STEROID FORMATION BY ADRENAL TISSUE FROM HYPERTENSIVES [1]

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The adrenal cortical hormones have been thought to play a role in the pathogenesis of hypertension for the following reasons: *1*) Hypertension is a characteristic finding in Cushing's disease; *2*) large doses of desoxycorticosterone (DCA) and salt administered to Addisonian patients produce hypertension (1); *3*) hypertensive patients who developed Addison's disease had blood pressure falls to normal limits which were restored to previous levels with DCA (2); and *4*) 80 per cent of the patients with severe hypertension have had significant improvement for three to seven years following 90 to 100 per cent adrenalectomy and limited sympathectomy (3). Moreover, animal experiments (4, 5) have shown that hypertension following renal artery occlusion cannot be produced in the absence of adrenal cortical steroids.

Despite these observations, altered adrenal function in hypertension has not been clearly demonstrated. While mean levels of cortical steroids in blood and urine (6–9) are not elevated in hypertensive patients, disturbances in salt and water metabolism observed in early hypertension prior to the development of renal damage by Green, Johnson, Bridges and Lehmann (10) and Braun-Menendez (11) indirectly support the idea that the pattern of adrenal corticoids is altered.

Brady (12) found that slices of canine adrenal tissue produce large quantities of steroids when incubated *in vitro* in autologous plasma and proposed the measurement of steroid formation *in vitro* as a direct assessment of the functional capacity of adrenal tissue (13). The use of adrenalectomy for the palliative management of advanced cancer (14, 15) and the treatment of severe hypertension (16) enabled us to apply this method to human adrenal tissue and to demonstrate that the amount of steroids formed by the human adrenal incubates is sufficiently large for semiquantitative estimation and identification.

In the present report we are presenting steroid analyses of adrenal tissue incubates from 3 normotensive cancer-free patients, 8 normotensive patients with carcinoma of the breast or prostate and 23 patients with severe hypertension. The differences in the steroid formation by the incubates from normotensive and hypertensive subjects and the relationship between clinical picture and steroid formation within the hypertensive group will be discussed. In addition, the steroid pattern in the incubates from hypertensive patients will be compared with that in the adrenal vein blood obtained at operation. Studies concerning the identification of the steroids will be reported elsewhere.

EXPERIMENTAL

1. Patients. The clinical data on the normotensive patients are listed in Table I. In two of the three cancer-free patients only a portion of one adrenal gland (200 to 600 mg.) was excised, while in the third a unilateral adrenalectomy was performed because of a hemorrhage occurring in the gland during the operation. No abnormalities were found by histological examination. The eight normotensive cancer patients were ambulatory and not nutritionally depleted at the time of adrenalectomy as evidenced by the satisfactory levels of serum proteins (Table I), although all had far advanced carcinoma of the breast or prostate. The adrenals from these patients were carefully examined grossly and microscopically for evidence of metastatic tumor. None was found.

Table II furnishes the clinical data of 14 of the 23 hypertensive patients studied. In these patients a com-

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TABLE I

Clinical data on normotensive patients

No.	Patient	Sex	Age	Height *in.*	Weight *lbs.*	Blood pressure *mm. Hg*	Diagnosis*	Duration of disease	Serum protein	Blood urea nitrogen	Urine albumin	Adrenal-ectomy
						A. Cancer-free patients						
1	E. O.	M	58	69	176	140/80	Sigmoid diverticulitis	10 yrs.		9	0	9/15/54
2	G. B.†	F	58	69	126	110/70	Congenital cystic dis. of kidney	6 mos.		12	trace	3/29/54
3	R. H.	M	49	67	167	130/80	Hematuria undiagnosed	1 yr.		11	trace	2/ 7/56
						B. Cancer patients‡						
1	A. E.	F	51	66	150	128/80	Breast	2 yrs.		9	0	2/17/54
2	D. B.	F	48	61.5	139	120/80	Breast	6 yrs.	7.2	13	0	12/20/54
3	L. F.	F	48	63	140	110/80	Breast	2 yrs.	6.0	12	trace	11/30/53
4	W. S.	M	62	61	135	130/60	Prostate	1–2 yrs.	6.1	13	0	4/ 8/54
5	J. B.§	M	57	64	125	132/80	Prostate	2 yrs.	5.4	16	0	1/22/54
6	G. M.§	M	58	67	173	114/68	Prostate	3 yrs.	5.4	16	1+	1/19/56
7	J. H.§	M	71	71	160	130/70	Prostate	3 yrs.	5.9	13	0	3/31/56
8	J. G.§	M	67	67	128	130/80	Prostate	4 yrs.	6.4	21	0	4/12/56

* Primary site of carcinoma in cancer cases.
† Subsequently found to have papilloma of bladder, six months hematuria prior to operation.
‡ All patients previously castrated (except those indicated by §).
§ Castrated at time of adrenalectomy.

plete chromatographic steroid analysis of the adrenal incubates was obtained. Blood pressure and adrenal weight records of the remaining eight patients in whom only the hydrocortisone values of the incubates are available are included with the latter values in Table V. The patients in Tables II and V are arranged according to ascending diastolic blood pressure. Those in Table II are divided for statistical analysis into three groups according to their clinical picture and diastolic blood pressure.

All patients were severe hypertensives who had been followed by the staff of the Hypertension Section of the Edward B. Robinette Foundation of the Hospital of the University of Pennsylvania. At the time adrenalectomy and a modified Adson type of sympathectomy were recommended they had failed to respond to several types of medical therapy and were showing progressive symptoms of the disease. All antihypertensive therapy had been stopped two weeks prior to operation. Twenty-one of the patients were on the usual hospital house diet without any restrictions. Two of the patients were in congestive heart failure as indicated in Table II and were on a salt-poor diet.

2. Preparation and extraction of the adrenal incubates. All adrenal tissue used in this study was obtained at the first stage of a bilateral adrenalectomy. No previous surgery had been done within one year in all cases studied.

Adrenal glands were dropped into an ice cooled beaker immediately after removal and transferred to a cold room (4° C.) where the periadrenal fat and capsule were removed. The glands were then weighed, a portion removed for histologic examination and the remainder sliced by means of a Stadie Riggs tissue slicer. Approximately 1.0 Gm. batches of slices were transferred to chilled 125 ml. glass-stoppered Erlenmeyer flasks equipped with gas inlet and outlet tubes in the stoppers. Ten ml. of the patient's heparinized plasma with 50,000 units of penicillin and 0.1 Gm. of streptomycin were added to each flask. The flasks were agitated in a water bath at 37.5° C. for 24 hours while a slow current of a gas containing 95 per cent oxygen and 5 per cent carbon dioxide was continuously passing through the gas space of the flasks. The shaking rate was 50 to 60 oscillations per minute through an amplitude of 4 to 5 cm. The time interval between removal of the gland and the beginning of incubation was 45 minutes or less. Five units of adrenocorticotropin (Armour) was added to selected flasks.

After incubation, the tissue and medium were quantitatively transferred to and homogenized in a Potter Elvehjem all-glass homogenizer. Five volumes of acetone was added to precipitate the protein which was separated by filtration and washed several times with additional acetone. The acetone was removed *in vacuo* in a water bath at a temperature not exceeding 45° C. The aqueous phase remaining was extracted twice with 50 ml. of ethyl acetate and twice with 50 ml. of chloroform. The combined organic extracts were washed twice with 25 ml. portions of 0.1 N sodium hydroxide and twice with 50 ml. portions of distilled water. The organic phase was dried over sodium sulfate and evaporated to dryness *in vacuo*. Adrenal vein blood was treated in a similar manner.

3. Separation of the steroids. The residue of the extracts of the adrenal incubates was subjected to paper chromatography by means of a modification of the method

TABLE II

Clinical data on 14 hypertensive patients

Group	No.	Patient	Sex	Age	Height	Weight	Blood pressure	Duration	Smith-wick group	Cardiac thoracic ratio	Electro-cardiogram	Blood urea nitro-gen	Phenol-sulfon-phthalein*	Urine albumin	Fundo-scopic grade†	Other
					in.	*lbs.*	*mm. Hg*	*yrs.*								
I	1	M. R.	M	40	69.8	141	172/80	11	1	0.49	Small. S-T elevation	28	15–25 2–65	1+	3–4	Severe diabetic
	2	C. B.‡	M	40	70.5	175	220/110	22	3	0.50	Left axis div.	13	15–20 2–60	0	4	Retinal hemorrhages
	3	M. U.	M	51	62	142	200/115	28	3	0.57	Normal	20	15–25 2–80	0	2	3 strokes; congest. heart failure
	4	A. R.	M	39	68	162	155/120	6	2	0.58	Normal	11	15–30 2–60	0	2	Headaches
	5	A. B.	F	44	66	148	190/120	15	2	0.51	Normal	10	15–35 2–75	0	2	
II	6	M. A.	F	45	64	176	210/125	17	3	0.52	T-wave changes	28	15–20 2–73	4+	3	Vertigo
	7	G. C.	M	50	72	180	220/130	0.5	3	0.56	Diphasic T-waves	18	15–25 2–50	2+	4	Headache
	8	M. M.	F	50	65.5	127	240/140	10	4	0.50	Left axis dev.	14	15–20 2–60	2+	3	Syncope
	9	F. McC.	M	50	66	180	250/140	6	4	0.54	Left axis dev.	13	15–20 2–65	1+	3	Headache
III	10	P. B.	F	25	68	115	200/146	1	4	0.46	Left axis dev.	39	15–15 2–45	3+	4	Stroke; congest. heart failure
	11	F. McL.	M	54	70.5	152	246/146	1.5	4		T-wave changes	23	15–5 2–15	1+	4	Headache
	12	J. K.	M	39	69	158	216/150	20	4	0.57	Left axis dev.	13	15–25 2–75	3+	3	Epistaxis
	13	J. D.‡	M	43	71	126	230/150	3	4	0.57	Left axis dev.	15	15–25 2–85	4+	4	Congest. heart failure
	14	T. O'C.	M	34	73	177	260/170	15	4	0.57	Left axis dev.	11	15–15 2–50	4+	4	

* Fifteen minutes and two hour per cent phenol-sulfonphthalein excretion.
† Keith Wagner classification.
‡ Not included in analyses of variance and corresponding figures.

of Burton, Zaffaroni and Keutmann (17). Three main fractions resulted. Fraction X consisted of material found on the paper in a toluene-propylene glycol system after 13 ml. of effluent had been collected per 0.5 inch width of strip. The effluent from fraction X was evaporated and rechromatographed until 3 ml. of effluent per half inch strip was collected to give fraction Y. The effluent from fraction Y was evaporated to dryness and rechromatographed in a methylcyclohexane-propylene glycol system until 17 ml. of effluent per 0.5 inch strip was collected to give fraction Z.

In all instances aliquots of the extract corresponding to approximately 0.15 Gm. of adrenal tissue were placed on each half inch width of chromatographic strip. The strips, after drying in air, were scanned at 245 mμ in a Beckman model D.U. spectrophotometer equipped with an adapter as described by Tennent, Whitla and Florey (18). The instrument was set to 100 per cent transmittance against an area of the paper strip one inch above the starting line. Representative strips were sprayed with blue tetrazolium reagent and read at 600 mμ for detection of α ketolic substances.

Absorption curves for all strips were obtained by plotting the optical density of each point against the distance from the starting line. Quantitative determinations of the amount of steroid were made by comparison of the area under the peak of absorption at 245 mμ with the area under the curve produced by known amounts of cortisol, chromatographed under identical conditions as fraction X. In nine determinations of hydrocortisone by this method, agreement with the Porter-Silber values on aliquots from the same zone was better than 95 per cent in all cases.

Results of the incubation experiments are expressed as μg. steroid formed per 24 hours per Gm. of tissue. The standard error of measurement computed from 20 duplicate determinations of adrenal incubates was plus or minus 20 μg. for the F position and plus or minus 40 μg. for the steroids of the Y and Z positions. This corresponds approximately to a reproducibility of plus or minus 5 per cent for the F position and plus or minus 8 per cent for the B-region. Recoveries of added cortisol were 95 to 105 per cent (13).

RESULTS

1. *Chromatographic pattern in adrenal incubates and adrenal vein blood*

The chromatograms shown in Figure 1 represent the absorption curves at 245 mμ from the extracts of the adrenal incubate and the adrenal vein blood of a patient. The pattern is characteristic for the entire group of patients studied. Five definite areas of absorption on the chromatograms from the adrenal incubates have been found. The first area, corresponding to material more polar than compound F, has been labeled the "before F" region. It contains several steroids not as yet identified. The second peak has been labeled as hydrocortisone on the basis of the following characteristics: The color reaction with blue tetrazolium gave a quantititative value agreeing with the degree of absorption of ultraviolet light at 245 mμ and the results of Porter-Silber determinations on selected zones. The mobility of the eluted zone in toluene-propylene-glycol and chloroform-formamide paper chromatograms as the free alcohol and as the acetate was the same as that of authentic hydrocortisone. The spectrum in sulfuric acid from 220 to 600 mμ was the same as that of the reference steroid under equivalent conditions. The characteristic green fluorescence was also shown by the eluted zones. The third peak has a mobility comparable to compound E in this system. Since several unidentified substances are present in this region, the area has been labeled the E-region.

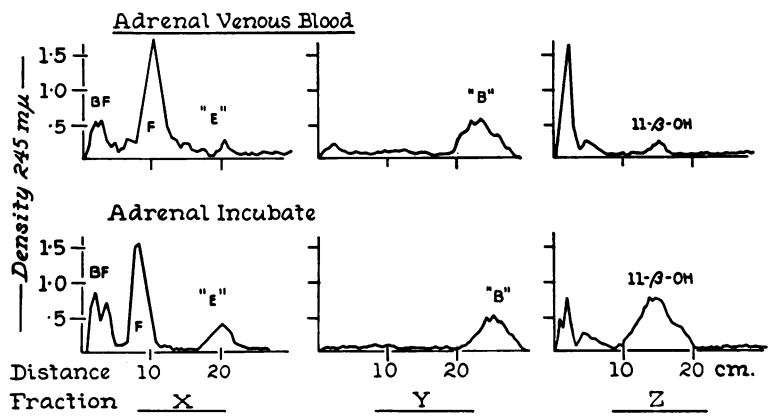

FIG. 1. CHROMATOGRAMS OF EXTRACTS OF ADRENAL VENOUS BLOOD AND ADRENAL INCUBATES

The fourth peak may be in either the Y or Z fraction and corresponds to the area in which compound B would separate. This area, designated the B-region, has recently been further separated by use of the dimethyl formamide-methylcyclohexane paper chromatography system and shown to consist of about 70 per cent corticosterone and 10 per cent Substance S [17α-hydroxydesoxycorticosterone (19)]. The remaining 20 per cent of this region has not been identified. The fifth area represents Δ^4-androstene-11β-ol-3,17-dione as has been previously reported by us (20).

The five areas representing the different steroids on the chromatograms from the adrenal vein blood are similar to those from the adrenal incubates. It is possible, however, that there are differences in the individual compounds which form the five areas since all of the substances have not been identified.

2. Steroid formation by adrenal incubates without added ACTH

a. Normotensive Group. Comparison of the values (Table III) for steroid formation by adrenal incubates from the three "normal" and eight carcinoma patients suggests that the latter do not differ greatly from those of the former, although the number of patients in the "normal" group is too small for statistical evaluation. While no comparative data on *in vitro* steroid formation of normal human adrenal tissue are available in the literature, Sweat (21) has obtained ratios of 2.8 to 3.0 for hydrocortisone to corticosterone in peripheral blood of normal subjects—a value similar to the averages of 2.62 for carcinoma and 3.09 for "normal" adrenal incubates in our series.

b) Hypertensive group. From Table IV it is seen that the pattern of steroid formation in the hypertensive group did not differ markedly from that in the normotensive series. In 9 of the 14 cases of Table IV, however, the rate of steroid formation was considerably higher than in adrenal incubates from the normotensive patients.

Steroid formation and diastolic blood pressure

To explore whether the differences in rate of steroid formation within the hypertensive groups were related to the severity of the disease, hydrocortisone formation by the incubates from the 23 patients of Tables IV and V was plotted against diastolic blood pressure. The results are illustrated in Figure 2. A significant negative correlation ($r = -0.64$; $p < 0.01$) was obtained.

In Tables II and IV the hypertensive patients have been divided into three equal sub-groups according to clinical criteria of the severity of the disease. Groups I and III comprise the least and most severe cases, respectively, at the time of operation while the intermediary cases make up Group II as recorded in Table II. In general this classification agrees well with the diastolic blood pressure arrangement, the mean diastolic pressures being 109, 134 and 156 mm. Hg for the three groups. All patients of Group III with the exception of P. B. have subsequently died. The grouping of our patients may require slight revision when a greater number of cases becomes available.

The mean hydrocortisone formation (372 μg.) by the incubates of Group I adrenal tissue was significantly greater than that (184 μg.) of the normotensive cancer patients (Tables III and VI), whereas the rate of hydrocortisone formation (184 μg.) by the Group III adrenal incubates was on the average not higher than that of the normotensive control group (the lower curve in Figure 3 and Table VI). The rate of hydrocortisone formation per unit weight of adrenal tissue thus decreased with increasing severity of hypertension.

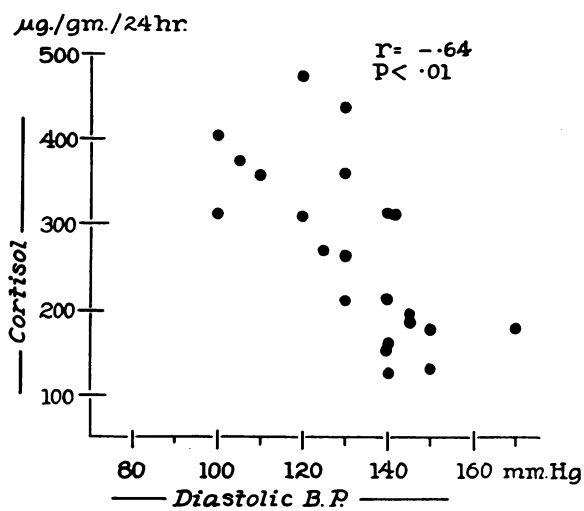

FIG. 2. RELATIONSHIP BETWEEN CORTISOL FORMATION AND DIASTOLIC BLOOD PRESSURE

TABLE III

Steroid formation by adrenal tissue from normotensive patients

No.	Patient	Adrenal weight (*Gm.*)	No ACTH *μg./24 hrs./Gm.* Before F	F	E	B	11-β-OH	Total	F/B	With ACTH *μg./24 hrs./Gm.* Before F	F	E	B	11-β-OH	Total	F/B
							A. Cancer-free patients									
1	E. O.		60	176	70	81	44	431	2.18							
2	G. B.	5.2	138	265	50	57	65	575	4.60	267	430	144	90	125	1,056	4.70
3	R. H.		30	222	106	90	120	573	2.50	82	410	245	150	180	1,067	2.70
	Mean	5.2	76	223	75	76	76	526	3.09	175	420	195	120	153	1,062	3.70
	S. E.*		±32.2	±25.8	±16.4	±9.85	±22.7	±54.9	±0.624							
							B. Cancer patients									
1	A. E.	4.80	66	313	108	138	124	750	2.27	138	338	64	86	267	893	3.93
2	D. B.	4.41	25	201	26	41	96	389	4.90	63	482	78	127	138	888	3.80
3	L. F.	4.89	51	124	37	117	102	431	1.07	138	345	145	272	181	1,081	1.27
4	W. S.	5.85	104	89	107	54	43	397	1.65	243	257	78	163	98	839	1.58
5	J. B.	4.30	51	155	40	41	43	330	3.78							
6	C. M.	4.57	54	267	70	67	79	537	3.99	119	429	115	327	274	1,264	1.31
7	J. H.	5.30	96	195	35	107	128	561	1.82	179	321	122	106	232	960	3.02
8	J. G.	3.80	70	131	65	91	91	448	1.44	55	176	51	155	104	541	1.14
	Mean	4.74	64	184	61	82	88	480	2.62	134	336	93	177	184	924	2.29
	S. E.	±0.223	±9.08	±26.8	±11.45	±13.88	±11.41	±47.0	±0.498	±22.6	±38.5	±13.0	±33.8	±28.1	±84.2	±0.472

* S. E. $= \sqrt{\frac{\Sigma(x_1 - \bar{x})^2}{n(n-1)}}$.

TABLE IV

Steroid formation by adrenal tissue from patients with hypertension—Cases with complete analyses

Group	No.	Patient	Adrenal weight (*Gm.*)	No ACTH *μg./24 hrs./Gm.* Before F	F	E	B	11-β-OH	Total	F/B	With ACTH *μg./24 hrs./Gm.* Before F	F	E	B	11-β-OH	Total	F/B
I	1	M. R.	6.06	68	341	99	187	74	769	1.82	94	618	88	194	99	1,093	3.19
	2	C. B.*	3.55	137	360	90	125	125	837	2.88	252	724	217	287	345	1,825	2.52
	3	M. U.	3.99	111	373	200	255	72	1,011	1.46	153	456	347	210	124	1,290	2.17
	4	A. R.	6.75	111	310	190	260	173	1,044	1.19	199	842	253	298	319	1,911	2.64
	5	A. B.	4.34	104	476	275	255	163	1,273	1.87	276	760	237	280	178	1,731	2.70
Mean	5		4.94	106	372	171	216	121	987	1.84	195	680	228	254	213	1,570	2.64
S. E.			±0.621	±11.1	±28.1	±34.5	±26.5	±21.1	±88.2	±0.287	±33.0	±66.6	±41.4	±21.5	±50.4	±160.1	±0.159
II	6	M. A.	4.80	62	270	144	218	74	768	1.24	113	466	262	298	144	1,283	1.56
	7	G. C.	5.02	123	361	165	130	170	949	2.78	141	625	328	262	199	1,555	2.39
	8	M. M.	4.22	109	314	207	318	200	1,148	0.99	177	648	352	422	285	1,884	1.54
	9	F. McC.	8.12	45	210	66	192	78	591	1.09	63	424	119	438	135	1,179	0.97
Mean	4		5.54	85	289	146	215	131	864	1.52	124	541	265	355	191	1,475	1.62
S. E.			±0.893	±18.6	±32.2	±29.6	±39.1	±32.1	±119.6	±0.456	±24.0	±56.1	±52.3	±44.0	±34.4	±157.6	±0.292
III	10	P. B.	5.81	67	196	117	140	56	576	1.40	94	230	72	252	132	780	1.74
	11	F. McL.	9.30	73	200	86	187	115	661	1.07	102	365	90	334	155	1,046	1.08
	12	J. K.	4.96	56	164	72	178	165	635	0.99	84	366	162	380	184	1,176	0.96
	13	J. D.*	6.58	57	131	51	130	110	479	1.00	79	109	96	200	79	563	0.55
	14	T. O'C.	6.74	69	175	134	180	130	688	0.97	93	462	74	336	249	1,214	1.38
Mean	5		6.68	64	173	92	163	115	608	1.09	90	306	99	300	160	956	1.14
S. E.			±0.728	±3.17	±12.5	±15.0	±17.6	±17.8	±37.1	±0.080	±4.03	±61.6	±16.4	±32.5	±28.2	±102.1	±0.200

* Omitted from analysis of variance and corresponding Figures 3, 4 and 6.

There was also a slight though less significant diminution (from 216 to 163 μg.) in the synthesis of steroids of the B region from Subgroups I to III. In all the three hypertensive groups, however, the rate of formation of B-region steroid was significantly higher than in the cancer group (*cf.* Figure 4 and Table VI). The increasing preponderance of formation of B-region steroids with increasing diastolic pressure can be most readily seen by inspecting the ratio of synthesis of hydrocortisone to synthesis of B-region steroids in Tables III and IV. This ratio declined from a mean of 2.6 in the group of normotensive patients with cancer to 1.6 in Group I of the hypertensive patients and, finally, to 1.1 in the patients with the most severe hypertension.

Steroid formation and adrenal weight

Inspection of the data in Tables IV and V reveals that the rate of hydrocortisone formation by incubates from glands weighing more than 6 Gm. was in 7 out of 9 instances below 200 μg. per Gm. per 24 hours, whereas only 2 out of 14 incubates from lighter glands displayed similarly low rates. In Figure 5 the regression of rate of hydrocortisone formation on weight of gland is depicted. The correlation proved to be significant statistically ($r = -0.65$; $p < 0.01$). Since the adrenal weights include both the weight of cortex and of medulla, it cannot be decided at present which ana-

TABLE V

Steroid formation by adrenal tissue from patients with hypertension—Cases with hydrocortisone determination only

Patient	Adrenal weight	Blood pressure	F
no.	*Gm.*	*mm. Hg*	*μg./24 hrs./Gm.*
15	3.31	200/100	312
16	5.30	198/105	405
17	4.00	200/130	440
18	3.67	245/130	210
19	5.85	270/130	263
20	5.76	210/140	314
21	10.49	220/140	112
22	7.14	240/140	127
23	6.52	240/140	156

TABLE VI

The differences in steroid formation and adrenal weight between hypertensive groups and cancer group

Group differences*	Adrenal weight (Gm.)	Before F (μg.)	F (μg.)	E (μg.)	B (μg.)	11-β-OH (μg.)	Total (μg.)
Without ACTH			Mean differences ± standard error†				
I—Cancer	0.12 ±0.557	41.6 ±10.45 p < .01	187.6 ±40.68 p < .01	109.8 ±30.31 p < .01	134.4 ±40.21 p < .01	33.3 ±21.91	506.4 ±90.97 p < .01
II—Cancer	0.80 ±0.681	20.1 ±16.29	104.4 ±44.42 p < .05	84.5 ±25.85 p < .01	132.5 ±32.29 p < .01	42.2 ±27.13	384.0 ±110.35
III—Cancer	1.94 ±0.629 p .02	− 0.2 ±11.92	−11.2 ±35.78	7.5 ±18.68	81.0 ±18.94 p < .01	26.9 ±19.99	128.0 ±73.61
With ACTH							
I—Cancer		61.2 ±38.51	344.6 ±71.90 p < .01	135.1 ±37.7 p < .01	77.2 ±44.3	28.1 ±36.61	646.3 ±166.8 p < .01
II—Cancer		−10.1 ±33.91	205.3 ±66.06 p < .02	172.0 ±41.77 p < .01	178.4 ±55.78 p < .02	5.9 ±45.50	551.5 ±161.3 p < .01
III—Cancer		−43.2 ±27.33	−29.0 ±68.85	4.5 ±20.69	123.8 ±48.71 p < .05	−25.1 ±40.99	32.1 ±108.3

* Number of experiments are 5, 4 and 5 in hypertensive groups I, II and III, respectively, 8 in cancer group without ACTH and 7 with ACTH.

† Standard error $= \pm \sqrt{\frac{\Sigma(x_i - \bar{x}_1)^2 + \Sigma(x_i - \bar{x}_2)^2}{n_1 + n_2 - 2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$. Unless p value is recorded, difference is not significantly different from zero statistically.

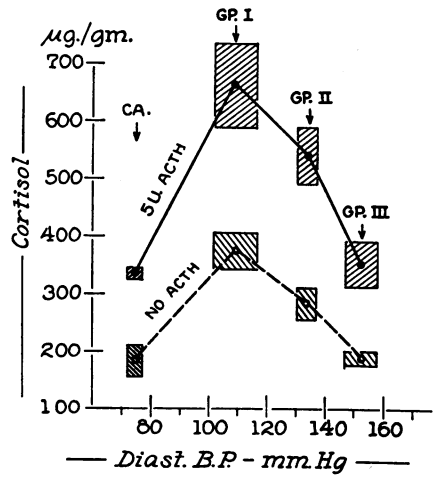

FIG. 3. CORTISOL FORMATION WITH AND WITHOUT ADDED ACTH—GROUPED DATA

The points in the center of the shaded rectangles represent the group means, the sides of the rectangles correspond to plus or minus one standard error of y and x, respectively. There are four cases in each hypertensive group as indicated in Table V. Analysis of variance of the data from the three hypertensive groups revealed a statistically highly significant association ($p < 0.01$) of cortisol formation and diastolic blood pressure and a highly significant ACTH effect ($p < 0.01$) in all three groups.

tomical constituent was responsible for the increase in weight of the whole gland.

The question arose to what extent the increased weight of the gland in severe hypertension compensated for the reduced rate of steroid formation per unit weight of adrenal tissue. To estimate the potential total capacity of steroid synthesis of the adrenal tissue present in the individual patient, the rates of steroid formation *in vitro* were multiplied by twice the weight of the gland excised. In Figure 6 the values thus computed for hydrocortisone and B-region steroid synthesis in normotensive cancer patients and the three groups of hypertensives have been plotted against diastolic blood pressure. The grand mean of total hydrocortisone output for the more severe hypertensive Groups II and III was significantly higher (931 μg.; $p < 0.05$) than that of the normotensive cancer group, whereas the rate of synthesis per unit weight of tissue had been significantly elevated only in Group I and II hypertensives. It is also seen that the total capacity to form B-region steroids did not diminish with increasing severity of the disease.

3. *Steroid formation by adrenal incubates in presence of added ACTH*

The addition of 5 units of ACTH to the incubates increased the total synthesis of steroids per Gm. of tissue by 92 per cent and 59 per cent in the normotensive cancer group and the three hypertensive groups, respectively. With the normotensive tissue the relative amounts of steroids in the different regions of the chromatograms remained essentially unchanged. Acceleration of the hydrocortisone formation by ACTH in the hypertensive groups (81 to 93 per cent) was of the same order as in the cancer group (82 per cent). However, rates of formation of E-region steroids in the hypertensive Groups I and III and of B-region steroids in Group I were not significantly stimulated by ACTH. In view of the small size of the groups, an interpretation of these comparatively slight deviations from the general response to ACTH stimulation should be postponed until confirmatory experimental evidence is available. ACTH did not alter greatly the differences between steroid formation of normotensive and hypertensive groups or within the hypertensive series. However, it appeared to augment the formation of B-region steroids in the adrenal incubates from the most severe hypertensive cases. About 31 per cent of the total steroid synthesis in

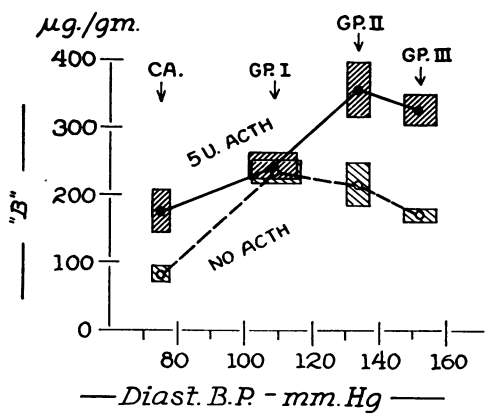

FIG. 4. FORMATION OF B-REGION STEROIDS WITH AND WITHOUT ACTH

Representation as in Figure 3. The slight decrease in steroid formation within the hypertensive groups was not significant statistically (analysis of variance).

Group III was due to steroids of the B-region as compared with 17 to 19 per cent in both Group I hypertensives and the normotensive cancer group after ACTH stimulation.

DISCUSSION

This study aimed at assessing the functional capability of normotensive and hypertensive adrenals by estimating *in vitro* steroid formation of tissue slices incubated in the autologous plasma. The close resemblance of the steroid patterns in adrenal vein blood and adrenal incubates supports the soundness of this method. The use of adrenals from the normotensive cancer patients as baseline for evaluating hypertensive alterations appeared justifiable as the data from the incubation of adrenal tissue from three normotensive cancer-free patients were in close agreement with the average of those from the cancer group.

The experiments yielded three findings of possible interpretative significance: *1*) a negative correlation between steroid formation per Gm. of tissue and diastolic blood pressure in the hypertensive group associated with a fall of the ratio of F/B steroids due to the fact that formation of B-region steroids declined significantly less with increasing diastolic blood pressure than hydrocortisone formation; *2*) a doubling of the rate of formation of all steroids, with the exception of 11β-hydroxyandrostene-3,17-dione, in the less severe hypertensive groups when compared with the normotensive group; and *3*) a positive correlation between adrenal weight and diastolic blood pressure compensating, in part, for the reduced rate of steroid formation per Gm. of tissue in advanced hypertension.

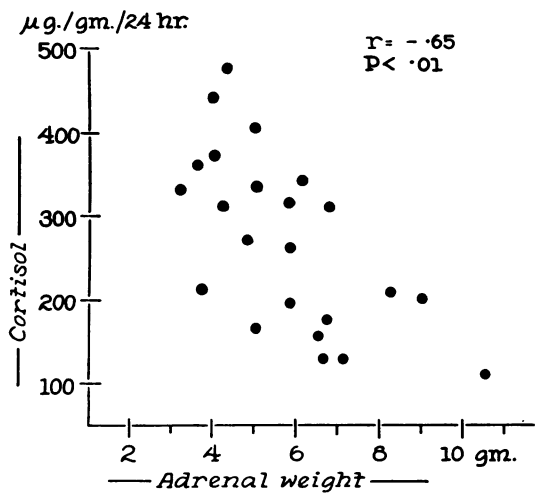

FIG. 5. RELATIONSHIP BETWEEN CORTISOL FORMATION AND ADRENAL WEIGHT

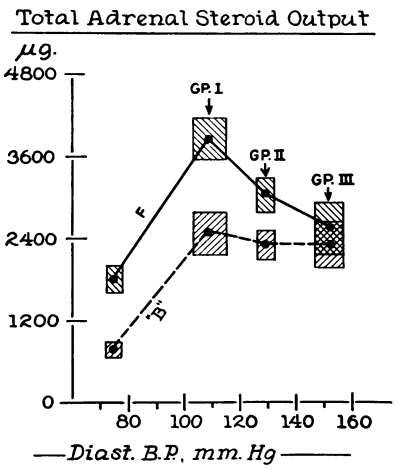

FIG. 6. TOTAL CAPACITY OF FORMATION OF CORTISOL AND B-REGION STEROIDS WITHOUT ACTH

Representation as in Figure 3. For details consult the text.

In evaluating the significance of the quantitative differences in steroid formation outlined above, consideration must be given to the fact that our assay system includes in the tissue slice and plasma two potentially independent variables. The question thus arises to what extent differences in the supply of substrates and ACTH by the individual plasmas contributed to the differences in rate of steroid synthesis.

If the difference in rate of steroid formation between the adrenal incubates from normotensive and hypertensive patients were due only to different amounts of corticotropin in the autologous plasmas used for the incubates, supplementation of the plasma medium with an excess of corticotropin should eliminate these differences. Exploratory experiments (13) have shown that the addition of 5 units of ACTH to the assay system produced maximum stimulation of steroid formation by both normotensive and hypertensive tissue. While hypertensive adrenal tissue was slightly though not significantly less stimulated than normotensive tissue by ACTH, the differences between the values from the hypertensive and normotensive groups as well as among the hypertensive groups

remained essentially unaltered when the plasma was supplemented with 5 units of ACTH.

The question whether the substrate concentration of the plasma was rate-limiting cannot be decided directly from the available experimental evidence. Whenever tested, steroid formation by the incubates appeared to proceed almost linearly during the first 12 hours and to decline but gradually during the second 12 hour period. Routine determinations of the time course which might have revealed instances of lack of substrate were not deemed advisable because of the limited amount of tissue available and the need for comparatively large quantities of steroids for separation and identification. The fact that in three exploratory experiments substitution of plasma from a normal donor for the hypertensive patient's plasma failed to alter significantly the rate of steroid formation by the incubates indicates a lack of substantial differences in substrate concentrations between the plasmas of the various groups. The fall of the F/B ratio with increasing severity of hypertension appears to be a significant observation. Since the steroids of these two regions arise from the same precursors, the shift of the ratio indicates changes in the functional capability of the adrenal cortical cells rather than differences in the supply of substrates.

Since corticosterone constitutes 70 per cent of the B-region steroids elaborated by the incubates, the fall of the F/B ratio suggests that there develops a deficiency of the 17-hydroxylase with progressing hypertension. To substantiate this interpretation it would be desirable to get quantitative information on the rate of synthesis of 17-hydroxycorticosterone (Substance S) and 18-aldocorticosterone (aldosterone), the formation of which should be affected by a deficiency in 17-hydroxylase in a similar manner as compounds F and B.

Our findings of increased adrenal weights in advanced hypertension, occasionally associated with nodularity of the cortex, corroborates the observations of earlier investigators [Rinehart, Williams and Cappeller (22); Sarason (23); Page and Corcoran (24)]. According to observations of Page and Corcoran which we can confirm, human adrenals in advanced hypertension frequently show arteriolar sclerosis. Reports in the literature (25) indicate that repeated trauma and chronic illness may cause hypertrophy of the adrenal cortex, increased weight of the glands and abnormally high rises in the plasma levels of corticosteroids following administration of ACTH. Whether incubates of adrenal slices from such glands would show alterations in the rate of steroid formation similar to those in the adrenal incubates from the enlarged glands of hypertensive patients remains an open question.

The decrease of the F/B ratio in the adrenal incubates from the patients with far advanced hypertension is of clinical interest since, besides aldosterone, corticosterone is the only salt-retaining steroid which can be detected in human adrenals (26). Perera and Pines (27), Woodbury, Rosenberg and Sayers (28), and Selye (29) suggested that decreased formation of glucocorticoids rather than over-production of mineralocorticoids might be the essential feature of hypertension, since in the dog the pathological changes produced by administration of the mineralocorticoid desoxycorticosterone could be prevented by simultaneous treatment with cortisone (28). There are several clinical reports of increased mineralocorticoid activity in essential hypertension. Genest and associates (30, 31) found increased urinary aldosterone excretion in 50 per cent of patients with advanced essential hypertension. It is of interest that aldosterone is metabolically related to corticosterone in not requiring 17-hydroxylation. The disturbances of the salt and water metabolism described by Green, Johnson, Bridges and Lehmann (10) and by Braun-Menendez (11) also support the concept of increased activity of mineralocorticoids. It should be noted, however, that the latter disturbances were manifest in the early hypertensives, whereas in the incubates the F/B ratio was markedly reduced only in the most severe cases. Precise information on the F/B ratio in the peripheral blood of hypertensive patients is as yet not available. This question is under investigation in our laboratory.

The increased rate of steroid formation in the adrenal incubates from the less severe hypertensive patients and the decline of the rate of cortisol synthesis with the progress of the disease might be the reason for the wide range of urinary corticoid excretion in the hypertensive patients, studied by other investigators. Hypercorticoiduria was observed by F. L. Selye (6), using bioassays, in 6

out of the 18 patients and by Corcoran, Page and Dustan (9) in 20 out of 46 hypertensives (formaldehydogenic steroids). The possibility of a relationship between urinary steroid excretion and diastolic blood pressure which might have explained the variability of the results, has not been taken into consideration. More direct information on the relationship between *in vitro* and *in vivo* synthesis of corticoids is provided by preliminary results from our laboratory (32) which indicated that the rate of cortisol output by adrenals *in situ*, computed from the rate of blood flow and the steroid concentration in the adrenal blood at operation prior to excision of the gland, showed a negative correlation to the diastolic blood pressure of the patient similar to that in the corresponding adrenal incubate.

Studies of the tissue metabolism *in vitro* reveal only potential cellular capabilities or deficiencies. The relevance of the *in vitro* findings for the function of the intact organ must be proved by *in vivo* observations. We have shown that the biochemical behavior of the slices from adrenal glands of hypertensive patients is consistent with some of the clinical observations on corticoid metabolism in essential hypertension. It should be re-emphasized, however, that our data on the rate of steroid formation of adrenal tissue *in vitro* concern only glands from hypertensive patients in whom the disease was progressing or had reached the malignant stage. While the alterations in the *in vitro* metabolism may be characteristic for the transition to the malignant stage of the disease, there is no evidence that they are specific for essential hypertension, since no similar series of adrenal glands from patients with other diseases producing chronic stress, except patients with advanced cancer of the breast and prostate, were available for comparison. Whether similar alterations of steroid metabolism *in vivo* are manifest in patients with various chronic diseases is being investigated.

SUMMARY

Steroid formation by slices of adrenal glands excised for the treatment of hypertension has been compared with that of glands from normotensive patients adrenalectomized for palliation of advanced cancer. The slices were incubated in the autologous plasma with and without addition of ACTH. The steroids were extracted from the incubates and separated by paper chromatography. Similar assays were done upon adrenal vein blood. The main results were:

1) On the chromatograms of all the incubates and samples of adrenal vein blood studied the steroids have been situated in five main areas designated as "before F" region, F position, E- and B-regions and Δ^4-androstene-11β-ol-3,17-dione position. The latter steroid and compound F have been identified. The B-region contains 70 per cent corticosterone.

2) The rate of steroid formation per unit weight of hypertensive gland decreases with increasing diastolic blood pressure; *i.e.*, with increasing severity of the hypertension. The decline is associated with a fall of the F/B ratio since hydrocortisone formation diminishes more than the synthesis of B-region steroids.

3) In the less advanced hypertensives the rate of formation of all steroids with the exception of Δ^4-androstene-11β-ol-3,17-dione, is about twice that in the normotensive cancer group. In advanced hypertension only B-region steroid formation is significantly augmented.

4) The weight of the adrenal gland increases with increasing diastolic blood pressure and apparently compensates, in part, for the reduction in the rate of steroid formation per unit weight of tissue.

5) The implications of the *in vitro* changes in steroid metabolism for the interpretation of the clinical findings in essential hypertension have been discussed.

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