Metabolism of C₁₉-Steroids by Homogenates of Normal Rat and Mouse Adrenal Tissue and of the Snell Transplantable Rat Adrenocortical Tumour 494

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C₁₉-steroid metabolism in homogenates of adrenal tissue from rats and mice has been studied. Production of these compounds from [7 α -³H]cholesterol by rat adrenal tissue appeared to follow a route independent of pregnenolone. The major products of [7 α -³H]-dehydroepiandrosterone metabolism by rat adrenal tissue were 5 α -reduced steroids, principally androsterone, epiandrosterone and 5 α -androstanedione. No differences in metabolism of [7 α -³H]dehydroepiandrosterone or [4-¹⁴C]pregnenolone were detected between adrenal tissue from Sprague–Dawley, Wistar and Osborne–Mendel rats, but experiments with the Snell rat adrenocortical tumour 494 showed that this tissue had low 5 α -reductase activity. In contrast, the major products of [7 α -³H]dehydroepiandrosterone metabolism by mouse adrenal tissue were 5 β -reduced steroids. Differences were observed between LACA and NH strains of mice in that there was a lower metabolism of androstenedione by NH mouse adrenal and a considerable difference in the proportions of aetiocholanolone and epiaetiocholanolone produced.

In this paper the following trivial names are used: adrenosterone, androst-4-ene-3,11,17-trione; aetiocholanolone, 3 α -hydroxy-5 β -androstan-17-one; 5 α -androstanedione, 5 α -androstan-3,17-dione; 5 β -androstanedione, 5 β -androstan-3,17-dione; androstenedione, androst-4-ene-3,17-dione; androsterone, 3 α -hydroxy-5 α -androstan-17-one; cholesterol, cholest-5-en-3 β -ol; corticosterone, 11 β ,21-dihydroxypregn-4-ene-3,20-dione; dehydroepiandrosterone, 3 β -hydroxyandrost-5-en-17-one; 5 α -dihydrotestosterone, 17 β -hydroxy-5 α -androstan-3-one; 5 β -dihydrotestosterone, 17 β -hydroxy-5 β -androstan-3-one; deoxycorticosterone, 21-hydroxypregn-4-ene-3,20-dione; epiaetiocholanolone, 3 β -hydroxy-5 β -androstan-17-one; testosterone, 17 β -hydroxyandrost-4-en-3-one; pregnenolone, 3 β -hydroxypregn-5-en-20-one; progesterone, pregn-4-ene-3,20-dione.

In Osborne–Mendel rats adrenocortical tumours arise spontaneously in old animals, and one such tumour occurring in a female animal of this strain has been maintained as a transplantable line (Snell & Stewart, 1959). Similar neoplasms develop in ageing female NH mice (Kirschbaum *et al.*, 1946).

The biosynthetic pathways by which C₁₉-steroids can be produced in rat adrenal tissue have been reported to follow a route through pregnenolone to dehydroepiandrosterone (Favino *et al.*, 1966), or directly from cholesterol to dehydroepiandrosterone (Jungmann, 1968). Recently, however, doubt has been cast on the existence of the 'direct' pathway, because

Burstein *et al.* (1971) were unable to demonstrate [25, 26-³H₂]cholesterol cleavage in such a manner as to give rise to significant quantities of a ³H-labelled C₈-fragment.

However, the metabolism of C₁₉-steroids in the rodent adrenal gland has not been investigated in any depth and a study of this in Sprague–Dawley rats and LACA mice was undertaken. A comparison was also made of the steroid-metabolizing enzymes in adrenal tissue from Sprague–Dawley and Wistar rats with that from Osborne–Mendel rats and the Snell transplantable tumour 494. A similar comparison was made between adrenal tissue from female LACA and NH mice. Such studies were designed to reveal any differences between 'normal' strains and those with a suspected general endocrine imbalance.

Materials and Methods

Materials

Animals. Rats of the Sprague–Dawley, Wistar and Osborne–Mendel strains, and mice of the NH strain were bred and maintained conventionally. LACA mice were purchased from Fisons Pharmaceutical, Loughborough, Leics., U.K.

The Snell adrenocortical tumour 494 (Snell & Stewart, 1959), generously supplied by Dr. Katharine C. Snell, was maintained in male Osborne–Mendel rats.

Reagents. All reagents including ethanol and methanol were of analytical grade. Other solvents were of laboratory-reagent grade and were redistilled before use.

Radioactive substrates. [7α - ^3H]Cholesterol (specific radioactivity 7.35 Ci/mmol), [7α - ^3H]dehydroepiandrosterone (specific radioactivity 500 mCi/mmol), [7α - ^3H]pregnenolone (specific radioactivity 6.9 Ci/mmol) and [4 - ^{14}C]pregnenolone (specific radioactivity 55.7 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The purity of these materials was checked by reverse isotope dilution with chromatography on thin layers of silica gel in a number of solvent systems before and after derivative formation.

Methods

Incubation procedure. Animals were killed by cervical dislocation and the adrenal glands or tumour removed and placed in a Petri dish on ice. The tissues were freed from fat (and decapsulated in the case of normal, non-tumorous, rat tissue) and a 20% homogenate prepared in 0.25 M-sucrose containing 0.12 M-nicotinamide. This was mixed with an equal volume of an incubation medium (Griffiths & Glick, 1966) containing cofactors such that the final concentration of each was: tris-HCl buffer, pH 7.4, 43.02 mM; KCl, 42.82 mM; MgSO_4 , 3.16 mM; potassium fumarate, 8.57 mM; ATP, 0.94 mM; NADP $^+$, 0.18 mM; glucose 6-phosphate, 17.60 mM; glucose 6-phosphate dehydrogenase, 0.5 Kornberg unit (Kornberg & Horecker, 1955)/ml.

A solution of the required radioactive precursor in benzene was placed in an incubation flask, together with propylene glycol (0.1 ml). After the benzene had been evaporated in a stream of air the adrenal preparation was added. Incubations were performed in a shaking water bath at 37°C in an atmosphere of air and were normally terminated by the addition of acetone (15 ml) containing 500 μg of each carrier steroid.

Extraction and fractionation of steroids. Incubation mixtures containing acetone and carrier steroids were homogenized in a Silverson mixer and filtered. Residues were then washed three times with 30 ml volumes of acetone and the pooled extract and washings evaporated almost to dryness under reduced pressure. These extracts were partitioned between light petroleum (b.p. 80–100°C) (20 ml) and aq. 80% (v/v) methanol (3 $\times$ 20 ml). The aqueous methanol fractions were combined and the methanol was evaporated under reduced pressure. This aqueous residue was then diluted to 20 ml with water and extracted with diethyl ether (3 $\times$ 20 ml). After evaporation of the ether the steroid fraction remaining was subjected to extensive paper and thin-layer chromatography as described below.

Derivative formation, chromatography and measure-

ment of steroids. Derivatives were prepared by the methods described by Griffiths *et al.* (1963), except that acetates of C_{19} -steroids were hydrolysed by addition of 0.1 M-KOH in aq. 70% (v/v) methanol (0.4 ml) to the steroid residue dissolved in methanol (0.2 ml) (Pierrepoint *et al.*, 1969). The mixture was kept overnight at room temperature and the steroid material was extracted with ethyl acetate (2 $\times$ 5 ml) after the addition of water (2 ml).

Paper chromatography was performed on Whatman no. 1 paper in the descending system methanol–water – light petroleum (b.p. 80–100°C) – benzene (70:30:67:33, by vol.; solvent PB21) or methanol–water – light petroleum (b.p. 80–100°C) (9:1:10, by vol.; solvent P10-M) (Griffiths *et al.*, 1963).

The procedures used for the identification of steroids and the establishment of their radiochemical purity involved extensive t.l.c. on Merck silica gel HF $_{254/366}$ in at least three systems that were selected from the following, the solvent proportions being by volume: (I) cyclohexane–ethyl acetate (1:1); (II) cyclohexane–ethyl acetate (7:3); (III) cyclohexane–ethyl acetate (4:1); (IV) chloroform–acetone (37:3); (V) chloroform–ethanol (19:1); (VI) diethyl ether–ethyl acetate (19:1); (VII) hexane–chloroform–acetone–diethyl ether (140:10:1:50); (VIII) cyclohexane–acetone–chloroform (10:3:3); (IX) (*n*-)butyl acetate; (X) benzene–ethyl acetate (9:1); (XI) chloroform–methanol–water (187:12:1).

G.l.c. was employed for the determination of Δ^5 - 3β -hydroxy steroids and ring-A-reduced steroids. The technique involved the use of a Pye 104 gas chromatograph with flame ionization detector (model 24) with internal standardization on a 3% OV-1 column 152 cm in length. The mass of Δ^4 -3-oxo steroids was measured by means of their selective absorption at 240 nm. Radioactivity was measured in a Nuclear-Chicago Mk II liquid-scintillation spectrometer. A portion of the steroid sample in ethanol solution was placed in a counting vial and the solvent was evaporated. The residue was then dissolved in toluene (5 ml) containing 0.3% 2,5-diphenyloxazole and 0.01% 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene. The counting efficiency was 45% for ^3H and 90% for ^{14}C in samples containing a single radioactive isotope. In samples containing both radioactive isotopes the efficiency was 40% for ^3H and 55% for ^{14}C in their respective channels. No spill-over of ^3H was observed in the ^{14}C channel and ^{14}C was counted with an efficiency of 18% in the ^3H channel. D.p.m. for each isotope were then calculated by the method of Okita *et al.* (1957). The observation that the specific radioactivities of a steroid and two of its derivatives differed by not more than 10% was taken as satisfactory evidence for radiochemical purity. The mean of the specific radioactivities was used to calculate the percentage conversion from the original steroid precursor.

Results

To demonstrate that rat adrenal tissue has the ability to produce C₁₉-steroids from cholesterol or pregnenolone under the standard conditions used throughout this investigation, an incubation was performed with a homogenate preparation by using [7 α -³H]cholesterol and [4-¹⁴C]pregnenolone as substrates. As can be seen from Table 1 small amounts of ³H were found in some of the androgens isolated, but in no case was any ¹⁴C detected. Thus under the conditions employed, cholesterol was apparently metabolized to the C₁₉-steroids examined without formation of pregnenolone as an intermediate.

Although the evidence so described does not establish the existence of a direct route from cholesterol to dehydroepiandrosterone, incubation of [7 α -³H]dehydroepiandrosterone with Sprague-Dawley rat adrenal tissue was undertaken to establish the identity of the C₁₉-steroid metabolites produced by such a preparation. Because the identity of the products was not known in this preliminary experiment, no carrier steroids were added at the end of the incubation period. The neutral steroids in the ether

fraction were subjected to paper chromatography in system PB21 (3.5h), after which three radioactive regions were detected on scanning.

The least polar zone (*R_F* 0.6–1.0) was eluted and chromatographed in system P10-M, which was over-run for 7.5h, when a further three distinct radioactive zones were found. These were characterized as 5 α -androstenedione, androsterone and epiandrosterone by comparison of their chromatographic mobilities with authentic compounds before and after derivative formation.

The mid-polar zone (*R_F* 0.4–0.6) was eluted and, after acetylation, re-run in the PB21 system (3.5h) whereupon two radioactive zones were detected. The acetylated material had chromatographic mobilities identical with those of the acetates of testosterone, 5 α -androstane-3 α ,17 β -diol and 5 α -androstane-3 β ,17 β -diol. These were again further characterized after derivative formation. The non-acetylated material was tentatively identified as 11 β -hydroxy-5 α -androstenedione, but lack of the authentic compound prevented complete characterization.

The most polar zone of the initial chromatogram (*R_F* 0.0–0.4) was eluted and then over-run in the PB21

Table 1. Conversion of 10 μ Ci of [7 α -³H]cholesterol and 2 μ Ci of [4-¹⁴C]pregnenolone simultaneously into various metabolites by a homogenate of 200mg of adrenal tissue from Sprague-Dawley rats, incubated for 2h

A zero indicates that radioactivity was not significantly above background.

Steroid investigated and derivative formed	Final solvent system	Specific radioactivities (d.p.m./nmol)		% of radioactivity found in isolated steroids	
		³ H	¹⁴ C	³ H	¹⁴ C
Pregnenolone	IV	376	155	1.93	5.03
20 β -Hydroxypregnenolone	IV	343	158		
20 β -Acetoxypregnenolone	X	339	162		
Androstenedione	I	51.3	0	0.32	0
Testosterone	IV	51.7	0		
Testosterone acetate	I	53.9	0		
11 β -Hydroxyandrostenedione	IV	11.7	0	0.07	0
11 β -Hydroxytestosterone	V	11.4	0		
Adrenosterone	IV	10.8	0		
5 α -Androstenedione	II	6.8	0	0.04	0
5 α -Androstane-3 β ,17 β -diol	VI	7.1	0		
5 α -Androstane-3 β ,17 β -diol diacetate	III	6.9	0		
Dehydroepiandrosterone	I	0.7	0	Trace	0
Androst-5-ene-3 β ,17 β -diol	IV	0.6	0		
Dehydroepiandrosterone acetate	VII	0.6	0		
Androsterone	I	0.5	0	Trace	0
5 α -Androstenedione	VII	0.6	0		
5 α -Androstane-3 β ,17 β -diol	II	0.7	0		
Epiandrosterone	I	0.4	0	Trace	0
5 α -Androstenedione	VII	0.5	0		
5 α -Androstane-3 β ,17 β -diol	II	0.5	0		

system for 7.5h, after which four radioactive zones were detected. Three of these were characterized as 11 β -hydroxyandrost-4-ene-dione, 11 β -hydroxyandrostosterone and 11 β -hydroxyepiandrosterone. The most polar region could not be properly identified, but was not an androstane-3,11,17-triol. No 5 β -reduced compounds could be detected in the incubation extract.

The above experiment was then repeated but, at the end of the incubation period, 500 μ g of the steroids identified as metabolites of [7 α -³H]dehydroepiandrosterone were added, thus enabling the conversion into these compounds to be accurately determined (Table 2). Incubation of tissue from the Snell adrenocortical carcinoma 494 with [7 α -³H]-dehydroepiandrosterone showed a complete contrast

Table 2. Conversion of 10 μ Ci of [7 α -³H]dehydroepiandrosterone into various metabolites by homogenates of 150mg of tissue of adrenal glands from 4-month-old Sprague-Dawley rats and from Snell adrenocortical carcinoma 494 incubated for 90 min

— indicates that the steroid was not examined.

Steroid investigated and derivative formed	Final solvent system	Specific radioactivities (d.p.m./nmol)		% of radioactivity found in isolated steroids	
		Rat adrenal tissue	Snell carcinoma 494	Rat adrenal tissue	Snell carcinoma 494
Dehydroepiandrosterone	I	93.2	521	0.8	3.74
Androst-5-ene-3 β ,17 β -diol	IV	101.6	574		
Dehydroepiandrosterone acetate	VII	94.2	537		
Androstenedione	I	15.4	9657	0.1	68.0
Testosterone	IV	13.9	10453		
Testosterone acetate	I	14.0	9861		
Testosterone	IV	4.1	—	0.03	—
Androstenedione	I	4.2	—		
Testosterone acetate	I	3.9	—		
5 α -Androstenedione	II	1255	520	11.1	3.6
5 α -Androstane-3 β ,17 β -diol	VI	1283	499		
5 α -Androstane-3 β ,17 β -diol diacetate	III	1374	539		
Androsterone	I	2467	644	21.9	4.3
Androsterone acetate	VII	2571	614		
5 α -Androstenedione	II	2534	632		
Epiandrosterone	I	3594	2289	30.9	15.1
Epiandrosterone acetate	VII	3523	2200		
5 α -Androstenedione	II	3822	2121		
5 α -Androstane-3 α ,17 β -diol	VI	396	—	3.2	—
5 α -Androstenedione	II	382	—		
5 α -Androstane-3 β ,17 β -diol	VI	366	—		
5 α -Androstane-3 β -17 β -diol	VI	750	—	6.7	—
5 α -Androstenedione	II	790	—		
5 α -Androstane-3 β ,17 β -diol diacetate	III	821	—		
11 β -Hydroxyandrostenedione	IV	616	36.7	5.1	0.3
11 β -Hydroxytestosterone	V	609	36.1		
Adrenosterone	IV	574	38.6		
11 β -Hydroxyandrosterone	V	94.1	—	0.74	—
11 β -Hydroxyandrosterone acetate	IV	88.4	—		
5 α -Androstane-3,11,17-trione	VIII	92.6	—		
11 β -Hydroxyepiandrosterone	V	352	—	2.9	—
11 β -Hydroxyepiandrosterone acetate	IV	378	—		
5 α -Androstane-3,11,17-trione	VIII	361	—		

in the proportions of the metabolites produced, the radioactivity being largely associated with androstenedione (Table 2).

A series of incubations with adrenal tissue from rats aged approximately 4 months and 15 months of both sexes of the Sprague-Dawley, Wistar and Osborne-Mendel strains showed no significant differences from one another in the metabolism of [7α-³H]dehydroepiandrosterone. [4-¹⁴C]Pregnenolone was incubated simultaneously with the [7α-³H]dehydroepiandrosterone in this series of experiments and again no marked differences between the strains, ages or sexes were observed.

When tissue from the tumour 494 was incubated with [7α-³H]pregnenolone a different pattern of metabolism was observed (Table 3). Approximately half of the substrate remained unchanged and most of the remainder was associated with deoxycorticosterone. By comparison, in an incubation of adrenal tissue from 4-month-old Sprague-Dawley rats, little substrate, progesterone or deoxycorticosterone was left (1%) and 50.7% of the radioactivity was associated with corticosterone (Table 3).

Identification of the metabolites of [7α-³H]dehydroepiandrosterone produced by adrenal tissue from female LACA mice was performed in a manner similar to that described for the rat. Paper chromatography of the neutral steroids in the PB21 system (3.5h) showed a similar tripartite distribution of radioactivity but on thin-layer chromatography in system IV most of the material of the least polar zone

was found to be associated with aetiocholanolone. The other androstanolones were detected after acetylation and t.l.c. in system VII and hydrolysis and rechromatography in system IV.

The acylable components of the mid-polar zone contained testosterone, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol. Non-acylatable material after oxidation was chromatographed in system VIII, after which two further regions of radioactivity were detected. These were associated with 5α-androstane-3,11,17-trione and 5β-androstane-3,11,17-trione and it was concluded that the incubation had contained steroids that were probably 11β-hydroxy-5α-androstanedione and 11β-hydroxy-5β-androstanedione. The most polar zone contained 11β-hydroxyandrostenedione in addition to small quantities of 11β-hydroxyaetiocholanolone, 11β-hydroxyandrosterone and 11β-hydroxyepiandrosterone.

Again, the above experiment was repeated, 500 μg of each steroid identified above being added as carrier compounds at the end of the incubation period. The conversion of [7α-³H]dehydroepiandrosterone into these compounds was thus estimated (Table 4) and, in contrast with the rat adrenal incubation in which only 5α-reduced steroids were found, 5α- and 5β-isomers were present although the major proportion was of the 5β-series. The results of one incubation with adrenal tissue from female NH mice (Table 4) revealed differences in the proportions of [7α-³H]dehydroepiandrosterone

Table 3. Conversion into various metabolites of 2 μCi of [4-¹⁴C]pregnenolone by a homogenate of 150 mg of adrenal tissue from 4-month-old Sprague-Dawley rats and of 10 μCi of [7α-³H]pregnenolone by a homogenate of 150 mg of tissue from Snell adrenocortical carcinoma 494 incubated for 90 min

Steroid investigated and derivative formed	Final solvent system	Specific radioactivities (d.p.m./nmol)		% of radioactivity found in isolated steroids	
		Rat adrenal tissue	Snell carcinoma 494	Rat adrenal tissue	Snell carcinoma 494
Pregnenolone	IV	18.7	7880	0.6	42.2
20β-Hydroxypregnenolone	IV	17.9	7804		
Pregnenolone	X	18.5	8176		
Progesterone	I	6.4	859	0.2	4.5
20β-Hydroxyprogesterone	IV	6.1	845		
20β-Acetoxyprogesterone	I	6.0	822		
Deoxycorticosterone	IV	5.8	8421	0.2	43.1
Deoxycorticosterone acetate	I	5.6	8632		
20β,21-Dihydroxypregn-4-en-3-one	V	5.9	8384		
Corticosterone	V	1844	635	50.7	3.1
Corticosterone acetate	IV	1808	670		
11β,20β,21-Trihydroxypregn-4-en-3-one	XI	1797	622		

Table 4. Conversion of 10 μ Ci of [7 α - 3 H]dehydroepiandrosterone into C_{19} -steroid metabolites by homogenates of 50 mg of adrenal tissue from female LACA and NH mice incubated for 90 min

— indicates that the steroid was not examined.

Steroid investigated and derivative formed	Final solvent system	Specific radioactivities (d.p.m./nmol)		% of radioactivity found in isolated steroids	
		LACA-mouse adrenal tissue	NH-mouse adrenal tissue	LACA-mouse adrenal tissue	NH-mouse adrenal tissue
Dehydroepiandrosterone	I	42.5	161	0.28	1.15
Androst-5-ene-3 β ,17 β -diol	IV	39.1	173		
Dehydroepiandrosterone acetate	VII	40.9	171		
Androstenedione	I	45.1	3252	0.30	22.0
Testosterone	IV	45.9	3101		
Testosterone acetate	I	41.9	3331		
Testosterone	IV	5.6	374	0.04	2.54
Androstenedione	I	5.9	370		
Testosterone acetate	I	5.6	378		
5 α -Androstenedione	II	80.0	140	0.51	0.97
5 α -Androstane-3 β ,17 β -diol	VI	74.3	144		
5 α -Androstane-3 β ,17 β -diol diacetate	III	74.2	144		
Androsterone	I	273	531	1.90	3.4
Androsterone acetate	VII	288	502		
5 α -Androstenedione	II	277	483		
Epiandrosterone	I	189	469	1.31	3.1
Epiandrosterone acetate	VII	196	471		
5 α -Androstenedione	II	193	475		
5 α -Dihydrotestosterone	IV	50.9	32.5	0.35	0.22
5 α -Androstenedione	II	49.5	29.9		
5 α -Androstane-3 β ,17 β -diol	VI	52.7	32.5		
5 α -Androstane-3 α ,17 β -diol	VI	169	27.5	1.15	0.19
5 α -Androstenedione	II	169	28.6		
5 α -Androstane-3 β ,17 β -diol diacetate	III	174	28.1		
5 α -Androstane-3 β ,17 β -diol	VI	146	58.4	0.96	0.39
5 α -Androstenedione	II	137	59.7		
5 α -Androstane-3 β ,17 β -diol diacetate	III	146	57.7		
5 β -Androstenedione	II	76.3	2957	0.50	20.0
5 β -Androstane-3 α ,17 β -diol	IX	75.2	3025		
5 β -Androstane-3 α ,17 β -diol diacetate	III	70.9	2902		
Aetiocholanolone	IV	5823	1649	41.1	11.4
Aetiocholanolone acetate	VII	6165	1690		
5 β -Androstenedione	II	6176	1681		
Epiaetiocholanolone	IV	53.4	2238	0.36	15.6
Epiaetiocholanolone acetate	VII	54.0	2240		
5 β -Androstenedione	II	52.2	2405		
5 β -Dihydrotestosterone	IV	78.2	191	0.56	1.28
5 β -Androstenedione	II	86.7	193		
5 β -Androstane-3 α ,17 β -diol	IX	80.6	179		
11 β -Hydroxyandrostenedione	IV	3275	628	20.2	4.20
11 β -Hydroxytestosterone	V	3056	673		
Adrenosterone	IV	3023	694		

Table 4 (*continued*)

11 β -Hydroxyandrosterone	V	40.2	—	}	0.27	—
11 β -Hydroxyandrosterone acetate	IV	43.6				
5 α -Androstane-3,11,17-trione	VIII	40.8				
11 β -Hydroxyepiandrosterone	V	247	—	}	1.69	—
11 β -Hydroxyepiandrosterone acetate	IV	270				
5 α -Androstane-3,11,17-trione	VIII	262				
11 β -Hydroxyaetiocholanolone	V	126	—	}	0.83	—
11 β -Hydroxyaetiocholanolone	IV	135				
5 β -Androstane-3,11,17-trione	VIII	124				

metabolites, but the compounds formed were identical with those produced by adrenal tissue from LACA mice.

Discussion

Although the spatial integrity of the cell is destroyed in homogenized tissue, such a preparation was used in these investigations so that comparative studies could be readily undertaken. Further, it is recognized that the cofactor medium included a system for the maintenance of a high concentration of NADPH that is different from the physiological state. Under these conditions the conversion of [7 α -³H]cholesterol, but not [4-¹⁴C]pregnenolone, into C₁₉-steroids by rat adrenal tissue (Table 1) supports the evidence of Jungmann (1968) for the existence of a pathway for dehydroepiandrosterone production from cholesterol without pregnenolone as an intermediate. However, the results of Burstein *et al.* (1971) caused them to conclude that pregnenolone was important in the biosynthesis of all rat adrenal steroid hormones. Throughout these studies, extremely low conversions were obtained for certain steroids. The biological significance of these values must be uncertain, but the constancy of the specific radioactivities of steroid and derivatives was taken as a further indication of the presence of the compounds in question.

The metabolites of [7 α -³H]dehydroepiandrosterone produced by rat adrenal tissue under the conditions used in the present studies were principally ring-A-reduced compounds of the 5 α -series (Table 2). With the exception of 11 β -hydroxyandrostenedione (5.1%) only small amounts of Δ^4 -3-oxo steroids were detected (<0.2%). Similarly production of 11 β -hydroxylated steroids was very small (approx. 10% of all metabolites investigated) but 3 α - and 3 β -hydroxy steroid dehydrogenases were both highly active, over 50% of the radioactivity being associated with androsterone, epiandrosterone and their 11 β -hydroxylated derivatives.

No significant difference in the activities of steroid-metabolizing enzymes was detected between strains, sexes or ages (up to 15 months) in rats of the Sprague-Dawley, Wistar or Osborne-Mendel strains. However, tissue from the transplantable adrenocortical tumour 494, which arose originally in a female Osborne-Mendel rat (Snell & Stewart, 1959) showed a greatly decreased capacity for 5 α -reduction as 68.0% of the radioactivity was associated with androstenedione. Pregnenolone metabolism was also greatly decreased (Table 3) owing to a markedly low 11 β -hydroxylase activity, a finding consistent with previous reports (Johnson *et al.*, 1961; Sweat & Bryson, 1964).

In contrast with the situation found in the strains of rats examined, there were substantial differences in the proportions of the metabolites produced by adrenal tissue from female NH-strain mice, to that produced by adrenal tissue from female LACA-strain mice. These differences are particularly noticeable in the ratio of 3 α - to 3 β -hydroxylated steroids. Thus approximately equal quantities of aetiocholanolone and epiaetiocholanolone were produced by NH mouse adrenal tissue, but very little epiaetiocholanolone by adrenal tissue from LACA mice (Table 4). The metabolism of androstenedione was also different, 22% of the radioactivity being associated with this steroid in the NH mouse adrenal incubation in contrast with 0.5% in the incubation with LACA mouse adrenal tissue. Further evidence is necessary to assess the significance of these differences, in particular of their relationship to the development of adrenocortical neoplasia.

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