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ANDROGEN RELEASE AND SYNTHESIS *IN VITRO* BY HUMAN ADULT ADRENAL GLANDS *

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It is well established that surviving human adrenal slices *1*) release corticosteroids into the media; *2*) increase their output in response to ACTH, and *3*) convert radioactive precursors to biologically active corticosteroids (1–11). In contrast, *in vitro* human adult androgen (C-19) production has not been well studied. Lombardo and Hudson (7) and Cooper and associates (3) isolated 11β-hydroxyandrostenedione [1] from the media after incubation of adrenal tissue from patients with metastatic breast carcinoma, prostatic carcinoma, and hypertension. Other C-19 steroids such as androstenedione or dehydroepiandrosterone were not identified by these workers,[2] although these steroids and 11β-hydroxyandrostenedione have been demonstrated in human adrenal vein blood (14–17). The adrenal origin of the aforementioned androgens is well documented by 17-ketosteroid secretory rate studies measured by isotope dilution (18, 19).

Furthermore, pathways of adrenal androgen biosynthesis in adult human tissues are also not well defined. Alternative pathways, two of which bypass progesterone have been postulated (20, 21) and are shown in Figure 1. Reactions 2 and 4 requiring a 3β-ol dehydrogenase and double-bond shift and reactions 1 and 3 requiring a 17α-hydroxylase are well recognized intermediate steps for mammalian and human corticosteroid biosynthesis. In 1957, Bloch, Dorfman, and Pincus reported the conversion of acetate-C^{14} to C^{14}-labeled dehydroepiandrosterone, androstenedione, and 11β-hydroxyandrostenedione by adrenal slices from a 28-year-old female with androgenital syndrome (22). Recently, Goldstein, Gut, and Dorfman (23) and Lipsett and Hökfelt (24) demonstrated dehydroepiandrosterone synthesis with adult human adrenal homogenates and slices respectively via reactions 1 and 5 (pregnenolone $\rightarrow$ 17α-hydroxypregnenolone $\rightarrow$ dehydroepiandrosterone). Other alternative pathways of androgen biosynthesis were not described in these studies.

However, Solomon, Lanman, Lind, and Lieberman (25) incubated human fetal adrenal homogenates with progesterone-4-C^{14} and observed 17-hydroxylation and side-chain cleavage (reactions 3 and 7, Figure 1) with the formation of androstenedione. The biosynthetic capacity of fetal adrenal slices incubated with sodium-C^{14} acetate was verified further by Bloch and Benirschke (26) who isolated C-21 intermediates pregnenolone and 17α-hydroxyprogesterone and C-19 steroids—dehydroepiandrosterone, androstenedione, and 11β-hydroxyandrostenedione.

Since androgen production and biosynthetic pathways were not completely resolved in adult adrenal tissue, an investigation was undertaken to study these aspects of C-19 metabolism with "normal," atrophic, "hypertensive," hyperplastic, adenomatous adrenal gland slices. Adrenal carcinomatous tissues from two children were also

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[1] Compounds referred to are: Pregnenolone: 3β-hydroxy-5-pregnene-20-one; 17α-hydroxypregnenolone: 3β,-17α-dihydroxy-5-pregnene-20-one; progesterone: 4-pregnene-3,20-dione; 17α-hydroxyprogesterone: 17α-hydroxy-4-pregnene-3,20-dione; 21-deoxycortisol: 11β,17α-dihydroxy-4-pregnene-3,20-dione; 11-deoxycortisol, (Compound S): 17α,21-dihydroxy-4-pregnene-3,20-dione; cortisol: 11β,17α,21-trihydroxy-4-pregnene-3,20-dione; dehydroepiandrosterone (DHEA): 3β-hydroxy-5-androstene-17-one; androstenedione (4-andro): 4-androstene-3,17-dione; 11β-hydroxyandrostenedione (11β-OH-4-andro): 11β-hydroxy-4-androstene-3,17-dione; testosterone: 17β-hydroxy-4-androstene-3-one; andrenosterone: 4-androstene-3,11,17-trione; 2,4-DNPH; 2,4-dinitrophenylhydrazone derivative; ACTH: adrenocorticotropin.

[2] Plantin, Diczfalusy, and Birke identified dehydroepiandrosterone after extraction (without incubation) of a virilizing adrenal tumor removed from a 62-year-old woman (12). In addition, Anliker, Rohr, and Marti isolated testosterone from a virilizing adrenal tumor (13).

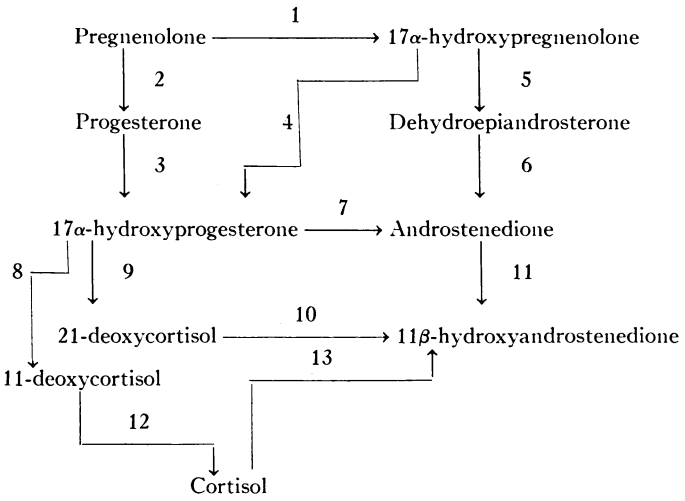

FIG. 1. POSSIBLE PATHWAYS OF ADRENAL ANDROGEN BIOSYNTHESIS.

investigated. This paper is a report of *1*) isolation, measurement, and identification of adrenal androgens released into the incubation media with slices alone and with the addition of radioactive steroid precursors and *2*) the *in vitro* effect of ACTH on androgen release and substrate conversion. Studies of corticosteroid production and biosynthesis by some of these tissue slices were reported previously (11, 27).

MATERIALS AND METHODS

Subjects. The clinical subjects included eight patients with Cushing's syndrome, two with adrenal virilism, one patient with malignant hypertension (E.W.), one with a suspected pheochromocytoma (J.K.), and one with carcinoma of the prostate (J.C.) (Table I). Normal adrenocortical function was observed in Patients E.W. and J.K. but not studied in Patient J.C.

The patients with Cushing's syndrome demonstrated the typical clinical manifestations associated with the

TABLE I

Clinical and pathological findings of patients with normal and hyperadrenal function

Patient	Sex	Age	Clinical diagnosis	Adrenal pathology	Symptoms and signs		Menstrual irregularities	Laboratory	
					Masculinization	Hirsutism		17-Ketosteroids	17-Ketogenic steroids
								mg/24 hrs	
				Normal adrenal function					
E.W.*	F	47	Malignant hypertension	Bilateral normal	Absent	Absent	Menopausal	14.0	8.6
J.K.*	M	41	Pheochromocytoma	Right normal	Normal	Normal		8.4	7.4
J.C.	M	63	Carcinoma of prostate	Bilateral normal	Normal	Normal			
				Adrenal hyperfunction					
L.H.*	F	30	Cushing's syndrome	Bilateral hyperplasia Right adenoma	Absent	Present	Amenorrhea	31.5	48.5
L.J.*	F	45	Cushing's syndrome	Left adenoma Left atrophy	Absent	Present	Oligomenorrhea	19.1	20.6
M.C.	F	28	Cushing's syndrome	Left adenoma Right atrophy	Present	Present	Amenorrhea	22.6	9.2
G.H.	F	35	Cushing's syndrome	Bilateral hyperplasia	Absent	Present	Amenorrhea	16.7	33.1
A.D.	F	39	Cushing's syndrome	Bilateral hyperplasia	Absent	Absent	Amenorrhea	19.8	28.0
B.H.	M	24	Cushing's syndrome	Bilateral normal	Normal male	Normal		10.1	48.0
T.N.*	M	12	Cushing's syndrome	Bilateral hyperplasia	Normal male	Present		16.7	30.5
P.P.	M	12	Cushing's syndrome	Bilateral hyperplasia	Normal male	Absent		9.4	19.7
C.S.†	F	3	Adrenal virilism	Right carcinoma Left atrophy	Present	Present		6.8	2.9
E.S.†	F	1½	Adrenal virilism	Right carcinoma	Present	Present		2.8	3.0

* The clinical features of these patients were summarized in a previous communication (11).
† Genetic studies indicated the patients were female. No clinical stigmata of Cushing's syndrome were evident.

disease. Laboratory studies revealed elevated 8:30 a.m. plasma cortisol levels (except L.J., whose basal level was within the normal range) and exaggerated cortisol responses to 5-hour infusions of ACTH (28). The 24-hour urinary 17-ketogenic steroids (29) usually mirrored the findings in the plasma. Elevated total 17-ketosteroids were observed in six (L.H., M.C., L.J., A.D., G.H., and T.N.) of the eight patients. Gradient elution column chromatography of the urinary 17-ketosteroids (30) of patients M.C. and L.H. revealed elevation of the 11-oxygenated 17-ketosteroids and etiocholanolone of the 11-deoxy-17-ketosteroid fraction similar to the observations of Jailer, Vande Wiele, Christy, and Lieberman (31).

The clinical impression of adrenal virilism (patients E.S. and C.S.) was corroborated by normal endogenous cortisol secretion (32) and studies of corticosteroid metabolism (27, 29). Urinary 17-ketosteroids were elevated, predominantly of the 11-deoxy-17-ketosteroid fractions, dehydroepiandrosterone and etiocholanolone. Adrenal cortical suppression tests were not performed.[3]

The patients (except L.J., whose small right adrenal gland was not removed) underwent total bilateral adrenalectomies at a one-stage operation (28); they received cortisone acetate or cortisol hemisuccinate the evening prior to and the morning of surgery. Blood and cortisol hemisuccinate were administered during the operative procedure. The pathological diagnoses of these glands are listed in Table I.

Incubation technique. The adrenal glands removed at operation were immediately iced and kept cold throughout pathological examinations and experimental procedures. The glands were weighed, sectioned, freed of fat, reweighed, and sliced with a Stadie-Riggs microtome. The medullary tissues were not separated. Slices from multiple areas of each adrenal were taken for each experimental flask. Incubation of weighed slices was performed in a Dubnoff metabolic incubator for 3 hours at 37° C in an atmosphere of 95 per cent oxygen, 5 per cent carbon dioxide in Krebs-Ringer bicarbonate buffer with 200 mg per 100 ml glucose. The incubations were started within 3 hours after excision of the adrenals. Ten units of ACTH[4] per gram of tissue was added to selected flasks.

Addition of radioactive substrates. Prior to the addition of buffer and slices, certain flasks received tracer quantities of either progesterone-16-H^3 (specific activity, SA, 0.506 mc per μmole), dehydroepiandrosterone-7α-H^3 (SA 1.25 mc per μmole), or 17α-hydroxypregnenolone-7α-H^3 (SA 0.863 mc per μmole) dissolved in absolute ethanol: methanol (1:1) or benzene: methanol (9:1). In one study, approximately equimolar amounts of 17α-hydroxypregnenolone-H^3 and progesterone-4-C^{14} (SA 16.2 μc per μmole) were added. In several experiments microgram quantities of carrier steroid were also added prior to incubation. The solvents were evaporated under nitrogen or air (at 37° C) and propylene glycol was added to facilitate solubilization of the substrates. The C^{14}-labeled progesterone was purified by sequential paper chromatography in the systems of Bush C (33), petroleum ether, 75 per cent methanol, and the cyclohexane: dioxane: methanol: water (100:100:50:25) system of Kliman and Peterson (34). Tritium-labeled progesterone was similarly processed after the addition of unlabeled progesterone.[5] The 17α-hydroxypregnenolone-7α H^3 was subjected to three separate solvent partitionings in *n*-hexane: ethanol: water (100:2:98 vol/vol) at 2° C, *n*-hexane: ethanol: water (100:20:80: vol/vol) at 25° C, and *n*-hexane: carbon tetrachloride: ethanol: water (30:70:47.5:52.5 vol/vol) at 25° C as described by Carstensen, Oertel, and Eik-Nes (35). The compound was stored in a frozen state in benzene: methanol (9:1) until used. Radiochemical homogeneity was observed after the addition of stable 17α-hydroxypregnenolone and paper chromatography in cyclohexane: benzene: methanol: water (100:50:100:25) and toluene: propylene glycol systems followed by reverse phase paper chromatography in the benzene: petroleum ether: methanol: water (20:10:40:10) system. A second minor peak of radioactivity estimated to contain less than 5 per cent of the radioactivity of the 17α-hydroxypregnenolone peak was noted in one system. The preparation and purification of dehydroepiandrosterone-7α-H^3 from the acetate derivative were described previously (36). Dehydroepiandrosterone-7α-H^3 was chromatographed in the heptane: 96 per cent methanol system (37) within 72 hours of use.

Steroid separation and measurement. After incubation, the media were carefully decanted, and the tissues and flasks were washed three times with cold isotonic saline. The appropriate media and washings were pooled. In order to correct for chemical losses incurred during manipulations, tracers of tritium-labeled dehydroepiandrosterone, SA 1.25 mc per μmole; androstenedione-4-C^{14}, SA 2.13 μc per μmole; and 11β-hydroxyandrostenedione-4-C^{14},[6] SA 1.4 μc per μmole, were added to some media prior to extraction (with the exception of the radioactive conversion studies). Constant specific activities of the free C-19 tracers were observed after reverse isotope dilution with paper chromatography.

The pooled washings and media were extracted twice with 3 volumes of dichloromethane which had been purified by passage through a silica-gel column. The dichloromethane extract was washed once with 1/10 volume of 0.05 N sodium hydroxide and twice with 1/10 volume of distilled water. The water was removed with anhydrous sodium sulfate and the extract evaporated *in*

[3] The exception was C.S., a 3-year-old girl with a clinical diagnosis of "adrenogenital syndrome." Twenty mg of prednisone was administered per day for 30 days without effecting a change of the total urinary 17-ketosteroid excretion.

[4] Armour beef adrenocorticotropin.

[5] The tritium-labeled steroids were purchased from the New England Nuclear Corp., Boston, Mass. Progesterone-4-C^{14} was generously donated by the Endocrine Study Section, National Institute of Health, Bethesda, Md.

[6] Dr. H. Leon Bradlow, Sloan-Kettering Institute, New York, N. Y. graciously made this isotope available.

vacuo at 37° C. The residues were applied to washed Whatman no. 1 filter paper and developed for 72 hours in a modification of the toluene: propylene glycol system (38).[7] The effluents were collected and chromatographed for 20 hours in the cyclohexane: dioxane: methanol: water system (100: 100: 50: 25) which separated corticosteroids (corticosterone and 11-deoxycortisol) (34) from androgens. Dehydroepiandrosterone and androstenedione ran off the paper and were collected. On occasion the more polar C-19 steroids were located at the bottom of the paper. The effluents and paper eluates[8] were dried *in vacuo* and transferred quantitatively with chloroform to washed Whatman no. 1 filter paper (28 × 1 inch strips with a common origin) in the heptane: 96 per cent methanol (1: 1) system of Bush and Willoughby. Fifteen to twenty μg each of reference compounds, dehydroepiandrosterone, androstenedione, 11β-hydroxyandrostenedione, and adrenosterone were applied together in a single spot on each side of the "extract area." The paper was equilibrated for 4 hours and the mobile phase allowed to develop for 14 to 16 hours,[9] after which the paper was air dried. The C-19 steroids with a Δ^4-3-ketone configuration were located on the chromatograms by the ultraviolet scanning technique and the Δ^5-3β-ol steroid (dehydroepiandrosterone standard) by Zimmermann reagent stain (alkaline *m*-dinitrobenzene). Appropriate areas corresponding to the standards and paper blanks were cut out and eluted with 4.0 ml of absolute ethanol as previously described (36).

Measurements of the isolated androgens with a Δ^4-3-ketone configuration were performed on portions of the eluates by the alkaline fluorescent method of Abelson and Bondy (39). Authentic androstenedione was used in construction of the standard curve. The C-19 steroids were determined also by absorbance (0.8 ml microcuvets with a 1.0 cm light path) at 240 mμ (ethanol) in a Beckman model DU spectrophotometer. The extinction coefficients at 240 mμ were used for the chemical determination. Dehydroepiandrosterone was converted to the 2,4-dinitrophenylhydrazone derivative and measured spectrophotometrically at 368 mμ (36).

A 0.5 ml portion of the ethanol eluate was counted to a standard error of ± 2 per cent in a liquid phosphor scintillation counter (Model LP-2, Technical Measurements Corp., New Haven, Conn.), which had a counting efficiency of 45 to 48 per cent for carbon-14. The tritium-labeled dehydroepiandrosterone 2,4-dinitrophenylhydrazone was plated at infinite thinness on aluminum planchets and counted to a ± 2 per cent standard error in a windowless gas-flow counter (Automatic Sample Changer, Model C110 B, Nuclear-Chicago Corp., Des Plaines, Ill.). The efficiency for tritium was 18 to 20 per cent. The final value was determined, taking into account the absorbance of the blank, chemical amount of the added carbon-14 tracer (0.20 μg or less), dilution factor, and the isotope recovery (40). Since the chemical amount of the added dehydroepiandrosterone tracer was less than 0.01 μg, the steroid was calculated as the free compound without chemical correction. The calculation of the androgens isolated from media of the radioactive conversion experiments was uncorrected for isotope recovery, although other results were so corrected.

Identification. The isolated androgens from the incubations *without* added radioactive substrates were pooled separately. The identification of Δ^4-3-keto C-19 steroids was substantiated by characteristic mobilities and constant specific activities in two additional paper chromatographic systems, heptane: propylene glycol (41) and ligroin: benzene: methanol: water (667: 333: 800: 200) (42). Areas of ultraviolet absorption and Zimmermann positive staining coincided with the radioactive peaks located by a gas-flow paper chromatogram scanner (Model C-100 B, Actigraph II, Nuclear-Chicago Corp., Des Plaines, Ill.). Androstenedione-C^{14} and 11β-hydroxyandrostenedione-C^{14} *bis*-2,4-dinitrophenylhydrazones were formed (prior acetylation was unsuccessful) and confirmed by characteristic absorption maxima of 378 to 380 mμ (22) in the Beckman DU spectrophotometer. The specific activities of the hydrazones were determined by absorbance at 378 mμ and calculated for the free compound. Radioactivity was measured by counting at infinite thinness on aluminum planchets to a standard error of ± 2 per cent in the Nuclear-Chicago, Model C-110 B micro-window gas-flow counter. The efficiency for C^{14} was 24 to 28 per cent by this technique.

Dehydroepiandrosterone was verified as the mono-2, 4-dinitrophenylhydrazone derivative by the following criteria: *1*) absorption maximum of 368 mμ (chloroform); *2*) characteristic position of only one radioactive peak on elution from micro-alumina columns (36); and *3*) mobility of the mono-2,4-dinitrophenylhydrazone (1 cm per hour) developed for 12 hours in the heptane: propylene glycol system (22). The appropriate yellow radioactive area and paper blanks were cut into thirds, eluted with chloroform, and specific activities determined by spectrophotometry and windowless planchet counting with the Nuclear-Chicago Model C-110 B gas-flow counter. The specific activities were calculated on the basis of free dehydroepiandrosterone.

Radioactive androgens from the precursor experiments were identified after paper chromatographic separation and spectrophotometric and isotope measurements. One hundred micrograms of carrier androstenedione, 11β-hydroxyandrostenedione, and dehydroepiandrosterone were added to pooled paper eluates from experiments with a common radioactive substrate. The separate eluates were dried *in vacuo* at 37° C and the dried residues of each Δ^4-3-ketone androgen product subjected to a 40-tube countercurrent distribution (E-C Apparatus Company,

[7] The stationary phase was propylene glycol diluted with methanol (1: 1) before application to the paper. The methanol was then allowed to evaporate.

[8] Radioactive C-19 tracers were added at this stage of the steroid isolation if omitted previously, in order to correct for losses in purification.

[9] Paper chromatography of the 17-ketosteroids was carried out in a room maintained at a constant temperature of 20° C and relative humidity of less than 50 per cent.

TABLE II

Androgen production by "normal," atrophic, and "hypertensive" adrenal glands

Patient	Pathology	Wt of incubated tissue	Steroids*		
			DHEA	Andro-stenedione	11β-OH-4 Andro
		g		μg/g/3 hrs†	
J.K.	"Normal"	1.661	2.6	2.9	1.5
J.C.	"Normal"	2.524	0.4	1.8	1.5
L.J.	Atrophic	0.984	0	1.1	2.0
	Atrophic + ACTH ‡	0.474	4.4	3.8	17.7
M.C.	Atrophic	0.700	14.3	0	0
	Atrophic + ACTH ‡	0.158	5.3	2.7	34.2
E.W.	Left hypertension	0.573	0	3.5	5.4
	Right hypertension	0.570	0	3.7	4.9

* DHEA, dehydroepiandrosterone; 11β-OH-4 andro, 11β-hydroxyandrostenedione.

† The total mean ±SD per cent recoveries of C^{14}- and tritium-labeled tracers added to media before extraction were 49 ± 8.2. The total per cent isotopic recoveries for tracers added to effluents and eluates from cyclohexane:dioxane:methanol:water system were 82 ± 10.1.

‡ The amount of ACTH added was 10 units per gram of tissue.

Swarthmore, Pa.) at room temperature. Theoretical countercurrent distribution curves were calculated according to the procedure of Way and Bennett (43). The solvent system employed for androstenedione (K = 0.397) and 11β-hydroxyandrostenedione (K = 0.178) was ethyl acetate : hexane : methanol : water (120 : 180 : 180 : 120). A 20-tube transfer was carried out for dehydroepiandrosterone in the ethyl acetate : cyclohexane : ethanol : water (30 : 70 : 55 : 45) system, K = 1.02, of Savard and collaborators (44). At the end of the distribution, the total contents of each tube (20 ml) were transferred to test tubes and dried in air at 37° C. After specific activities were determined as described, the residues were dissolved in ethanol, and the peak tube and the two adjoining tubes on both sides of the peak pooled for further identification. Mobilities and specific activities of the free steroids and 2,4-dinitrophenylhydrazone derivatives were calculated after paper (Bush and Zaffaroni systems) and micro-alumina column chromatographic separation, respectively.[10] In addition, a portion of the pooled transfers from the countercurrent distribution of 11β-hydroxyandrostenedione from one series of incubations with 17α-hydroxypregnenolone-H^3 was oxidized with chromic acid

[10] Only dehydroepiandrosterone 2,4-dinitrophenylhydrazone was chromatographed on alumina columns.

TABLE III

Androgen production by hyperplastic, "normal," adenomatous, and carcinomatous tissue from patients with adrenal hyperfunction

Patient	Pathology	Wt of incubated tissue	Steroids*			Wt of incubated tissue	Steroids (with ACTH†)		
			DHEA	4-Andro	11β-OH-4 Andro		DHEA	4-Andro	11β-OH-4 Andro
		g		μg/g/3 hrs		g		μg/g/3 hrs	
						Cushing's syndrome			
L.H.	R. hyperplasia	1.005	0	3.1	11.3	1.010	0	4.1	25.0
	L. hyperplasia	1.025	0	3.6	8.7	.500	0	12.6	47.4
	R. adenoma	1.845	2.3	0.9	3.4	1.200	2.5	11.7	15.0
T.N.	R. hyperplasia	2.056	0	0	4.3	2.008	0	1.0	6.3
	L. hyperplasia	2.482	0	0	3.8	2.532	0	0	5.2
P.P.	R. hyperplasia	0.984	1.3	0	4.3	1.326	0.7	0	2.6
	L. hyperplasia	1.027	0	1.2	1.2	.986	0.8	1.6	2.1
A.D.	L. hyperplasia	1.019	0	1.3	0.9	1.008	0	0.7	1.3
B.H.	R. "normal"	1.033				1.010	0	1.2	4.6
	L. "normal"	0.977	0	4.5	0	.992	1.6	0.6	4.2
L.J.	Adenoma	1.554	0	1.3	3.0	1.544	1.5	0	6.6
M.C.	Adenoma	2.380	0	3.3	7.4	2.406	0	1.8	6.3
						Adrenal virilism			
C.S.	Carcinoma	1.504	2.7	0	0	1.508	4.5	2.7	0
E.S.	Carcinoma	1.007	0	0	8.0	.940	3.5	8.9	12.9

* DHEA, dehydroepiandrosterone; 4-andro, androstenedione; 11β-OH-4 andro, 11β-hydroxyandrostenedione.

† 10 units of ACTH per gram of tissue.

according to the method of Lieberman and collaborators (45). The identity of the radioactive product, adrenosterone, was verified by its characteristic mobility on paper in the heptane : propylene glycol and 96 per cent methanol : heptane systems. The identical ultraviolet light absorbing and radioactive zone from each chromatogram was eluted and specific activities determined by planchet counting and absorbance at 240 mμ as described.

RESULTS

A. Adrenal androgen production

Three adrenal androgens, dehydroepiandrosterone, 11β-hydroxyandrostenedione, and androstenedione were isolated from the media after 3-hour incubation. Adrenosterone was not detected. In Table II is listed the steroid production (μg per gram of slice) by tissue slices from patients with normal and with atrophic adrenals. The results of Patients J.K. and J.C. represent the pattern of production by "normal" tissues. The functional capacity of the atrophic glands (L.J., ipsilateral, and M.C., contralateral) is indicated by the endogenous and ACTH-stimulated androgen release, especially the striking increase in 11β-hydroxyandrostenedione. The "hypertensive" glands of Patient E.W. produced 11β-hydroxyandrostenedione and androstenedione at a rate comparable with that of adrenal tissue from some patients with Cushing's syndrome.

The androgen production by adrenal slices from patients with Cushing's syndrome and adrenal virilism is listed in Table III. Small amounts of endogenous dehydroepiandrosterone were detected in incubations of adenomatous (L.H.), hyperplastic (P.P.), and carcinomatous tissues (C.S.). The addition of ACTH to the system did not stimulate dehydroepiandrosterone production consistently. However, ACTH stimulated the carcinomatous tissues (C.S. and E.S.) to produce amounts of dehydroepiandrosterone in the range of the atrophic tissues of L.J. and M.C. (Table II).

On the other hand, incubation of "Cushing adrenal tissue" (Table III) in the presence of ACTH resulted in a one and a half to sixfold increment of 11β-hydroxyandrostenedione production. Nevertheless, the most exaggerated 11β-hydroxyandrostenedione response to ACTH is found in the atrophic tissues (Table II), associated clinically with adenomata.

The effect of ACTH on androstenedione release

TABLE IV

Total androgen production per gland

Patient	Pathology	Total weight of tissue*	Steroids†				Steroids (with ACTH)			
			DHEA	4-Andro	11β-OH-4-Andro	Total	DHEA	4-Andro	11β-OH-4-Andro	Total
		g	*μg/gland/3 hrs*				*μg/gland/3 hrs*			
J.K.	Normal	8.3	21.6	24.1	12.5	58.2				
J.C.	Normal	4.7	1.9	8.5	7.1	17.5				
E.W.	L. hypertension	16.0	0	56.0	86.4	142.4				
	R. hypertension	12.5	0	46.3	61.3	107.4				
L.H.	L. hyperplasia	13.5	0	48.6	117.5	166.1	0	170.1	639.9	810
	R. hyperplasia	12.5	0	38.8	141.3	180.1	0	51.3	312.5	363.8
	R. adenoma	32.0	73.6	28.8	108.8	211.2	80	374.4	480.0	934.4
L.J.	L. adenoma	22.02	0	28.6	66	94.6	33	0	145.2	178.2
	R. atrophic	3.0	0	3.3	6	9.3	13.2	11.8	53.1	77.7
M.C.	L. adenoma	51.9	0	171.3	384.1	555.4	0	93.4	327	420.4
	R. atrophic	0.7	10.0	0	0	10.0	3.7	1.9	23.9	29.5
A.D.	L. hyperplasia	18.0	0	23.4	16.2	39.6	0	12.6	23.4	36
B.H.	L. normal	4.0	0	18.0	0	18.0	6.4	2.4	16.8	25.6
	R. normal	5.2					0	6.2	23.9	30.1
T.N.	L. hyperplasia	11.5	0	0	43.7	43.7	0	0	59.8	59.8
	R. hyperplasia	14.5	0	0	62.4	62.4	0	14.5	91.4	105.9
P.P.	L. hyperplasia	7.7	0	9.2	9.2	18.4	6.2	12.3	16.2	34.7
	R. hyperplasia	6.7	8.7	0	28.8	37.5	4.7	0	17.4	22.1
C.S.	R. carcinoma	9.0	24.3	0	0	24.3	40.5	24.3	0	64.8
E.S.	R. carcinoma	7.2	0	0	57.6	57.6	25.2	64.1	92.9	182.2

* These values represent weights of the trimmed pathological specimen.
† Abbreviations as described in Tables II and III.

TABLE V

Specific activities of pooled androgens obtained from human adrenal slice incubations

Steroid *	Method	Solvent system		Specific activity Dehydroepiandrosterone†
				cpm/μmole
Mono-2,4-DNPH derivative‡				20,818‖
	Column chromatography§			
	3.9% Hydration	Benzene:chloroform (4:1)		20,523
	5.0% Hydration	Benzene:chloroform (4:1)		20,469
	Paper chromatography			
		Upper third		20,364
	Heptane:propylene glycol	Middle third		20,148
		Lower third		20,287
			11β-OH-4-androstenedione	Androstenedione
Free compounds			8,048‖	10,708‖
	Paper chromatography			
		96% Methanol:benzene (1:1)	7,748	10,255
		Heptane:propylene glycol	7,508	10,340
		Ligroin:benzene:methanol:water (667:333:800:200)	7,547	10,028
Bis-2,4-DNPH derivative¶			7,540	10,001

* The chemical amount of the steroid 2,4-DNPH was measured at 368 mμ (mono) and 378 mμ (bis) in a spectrophotometer. Radioactive measurements were performed with a windowed (C^{14}) and windowless (H^3) gas-flow counter. The efficiency for C^{14} was 24 to 28 per cent.
† The specific activity is calculated on the basis of the free compound.
‡ 2,4-DNPH, 2,4-dinitrophenylhydrazone.
§ The steroid 2,4-DNPH was chromatographed on 1,000 mg of neutral aluminum oxide (Woelm) 12.5 ×0.3 cm column.
¶ Specific activity of free androgen.
‖ Specific activity of pooled androgens prior to identification procedures.

is marked in the left hyperplastic and adenomatous tissues of L.H. and carcinomatous slices from Patient E.S. (Table III).

The amounts of androstenedione are decreased rather than increased in most other incubations with ACTH. The total androgen production per gland by all tissues is shown in Table IV. These results are based on the assumption that the incubated tissue is representative of the entire gland. The total production of 11β-hydroxyandrostenedione in the absence of ACTH by the hyperplastic, adenomatous, and carcinomatous tissues (except Patients C.S., B.H., and P.P.) is consistently greater than that of the "normal" and atrophic glands. The total androgen production of the "hypertensive" glands (E.W.) is comparable to "Cushing tissue."

The elevated total urinary 17-ketosteroid levels of Patients L.H., M.C., L.J., and T.N. (Cushing's syndrome) correlated well with the increased total adrenal androgen production *in vitro*. A similar correlation is present in Patients C.S. and E.S. with adrenal carcinoma and virilism. The functional *in vitro* capacity of the contralateral atrophic glands of C.S. and E.S. is not known.

Identification of adrenal androgens without addition of radioactive precursors. After chemical and radioactive measurements were performed,

TABLE VI

Incubation of adrenal slices with progesterone-16-H^3

Patient	Pathology	Substrate Progesterone-16-H^3 SA	Substrate Progesterone-16-H^3 Amount added	Weight of tissue	Products: Androstenedione μg/g/3 hrs	Androstenedione μc/μmole	Androstenedione % conv.*	Androstenedione Ratio SA†	11β-Hydroxyandrostenedione μg/g/3 hrs	11β-Hydroxyandrostenedione μc/μmole	11β-Hydroxyandrostenedione % conv.	11β-Hydroxyandrostenedione Ratio SA
		μc/μmole	*μc*	*g*								
J.K.	"Normal"	50.3	27	2,734	2.5	1.34	0.12	0.027	1.8	3.29	0.20	0.065
L.H.	Right hyperplasia	72.6	4.6	510	2.4	0.32	0.03	0.0044	8.5	1.96	0.061	0.027
	Adenoma	72.6	9.12	1,200	2.8	2.46	0.32	0.034	2.1	4.34	0.40	0.059
L.J.	Adenoma	40.8	5.3	1,010	1.2	0.21	0.016	0.0051	2.5	5.05	0.80	0.124

* Per cent conversion of radioactive substrate to androstenedione and 11β-hydroxyandrostenedione measured after paper chromatography in the heptane:96 per cent methanol system.
† Ratio SA is the ratio of the specific activity of the product to the specific activity of the substrate.

TABLE VII

Conversion of 17α-hydroxypregnenolone-7α-H³ to androgens by adenomatous and hyperplastic adrenal slices

Patient	Pathology	Wt of incubated tissue	Substrate 17α-Hydroxypregnenolone-H³		Products											
					Dehydroepiandrosterone				Androstenedione				11β-Hydroxyandrostenedione			
			SA	Amt. added		SA	% Conv.*	Ratio† SA		SA	% Conv.	Ratio SA		SA	% Conv.	Ratio SA
		g	*μc/μmole*	*μc*	*μg/g/3 hrs*	*μc/μmole*			*μg/g/3 hrs*	*μc/μmole*			*μg/g/3 hrs*	*μc/μmole*		
M.C.	Adenoma	7.991	1.196×10^2	13.56	2.0	3.9	1.6	0.033	15.6	2.1	6.8	0.018	18.9	1.9	7.2	0.016
P.P.	Hyperplasia	0.992	1.196×10^2	3.93	1.1	14.3	1.4	0.120	5.0	12.5	5.6	0.104	6.2	13.0	6.8	0.108

* Per cent conversion is the minimal per cent conversion of substrate to respective products. No correction for manipulative losses was applied. Measurements were determined after paper chromatography in the heptane:96 per cent methanol system.
† Ratio SA is the ratio of the specific activity of the product to the specific activity of the substrate.

TABLE VIII

Conversion of 17α-hydroxypregnenolone-7α-H³ and progesterone-4-C¹⁴ to androgens with hyperplastic human adrenal slices

Patient	Pathology	Experiment	Wt of incubated tissue	Substrates			Products									
							DHEA			Androstenedione				11β-Hydroxyandrostenedione		
				Mixture	SA	Amt. added	$H^3:C^{14}$ ratio	% Conv.		SA	% Conv.†	$H^3:C^{14}$ ratio‡		SA	% Conv.	$H^3:C^{14}$ ratio
			g		*μc/μmole*	*μc*		*μg/g/3 hr*		*μg/g/3 hr*	*μc/μmole*			*μg/g/3 hr*	*μc/μmole*		
A.D.	Hyperplasia	Control	2.433	17α-Hydroxypregnenolone-H³	4.64	5.67	2.06	0	2.1	6.0	4.44	3.9	15.8	7.9	6.38	7.2	22.0
				Progesterone-C¹⁴	1.57	2.75					0.28	0.51			0.29	0.68	
A.D.	Hyperplasia	ACTH §	1.946	17α-Hydroxypregnenolone-H³	4.64	4.54	2.06	0	2.3	8.6	6.75	8.8	16.1	15.9	4.54	10.3	25.2
				Progesterone-C¹⁴	1.57	2.20					0.42	1.1			0.18	0.86	

* DHEA is an abbreviation for dehydroepiandrosterone.
† Percentage conversion of radioactive substrate to respective C-19 steroids.
‡ Tritium to carbon-¹⁴ ratios of product specific activities.
§ ACTH is lyophilized Armour adrenocorticotropin, 10 units per gram of slice.

TABLE IX

The conversion of dehydroepiandrosterone-H³ to androstenedione-H³ and 11β-hydroxyandrostenedione-H³ by hyperplastic adrenal slices

Patient	Pathology	Wt. of tissue	Products							
			Dehydroepiandrosterone-H³		Androstenedione			11β-Hydroxyandrostenedione		
			SA	Amt. added		SA	% Conv.		SA	% Conv.
		g	*μc/μmole*	*μc*	*μg/g/3 hr*	*μc/μmole*		*μg/g/3 hr*	*μc/μmole*	
G.H.	Right hyperplasia	2.997	31.53	6.43	23.2	2.55	9.7	38.5	2.76	16.5

the remainder of the eluates (from the 96 per cent methanol : heptane system) were pooled and specific activities determined. Radiochemical homogeneity of the isolated androgens is demonstrated by the data in Table V.

B. Radioactive precursor conversion studies

In Table VI are listed the data from the incubation of "normal" and pathological adrenal slices with progesterone-16-H³. Radioactive androstenedione and 11β-hydroxyandrostenedione were isolated from the media. The percentage conversion of progesterone to 11β-hydroxyandrostenedione was higher than the percentage conversion of this precursor into androstenedione. The data demonstrate that less than one per cent (uncorrected for manipulative losses) of progesterone-16-H³ was converted to the C-19 steroids measured.

The "normal," hyperplastic, and adenomatous tissues released approximately the same amount of androgen in the presence of progesterone compared to similar incubations without this substrate (Tables II, III). In contrast, incubation of 17α-hydroxypregnenolone-7α-H³ with adenomatous and hyperplastic slices resulted in conversion of 6 and 7 per cent of the substrate to androstenedione and 11β-hydroxyandrostenedione, respectively (Table VII). In addition, 1.6 and 1.4 per cent of this substrate was converted to tritium-labeled dehydroepiandrosterone. There was a slight decrease in the specific activity (SA) between dehydroepiandrosterone, androstenedione, and 11β-hydroxyandrostenedione with the adenomatous slices of M.C. On the other hand, the SA of the androgens synthesized by the hyperplastic slices of P.P. are of the same order of magnitude, 14.3, 12.5, and 13.0 μc per μmole (Table VII).

Since these experiments suggested that 17α-hydroxypregnenolone alone appeared to be a better substrate than progesterone for adult adrenal androgen biosynthesis, an incubation of hyperplastic adrenal slices was carried out with a mixture of tritium-labeled 17α-hydroxypregnenolone and C^{14}-labeled progesterone. Ten units of ACTH per gram of slices was added to alternate flasks. In Table VIII the results of this study are listed. The percentage conversion of tritium-labeled 17α-hydroxypregnenolone to androstenedione and 11β-hydroxyandrostenedione greatly exceeded that of C^{14}-labeled progesterone. The percentage conversion of the 17α-hydroxypregnenolone to dehydroepiandrosterone was about the same (2.1) as the values observed in previous incubations with 17α-hydroxypregnenolone, 1.6 and 1.4 per cent (Table VII).

The low percentage conversion of 17α-hydroxypregnenolone to dehydroepiandrosterone compared to androstenedione and 11β-hydroxyandrostenedione (Tables VII and VIII) prompted a study of the 3β-ol dehydrogenase system (and double-bond shifting from Δ^5 to Δ^4) in fresh hyperplastic adrenal slices. The Δ^5-3β-ol substrate dehydroepiandrosterone was readily converted to the Δ^4-3-ketone androgens androstenedione and 11β-hydroxyandrostenedione as shown in Table IX.

Identification of adrenal androgens from incubations with radioactive substrates. After chemical determinations were performed, the C-19 steroids derived from a common precursor were pooled separately and carrier androstenedione, 11β-hydroxyandrostenedione, and dehydroepiandrosterone were added. (Carrier dehydroepiandrosterone was not added to paper eluates from the incuba-

TABLE X

Specific activities of pooled radioactive adrenal androgens from incubations with labeled substrates

Substrate	Experimental method	Products*							
		Dehydroepiandrosterone SA	Androstenedione SA			11β-Hydroxyandrostenedione SA			
		μc/μmole	μc/μmole			μc/μmole			
Progesterone-16-H^3			0.90			2.52			
	CCD in System 1*		0.86, 0.84, 0.84, 0.86, 0.78			2.46, 2.48, 2.46, 2.50, 2.44			
	Paper chromatography								
	Ligroin, benzene, methanol, water†		0.88			2.47			
	Heptane:propylene glycol		0.83			2.48			
	2,4-DNPH derivative‡		0.82			2.42			
	Neptane:propylene glycol		0.78, 0.84, 0.69			2.44, 2.40, 2.41			
17α-Hydroxypregnenolone-H^3		5.02	3.92			4.26			
	CCD in System 1		3.76, 3.78, 3.82, 3.80, 3.81			4,20, 4.16, 4.10, 4.18, 4.09			
	Chromic acid oxidation product					4.17, 4.0‖			
	CCD in System 2 §	5.10, 5.12, 5.08, 5.06							
	Paper chromatography								
	Ligroin, benzene, methanol, water	5.06	3.81			4.14			
	Heptane:propylene glycol	4.92	3.76			4.10			
	2,4-DNPH derivative	4.88	3.72			4.02			
	3.9% hydrated micro-alumina column¶	4.86, 4.90, 4.84							
	Heptane:propylene glycol paper system		3.72, 3.68, 3.71			4.00, 4.06, 4.04			
Dehydroepiandrosterone-H^3			1.70			1.82			
	CCD in System 1		1.62, 1.60, 1.64, 1.63, 1.66			1.81, 1.80, 1.76, 1.78			
	Paper chromatography								
	Ligroin, benzene, methanol, water		1.66			1.78			
	Heptane:propylene glycol		1.64			0.74			
		H^3	H^3	C^{14}	$H^3:C^{14}$	H^3	C^{14}	$H^3:C^{14}$	
17α-Hydroxypregnenolone-H^3 Progesterone-4-C^{14}		0.34	4.76	0.30	15.9	4.68	0.20	23.4	
	CCD in System 2	0.32, 0.36, 0.35, 0.32, 0.33							
	CCD in System 1		5.24**	0.35	14.9	4.61**	0.22	20.9	
	Paper chromatography								
	Heptane:propylene glycol	0.33	5.20	0.34	15.3	4.58	0.19	24.1	
	2,4-DNPH derivative	0.31	5.21	0.36	14.5	4.52	0.18	25.1	
	3.9% hydrated micro-alumina column	0.31, 0.32, 0.29							
	Heptane:propylene glycol	0.30	5.18††	0.35	14.8	4.56††	0.19	24.0	

* Forty-tube countercurrent distribution (CCD) in System 1. Ethyl acetate:hexane:methanol:water (120:180:180:120 vol/vol). The values are the SA of the peak and two adjoining tubes on both sides of the peak.

† The proportions of the solvents were 667:333:800:200, respectively.

‡ 2,4-DNPH is an abbreviation for 2,4-dinitrophenylhydrazone. Androstenedione, 11β-hydroxyandrostenedione, and dehydroepiandrosterone form bis and mono derivatives, respectively. The specific activities of the hydrazone derivatives were calculated on the basis of the free compounds.

§ A 20-tube distribution was carried out in System 2. Ethyl acetate:cyclohexane:ethanol:water (30:70:55:45 vol/vol).

‖ SA of adrenosterone after paper chromatography in the heptane:propylene glycol and heptane: 96 per cent methanol systems, respectively.

¶ The stationary phase was benzene:petroleum ether (1:1). Elution was carried out with benzene:chloroform (4:1). The SA represent the peak and one adjoining fraction on both sides of the peak.

** Mean SA of peak tube and two adjoining tubes.

†† Mean SA of 3 one-third portions of the radioactive area.

tion experiments with progesterone-H^3).[11] Identification was verified by identical observed and theoretical distributions of the free androgens in the countercurrent systems and characteristic mobilities in two different paper chromatographic systems. The reported column (dehydroepiandrosterone only) and paper chromatographic mobilities and spectrophotometric spectra of the steroid 2,4-dinitrophenylhydrazone derivatives were observed, further substantiating the identification of the isolated androgens. The SA of the pooled androgens (and carriers) from individual substrate experiments (Tables VI–IX) along with the experimental conditions used for identification are listed in Table X. Constant SA were maintained, indicating radiochemical purity of the measured compounds.

DISCUSSION

Androgen release by adult human adrenal gland slices. The use of an *in vitro* incubation technique with human adrenal slices affords a direct approach to an understanding of adrenal androgen metabolism. The data from the present incubations (Tables II–IV) indicate that the slices *1*) release detectable amounts of free androgens, dehydroepiandrosterone, androstenedione, and 11β-hydroxyandrostenedione into the media and *2*) respond to adrenocorticotropin (ACTH) *in vitro*.

In the present study the amounts of dehydroepiandrosterone and androstenedione released per gland per three hours *in vitro* are less than the quantities found by Hirschmann, deCourcy, Levy, and Miller (17) in the adrenal vein blood of two patients with breast carcinoma and one patient with essential hypertension. In contrast, the adrenal venous blood levels of 11β-hydroxyandrostenedione are of the same order of magnitude found by the *in vitro* tissue slice method (Table II). There is also a marked discrepancy between the low dehydroepiandrosterone levels determined by the *in vitro* technique (and analysis of adrenal vein blood) (17) compared to the normal *in vivo* secretory rates of this C-19 steroid (15 to 25 mg per 24 hours) determined by Vande Wiele and Lieberman (18).[12]

On the other hand, the adrenal tissue from three patients with Cushing's syndrome, L.H., L.J., and M.C. (Table IV) released 11β-hydroxyandrostenedione, only in the presence of ACTH, commensurate with normal secretory rates of 2.0 mg per 24 hours reported by Bradlow and Gallagher (19). The comparison between the two methods is made on the assumption that the 3-hour *in vitro* release rate projected to 24 hours is constant over that interval. The data of Cooper and associates (3) substantiate the validity of this speculation for C-21 steroids and 11β-hydroxyandrostenedione.

The stimulatory effect of ACTH on 11β-hydroxyandrostenedione production *in vitro* (Table III) is in agreement with the data of Dyrenfurth, Lucis, Beck, and Venning (6); Touchstone, Glazer, Cooper, and Roberts (46); and Cooper and associates (3), despite the differences of glandular weights and pathological findings of the adrenal tissues. The most striking increase of 11β-hydroxyandrostenedione production occurred with ACTH in the atrophic glands of L.J. and M.C. (Table II), resulting in 8- and 34-fold increases, respectively. The functional capacity of these two tissues is demonstrated also by the effect of ACTH on dehydroepiandrosterone and androstenedione production. There is a slight rise of the androstenedione level in both tissues. However, the elevated (control) dehydroepiandrosterone in J.C. fell with ACTH, 14.3 to 5.3 μg per g per 3 hours, and rose in L.J. from a control value of 0 to 4.4 μg per g per 3 hours. The effect of ACTH on dehydroepiandrosterone release is ambiguous with other glands from patients with Cushing's syndrome. The failure to detect testosterone in the incubation media implies that this steroid is not usually one of the adrenal androgens produced.

Adrenal androgen biosynthesis. The studies demonstrate the conversion of radioactive steroid

[11] In the 17α-hydroxypregnenolone-H^3 studies, 100 μg of carrier testosterone was added to a portion of the overflow from the cyclohexane : dioxane : methanol : water system. After acetylation, the extract was chromatographed in the heptane : propylene glycol system. The isolated testosterone acetate was devoid of radioactivity.

[12] Recent observations (47) by the authors indicate that human adrenal tissues form dehydroepiandrosterone sulfate from 17α-hydroxypregnenolone and dehydroepiandrosterone. It is certain that our extraction procedures would not remove steroid sulfates from the media, which may possibly explain the low observed unconjugated dehydroepiandrosterone values.

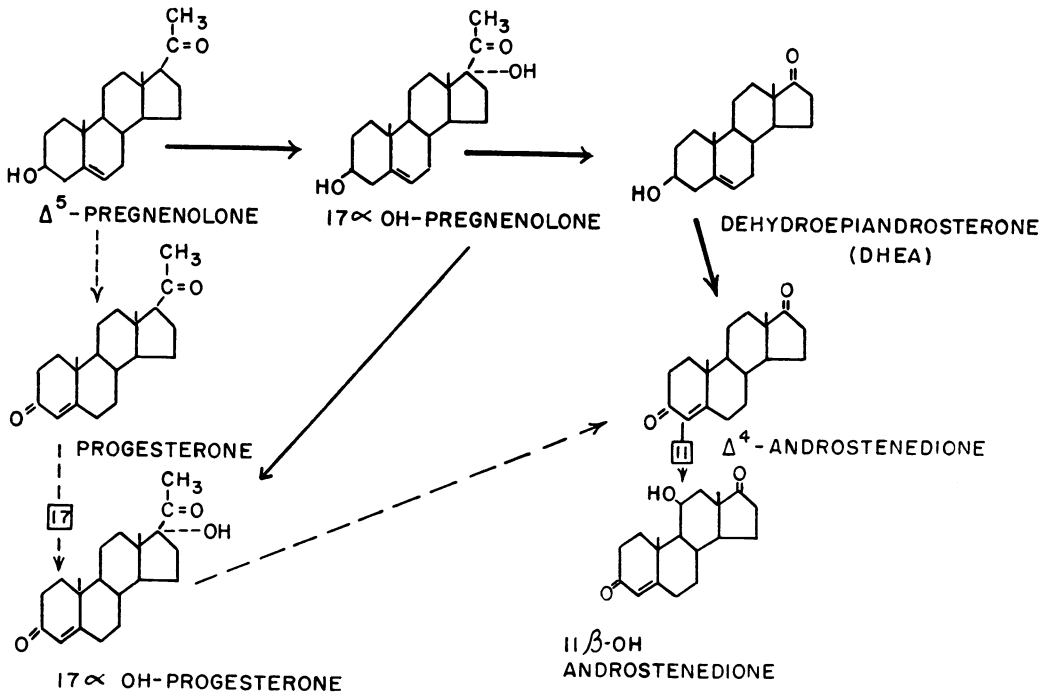

FIG. 2. PATHWAYS OF ADULT ADRENAL ANDROGEN BIOSYNTHESIS. The solid line indicates the major pathway in the present study.

substrates to C-19 steroids with "normal," hyperplastic, and adenomatous tissue slices. The data from Tables VI to IX suggest the probable biosynthetic sequence of androgen production shown in Figure 2.

The biosynthesis of dehydroepiandrosterone from 17α-hydroxypregnenolone with adenomatous and hyperplastic adrenal slices (Table VII) confirms previous observations of Lipsett and Hökfelt (24). Their results, however, seem to indicate that 17α-hydroxypregnenolone is not the important "intra-adrenal" precursor of dehydroepiandrosterone in the "normal" adrenal gland slice. The *in vitro* data in Table VII and VIII show that adrenal tissues from three patients with Cushing's syndrome are capable of this conversion, substantiating *in vivo* observations of this pathway in a patient with metastatic adrenal carcinoma (48).[13] Although this pathway is now well documented, an alternative pathway from cholesterol to dehydroepiandrosterone not involving C-21 intermediates, pregnenolone and 17α-hydroxypregnenolone, has been suggested by Dorfman (50).

The formation of dehydroepiandrosterone from pregnenolone and 17α-hydroxypregnenolone is closely associated with the hypothesis that androstenedione may arise from these steroids via an alternative pathway without the intermediate progesterone (Figure 2). Since a 3β-ol dehydrogenase system and double-bond shift are required for the conversion of the Δ^5-3β-ol steroids to the corresponding Δ^4-3-one derivatives (51, 52), studies were designed to investigate this hypothesis. The data in Tables VI to VIII indicate that adult adrenal tissues convert a higher percentage of the Δ^5-3β-ol substrate, 17α-hydroxypregnenolone, than the Δ^4-3-one precursor, progesterone, to the product androstenedione. A common intermediate to both these reactions may be 17α-hydroxyprogesterone as seen in Figure 2. Also, 17α-hydroxypregnenolone can be converted to androstenedione via dehydroepiandrosterone. Villee, Engel, Loring, and Villee observed that fresh human fetal adrenal tissues formed androstenedione from progesterone 4-C^{14} early in gesta-

[13] Recently, Roberts, Vande Wiele, and Lieberman (49) demonstrated that only 0.5 per cent of 17α-hydroxypregnenolone was converted to dehydroepiandrosterone in a patient with a virilizing adrenal adenoma, strongly suggesting the *adrenal* rather than *peripheral* origin of this androgen.

tion but not after 12 to 15 weeks gestational age (53). These investigators suggested the 21-hydroxylation appeared to reach a maximum at 12 to 15 weeks gestation with concomitant diminished 17-hydroxylation and disappearance of androstenedione. These findings are opposite to those of Solomon, Lanman, Lind, and Lieberman, who described 17α-hydroxyprogesterone and androstenedione conversion from progesterone-4-C^{14} with frozen fetal adrenal tissues of 10 to 22 weeks gestation (25). Differences in co-factor additions (Solomon added diphosphopyridine nucleotide and adenosine triphosphate and Villee, triphosphopyridine nucleotide and glucose 6-phosphate) and the status of the tissues may account for the differences of the results. Although 17α-hydroxyprogesterone is present in normal and hyperplastic adult adrenal tissues (7, 28), the factors regulating subsequent 21-hydroxylation of 17α-hydroxyprogesterone to 11-deoxycortisol (Compound S) compared to side-chain cleavage to androstenedione may determine the predominant direction of the pathway. When a mixture of tritium-labeled 17α-hydroxypregnenolone and C^{14}-labeled progesterone are incubated with hyperplastic adrenal slices of A.D. (Cushing's syndrome, Table VIII), the $H^3 : C^{14}$ ratios of androstenedione (15.8:1) compared to cortisol (1.3:1) (28) are observed. The high $H^3 : C^{14}$ in androstenedione, despite the fact that much less androstenedione was produced compared to cortisol, suggests that the major proportion of this androgen and 11β-hydroxyandrostenedione are probably derived via a pathway which does not utilize progesterone, presumably by way of 17α-hydroxypregnenolone → DHEA → androstenedione → 11β-hydroxyandrostenedione. However, further studies are necessary to determine the significance of 17α-hydroxyprogesterone as an intermediate for adrenal androgen biosynthesis.

In addition, the high $H^3 : C^{14}$ ratio of 11β-hydroxyandrostenedione (22:1) compared to cortisol (28) implies that "intra-adrenal" side-chain cleavage of cortisol is an unlikely pathway for 11β-hydroxyandrostenedione formation. The data indicate that 11-hydroxylation of androstenedione is the more plausible explanation for the biosynthesis of 11β-hydroxyandrostenedione in the tissues studied.

The results of substrate conversions with adrenal slice incubations must be interpreted with reservation. Tissue membrane permeability, availability of intracellular co-factors, endogenous pools of steroid intermediates and products, and enzymatic activity are limiting factors not controlled by the tissue slice method. Further studies are thus necessary with purified human adrenal enzyme preparations to elucidate the conditions which regulate the pathways of pregnenolone metabolism to the corticosteroids, or androgens, or both.

SUMMARY

These studies demonstrate that fresh human adult "normal," atrophic, "hypertensive," hyperplastic, adenomatous, and carcinomatous tissue slices release dehydroepiandrosterone, androstenedione, and 11β-hydroxyandrostenedione into the media after three hours of incubation in Krebs-Ringer bicarbonate buffer, fortified with glucose. The addition of ACTH to the system increases 11β-hydroxyandrostenedione release in all tissues. ACTH did not stimulate dehydroepiandrosterone and androstenedione release.

In addition, data are presented for the pathways of adrenal androgen synthesis in "normal," hyperplastic, and adenomatous tissues. The data suggest that adrenal androgens are converted predominantly from 17α-hydroxypregnenolone → dehydroepiandrosterone → androstenedione → 11β-hydroxyandrostenedione rather than from progesterone → androstenedione → 11β-hydroxyandrostenedione.

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