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COMPARISON OF STEROIDOGENESIS IN ADRENAL TISSUE AND ADRENAL ADENOMA FROM A CASE OF PRIMARY ALDOSTERONISM*

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SUMMARY

The patterns of progesterone-4-^{14}C conversion by adrenal tissue and adenoma from a case of dexamethasone sensitive primary aldosteronism were compared *in vitro*. Morphologically the adrenal tissue appeared normal and the tumor was composed of cells resembling those of the zona fasciculata. Adrenal cortical tissue and the adenoma of the diseased gland converted labeled progesterone to deoxycorticosterone, 18-hydroxy-11-deoxycorticosterone, aldosterone, 18-hydroxycorticosterone, 17α-hydroxyprogesterone, cortisol and cortisone. The biosynthesis of labeled C_{19} steroids was not detected. Per unit of tissue weight the adenoma converted progesterone-4-^{14}C to aldosterone and 18-hydroxycorticosterone more efficiently than the adrenal tissue. Both types of tissue on timed incubation showed a stepwise utilization of labeled progesterone predominantly via 17-deoxysteroid pathway. The adenoma formed cortisol-^{14}C to a lesser extent that the adrenal cortical tissue. Biochemically the adenoma showed the presence of enzyme systems which are commonly found in the cells of the adrenal zona glomerulosa and fasciculata.

INTRODUCTION

PRIMARY aldosteronism characterized by hypertension, hypokalemic alkalosis and an increased aldosterone secretion was first recognized as a clinical syndrome by Conn[1]. Since that time, primary aldosteronism has been diagnosed with increasing frequency [2–4]. In most of the cases the underlying pathology has been an adrenal cortical adenoma. According to Neville and Symington [5] these tumors occur more frequently in females than in males normally between the ages of 31 and 50 years. The histological structure of the adenomas has been very variable. Commonly seen tumors are composed of large lipid laden clear cells similar to those of the zona fasciculata [6,7,5]. Adenomas composed of several cell types are occasionally found; less often, however, the tumors are of glomerulosa cell type only. In very rare instances, primary aldosteronism has been caused by adrenocortical carcinoma [5]. Recently the syndrome of primary aldosteronism has also been linked with hyperplasia of the adrenal zona glomerulosa [8–10]. Biochemical studies with adrenal adenoma tissue from cases of primary aldosteronism indicate that the tumor tissue *in vitro* can synthesize not only aldosterone but also deoxycorticosterone (DOC),† 18-hydroxy-deoxycorticosterone

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†The following trivial names and abbreviations are used in this paper.

Steroid hormones

18-hydroxycorticosterone (18-OH-B): 11β,18,21-trihydroxy-4-pregnene-3,20-dione; cortisol: 11β, 17α,21-trihydroxy-4-pregnene-3,20-dione; cortisone: 17α,21-dihydroxy-4-pregnene-3,11,20-trione; aldosterone: 11β,21-dihydroxy-4-pregnene-3,20-dion-18-al; 18-hydroxydeoxycorticosterone(18-OH-DOC): 18,21-dihydroxy-4-pregnene-3,20-dione; corticosterone: 11β,21-dihydroxy-4-pregnene-3,20-dione; 11-dehydrocorticosterone: 21-hydroxy-4-pregnene-3,11,20-trione; 11-deoxycortisol:

(18-OH-DOC), 18-hydroxycorticosterone (18-OH-B), corticosterone, cortisol, cortisone, and 11β-hydroxy-androstenedione (11β-OH-A)[6, 8, 11–14]. In spite of the presence of enzyme systems for cortisol and androgen biosynthesis in the adrenal adenomas the principal abnormality manifested *in vivo* is an overproduction of mineralocorticoids. In the present investigation the qualitative and quantitative biosynthetic patterns of adrenocortical steroids by an adenoma tissue were compared with histologically normal adrenal tissue from the same gland.

MATERIALS AND METHODS

Case history

A white female, 41 years old, who was well until the age of 37 developed headaches, dizzy spells and insomnia. Clinically the patient had diastolic hypertension of 120–130 mm Hg. Her serum potassium was in the low normal range and the urinary excretion of potassium was increased. Urinary 17-hydroxycorticosteroids and 17-keto-steroids were within normal limits. Serum thyroxine and protein-bound iodine were also normal. Urinary excretion of aldosterone conjugate on a normal sodium diet as assayed by New England Nuclear Biomedical Laboratories ranged from 27·8 to 31·1 μg/24 hr (normal 5–20 μg/24 hr). Treatment with 0·75 mg dexamethasone q.i.d. for 2 days reduced urinary aldosterone excretion to 0·9 and 1·8 μg/24 hours on two occasions. The renin activity in peripheral venous plasma was assayed by Dr. R. Boucher, Clinical Research Institute of Montreal and no renin activity was detected when the patient was on 100 mEq of sodium intake or on 10 mEq of sodium intake. Venogram of the renal veins revealed a mass in the region of the left adrenal gland. Adrenalectomy was performed and the surgical specimen weighed 12 g. Near one pole there was a globular tumor measuring 2·5 × 2·5 × 2 cm and the dimensions of the adrenal were 5 × 3 × 1·5 cm. The cut surface of the tumor was deep yellow–orange in color with faint lobular architecture. The gross appearance of the sectioned tumor is shown in Fig. 1. Histologically the tumor was composed of trabecular and alveolar cellular arrangement with abundant vascular channels between groups of cells. The tumor cells were large with pale, vacuolated cytoplasm and small central round and uniform nuclei Fig. 2. A fibrous capsule enclosed the entire tumor. Frozen section of the tumor revealed abundant lipid in the cytoplasm as well as the presence of cholesterol type of crystals. The histology of the adrenal tissue was normal in appearance (Fig. 3).

17α,21-dihydroxy-4-pregnene-3,20-dione; deoxycorticosterone (DOC): 21-hydroxy-4-pregnene-3,20-dione; progesterone: 4-pregnene-3,20-dione; 11β-hydroxyprogesterone: 11β-hydroxy-4-pregnene-3, 20-dione; 17α-hydroxyprogesterone (17α-OH-P): 17α-hydroxy-4-pregnene-3,20-dione; pregnenolone: 3β-hydroxy-5-pregnen-20-one; testosterone: 17β-hydroxy-4-androsten-3-one; androstenedione (andr. -dione): 4-androstene-3,17-dione; 11β-hydroxyandrostenedione (11β-OH-A): 11β-hydroxy-4-androstene-3,17-dione; dexamethasone: 11β,17α,21-trihydroxy-pregn-1,4-diene-3,20-dione-9α-fluoro-16α-methyl.

Chromatographic systems

LPG	ligroine/propylene glycol
TEG	toluene/ethylene glycol
BF	benzene/formamide
Bush A	petroleum ether/methanol: water (100/80: 20)
Bush C	toluene: ethyl acetate/methanol: water (90: 10/50: 50)
BL_1	petroleum ether: benzene/methanol: water (30: 70/50: 50)
E_2B	isooctane/*t*-butanol: water (100/50: 90).

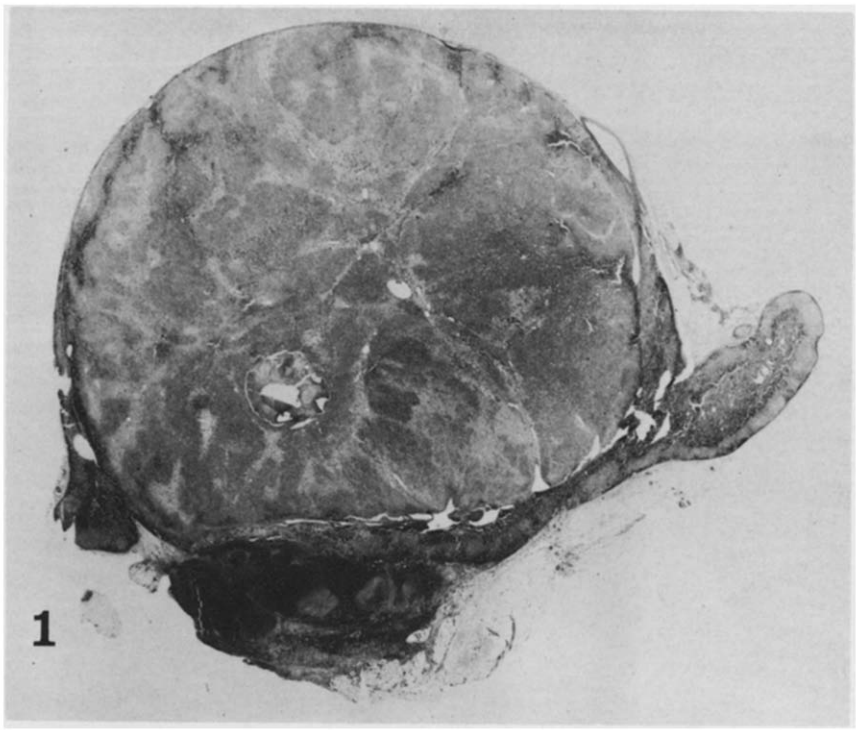

Fig. 1. Cross section of the adrenal tumor and the adrenal tissue. Hematoxylin and eosin, × 4.

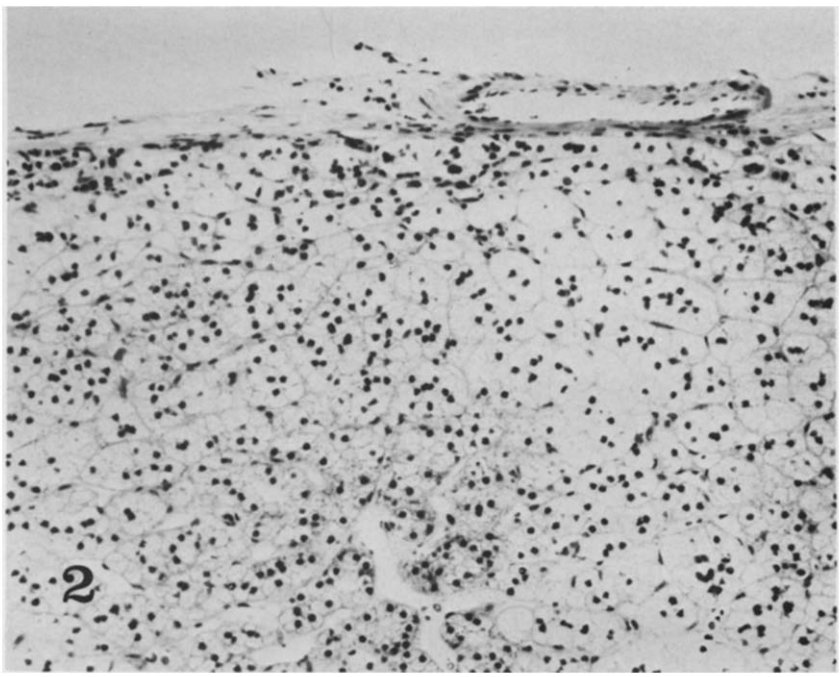

Fig. 2. Histological structure of the adrenal tumor tissue. Hematoxylin and eosin, × 400.

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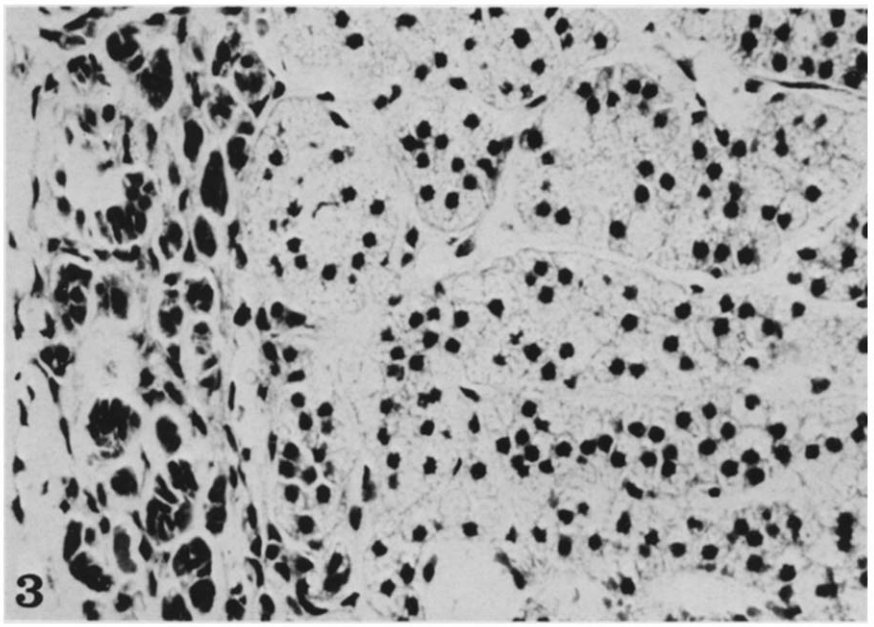

Fig. 3. Histological structure of the adrenal tissue. Hematoxylin and eosin, × 800.

Incubation of the adrenal tissue

A part of the surgical specimen was kept in Krebs–Ringer bicarbonate buffer, pH 7·4 cooled over ice. The tumor tissue was dissected and separated from the adrenal gland. Each preparation was minced with scissors. Aliquots of 0·5 g of wet tissue were placed in incubation flasks, containing 10 ml Krebs–Ringer bicarbonate buffer + 20 mg glucose and progesterone-4-^{14}C (0·83 μg; 1·15 μCi) which were preheated to 37·5°C. The incubation was carried out under atmospheric air at 37·5°C for 30, 60, 120 and 240 min. After incubation the buffer solution was separated from the tissue by filtration through glass wool and the tissue was washed twice with 5 ml of fresh buffer solution. The washings were combined with the incubation medium. Steroids present in the tissue were extracted with acetone and the buffer solution was extracted with ethyl acetate [15]. For further analysis only extracts from the incubation media were used. Carbon-14 was assayed in liquid scintillation spectrometer Packard Model 3365 as previously described[15, 16]. Before chromatography the crude extracts were combined with non-radioactive carriers; aldosterone, corticosterone, cortisol, 17α-hydroxyprogesterone, deoxycorticosterone and progesterone, using 50 μg of each. The extracts were separated by a modified serial paper chromatography of Lucis *et al.*[16, 17] as shown in the flow diagram in Fig. 4. Radioactive areas

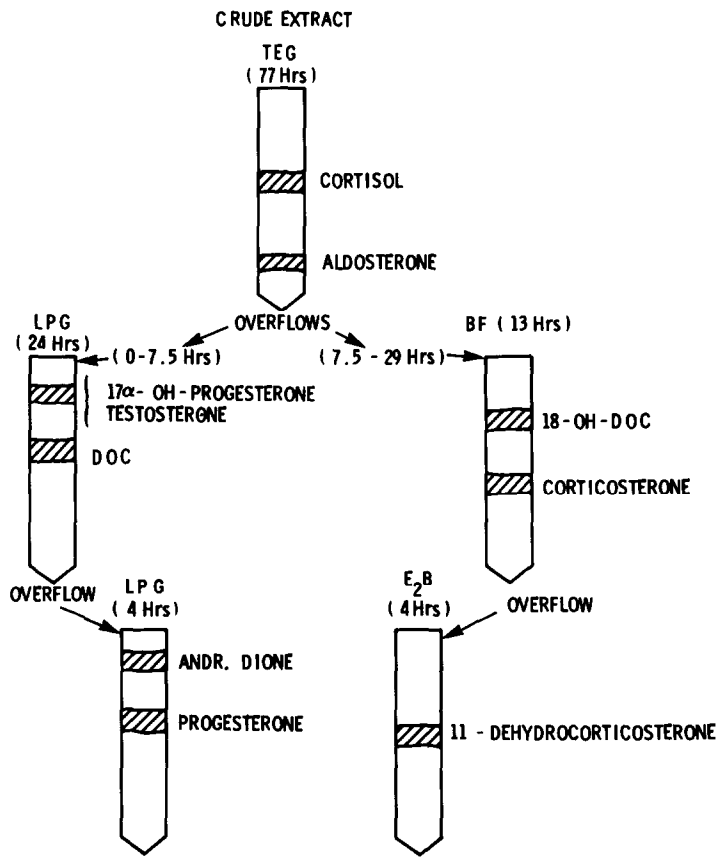

Fig. 4. Sequential chromatography of the extracts. See footnote on page 19 for abbreviations.

on the chromatograms were detected by autoradiography on X-ray film and the carriers were visualized under ultraviolet light (2530 Å). The separation of 17α-hydroxyprogesterone from testosterone was achieved by acetylation and rechromatography of the products on Bush A system[17]. Carbon-14 labeled progesterone used in this study was purchased from the New England Nuclear Corporation, Boston, Massachusetts. The radio-chemical purity of the material was verified by chromatography in ligroine-propylene glycol (LPG) paper chromatographic system. Authentic crystalline 18-hydroxydeoxycorticosterone and 18-hydroxycorticosterone were kindly donated by Dr. E. H. Venning, McGill University, Montreal, Canada. Other steroid reference standards were purchased from Ikapharm, Ramat Gan, Israel and Steraloids Inc., U.S.A.

RESULTS

In the timed incubation of the adrenal adenoma and the adrenal tissue the aim was to establish the exchange of radioactive products between the preserved cells within the tissue mince and the incubation medium. As shown in Table 1 there are notable differences in the uptake and release of the labeled products not only with respect to time of incubation but also with regard to the type of tissue. The patterns of labeled product exchange for the adenoma deviated from those of the adrenal tissue. In the case of the adrenal adenoma tissue the concentration of ^{14}C remained at a high level during first 60 min of incubation. On longer incubation the tissue showed a rapid diminution of extractable ^{14}C and a decrease in ^{14}C tissue/medium ratio. The adrenal tissue during the course of incubation exhibited a gradual and less pronounced diminution in ^{14}C concentration. Calculated ^{14}C tissue/medium ratio remained constant during incubation for 120 min and decreased notably after 240 min. The protein binding of ^{14}C labeled steroids and the possible esterification of the radioactive products was not in-

Table 1. Extractable radioactivity after timed incubation

	Incubation medium (dpm/ml × 10^{-3})	Tissue (dpm/g × 10^{-3})	Ratio tissue medium
Initial	256·9	0	0
Tumor (0·5 g)			
Time of incubation			
30 min	156·6	1202	7·7
60 min	166·6	1210	7·3
120 min	143·7	798	4·8
240 min	131·2	610	4·7
Adrenal (0·5 g)			
30 min	159·5	1054	6·6
60 min	147·7	980	6·7
120 min	147·0	970	6·6
240 min	147·2	798	5·5

vestigated. Analysis of the extracted conversion products was limited to the incubation media only. Figure 5 shows that the tumor rapidly utilized labeled progesterone which was accompanied by appearance of corticosterone and DOC. Nearly equal quantities of labeled DOC and corticosterone appeared in the medium after 30 min. As the time of incubation increased corticosterone reached its maximal concentration after 2 hr which was followed by a decrease on longer incubation. An entirely different pattern was observed with labeled DOC. This

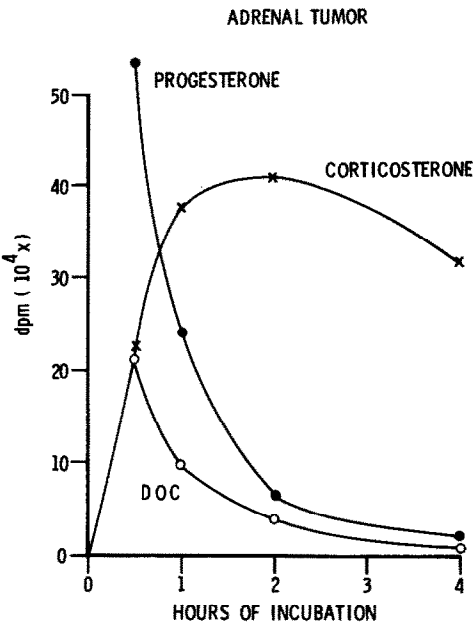

Fig. 5. Changes in radioactive steroid concentrations during timed incubation of the adrenal tumor tissue.

steroid had a maximum after 30 min and then diminished on prolonged incubation. Table 2 shows that 18-hydroxydeoxycorticosterone produced by the tumor tissue reached its highest level after 2 hr and then decreased in a pattern similar to corticosterone. Other steroids of the 17-deoxy series: 18-hydroxycorticosterone and alsosterone increased continuously as the time of incubation progressed. In terms of efficiency the tumor tissue converted progesterone to 18-hydroxycorticosterone more than to aldosterone. The presence of 11-dehydrocorticosterone was demonstrable after 30 min and the quantity of this steroid remained low through the time of incubation. Since in steroidogenesis of 17-deoxycorticosteroids from progesterone precursor 11β-hydroxyprogesterone may act as an intermediate, a search was made for this product. The presence of labeled 11β-hydroxyprogesterone could not be demonstrated. Apart from steroids of the 17-deoxy series the tumor tissue also formed 17α-hydroxycorticosteroids. Figure 6 shows that the conversion of progesterone to 17α-hydroxyprogesterone proceeded most rapidly during the first 30 min of incubation and as the time of incubation was prolonged the quantity of labeled 17α-hydroxyprogesterone diminished. Cortisol was formed most rapidly during the first hour and later at a diminished rate. The yield of labeled cortisol in comparison with corticosterone was considerably less. As

Table 2. Conversion of progesterone-4-^{14}C by adrenal tumor. Tissue weight, 0·5 g; substrate, progesterone-4-^{14}C (1·15 μCi/0·83 μg)

Time of incubation (min) Radioactivity	30 (dpm × 10^{-4})	60 (dpm × 10^{-4})	120 (dpm × 10^{-4})	240 (dpm × 10^{-4})
Chromatographically separated products				
Progesterone	53·30	24·00	6·53	2·02
DOC	21·00	9·89	4·18	0·80
18-OH-DOC	1·05	1·08	1·14	0·78
Corticosterone	22·60	37·80	41·10	31·60
18-OH-B	1·75	3·02	4·19	9·52
11-dehydrocorticosterone	0·08	0·11	0·11	0·17
Aldosterone	0·92	1·60	3·17	3·61
17α-OH-progesterone	2·83	1·23	0·20	0·08
Cortisol	3·46	7·29	8·48	8·83
Cortisone	0·20	0·35	0·60	0·96

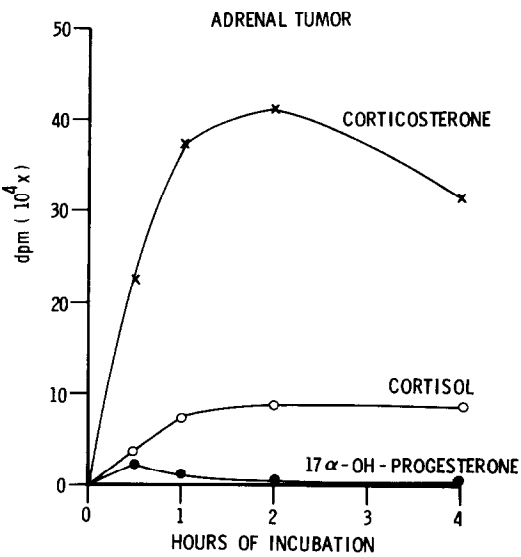

Fig. 6. Formation of corticosterone, cortisol and 17α-OH-progesterone by adrenal tumor tissue.

shown in Table 2, along with cortisol the tumor tissue produced small quantities of labeled cortisone which increased progressively with the time of incubation. The biochemical conversion reactions of 17α-hydroxyprogesterone to cortisol apparently proceeded very rapidly and no labeled 11-deoxycortisol was detected in the incubation media. The tumor tissue also failed to show the steroid 17-lyase activity which is involved in conversion of 17α-hydroxyprogesterone to C_{19} steroids.

Adrenal tissue preparations incubated under identical conditions to those of the tumor qualitatively showed the presence of the same enzyme systems. The steroidogenesis from labeled progresterone by the adrenal preparations had a quantitatively different trend. Formation of corticosterone and changes in DOC

are shown in Fig. 7 and Table 3. Within 1 hr of incubation the level of labeled corticosterone reached a maximum which remained as a plateau up to 4 hr. The pronounced rise in DOC after 30 min was followed by a rapid decline. In comparison with the tumor tissue the adrenal preparation utilized the exogenous progesterone with a greater efficiency particularly via the 17α-hydroxy steroid pathway. As shown in Fig. 8 the formation of cortisol increased progressively with time in a pattern quite different from that of corticosterone. The biosynthesis of 17α-hydroxyprogesterone had the highest level after 30 min and was followed by a decline on longer incubation. The *in vitro* system used for tumor and adrenal tissue incubation reveals that the intermediate steroids DOC and

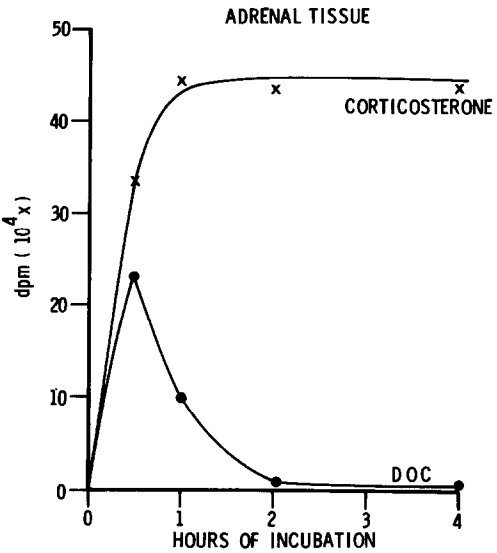

Fig. 7. Formation of corticosterone and DOC by adrenal cortical tissue.

Table 3. Conversion of progesterone-4-^{14}C by adrenal tissue. Tissue weight, 0·5 g; substrate progesterone-4-^{14}C (1·15 μCi/0·83 μg)

Time of incubation (min) Radioactivity	30 (dpm × 10^{-4})	60 (dpm × 10^{-4})	120 (dpm × 10^{-4})	240 (dpm × 10^{-4})
Chromatographically separated products				
Progesterone	27·80	6·75	1·82	0·63
DOC	22·90	9·99	1·46	0·45
18-OH-DOC	0·80	1·27	1·10	0·97
Corticosterone	33·40	44·20	43·20	43·60
18-OH-B	0·47	1·04	1·56	2·94
11-dehydrocorticosterone	0·17	0·19	0·17	0·21
Aldosterone	0·16	0·41	0·70	0·79
17α-OH-progesterone	5·89	1·51	0·17	0·07
Cortisol	6·06	11·60	14·40	20·10
Cortisone	0·22	0·50	0·64	0·66

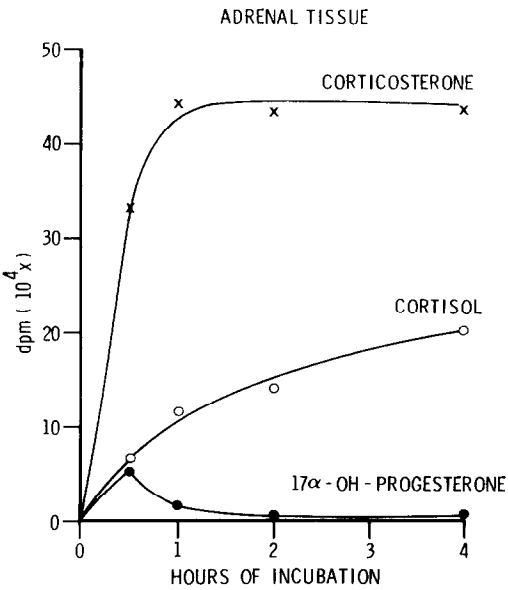

Fig. 8. Comparison of corticosterone, cortisol and 17α-OH-progesterone production by adrenal cortical tissue.

17α-hydroxyprogesterone are formed in a distinctively different pattern from other products. An excessive biosynthesis of these hormones within 30 min of incubation was followed by a reutilization of these products during subsequent time intervals. Similarly to the adrenal adenoma, formation of 18-hydroxycorticosterone by the adrenal tissue exceeded that of aldosterone; however, in terms of efficiency both of these hormones were produced in a lower yield than by the tumor. On the other hand the adrenal formed more cortisol than the tumor. In common with the tumor tissue the adrenal failed to produce labeled C_{19} steroids and 11β-hydroxyprogesterone.

Radioactive steroids isolated from the incubation media were identified by crystallization with authentic carriers. As shown in Tables 4 and 5 the specific activities of the crystals in 3 successive crystallizations for aldosterone, DOC, corticosterone, 17α-hydroxyprogesterone and cortisol remained constant. The isolated labeled 18-hydroxydeoxycorticosterone was combined with 200 μg of authentic carrier and chromatographed in 4 successive paper chromatographic systems. After each chromatography the steroid was eluted and the quantity calculated from the ultraviolet light absorption spectrum in ethanol. Data presented in Table 6 indicate the R_F and specific activities of 18-hydroxydeoxycorticosterone obtained after each chromatography. Shortage of authentic 18-hydroxycorticosterone precluded a detailed identification and this steroid was characterized by chromatographic mobility only. Cortisone separated from the incubation media was also characterized by its chromatographic properties.

DISCUSSION

In vivo as well as *in vitro* the steroid biosynthesis in human adrenal cortex follows three distinct pathways[6, 11, 13, 14, 18]. Zonation of the normal adrenal cortex by separation of the zona glomerulosa from the innermost layers reveals

Table 4. Crystallization of conversion products

Steroid	Solvents	Specific activity (dpm/mg)	
Aldosterone		Adrenal	Tumor
Initial		991	3557
1st crystals	Methanol/H_2O	939	3525
2nd crystals	Acetone/hexane	920	3634
3rd crystals	Acetone/H_2O	840	3628
DOC		Adrenal	Tumor
Initial		5329	5445
1st crystals	Methanol/H_2O	4463	4710
2nd crystals	Acetone/hexane	4613	4503
3rd crystals	Acetone/H_2O	4540	4491
Corticosterone		Adrenal	Tumor
Initial		4709	5560
1st crystals	Methanol/H_2O	3739	4703
2nd crystals	Acetone/hexane	3459	4675
3rd crystals	Acetone/H_2O	3374	4554

Table 5. Crystallization of conversion products

Compound	Solvents	Specific activity (dpm/mg)	
17α-OH-progesterone		Adrenal	Tumor
Initial		2897	1134
1st crystals	Methanol/H_2O	2445	909
2nd crystals	Acetone/hexane	2449	906
3rd crystals	Acetone/H_2O	2453	887
Cortisol		Adrenal	Tumor
Initial		4602	2167
1st crystals	Methanol/H_2O	4526	2221
2nd crystals	Acetone/hexane	4783	2291
3rd crystals	Acetone/H_2O	4840	2263

Table 6. Properties on repeated chromatography of 18-OH-DOC isolated from adrenal and tumor incubation media

Compound	System	R_F	Specific activity (dpm/μg)
Adrenal			
18-OH-DOC	BuC	0·62	30·1
18-OH-DOC	E_2B	0·38	24·8
18-OH-DOC	BL_1	0·41	26·7
18-OH-DOC	BuC	0·62	26·4
Tumor			
18-OH-DOC	BuC	0·62	35·9
18-OH-DOC	E_2B	0·38	32·4
18-OH-DOC	BL_1	0·41	32·1
18-OH-DOC	BuC	0·62	35·5

that the former lacks progesterone 17α-hydroxylase system and consequently forms steroids of 17-deoxy series. Apart from this the zona glomerulosa also contains 18-hydroxylase and 18-oxidase enzymes essential for synthesis of aldosterone. The innermost adrenal layers, zona fasciculata and reticularis, lack 18-oxidase but contain 17α-hydroxylase. The latter enzyme system is essential for biosynthesis of cortisol and also for steroids of the C_{19} series. The biosynthetic pathways of steroid formation from progesterone are outlined in Fig. 9. Timed incubation of the adrenal adenoma and the adrenal cortex with labeled progesterone has shown that the conversion of the tracer proceeds stepwise and the yield of endproducts increases proportionally to the time of incubation. Exchange of

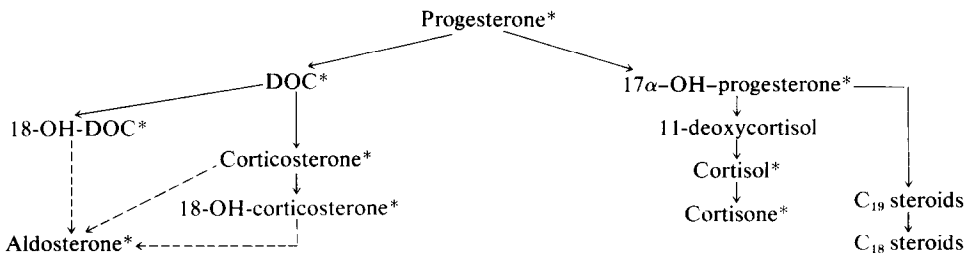

Fig. 9. Biosynthetic pathways of steroid formation demonstrable in the adrenal tumor and adrenal cortical tissue.

*Labeled steroids isolated and measured.

labeled steroids between the incubation medium and the tissue cells varies with the type of tissue and with the time of incubation. Both adrenal adenoma and adrenal cortex showed the presence of 17-deoxycorticosteroid pathway which predominated over the 17α-hydroxy pathway.

Recent studies with minced human adrenal fasciculata and reticularis tissue by Cameron *et al.*[19] have shown that pregnenolone was converted more efficiently to cortisol than progesterone. In their system progesterone contributed more towards the biosynthesis of 17-deoxycorticosteroids whereas pregnenolone yielded predominantly steroids of the 17α-hydroxy series. A similar relationship was demonstrated also in timed incubations of a clear cell adenoma from a patient with Cushing's syndrome[20]. The patterns of endogenous steroid biosynthesis by human adrenal tissue *in vitro* reported in earlier studies[18] have shown that from the Δ_4-3 keto steroids formed cortisol predominated over corticosterone in adrenal glands from patients with Cushing's syndrome, virilism, primary aldosteronism and carcinoma of the breast. All adrenal glands produced *in vitro* measurable quantities of aldosterone and 11β-hydroxyandrostenedione.

In the present study the steroid biosynthesis from endogenous sources was not determined but the patterns of labeled progesterone transformation were compared.

Results obtained suggest that the tumor tissue contains enzyme systems which are normally found in the adrenal zona glomerulosa as well as enzyme systems associated with the cells of the zona fasciculata. These biochemical properties correspond very well with the morphological appearance of the tumor cells. The adrenal tumor may have taken its origin from the cells of zona fasciculata where by an unexplained mechanism the normally suppressed genes responsible for the

synthesis of 18-oxygenases have become activated and consequently the biosynthetic pathway for aldosterone was established. The adrenal cortex of this diseased gland also shows a biochemical abnormality by failing to convert 17α-hydroxyprogesterone to C_{19} steroids. The aldosterone-secreting tumor exhibits mixed biochemical properties of the zona glomerulosa and fasciculata. Based on unit weight of wet tissue the adrenal tumor was considerably more efficient in biosynthesis of aldosterone than the adrenal itself (Fig. 10). In comparison with aldosterone the production of 18-hydroxycorticosterone was considerably higher. This observation is in agreement with the findings *in vivo* that

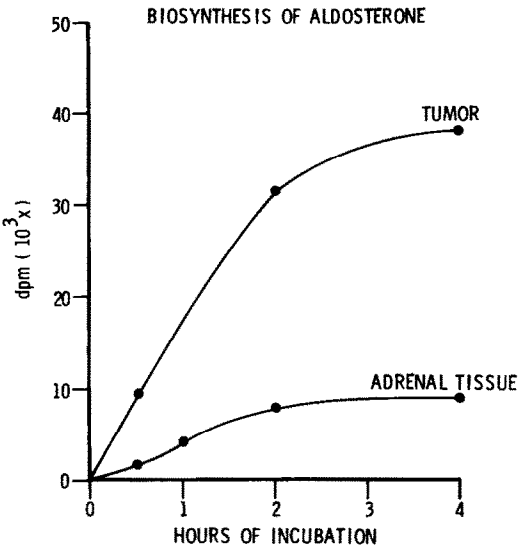

Fig. 10. Comparison of aldosterone formation by adrenal cortical and tumor tissue.

the secretion of 18-hydroxycorticosterone exceeds that of aldosterone in cases of tumorous and non-tumourous adrenal glands[4, 21]. Previous *in vitro* studies with adrenal tumors and adrenal tissue from cases of primary aldosteronism show a wide variation in steroid production which can be attributed to the nature of the tissue and also to the incubation methods used[6, 8, 11, 12, 14]. All of these studies show that the preparations produce aldosterone. The detailed investigations of Raman *et al.*[12] using homogenate and subcellular fractions of aldosterone secreting adenoma have revealed some interesting findings. In their system using labeled progesterone and DOC it was observed that DOC gave a greater yield of 18-hydroxycorticosterone and aldosterone than progesterone, suggesting that 21-hydroxylation facilitates further steps in aldosterone biosynthesis. Conversion of DOC to 18-hydroxycorticosterone and aldosterone has been demonstrated with mitochondrial preparations; however, the whole homogenate was more efficient in aldosterone synthesis, indicative that some other component present in the homogenate facilitated this reaction. In spite of the formation of 18-hydroxycorticosterone the homogenate failed to produce 18-hydroxydeoxycorticosterone. This was explained by the authors on grounds of substrate specificity of steroid C_{18}-hydroxylase. If their hypothesis is accepted, then the

tumor tissue and the adrenal cortex in the present case should contain at least two 18-hydroxylases, one for the formation of 18-hydroxydeoxycorticosterone, the other for 18-hydroxycorticosterone.

The *in vivo* function of the diseased adrenal was sufficiently abnormal to cause the signs and symptoms of primary aldosteronism. Urinary excretion of aldosterone conjugate was only moderately elevated and it could be suppressed by dexamethasone. This suggests that the function of the adenoma is under some influence of pituitary corticotropin. Clinical investigations by Slaton *et al.*[22] in patients with aldosterone-producing adenomas have shown that 6 out of 14 patients responded to dexamethasone with a diminution in aldosterone production. The responsiveness of aldosterone producing adenomas to exogenous ACTH was demonstrated in 15 out of 18 patients. From their studies these authors conclude that potassium balance and ACTH play important regulatory roles in production of aldosterone by these tumors. It is well known that in healthy subjects the secretion of aldosterone increases following ACTH stimulation and also after administration of angiotensin. The responsiveness to exogenous angiotensin in patients with aldosterone secreting tumors differs from healthy subjects. Patients with hyperaldosteronism due to adrenal adenoma show an increased sensitivity towards pressor effect and a relative insensitivity towards changes in aldosterone excretion[22]. These effects were not investigated in the presently described case. Primary aldosteronism as described by Sutherland *et al.*[23] may also be associated with nodular adrenocortical hyperplasia and in this condition the diseased glands responded to dexamethasone suppression as well as to ACTH stimulation. The clinical and also biochemical findings in cases with aldosterone secreting adenomas and nodular adrenocortical hyperplasia suggest that the abnormal adrenocortical cells have a tendency to retain their functional responsiveness to corticotropin rather than the responsiveness to angiotensin. In spite of ACTH dependence these cells do not maintain an augmented steroid biosynthetic pathway towards cortisol production but show an increased enzymatic activity in synthesis of aldosterone and 18-hydroxycorticosterone. A further elucidation of the deviations in steroid production at the cellular level by aldosterone producing tumors and the adrenocortical tissue would be desirable in the future.

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