Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 277–284. Pergamon Press. Printed in Great Britain

METABOLISM OF DEHYDROISOANDROSTERONE AND ANDROSTENEDIONE BY THE HUMAN LUNG *IN VITRO*

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(*Received* 15 *August* 1976)

SUMMARY

3β-Hydroxysteroid oxidoreductase-$\Delta^{(5\rightarrow4)}$-isomerase and 7α-hydroxylase activities are present in human lung and this is reflected in the rates of formation at initial velocities of androstenedione (~110 pmol/100 mg protein/h) and of 7α-hydroxydehydroisoandrosterone (~100 pmol/100 mg protein/h) as the principal metabolites obtained from incubations of human lung slices with [7-^{3}H]-dehydroisoandrosterone. The 5α-reduced metabolites 5α-androstanedione and androsterone were formed with total conversion rates of ~75 pmol/100 mg protein/h, and in addition, the 17β-hydroxysteroids 5-androstene-3β,17β-diol and testosterone were also formed (~25 pmol/100 mg protein/h and ~5 pmol/100 mg protein/h, respectively). The incubation of human lung slices with [1,2,6,7-^{3}H]-androstenedione resulted in the formation of 5α-androstanedione, androsterone and isoandrosterone with total conversion rates of ~44 pmol/100 mg protein/h, and of testosterone (~22 pmol/100 mg protein/h). 5β-Reduced-C_{19}-steroids were not identified among the metabolites. The human lung is a potential site for the conversion of circulating dehydroisoandrosterone to androstenedione, to testosterone and to 5α-reduced steroids. The resulting steroids may exert their activities *in situ* or elsewhere, conceivably after further metabolism to more potent androgens.

INTRODUCTION

Considering its massive blood supply and large capillary surface area, the lung may inactivate many substances in the pulmonary circulation or, alternatively, may serve as a metabolic source of more biologically-active compounds that could exert their activity systemically [1]. Huhtaniemi [2] carried out *in vitro* studies with minces of 11–17 week fetal human lung demonstrating the extensive metabolism of dehydroisoandrosterone and the presence of sulfokinase, 7α-hydroxylase, 16α-hydroxylase, and 17β-hydroxysteroid oxidoreductase enzymes in these tissues. Siiteri and Wilson [3] found that lung tissue obtained from human fetuses transformed testosterone to dihydrotestosterone,† thus establishing the presence of 5α-reductase activity in lung at early stages of development. Perfusion studies have been reported which indicate that the midterm human placenta may secrete androstenedione, which is partially converted by the fetus to testosterone [4, 5]. From a consideration of these studies it was inferred that one of the sites for this conversion was the fetal lung; however, the reverse reaction, *viz.* testosterone to androstenedione, was apparently favored [5]. Furthermore, from these studies it was suggested that the major metabolites of androstenedione in the fetal human lung were 5α-androstanedione and androsterone.

Steroid metabolism in adult human lung tissue has not been studied. To investigate the possibility that the human lung may serve a significant role in the extraglandular conversion of dehydroisoandrosterone and androstenedione to more potent androgens, *viz.* testosterone and dihydrotestosterone (6–9), we incubated adult human lung slices with tritium-labeled dehydroisoandrosterone and androstenedione, and characterized the metabolites in time course experiments.

EXPERIMENTAL

A sample of normal lung tissue was obtained from the middle lobe of a 57 year old white woman undergoing right pneumonectomy for squamous cell carcinoma confined to the right lower lobe. The lung tissue was rinsed in chilled 0.9% sodium chloride solution, blotted, and cut into slices 1.5 mm thick weighing approximately 100 mg each. These slices

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† The following trivial names and abbreviations are used: 5α-androstanedione, 5α-androstane-3,17-dione; dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one; isoandrosterone, 3β-hydroxy-5α-androstan-17-one; 7α-hydroxydehydroisoandrosterone, 3β,7α-dihydroxy-5-androsten-17-one; 7β-hydroxydehydroisoandrosterone, 3β,7β-dihydroxy-5-androsten-17-one; 19-hydroxydehydroisoandrosterone, 3β,19-dihydroxy-5-androsten-17-one; 16α-hydroxydehydroisoandrosterone, 3β,16α-dihydroxy-5-androsten-17-one.

were used for incubation. Approximately one hour elapsed between the surgical removal of the tissue and the beginning of the incubations.

Solvents and reagents. Anhydrous ethyl ether, petroleum ether (20°C–40°C), acetone of analytical reagent grade, and ethyl acetate, 2,2,4-trimethylpentane (isooctane), methanol, acetone, dichloromethane and chloroform of nanograde quality were obtained from Mallinckrodt Chemical Works. Ethylene glycol (chromatoquality) was obtained from Matheson Coleman & Bell, and celite was purchased from Johns–Manville Co.

Steroid sources. [1,2,6,7-^{3}H]-Androstenedione (S.A. 85 Ci/mmol) was synthesized [10]. [1,2,6,7-^{3}H]-Testosterone (S.A. 85 Ci/mmol), [7-^{3}H]-dehydroisoandrosterone (S.A. 10 Ci/mmol), [4-^{14}C]-testosterone and [4-^{14}C]-androstenedione (S.A. 55 mCi/mmol), [4-^{14}C]-dehydroisoandrosterone, [4-^{14}C]-5α-dihydrotestosterone, [4-^{14}C]-estrone and [4-^{14}C]-estradiol (S.A. 50 mCi/mmol) were obtained from New England Nuclear. The tritiated steroids were purified by column chromatography on celite–ethylene glycol as described below. The following ^{14}C labeled steroids with a specific activity of 50 mCi/mmol were synthesized*: [4-^{14}C]-5α-androstanedione, [4-^{14}C]-androsterone, [4-^{14}C]-isoandrosterone and [4-^{14}C]-5-androstene-3β,17β-diol. In brief, [4-^{14}C]-androstanedione was prepared by oxidation of [4-^{14}C]-dihydrotestosterone with Jones reagent [11]; [4-^{14}C]-isoandrosterone was prepared from [4-^{14}C]-dihydrotestosterone using a synthetic sequence that involved the following steps: (a) reaction with dihydropyran in presence of *p*-toluenesulfonic acid to give [4-^{14}C]-dihydrotestosterone-17β-tetrahydropyranyl ether; (b) reduction with sodium borohydride to give [4-^{14}C]-3β-hydroxy-5α-androstane-17β-tetrahydropyranyl ether; (c) acetylation with pyridine-acetic anhydride to give [4-^{14}C]-3β-acetoxy-5α-androstane-17β-tetrahydropyranyl ether; (d) acid hydrolysis with methanol–hydrochloric acid to yield [4-^{14}C]-3β-acetoxy-5α-androstan-17β-ol; (e) oxidation with Jones reagent [11] to give [4-^{14}C]-isoandrosterone acetate; and (f) hydrolysis with a solution of potassium hydroxide in methanol water to yield [4-^{14}C]-isoandrosterone. The [4-^{14}C]-androsterone was prepared by the enzymatic reduction of [4-^{14}C]-5α-androstanedione using purified rat prostate 3α-hydroxy-steroid oxidoreductase in the presence of NADPH [12]. [4-^{14}C]-5-Androstene-3β,17β-diol was synthesized by sodium borohydride reduction of [4-^{14}C]-dehydroisoandrosterone in isopropanol. Commercially available nonradioactive steroids were obtained from Steraloids, Inc. 7β-Hydroxydehydroisoandrosterone, 7α-hydroxydehydroisoandrosterone, 7α-hydroxydehydroisoandrosterone diacetate, and 19-hydroxydehydroisoandrosterone from the M.R.C. Steroid Reference Collection were generously provided by Professor W. Klyne and Dr. D. N. Kirk.

Incubation procedure. Each tissue aliquot was incubated in a 25 × 150 mm Teflon-capped tube with a standard incubation mixture that consisted of either (1) [1,2,6,7-^{3}H]-androstenedione (114 nM, containing 5.31×10^7 c.p.m.), glucose (3.7 mM) and Krebs–Ringer phosphate buffer, pH 7.4, in a total vol. of 2.5 ml, or (2) [7-^{3}H]-dehydroisoandrosterone (142 nM, containing 7.83×10^6 d.p.m.), glucose (3.7 mM) and Krebs–Ringer phosphate buffer, pH 7.4, in a total vol. of 2.5 ml. No cofactors were added. The incubations were carried out using concentrations of labeled androstenedione and dehydroisoandrosterone that exceeded the physiological plasma levels of these hormones 22–32 times and 6 times respectively. These concentrations were chosen arbitrarily to minimize the effect of possible endogenous hormones present in the lung slices.

Incubations containing tissue heated in boiling water for 10 min and incubation mixtures as above were used as the blanks. The samples were gassed for 20 s with a mixture of oxygen–carbon dioxide (95:5), the tubes were capped, and incubated at 37 C with shaking for varying periods of time from 0–4 h. The reactions were stopped by immersing the tubes in an ice slurry and adding 20 ml of a mixture of chloroform–methanol (2:1). The following carbon-14 marker steroids (~5000 c.p.m. each) were added and thoroughly mixed with the reaction mixtures: [^{14}C]-5α-androstanedione, [^{14}C]-dihydrotestosterone, [^{14}C]-estradiol and [^{14}C]-estrone; to the samples obtained from incubations with [^{3}H]-dehydroisoandrosterone, [^{14}C]-dehydroisoandrosterone was also added. The chloroform layers were transferred to clean tubes and the remaining aqueous–tissue mixtures were reextracted with 20 ml chloroform. The pooled chloroform extracts were backwashed twice with 5 ml water and evaporated with nitrogen at 40°C, and the residues were redissolved in 10 ml ethyl acetate. Chilled acetone (3 vols) was added to the residual tissues and the mixtures were left at 4°C for 15 h. After centrifugation, the residual pellets were digested with 2.5 N sodium hydroxide solution and protein was determined by the method of Lowry *et al.*[13].

Steroid separation techniques. One-tenth aliquots of the extracted samples were set aside for thin-layer chromatographic (t.l.c.) analysis, and aliquots thereof were mixed with the following authentic carrier steroids (20 μg each): 5α-androstanedione, androstenedione, androsterone, isoandrosterone, dihydrotestosterone, testosterone, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol. To the samples obtained from incubations with [7-^{3}H]-dehydroisoandrosterone 20 μg of dehydroisoandrosterone was also added. The mixtures were taken to dryness with nitrogen and redissolved in 50 μl chloroform. The samples were spotted on 20 × 20 cm thin-layer plastic sheets (Polygram Sil G-HY, 0.25 mm thick; Brinkman Instru-

* Milewich, L. and H. J. Schweikert. *J. Labelled Compds. Radiopharmaceuticals* (In press).

ments, Inc.) and developed using the solvent systems chloroform–methanol (99.7 : 0.3, v/v, 10 ascents) or methylene chloride–ethyl acetate–methanol (85:15:1, by vol., 1 ascent). The plates were air dried. The carrier steroids were visualized with a water spray or by staining with an acid spray consisting of acetic acid (100 ml), sulfuric acid (2 ml) and anisaldehyde (1 ml) followed by heating at 105°C, and then were assessed for radioactivity as described previously [14].

For column chromatographic separation the remaining extracts [9, 10] were transferred to celite–ethylene glycol columns and eluted as described [15]. Three-ml fractions were collected, and 0.3 ml aliquots of alternate fractions were counted in mini-vials with a Packard Tri Carb Liquid Scintillation Spectrometer Model 3300, using 4 ml of a mixture made of 15.0 g Omnifluor (New England Nuclear), 3.8 l toluene and 76 ml methanol as the scintillation fluid.

The identity of some of the labeled metabolites obtained from the incubations of lung with [^{3}H]-androstenedione and with [^{3}H]-dehydroisoandrosterone was further confirmed by subjecting appropriately pooled fractions obtained by column chromatography to additional thin-layer chromatographic steps, either as free steroids as described above, or as the corresponding acetate derivatives using for development the solvent system methylene chloride–ethyl acetate (99:1, v/v, 3 ascents).

Quantification of steroid metabolites. In addition to metabolite quantification by t.l.c. as described [14], in the experiment involving incubation with [^{3}H]-dehydroisoandrosterone quantification was also achieved by integration of the peaks of radioactivity obtained by column chromatography, expressing the values obtained for each peak as a fraction of the total radioactivity. This was done in this particular experiment because most ot the peaks were resolved, except for dehydroisoandrosterone and isoandrosterone, which could not be separated (data not shown).

Some of the metabolites isolated by column chromatography were further characterized and quantitated by crystallization to constant ^{3}H:^{14}C ratio as follows. To the isolated bands of radioactivity containing the metabolites 5α-androstanedione, androstenedione, androsterone, isoandrosterone, 5-androstene-3β,17β-diol and testosterone, authentic ^{14}C labeled steroids: [^{14}C]-androstenedione (10,000 c.p.m.), [^{14}C]-androsterone (1,300 c.p.m.), [^{14}C]-isoandrosterone (1,300 c.p.m.), 5-androstene-3β,17β-diol (1,200 c.p.m.) and testosterone (2,500 c.p.m.) and 100 μg each of the corresponding non-radioactive steroids were added. The metabolites were purified using either two or three consecutive thin-layer chromatographies as follows. 5α-Androstanedione: t.l.c., chloroform–methanol (99.5:0.5, v/v, 1 ascent); t.l.c. II, ethyl ether–isooctane (75:25, v/v, 1 ascent). Androstenedione: t.l.c. I, chloroform–methanol (300:1, v/v, 3 ascents); t.l.c. II, methylene chloride–ethyl acetate–methanol (85:15:1, v/v, 1 ascent). Androsterone: t.l.c. I, chloroform–methanol (300:1, v/v, 3 ascents); t.l.c. II, methylene chloride–ethyl acetate (95:5, v/v, 5 ascents); t.l.c. III, methylene chloride–ethyl ether (95:5, v/v, 5 ascents). Isoandrosterone: t.l.c. I, methylene chloride–ethyl acetate (95:5, v/v, 5 ascents). The isoandrosterone samples were acetylated after the first t.l.c. and then rechromatographed twice: t.l.c. II, methylene chloride–ethyl acetate (99:1, v/v, 3 ascents); t.l.c. III, methylene chloride–ethyl ether (95.5:0.5, v/v, 5 ascents). 5-Androstene-3β,17β-diol: t.l.c. I, benzene–ethyl ether (2:1, v/v, 1 ascent); after acetylation: t.l.c. II, methylene chloride–ethyl ether (97:3, v/v, 1 ascent); t.l.c. III, methylene chloride–ethyl acetate (98:2, v/v, 1 ascent). Testosterone: t.l.c. I, ethyl acetate–isooctane (1:1, v/v, 1 ascent); after acetylation: t.l.c. II, methylene chloride–ethyl ether (96:4, v/v, 1 ascent); t.l.c. III, methylene chloride–ethyl acetate (94:6, v/v, 1 ascent). The steroid markers were detected using a water spray and the areas containing the steroids were scraped and eluted with 5 ml of ethyl acetate, and 0.1 ml aliquots were counted for radioactivity at every t.l.c. purification step. After reaching constant ^{3}H:^{14}C ratios, carrier steroids (40 mg) were added to the corresponding metabolites and the samples were crystallized 5 times (Tables 1 and 2). The following solvent systems were used for crystallization: 5α-androstanedione, ethyl acetate–petroleum ether; androstenedione and androsterone, acetone–petroleum ether; isoandrosterone acetate, methanol; testosterone acetate and 5-androstene-3β,17β-diol diacetate, ethyl ether–petroleum ether.

RESULTS

T.l.c. of aliquots of chloroform extracts obtained from the incubations with [^{3}H]-androstenedione, indicated that tritiated 5α-androstanedione, androsterone, isoandrosterone and testosterone, but not dihydrotestosterone, 5α-androstane-3α,17β-diol or 5α-androstane-3β,17β-diol were formed. A polar metabolite that migrated the closest to the origin on t.l.c. was also detected on column chromatography; this metabolite, however, has not been identified.

By t.l.c. of the incubation products of [^{3}H]-dehydroisoandrosterone we found that radioactivity was associated with the following carrier steroids; 5α-androstanedione, androstenedione, androsterone, dehydroisoandrosterone (which could not be separated from isoandrosterone), testosterone, 5-androstene-3β,17β-diol and with some other more polar metabolites. By column chromatography we also found a major polar metabolite.

The t.l.c. findings, using the total metabolite mixtures, were substantiated by results obtained following column chromatographic separation. The ^{14}C steroid markers, added for monitoring the relative positions of the metabolites when changing the eluting solvent systems [15], indicated that there was coincidence of ^{3}H and ^{14}C radioactivity only with 5α-androstanedione, and not between any other ^{3}H

Table 1. Radiochemical homogeneity criteria used for the characterization of metabolites isolated from incubations of [1,2,6,7-^{3}H]-androstendione with human lung slices, and their quantitation*

Metabolite	Time of incubation† (min)	^{3}H:^{14}C ratios: Thin layer chromatography I	II	III	Crystallization ML1	ML2	ML3	Final crystals	pmol of metabolite per 100 mg protein: From crystallization data	From t.l.c.‡ data	% of substrate converted to metabolites§
5α-Androstanedione	0	—	—	—	—	—	—	—	—	—	—
	5	0.23	0.32	—	0.36	0.32	0.29	0.29	0.7	0.7	0.03
	30	4.18	4.08	—	3.92	3.87	3.88	3.87	11.2	14.0	0.34
	60	16.2	14.6	—	14.4	14.2	14.1	14.5	32.6	33.0	1.24
	120	31.9	29.7	—	29.1	28.5	28.9	29.2	70.6	73.0	2.51
	240	75.9	70.1	—	69.8	66.0	68.7	68.8	150	177	5.92
Androsterone	0	—	—	—	—	—	—	—	—	—	—
	5	4.14	3.06	3.04	3.07	2.66	2.54	2.42	1.1	—	0.04
	30	12.3	11.4	9.31	9.36	9.15	8.01	8.24	4.4	—	0.13
	60	17.7	19.4	20.3	17.8	18.4	19.1	18.8	7.5	—	0.28
	120	26.4	23.2	21.8	26.3	24.9	24.3	24.3	10.8	—	0.38
	240	67.5	59.1	62.2	63.5	62.9	63.9	67.1	24.6	—	0.97
Isoandrosterone	0	1.40	—	—	—	—	—	—	—	—	—
	5	1.20	0.08	—	0.10	0.10	0.04	0.10	0.0001	—	—
	30	6.70	6.96	5.47	5.38	4.95	5.31	5.59	1.0	—	0.03
	60	31.4	30.9	23.0	25.6	25.5	24.5	25.3	3.6	—	0.14
	120	66.8	70.9	57.2	53.6	50.2	55.2	54.6	8.2	—	0.29
	240†	—	—	—	—	—	—	—	—	—	—
Testosterone§§	0	1.29	—	—	—	—	—	—	—	—	—
	5	3.11	1.85	1.69	1.99	1.96	1.97	2.08	0.67	0.7	0.02
	30	24.7	22.1	23.9	24.7	22.8	22.7	23.3	10.8	17.0	0.30
	60	65.3	58.4	54.3	62.9	61.7	57.9	60.7	22.3	26.0	0.77
	120	128	124	116	133	123	120	133	50.1	40.0	1.62
	240	152	151	150	163	154	151	155	55.2	66.0	1.98

* Following celite column chromatography the corresponding bands of radioactivity were purified using two or three consecutive thin layer chromatographies. After addition of non-radioactive carriers the samples were crystallized 5 times. The ^{3}H:^{14}C ratios of the last 3 mother liquors and final crystals are reported. † Sample lost. ‡ Androsterone and isoandrosterone were not separated by t.l.c. and were measured together (See text). § Calculated from the crystallization data. §§ After the first t.l.c. testosterone was acetylated and further purified and crystallized as the acetate derivative.

Table 2. Radiochemical homogeneity criteria used for the characterization of metabolites isolated from incubations of [7-^{3}H]-dehydroisoandrosterone with human lung slices, and their quantitation*

Metabolite	Time of incubation† (min)	^{3}H:^{14}C ratios: Thin layer chromatography I	II	III	Crystallization ML1	ML2	ML3	Final crystals	pmol of metabolite per 100 mg protein: From crystallization data	From t.l.c. data	% of substrate converted to metabolites§
Androstenedione	0	—	—	—	—	—	—	—	—	—	—
	5	0.23	0.06	—	0.07	0.08	0.02	0.07	0.45	1.0	0.01
	30	10.2	9.50	—	9.56	9.44	9.01	9.41	58.7	55.0	1.61
	120	68.2	66.5	—	66.5	64.6	64.9	65.6	439	260	11.4
	240	153	147	—	148	141	142	146	986	810	25.3
5α-Androstanedione	0	—	—	—	—	—	—	—	—	—	—
	5	—	—	—	—	—	—	—	—	—	—
	30	0.66	0.50	—	0.56	0.54	0.42	0.60	9.1	8.0	0.25
	120	4.54	4.14	—	4.01	4.15	4.29	4.22	76.1	75.0	1.99
	240	9.67	8.63	—	9.16	9.22	8.89	9.35	170	160	4.36
Androsterone	0	—	—	—	—	—	—	—	—	—	—
	5	—	0.42	—	0.46	0.41	0.31	0.41	0.7	1.0	0.02
	30	11.3	11.1	—	10.9	11.1	11.1	11.2	16.3	12.0	0.46
	120	71.1	58.8	—	57.5	55.5	58.7	58.7	90.5	88.0	2.36
	240‡	—	—	—	—	—	—	—	—	—	—
5-Androstene-3β,17β-diol§§	0	—	—	—	—	—	—	—	—	—	—
	5	0.71	0.55	0.66	0.63	0.62	0.60	0.61	1.11	—	0.03
	30	8.73	7.92	7.42	8.20	8.18	7.72	8.06	12.5	—	0.32
	120	35.9	38.5	34.5	34.2	34.9	36.4	35.2	58.6	—	1.39
	240	50.6	46.7	46.7	49.1	49.4	46.7	47.8	81.8	—	1.91
Testosterone§§	0	—	—	—	—	—	—	—	—	—	—
	5	0.11	0.07	—	—	—	—	—	—	—	—
	30	0.27	0.27	0.22	0.20	0.20	0.20	0.31	0.77	0.30	0.22
	120	5.87	6.41	5.41	5.77	5.81	5.73	6.23	21.5	20.0	6.05
	240	12.6	11.9	12.2	11.8	12.8	12.9	13.5	47.2	53.0	13.3

* Following celite column chromatography the corresponding bands of radioactivity were purified using two or three consecutive thin layer chromatographies. After addition of non-radioactive carriers the samples were crystallized 5 times. The ^{3}H:^{14}C ratios of the last 3 mother liquors and final crystals are reported. † The 60 min incubation sample was lost. ‡ Sample lost. § Calculated from the crystallization data. §§ After the first t.l.c., 5-androstene-3β,17β-diol and testosterone were acetylated and further purified and crystallized as the acetate derivatives.

Table 3. [^{3}H]-7α-Hydroxydehydroisoandrosterone isolated from incubations of [7-^{3}H]-dehydroisoandrosterone with human lung slices, and its quantification by t.l.c.*

Metabolite	Time of incubation (min)	pmol of metabolite per 100 mg protein	% of substrate converted to metabolite
7α-Hydroxydehydroisoandrosterone	0	—	—
	5	—‡	—
	30	42	1.2
	120	252	6.6
	240	422	10.8

* See text for experimental procedure. ‡ Sample lost.

peak and any of the added ^{14}C labeled steroids. In order to find the relative positions of the identified metabolites androsterone and isoandrosterone, a mixture of [^{14}C]-5α-androstanedione, [^{14}C]-androsterone, [^{14}C]-isoandrosterone, [^{14}C]-testosterone and [^{3}H]-androstenedione was chromatographed on a celite–ethylene glycol column, as above (data not shown): there was good correspondence between their mobilities and those of the identified tritiated metabolites. Aliquots of the tritiated metabolites isolated by column chromatography, mixed with the corresponding authentic steroids, were developed on t.l.c. as above. This allowed for the further identification of the metabolites 5α-androstanedione, androstenedione, androsterone, isoandrosterone, testosterone and 5-androstene-3β,17β-diol.

The stereochemistry at C-5 of the metabolite 5α-androstanedione was established as follows. Aliquots of the metabolite isolated by column chromatography were mixed with the carrier steroids 5α-androstanedione, 5β-androstanedione and androstenedione and separated by t.l.c. using a mixture of benzene–ethyl acetate (9:1, v/v, 3 ascents) as the developing system. The distribution of radioactivity, determined as indicated above, established that it was associated exclusively with 5α-androstanedione.

Definitive proof for the radiochemical homogeneity of the metabolites 5α-androstanedione, androstenedione, androsterone, isoandrosterone, testosterone and 5-androstene-3β,17β-diol was obtained from data of crystallization to constant ^{3}H:^{14}C ratios (Tables 1 and 2).

The most polar metabolite of [^{3}H]-dehydroisoandrosterone was identified and quantified by t.l.c. using the solvent system ethyl acetate–benzene (3:1, v/v, 3 ascents) (Table 3). Aliquots of the metabolite, isolated by column chromatography, were chromatographed using the carriers 7α-hydroxydehydroisoandrosterone, 7β-hydroxydehydroisoandrosterone, 16α-hydroxydehydroisoandrosterone, 16β-hydroxydehydroisoandrosterone, 5-androstene-3β,16α,17β-triol and 19-hydroxydehydroisoandrosterone. It was found that the radioactivity was associated only with 7α-hydroxydehydroisoandrosterone. An aliquot of the metabolite was acetylated and developed on t.l.c. using the solvent system methylene chloride–ethyl acetate (98:2, v/v, 4 ascents), after mixing with the authentic standard 7α-hydroxydehydroisoandrosterone diacetate; here too, the radioactivity migrated with the carrier compound. Another aliquot of the metabolite [7-^{3}H]-7α-hydroxydehydroisoandrosterone, purified both by column chromatography and by t.l.c., was oxidized using Jones reagent [11]. Aliquots of the water-backwashed ethyl ether extract and of the distilled aqueous phase were counted. The results shown on Table 4 indicate that 82% of the

Table 4. Tritium distribution in [7-^{3}H]-7α-hydroxydehydroisoandrosterone, oxidation experiment*

Metabolite and transformation products	Tritium	
	Total radioactivity recovered (c.p.m.)	%
[7-^{3}H]-7α-hydroxydehydroisoandrosterone	4,446	100
After oxidation:		
Ethyl ether phase	528	11.9
Water phase	3,665	82.4

* A 1/10 aliquot of purified metabolite [^{3}H]-7α-hydroxydehydroisoandrosterone in ethyl acetate was assayed for radioactivity and the remaining 9/10 was evaporated to dryness and redissolved in 1 ml acetone. Fifty μl of Jones reagent (chromium trioxide 1.03 g, concentrated sulfuric acid 0.87 ml, and water 3 ml) was added. The mixture was stirred and left at room temperature for 10 min, and this was followed by the addition of 2.5 ml of water. The solution was extracted 5 times with 10 ml of ethyl ether. The ethyl ether extract was backwashed 6 times with 5 ml of water. The ethyl ether solution was evaporated to dryness and the residue was redissolved in 1 ml of ethyl acetate; 2 × 400 μl aliquots were counted. The residual aqueous layer was backwashed 3 times with 10 ml of ethyl ether and then neutralized with 5% sodium bicarbonate solution. The aqueous layer was distilled, and 3 × 400 μl aliquots of distilled water were counted. All samples were counted using Insta-gel emulsifier (Packard Instrument Company) as the scintillation fluid.

recovered radioactivity was in the water phase while only 12% was in the organic phase extract. This clearly shows that the isolated metabolite [7-^{3}H]-7α-hydroxydehydroisoandrosterone contains most of the tritium (at least 82%) at the 7β-position and that the remaining tritium is randomly distributed at other sites of the steroid nucleus.

The time course results expressing conversion rates which were obtained by using either t.l.c. or column chromatography (data not shown), indicate that the enzymatic activities were approximately linear up to 4 h of incubation. The results obtained by these techniques agreed within 35%. Conversion rates at initial velocities and thus totally dependent upon the substrate concentrations used were derived from data obtained by crystallization to constant ^{3}H:^{14}C ratios which are presented in Tables 1 and 2. These values are minimal since the ^{14}C steroids used for recovery purposes were added to the corresponding tritiated metabolites at a later stage, after column chromatographic separation. This study shows that under the experimental conditions used, adult human lung can convert dehydroisoandrosterone to androstenedione (~110 pmol/100 mg protein/h), 7α-hydroxydehydroisoandrosterone (~100 pmol/100 mg protein/h), androsterone (~45 pmol/100 mg protein/h), 5α-androstanedione (~30 pmol/100 mg protein/h), 5-androstene-3β,17β-diol (~25 pmol/100 mg protein/h), and testosterone (~5 pmol/100 mg protein/h); conversion rates obtained when labeled androstenedione was the substrate were as follows: androsterone (~7.5 pmol/100 mg protein/h), isoandrosterone (~3.6 pmol/100 mg protein/h), 5α-androstanedione (~33 pmol/100 mg protein/h), and testosterone (~22 pmol/100 mg mg protein/h). Protein determination on the residual incubation tissues indicated that it constituted between 8.8% and 9.1% of the wet lung weight.

DISCUSSION

The C_{19}-steroids androstenedione and dehydroisoandrosterone are found in the human peripheral circulation at concentrations of 1.0–1.5 ng/ml and ~7.0 ng/ml, respectively, and are weakly bound to plasma proteins, thus becoming potential substrates for metabolic transformation by the lung. In an attempt to gain some insight into androgen metabolism by the human lung we undertook an *in vitro* study of the metabolism of [^{3}H]-dehydroisoandrosterone and of [^{3}H]-androstenedione in a time course study. The human lung, under the conditions of the experiment, has the capability to metabolize dehydroisoandrosterone by two principal pathways, namely 3β-hydroxysteroid oxidoreductase-$\Delta^{(5\rightarrow4)}$-isomerase to form androstenedione, and 7α-hydroxylase to yield 7α-hydroxydehydroisoandrosterone (Fig. 1). It is noteworthy that the 3β-hydroxysteroid oxidoreductase-$\Delta^{(5\rightarrow4)}$-isomerase system is not expressed in the fetal human lung, as shown earlier by Huhtaniemi[2] in incubations of dehydroisoandrosterone with fetal lung

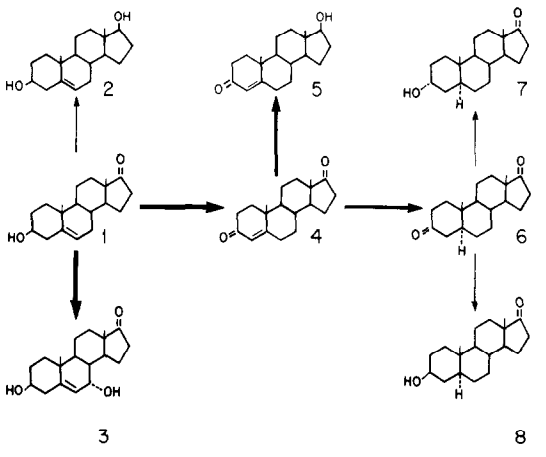

Fig. 1. Biosynthetic sequence in the formation of metabolites of [^{3}H]-dehydroisoandrosterone by human lung slices: (1) dehydroisoandrosterone; (2) 5-androstene-3β,17β-diol; (3) 7α-hydroxydehydroisoandrosterone; (4) androstenedione; (5) testosterone; (6) 5α-androstanedione; (7) androsterone; (8) isoandrosterone.

minces: the characterized metabolites were all C_{19}-5-ene steroids, and no androstenedione or any other 4-ene-3-oxo steroid metabolite was detected. This situation with the fetal human lung appears to be similar to that which prevails with the fetal adrenal gland [16–18], in which this enzymatic activity is also not fully expressed.

The isolation and identification of 7α-hydroxydehydroisoandrosterone was initially puzzling because the substrate [7-^{3}H]-dehydroisoandrosterone supposedly is labeled mainly at the 7α-position. Commercially available [7-^{3}H]-dehydroisoandrosterone has been reported to contain 84–89% of tritium at the C-7 position of which, depending on the batch, between 51–98% is present at the 7α-position and between 2–49% at the 7β-position [19]. Therefore, the substrate, [7-^{3}H]-dehydroisoandrosterone could conceivably contain a high proportion of [7β-^{3}H]-dehydroisoandrosterone. The enzymatic hydroxylation of steroids takes place with retention of configuration [20] thus explaining the formation of [7β-^{3}H]-7α-hydroxydehydroisoandrosterone in our experiments. We showed by an oxidation experiment that the isolated metabolite was labeled with tritium mainly at the 7β-position (Table 4); therefore, if only a fraction of the hydroxylated metabolite were detected (the one containing 7β-^{3}H), the measured conversion rates of ~100 pmol/100 mg protein/h (Table 3) would be a minimum value. Recently Couch *et al.* [21] reported the isolation of [7β-^{3}H]-7α-hydroxydehydroisoandrosterone from incubations of human mammary tissues with [7-^{3}H]-dehydroisoandrosterone. They did not feel that direct tritium retention in the 7α-hydroxylation process took place; however, this appears to have been quite likely. It is noteworthy that 7α-hydroxydehydroisoandrosterone has been isolated as a metabolite of dehydroisoandrosterone in a variety of *in vitro* experiments with human tissues, *viz.*, adrenal

glands [22], liver [24], skin [23], mammary tissue [21], testis [24], epididymis [24], placenta [25, 26], fetal adrenal gland [25], and fetal liver [25]. This metabolite also has been isolated from human urine [27–29]. The presence of the 7α-hydroxylated metabolite supports the findings of Oppelt *et al.*[30] who showed that cytochromes b_5 and P-450, required for hydroxylation, are present in lung microsomes in several species.

The metabolites 5α-androstanedione and androsterone were identified as products of incubation with [^{3}H]-dehydroisoandrosterone. Since it is known that 5α-reduced metabolites are derived from 4-ene-3-oxo-precursors and not by the direct reduction of 3β-OH-5-ene steroids, it may be concluded that androsterone is a transformation product of androstenedione, via 5α-androstanedione (Fig. 1).

The results obtained with adult human lung were in agreement with those reported by Huhtaniemi in studies of human fetal lung, where 5-androstene-3β,17β-diol was isolated as a metabolite of dehydroisoandrosterone [2]. Testosterone was also identified as a minor metabolite of [^{3}H]-dehydroisoandrosterone by adult human lung.

The metabolism of [^{3}H]-androstenedione by the lung gave rise to several products, among which 5α-androstanedione, androsterone, isoandrosterone and testosterone were unequivocally identified. Our findings imply that, under the *in vitro* experimental conditions used, the rate of reduction of 17-oxo-steroids is lower than the rate of oxidation, so that 17-oxo- rather than 17β-hydroxy-steroids are the major metabolites produced by the lung of the human adult. The equilibrium of the 17β-hydroxysteroid oxidoreductase favors the formation of 17-oxo-steroids and the equilibrium of the 5α-reductase reaction favors the formation of 5α-reduced steroids [31, 32]. Therefore, the principal intracellular C_{19}-steroid metabolites in the human lung appear to be dehydroisoandrosterone, androstenedione, 5α-androstanedione, androsterone, isoandrosterone, 5-androstene-3β,17β-diol, testosterone, and 7α-hydroxydehydroisoandrosterone. This pattern of metabolism is considerably different from the one observed in androgen target tissues in which dihydrotestosterone [33] or 5α-androstane-3α,17β-diol [34] are the predominant intracellular androgens in the steady state.

In general, the remaining phenomena noted following incubation of adult human lung slices with dehydroisoandrosterone and with androstenedione were similar. For example, the human lung *in vitro*, exhibited 5α-reductase activity as well as 3β-hydroxysteroid, 3α-hydroxysteroid and 17β-hydroxysteroid oxidoreductase activities, thus indicating that required cofactors were available in the incubations for up to 4 h (Tables 1 and 2).

To what extent the metabolic capabilities demonstrated with human lung slices are operative in the intact perfused organ or *in vivo* remains to be clarified. It is conceivable, however, that the lipid-soluble steroids, loosely bound to proteins, may find their way from plasma in the vascular compartment to distant pulmonary intracellular enzymes, and thus give rise to the local formation of androstenedione and of 5α-reduced steroids as well as of testosterone; these may exert their activities either *in situ* or elsewhere, after being secreted and further metabolized in other glands or peripheral tissues to more potent biological compounds. Adult human lungs weigh between 600 and 1,000 g, with a surface area in capillary bed of about 60–70 m^2; therefore, taking into consideration the *in vitro* data, the mass of tissue, and the blood-endothelium contact area, one could expect the lung to be a potential contributor to circulating levels of steroid hormones.

Acknowledgements—This study was supported by U.S. Public Health Service Grants AM06912 and T101 H D00256 (A.J.W.). The authors wish to thank Dr. Melvin R. Platt for making available the lung tissue used in this experiment.

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