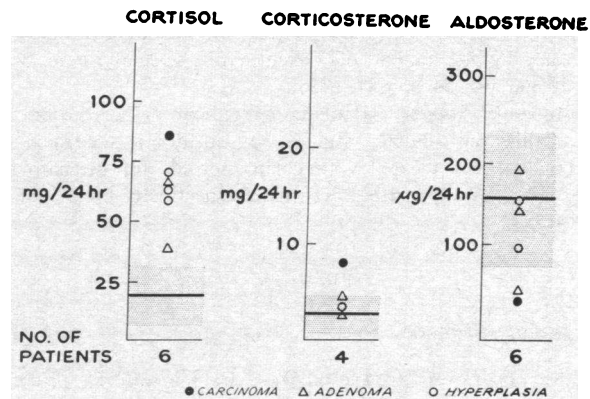


FIG. 2. SECRETORY RATES IN CUSHING'S SYNDROME. Stippled areas indicate 2 SD in normal subjects.

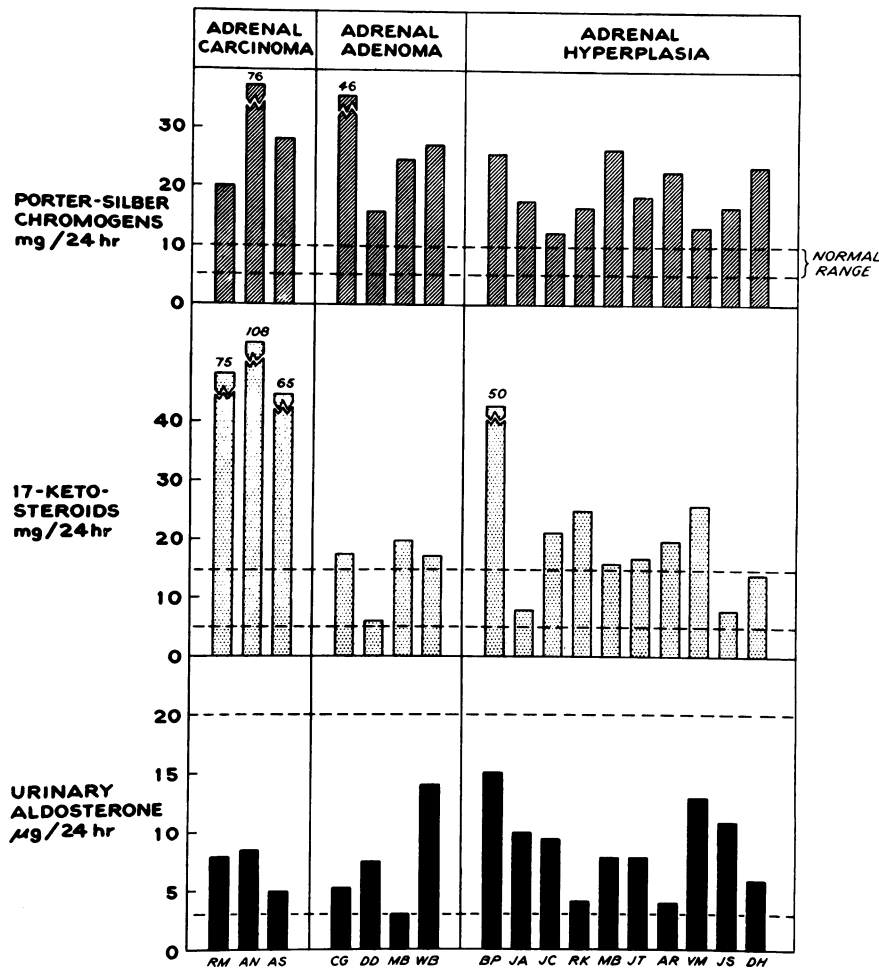


FIG. 3. TWENTY-FOUR HOUR EXCRETION OF PORTER-SILBER CHROMOGENS, 17-KETOSTEROIDS, AND ALDOSTERONE IN 17 PATIENTS WITH CUSHING'S SYNDROME. Dotted lines indicate normal range.

Peterson (normal range, 4 to 22 μg per 24 hours) (20), urinary Porter-Silber chromogens by a modification of the method of Reddy, Jenkins, and Thorn (normal range, 5 to 10 mg per 24 hours) (22), and urinary 17-ketosteroids by the method of Vestergaard (normal range, 5 to 15 mg per 24 hours) (23).

Metabolic studies. Patients were kept on a constant metabolic diet during studies in Cushing's syndrome. Two had been treated with corticosteroids for dermatomyositis and rheumatoid arthritis, respectively, for 9 and 10 years.

RESULTS

Normal subjects

Secretory rates are shown in Figure 1. For *cortisol*, in 19 subjects the mean secretory rate was 18.8 mg per 24 hours ($\text{SD} \pm 6.5$), with a

range of 9 to 31 mg per 24 hours. For *corticosterone*, in 17 subjects the mean secretory rate was 2.7 mg per 24 hours ($\text{SD} \pm 0.9$), with a range of 0.9 to 4.4 mg per 24 hours. For *aldosterone*, in 8 subjects the mean secretory rate was 150 μg per 24 hours ($\text{SD} \pm 34 \mu\text{g}$), with a range of 100 to 205 μg per 24 hours.

Patients with Cushing's syndrome

Secretory rates are shown in Figure 2. For *cortisol*, the secretory rate was increased in six patients, the most in a patient with adrenocortical carcinoma. Values ranged from 37 to 82 mg per 24 hours. For *corticosterone*, the secretory rate was normal in all patients except in one with adrenocortical carcinoma, in whom the secretory

TABLE I
Aldosterone excretion in Cushing's syndrome after salt restriction and administration of ACTH

Patient	Urinary aldosterone		
	Control	Day 4 of 9 mEq Na diet	After ACTH*
	µg/24 hours	µg/24 hours	µg/24 hours
Adrenal carcinoma			
A.N.	9.0	35.0	8.0
R.M.	6.0	49.0	
Adrenal hyperplasia			
T.M.	7.9	20.2	
B.P.	15.2	37.5	
B.P.†	15.0	39.8	
J.A.	9.6	22.5	73.0‡
Adrenal adenoma			
C.G. (before operation)	4.0	13.0	16.0
C.G. (after operation)	6.0	19.0	40.0‡
A.E.	8.0	30.0	

* 25 U in 8-hour infusion.
† Three weeks after unilateral adrenalectomy.
‡ ACTH administered on day 5 of 9 mEq Na diet.

rate was 9.0 mg per 24 hours. The secretory rate of aldosterone was normal in the patients with Cushing's syndrome who had no tumors. The secretion of aldosterone was depressed in one of the three patients with adrenocortical carcinoma and in one with an adenoma.

Urinary excretion of steroids. The basal urinary excretion of aldosterone was within normal limits in the 17 patients despite increased excretion of Porter-Silber chromogens in all and increased excretion of 17-ketosteroids in 13 of the 17, regardless of the cause of their Cushing's syndrome (Figure 3). Sodium deprivation in three patients with hyperplasia, two with carcinoma, and two with adenoma effected a threefold increase in urinary aldosterone without change in Porter-Silber chromogens or 17-ketosteroids (Table I). Normal subjects have a two- to tenfold increase in urinary aldosterone on the fourth day of sodium deprivation. In patient C.G., the increase of aldosterone in response to sodium deprivation was essentially the same both before and 3 weeks after unilateral adrenalectomy for tumor during replacement therapy. ACTH produced a prompt increase in urinary aldosterone and Porter-Silber chromogens before operation, but only in aldosterone afterwards. B.P. also had similar increases in urinary aldosterone before and after unilateral adrenalectomy for bilateral hyperplasia. No response to ACTH was observed in one patient with an adrenocortical carcinoma.

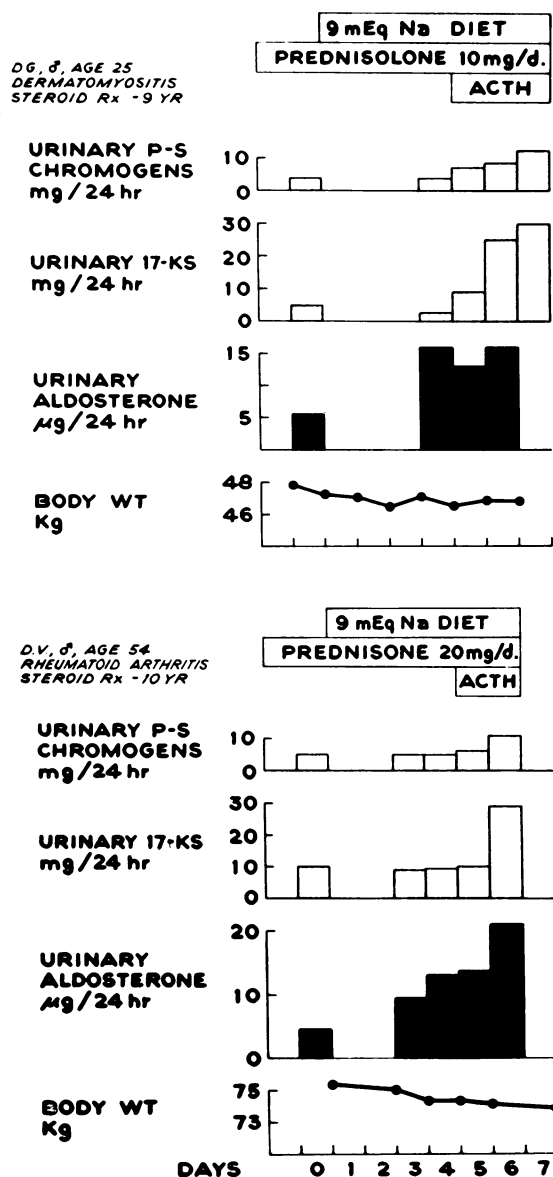


FIG. 4. CORTICOSTEROID EXCRETION AFTER SALT DEPRIVATION AND ADMINISTRATION OF ACTH IN TWO PATIENTS TREATED WITH EXOGENOUS CORTICOSTEROIDS FOR MORE THAN 9 YEARS.

terone afterwards. B.P. also had similar increases in urinary aldosterone before and after unilateral adrenalectomy for bilateral hyperplasia. No response to ACTH was observed in one patient with an adrenocortical carcinoma.

Prolonged administration of steroids. Two patients who had been continuously treated with exogenous steroids for more than 9 years (Figure 4) showed low normal basal excretion of aldosterone

TABLE II
Adrenal tissue analysis, steroid secretory rates, and steroid excretion in primary aldosteronism

Patient	Adrenal gland content				Adrenal tumor content				Secretory rates			Urinary steroids			
	Cortisol μg/g	11-Des-oxycorti- sol μg/g	Aldo- sterone μg/g	Corticos- terone μg/g	Desoxy- corticos- terone μg/g	Cortisol μg/g	11-Des- oxycorti- sol μg/g	Aldo- sterone μg/g	Corticos- terone μg/g	Desoxy- corticos- terone μg/g	Cortisol mg/24 hr	Corticos- terone μg/24 hr	Aldo- sterone μg/24 hr	Porter- Silber chromo- gens	17-Keto- steroids μg/24 hr
L.B.	8.4	0.45	6.0	1.01	10.5	1.75	16.0	1.72	300*	10.2	7.5	29			
R.T.	10.5	0.30	7.5	3.6	5.5	1.00	10.1	400*		8.6	12.6	40			
T.P.	10.0	0.33	3.6	0.60	6.0	0.98	11.1								
C.H.	4.5	0.51	7.8	0.14	3.9	0.51	17.5	1.30	29.2	3.8	706	110			
R.G.	2.5	0.10	0.05	0.14	4.3	0.30	1.8	0.76	17.8	3.1	632	57			
M.J. (left)	6.7	0.15	0.42	0.12	4.2	0.50	24.0	0.64	18.0	8.0	560	50			
(right)	2.1	0.56	0.65	0.20											
V.M. (left)†	1.3	0.12	0.02	0.12	5.1	0.70	1.2	1.20	19.5	3.2	1200	57			
(right)†	8.3	0.28	0.06	1.5	5.6	0.20	0.51	0.33	11.0	2.9	480	43			
M.P.	7.5	0.18	0.07	2.4	3.7	0.26	0.55	1.50	17.5	5.2	400	46			
R.V.	11.5	0.27	0.16	2.4	3.8	0.44	0.95	1.50	25.1	16.1	685	28			
C.T.									19.7	6.0	596	49			
K.L.									9.8	1.12					
I.M.									5.3	0.42	0.82	9.8	1.12		
Mean	6.7	0.27	0.27	4.3	0.32	5.3	0.42	0.82	9.8	1.12	5.3	0.42	0.82	9.8	1.12

* Randomly labeled, tritiated *dl*-aldosterone; result divided by 2.

† Nodular hyperplasia.

with a threefold increase in excretion after salt restriction. Response to added ACTH was lacking in D.G. and delayed in D.V. Porter-Silber chromogens and 17-ketosteroids did not change during salt restriction and showed a delayed increase during ACTH administration.

Patients with primary aldosteronism (12 cases)

Secretory rates of cortisol, corticosterone, and aldosterone (Table II). In seven patients, the secretion of cortisol was normal, and in ten, so was the urinary excretion of Porter-Silber chromogens. In three of seven patients, secretion of corticosterone was increased. Secretion of aldosterone was measured in nine patients, and the increases ranged from 300 to 1200 μg per 24 hours. Urinary excretion was increased in eleven patients 28 to 110 μg per 24 hours (normal range, 4 to 22 μg per 24 hours). In two of the patients in whom randomly labeled, tritiated *dl*-aldosterone was used, the secretory rates were from 600 to 800 μg per 24 hours; this corresponds to a secretory rate of 300 and 400 μg per 24 hours of *d*-aldosterone, since *l*-aldosterone is not metabolized.

Tissue analysis

Cushing's syndrome (Table III). In *hyperplastic glands*, the mean content of cortisol, 11-

desoxycortisol, desoxycorticosterone, aldosterone, and corticosterone were essentially the same as in normal adrenal tissue. In *adenomas*, although the mean cortisol, 11-desoxycortisol, and desoxycorticosterone content were essentially normal, corticosterone content was decreased and aldosterone was actually absent in one adenoma. In the *suppressed adrenal gland* (contiguous to tumor), mean cortisol and corticosterone content were extremely low. Aldosterone and desoxycorticosterone content were, however, within normal limits.

Primary aldosteronism (Table II). The size and histologic appearance of the contiguous *adrenal gland* in all patients with adrenocortical adenoma were within normal limits except for areas of nodular hyperplasia in V.M. The mean content in micrograms per gram wet weight was 6.7 for cortisol, 4.3 for corticosterone, 0.27 for aldosterone, 0.27 for 11-desoxycortisol, and 0.32 for desoxycorticosterone. These values were used as a standard of comparison for pathologic adrenal tissue and compare favorably with those reported by Neher (10). In the group of *adrenocortical tumors*, mean cortisol content of all the adenomas was normal at 5.3 μg per g. Mean content of corticosterone and aldosterone was increased to 9.8 and 0.82 μg per g, respectively. Mean desoxycorticosterone content was increased threefold, and 11-desoxycortisol was only slightly increased in four tumors. In all cases, corticosterone content was higher in the tumor. The highest corticosterone content, found in the tumor of patient M.J., was associated with an increased secretory rate of corticosterone. In one instance, aldosterone content of the tumor showed no increase over that of the contiguous adrenal gland.

TABLE III
Analysis of corticosteroids in adrenal gland and adrenal tumor

Patient	Cortisol	11-Desoxy-cortisol	Aldosterone	Corticosterone	Desoxycorticosterone
	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Cushing's syndrome due to hyperplasia					
a) Adrenal gland					
B.P.	7.8		0.22	8.0	
M.T.	8.2	0.63	0.20	4.7	0.45
C.W.	3.5	0.46	0.27	4.2	0.46
Mean	6.5	0.55	0.23	5.6	0.46
Cushing's syndrome due to adenoma					
a) Adrenocortical adenoma					
C.G.	8.9		0	1.6	
M.E.	6.7	0.48	0.02	0.5	0.42
W.B.	6.2	0.33	0.10	1.2	0.26
Mean	7.3	0.40	0.04	1.1	0.34
b) Contiguous adrenal gland					
C.G.	0.7		0.58	0	
M.E.	2.0	0.18	0.05	1.0	0.35
W.B.	1.6	0.37	0.26	4.0	0.55
Mean	1.4	0.28	0.30	1.7	0.45

Adrenal vein plasma concentration (Table IV)

The mean concentration of cortisol in Cushing's syndrome (hyperplasia) was greater than in primary aldosteronism (mean, 5.5 against 3.3 μg per ml). Corticosterone concentrations were virtually similar in both Cushing's syndrome and primary aldosteronism. Mean aldosterone concentration was greater in primary aldosteronism than in Cushing's syndrome (mean, 0.53 against 0.05 μg per ml). In one case, the concentration of aldosterone on the pathologic side showed a slight increase over that of the contralateral adrenal vein.

TABLE IV
Concentration of corticosteroids in adrenal vein at time of surgical operation in Cushing's syndrome and primary aldosteronism

Patient	Cortisol $\mu\text{g/ml}$	Corticosterone $\mu\text{g/ml}$	Aldosterone $\mu\text{g/ml}$
Cushing's syndrome			
V.M.	5.2	1.0	0.07
M.T.	5.7	2.0	0.02
Mean	5.5	1.5	0.05
Primary aldosteronism			
L.B.			0.7
R.T.			0.2
C.H.	3.0	0.2	0.12
R.G.	1.8	1.2	0.45
M.P.	6.7	3.2	1.56
M.J. (right)	2.5		0.02
(left)*	2.1	3.1	0.04
Mean	3.3	1.9	0.53

* Side of adenoma.

DISCUSSION

In our current concepts of the biosynthetic pathways of adrenocortical steroidogenesis, a dichotomy between cortisol and aldosterone synthesis exists. Cushing's syndrome and primary aldosteronism are clinical expressions of the predominance of one pathway or the other.

The secretory rate of cortisol in Cushing's syndrome is well above the normal range. The secretory rate of corticosterone, however, was normal in our patients, with the exception of an elevation in the patient with adrenocortical carcinoma. Secretory rates and urinary excretion of aldosterone were normal, results which agree with those of others (24-26), although hypertension was present in 14 of 17 patients and hypokalemia was seen in but one. Two significantly lowered secretory rates were observed, however, one in a patient with adenoma, the other, in one with carcinoma.

Despite the inherent difficulties in interpreting a momentary measurement of concentration in these active pathophysiologic states, the analysis of pathologic tissue in Cushing's syndrome revealed interesting correlations. For this study, we used as normal content of adrenal tissue the ipsilateral adrenal gland of the patients with primary aldosteronism. These glands were all normal in weight, no abnormalities were seen upon

histologic study, except for areas of nodular hyperplasia in one patient, and steroid values were similar to those of normal adrenal tissue (10). In both groups of Cushing's syndrome, hyperplasia and adenoma, cortisol content was not increased, although its secretory rate was, and the content of corticosterone was normal in hyperplasia, but decreased in adenoma. The content of aldosterone was virtually normal in hyperplastic tissue, as well as in the suppressed adrenal gland associated with an adenoma, but was decreased in tumor tissue itself. On analysis, the suppressed adrenal gland was almost devoid of cortisol and corticosterone, but aldosterone, 11-desoxycortisol, and desoxycorticosterone were measurable.

These observations are of particular significance because the suppressed adrenal gland remaining after operation in patient C.G. was able to increase aldosterone secretion after salt restriction without concomitant increase in cortisol secretion; aldosterone excretion increased promptly with ACTH in C.G., whereas excretion of Porter-Silber chromogens was considerably delayed; and four patients with hyperplasia and two with carcinoma were also able to increase aldosterone secretion after salt deprivation. In addition, two patients with profound adrenal suppression due to prolonged corticoid therapy were similarly able to increase aldosterone secretion promptly after the potent stimulus of salt restriction, whereas response in Porter-Silber chromogens and 17-ketosteroids was delayed after ACTH. The second day of ACTH did not increase urinary aldosterone in D.G., but did so in D.V.; apparently, maximal response had been achieved by salt deprivation in D.G. B.P., a patient with adrenal hyperplasia, had normal aldosterone excretion and normal responses to salt deprivation before and after unilateral adrenalectomy. This again shows that in the presence of increased endogenous or exogenous cortisol, aldosterone secretion responds normally. In a further contrast of primary aldosteronism and Cushing's syndrome, mean adrenal vein concentration of cortisol at surgery was higher in Cushing's syndrome than in primary aldosteronism, and conversely, mean concentration of aldosterone was higher in primary aldosteronism than in Cushing's syndrome. Mean corticosterone concentration showed no difference.

These studies in nonmalignant Cushing's syn-

drome show that secretion and excretion of aldosterone and secretion of corticosterone are normal in the presence of increased cortisol secretion. The virtual absence of corticosterone and aldosterone in adrenocortical tumors could be interpreted to mean that these tissues are producing predominantly cortisol. There were no marked differences in the 11-desoxycortisol content between the tumors and the glands in either Cushing's syndrome or primary aldosteronism.

Preservation of normal secretory and excretory aldosterone patterns and normal adrenal gland concentration of aldosterone is consistent with the observation that prolonged cortisol administration effects no changes in aldosterone excretion (27, 28). The site of aldosterone production is not clear in hyperplasia, but in Cushing's syndrome due to adenoma, it may reside in the atrophic adrenal gland.

In primary aldosteronism, increased aldosterone secretion occurred in the presence of normal cortisol secretion, but in two of five patients, increased secretion of corticosterone was observed. In contrast to Cushing's syndrome, mean concentration of corticosterone and aldosterone of eight tumors showed a two- and threefold increase, respectively, over the adjacent adrenal gland. The higher concentrations of corticosterone were associated with higher secretory rates of corticosterone.

The content of aldosterone in tumor tissue was not related to the level of urinary aldosterone or its secretory rate, as previously noted (11). In every case but one (C.H.), aldosterone content of the adrenocortical tumor was greater than that of the adjacent adrenal gland. The mean difference in concentration in tumor and gland was threefold. Others have reported similar increases in corticosterone and aldosterone (10-13).

In vitro incubation of pathologic tissues producing Cushing's syndrome and primary aldosteronism has at times demonstrated the propensity of these tissues to produce cortisol and aldosterone more rapidly than normal tissue (29). *In vivo* secretory rates of these studies, however, delineate the secretory patterns more clearly. In contrast to Cushing's syndrome, almost uniform agreement is observed between increased aldosterone content in the tumor and increased secretory rate in primary aldosteronism.

It is of particular importance that the increased content of corticosterone in tumors producing primary aldosteronism is usually not apparent as increased secretion. This, together with the finding that desoxycorticosterone content in tumors is increased in the same ratio as aldosterone, suggests their participation in the overproduction of aldosterone.

The absence of clear-cut increases of precursor steroids in tissue producing Cushing's syndrome, as compared with tissue giving rise to primary aldosteronism, is difficult to explain from these studies. The *in vivo* secretory studies clearly show the differences that exist, especially in the secretion of corticosterone. These studies again demonstrate the independence and preservation of aldosterone secretion in states of excessive endogenous cortisol production or exogenous administration of corticosteroids.

SUMMARY

In Cushing's syndrome, cortisol secretion was consistently elevated. Corticosterone secretion was normal, except in a single case of malignancy. Aldosterone secretion ranged from normal to low. Analysis of hyperplastic glands showed normal concentrations of cortisol, 11-desoxycortisol, aldosterone, corticosterone, and desoxycorticosterone. Analysis of adenomas revealed normal cortisol content, lowered corticosterone content, and low to absent aldosterone content. The suppressed ipsilateral adrenal gland revealed low cortisol and corticosterone content, but normal amounts of aldosterone, desoxycorticosterone, and 11-desoxycortisol. Patients with Cushing's syndrome had normal urinary aldosterone. The suppressed adrenal tissue remaining in patients who had adenomas surgically removed, as well as that in two patients who had prolonged steroid therapy, was capable of secreting normal amounts of aldosterone.

In primary aldosteronism, cortisol secretion was normal. In three of seven patients, there was an increase in corticosterone secretion. Aldosterone secretion was greatly increased. The tumor's content of cortisol was normal, but there was increased content of corticosterone, desoxycorticosterone, and aldosterone. Normal aldosterone secretion and responses are preserved in the presence of exogenous or endogenous cortisol excess.

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