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COMPARISON OF BILIARY METABOLITES OF ANDROSTERONE GLUCURONIDE AND ANDROSTERONE SULPHATE IN FEMALE RATS

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SUMMARY

The metabolic fate of [^{3}H]-androsterone glucuronide and [^{3}H]-androsterone sulphate was investigated in female rats with biliary fistulas. The injected steroids were predominantly excreted in the bile. The [^{3}H]-androsterone glucuronide was excreted rapidly and almost entirely in the unchanged form; very small amounts of 5α-androstane-3α,17β-diol were found in the monoglucuronide fraction. In contrast, [^{3}H]-androsterone sulphate was recovered in small amounts from the bile and the injected sulphate was metabolized to monosulphates of 3α,11β-dihydroxy-5α-androstan-17-one, 5α-androstane-3α,15α,17β-triol and -3α,16α,17β-triol, and disulphates of 5α-androstane-3α,17β-diol, 3α,17β-dihydroxy-5α-androstan-16-one, 5α-androstane-3α,15α,17β-triol and -3α,16α,17β-triol.

INTRODUCTION

Recent studies from this laboratory [1] demonstrated large variations in biliary metabolites of androsterone in female rats. It was found that some rats excreted androsterone metabolites very rapidly into bile (HE rats), whereas other rats excreted the metabolites at a much slower rate (LE rats). The HE rats formed mainly androsterone glucuronide, while the LE rats converted the androsterone to the monosulphates of androsterone and 3α,7α- and 3α,11β-dihydroxy-5α-androstan-17-ones. Siiteri *et al.*[2] administered ^{3}H labelled androsterone glucuronide to humans and found that this conjugate was rapidly eliminated in urine without undergoing further metabolism. Based on these observations, we speculated that the UDP-glucuronyltransferase system might be very active in the HE rat. Thus, the injected androsterone would be rapidly conjugated with glucuronic acid and excreted in the bile. In contrast, low activity of UDP-glucuronyltransferase or high activity of sulphotransferase in the LE rat might result in the further metabolism of androsterone or androsterone sulphate. However, little is known about the metabolism of androsterone glucuronide and androsterone sulphate in the rat.

In the present paper, [^{3}H]-androsterone glucuronide and [^{3}H]-androsterone sulphate were administered intraperitoneally into female rats and the biliary metabolites were isolated and identified by gas chromatography–mass spectrometry.

EXPERIMENTAL

Melting points were determined with a Kofler hot-stage apparatus. Nuclear magnetic resonance spectra (n.m.r.) were measured for solutions in deuteriochloroform with tetramethylsilane as internal standard on a JEOL JNM-MH-100 spectrophotometer. Chemical shifts are expressed in δ (ppm): s, singlet; m, multiplet.

Materials. [1,2-^{3}H]-Androsterone (44.5 Ci/mmol) was purchased from New England Nuclear Corp., and radiochemical purity was confirmed by t.l.c. shortly before use. Androsterone and 3α,11β-dihydroxy-5α-androstan-17-one were obtained from Sigma Chemical Co. Sodium borohydride reduction of androsterone gave 5α-androstane-3α,17β-diol. Preparation of 5α-androstane-3α,15α,17β-triol was described previously [3]. 5α-Androstane-3α,16α,17β-triol was prepared as described by Lieberman *et al.*[4], and 3α,17β-dihydroxy-5α-androstan-16-one by the procedure of Huffman *et al.*[5]. 3β,17β-Dihydroxyandrost-5-en-16-one, 3β,16α-dihydroxyandrost-5-en-17-one and 3β,16β-dihydroxyandrost-5-en-17-one were prepared according to the procedures described by Aoki *et al.*[6], Okada *et al.*[7] and Mattox *et al.*[8], respectively. Sodium androsterone sulphate was synthesized as described by Mumma *et al.*[9]. Sodium salt of androsterone glucuronide was prepared by the procedures reported previously [10].

3α,16α-Dihydroxy-5α-androstan-17-one. Following the procedure described for the synthesis of 3β,16α-diacetoxy-5α-androstan-17-one [11], 5α-androst-16-ene-3α,17-diol diacetate (1.0 g, m.p. 162–165°) [12] was treated with perbenzoic acid and the resultant 16α,17α-epoxy-5α-androstane-3α,17β-diol diacetate was rearranged to the 3α,16α-diacetoxy-17-oxosteroid by chromatography on a column (700 × 40 mm I.D.) of silica gel 60 (Merck, 150 g) using chloroform–acetone (20:1, v/v) as solvent, and crystallized from methanol to give 3α,16α-diacetoxy-5α-androstan-17-one (340 mg), m.p. 165–170° (Found: C, 71.02; H, 8.84. $C_{23}H_{34}O_5$ requires C, 70.74; H, 8.78%). n.m.r. δ 0.81 (3H, s, 19-H_3), 0.95 (3H, s, 18-H_3), 2.02 (3H, s, 3-$OCOCH_3$), 2.09 (3H, s, 16-$OCOCH_3$), 5.02 (1H,

m, 3-H), and 5.42 (1H, m, 16-H). Removal of the acetate groups of 3α,16α-diacetoxy-5α-androstan-17-one (247 mg) was performed in methanol–6N H_2SO_4 (75 ml, 2:1, v/v) [7], and the product was crystallized from acetone to give 3α,16α-dihydroxy-5α-androstan-17-one (119 mg), m.p. 193–201° (Found: C, 74.51; H, 10.14. $C_{19}H_{30}O_3$ requires C, 74.47; H, 9.87%). n.m.r. δ 0.81 (3H, s, 19-H_3), 0.95 (3H, s, 18-H_3), 4.12 (1H, m, 3-H), and 4.44 (1H, m, 16-H). Sodium borohydride reduction of 3α,16α-dihydroxy-5α-androstan-17-one yielded 5α-androstane-3α,16α,17β-triol, identical (mixed m.p., t.l.c., and GC) with an authentic sample.

Sodium salt of [1,2-^{3}*H*]-*androsterone glucuronide.* A mixture of [^{3}H]-androsterone (29.3 μCi), carrier androsterone (3.4 μmol), methyl (tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyran)uronate (250 μmol), and freshly prepared silver carbonate (360 μmol) in anhydrous benzene (3.0 ml) was stirred at 20°C for 16 h. The reaction mixture was filtered, and washed with benzene. The filtrate was evaporated *in vacuo* and purified by t.l.c. on silica gel GF (Merck), using chloroform–acetone (20:1, v/v) as solvent. The radioactive zones were scraped off and steroids eluted with methanol. Evaporation afforded methyl ([^{3}H]-17-oxo-5α-androstan-3α-yl tri-O-acetyl-β-D-glucopyranosid) uronate (14.5 μCi), which was then dissolved in 0.25N NaOH–methanol (3 ml). The reaction mixture was allowed to stand at 20°C for 16 h and evaporated *in vacuo* to dryness. The residue was dissolved in water (5 ml) and passed through a column (700 × 20 mm I.D.) packed with Amberlite XAD-2 resin (100 g). The column was washed with 400 ml of water, followed by 400 ml of methanol. The methanol was evaporated to dryness *in vacuo* and purified by t.l.c. on silica gel GF with solvent system chloroform–isopropanol–formic acid (15:5:3, by vol.). The radioactive zones corresponding to androsterone glucuronide were scraped off and eluted with methanol. Following evaporation of the solvent, the residue was dissolved in 0.25N NaOH (5 ml) and processed with Amberlite XAD-2 resin as described above to give sodium salt of [^{3}H]-androsterone glucuronide (7.2 μCi). The purity was confirmed by adding 10.20 mg of the unlabelled sodium salt of androsterone glucuronide to [^{3}H]-androsterone glucuronide (10.2 × 10^4 d.p.m.) and recrystallizing the mixture from methanol–ether to constant S.A.: 9830 d.p.m./mg, calculated 10,000 d.p.m./mg (98% purity). T.l.c. examination with solvent system chloroform–isopropanol–formic acid (15:5:3, by vol) showed 99% of the radioactivity in the glucuronide fraction. [^{3}H]-Androsterone glucuronide (3.6 μCi) was mixed with unlabelled sodium salt of androsterone glucuronide (20 μmol), dissolved in 1.0 ml of ethanol, and used for animal experiments.

Sodium [1,2-^{3}*H*]-*androsterone sulphate.* A mixture of [^{3}H]-androsterone (29.3 μCi), carrier androsterone (1.7 μmol), and dicyclohexyl carbodiimide (480 μmol) in dimethylformamide (1.5 ml) was treated with 95% H_2SO_4 (10 μl) in dimethylformamide (1.0 ml) for 20 min with cooling in ice water, followed by purification on a DEAE-cellulose column (90 × 20 mm I.D., chloride form) and Dowex-50 column (300 × 10 mm I.D., sodium form), as described for the unlabelled sulphate [9]. The purified product contained 11.0 μCi of radioactivity. The purity was determined by adding 12.17 mg of unlabelled sodium androsterone sulphate to [^{3}H]-androsterone sulphate (9.71 × 10^4 d.p.m.) and recrystallizing the mixture from methanol–ether to constant S.A.: 7510 d.p.m./mg, calculated 7980 d.p.m./mg (94% purity). Examination by t.l.c. on silica gel GF, using ethyl acetate–methanol (3:1, v/v) as solvent, revealed the appearance of 99% of the radioactivity in the sulphate fraction. [^{3}H]-Androsterone sulphate (4.15 μCi) was mixed with unlabelled sodium androsterone sulphate (26 μmol), dissolved in 1.0 ml of ethanol, and used for animal experiments.

Conditions of animal experiments. The common bile duct was cannulated in female rats of the Wistar strain (180–210 g: Matsumoto Experimental Animal Lab., Tokyo, Japan) as described previously [1]. After operation, the rats were kept in restraining cages with free access to water and food pellets. Ethanol solution of [^{3}H]-androsterone glucuronide (0.25 ml, 0.90 μCi, 5.11 μmol) or [^{3}H]-androsterone sulphate (0.20 ml, 0.83 μCi, 5.24 μmol) was diluted with 0.05 or 0.10 ml of saline, respectively, and injected intraperitoneally 18–20 h after operation into female rats. Bile was collected at 0–1, 1–2, 2–4, 4–6, 6–24, and 24–48 h.

Extraction and purification of biliary metabolites. The bile sample collected for 0–24 h was processed separately for each female rat. The bile was extracted with ether and the ether extract was washed with water, dried, and evaporated *in vacuo* (free steroid fraction). The aqueous fraction was evaporated *in vacuo* to a vol. of 20 ml and then passed through a column (700 × 20 mm I.D.) of Amberlite XAD-2 resin (100 g). The column was washed with 100 ml of water, followed by 400 ml of methanol [13]. The methanol was evaporated *in vacuo* to afford the conjugate fraction. The conjugate fraction was dissolved in 3 ml of chloroform–methanol (1:1, v/v), containing 0.01M NaCl, and applied on Sephadex LH-20 column (20 g, 800 × 15 mm I.D.) [14]. The column was eluted with 200 ml of the same solvent system, followed by 200 ml of methanol and afforded monoglucuronide, monosulphate, and diconjugate fractions.

Hydrolysis of conjugate fractions. The hydrolytic procedures have been described previously in detail [15]. The monoglucuronide fraction was hydrolyzed by incubation with β-glucuronidase (Ketodase), the monosulphate fraction was solvolyzed in acidified ethyl acetate, and the diconjugate fraction was hydrolyzed by solvolysis and by incubation with β-glucuronidase.

Thin-layer chromatography (t.l.c.). The liberated steroids were purified by t.l.c. on plates coated with silica gel GF (Merck), using chloroform-acetone (29:1, v/v) as solvent. Radioactive zones were detected with a Packard Model 7201 autoscanner,

scraped off, and eluted with methanol as previously described [15]. Polar steroid fractions remained near the starting line on the t.l.c. plates were further separated with the solvent system cyclohexane–ethyl acetate (2:3, v/v). In general, t.l.c. plates were developed three or four times in the same solvent system.

Gas chromatography (GC) and gas chromatography–mass spectrometry (GS–MS). Metabolites separated from t.l.c. plates were trimethylsilylated [16] and analyzed by GC and GC–MS. GC was performed on a Shimadzu GC-4BM chromatograph equipped with a flame ionization detector using 0.5% CHDMS (2.0 m × 3 mm; column 200°C; detector and flash heater 240°C) and 1.5% SE-30 (1.5 m × 3 mm; column 230°C; detector and flash heater 250°C) as the stationary phase. Nitrogen gas flow-rate was 40 ml/min. Retention times were calculated relative to 5α-cholestane. In general, the peaks were quantitated by peak-height measurement, employing known amounts of 5α-cholestane as internal standard. GC–MS was carried out on a JEOL JMS-D100 spectrometer using 1.5% SE-30 column (2.0 m × 3 mm, column 260°C; detector and flash heater 270°C). Helium gas flow-rate was about 30 ml/min. The temperatures of the molecular separator and ion source were 200°C. Mass spectra were recorded with a bombarding electron energy of 24 eV and filament current of 300 μA.

Measurement of radioactivity. Radioactivity was counted in an Aloka LSC-502 liquid scintillation spectrometer in a toluene medium as previously described [15]. Efficiency of ^3H counting was about 40%. Results are expressed in d.p.m.

RESULTS

Distribution of radioactivity

Table 1 shows the excretion of the radioactivity in the bile following intraperitoneal injection of [^{3}H]-androsterone glucuronide and [^{3}H]-androsterone sulphate into female rats with bile fistulas. The rats dosed with [^{3}H]-androsterone glucuronide eliminated about 62% of the ^3H during the first h and the radioactivity was quantitatively recovered in the bile within 24 h. On the other hand, the biliary excretion of the ^3H after administration of [^{3}H]-androsterone sulphate was about 6% in the first h and 74% in 24 h. Less than 1% of the injected radioactivity appeared in the 24–48 h bile fraction.

Biliary radioactivity was found entirely in the conjugate fraction. Table 2 shows the distribution of the recovered radioactivity in the monoglucuronide, monosulphate, and diconjugate fractions. The monoglucuronide was almost the sole conjugate in the rats dosed with [^{3}H]-androsterone glucuronide, whereas monosulphates and diconjugates predominated in the rats after [^{3}H]-androsterone sulphate injection.

Hydrolysis of conjugates

After β-glucuronidase hydrolysis of the monoglucuronide fraction from the rats dosed with [^{3}H]-androsterone glucuronide, approximately 96% of the radioactivity was extracted with ether. By solvolysis of the monosulphate and diconjugate fractions from the rats injected with [^{3}H]-androsterone sulphate, about 86 and 68% of the ^3H appeared in the ethyl acetate extract, respectively. Following solvolysis of the diconjugate fraction, the resultant aqueous fraction was hydrolyzed with β-glucuronidase. About 28% of the ^3H present in the aqueous fraction was extracted with ether. By direct hydrolysis of the diconjugate fraction with the β-glucuronidase, which contains no sulphatase, the radioactivity was not extracted with ether and subsequent solvolysis of the resultant aqueous fraction resulted in about 69% recovery of the ^3H in the ethyl acetate extract. These results clearly indicate that the diconjugate was probably predominantly in the form of disulphates and to a minor extent in the form of sulphoglucuronides.

Identification of steroids

Following separation of the liberated steroids by t.l.c., the metabolites were trimethylsilylated and analyzed by GC and GC–MS (Table 3). The identified metabolites provided relative retention times and mass spectra identical with those of the respective reference steroids.

Table 4 shows the percentage conversions of the identified metabolites, which were calculated from the injected dose. However, these values are conservative estimates in that no assessment of the procedural losses can be made. The total recovery of individual meta-

Table 1. Biliary excretion of radioactivity (% dose) following intraperitoneal injection of [^{3}H]-androsterone glucuronide and [^{3}H]-androsterone sulphate into female rats*

Bile (h)	[^{3}H]-Androsterone glucuronide	[^{3}H]-Androsterone sulphate
0–1	61.8 ± 12.1†	5.9 ± 3.6†
1–2	19.7 ± 5.2	16.1 ± 3.2
2–4	12.4 ± 6.4	25.3 ± 1.5
4–6	3.9 ± 0.8	12.9 ± 2.6
6–24	2.5 ± 0.8	14.2 ± 4.2
24–48	0.1 ± 0.1	0.6 ± 0.1
Total	100.4 ± 2.3	74.9 ± 11.2

* Dose: [^{3}H]-androsterone glucuronide (0.90 μCi, 5.11 μmol). [^{3}H]-androsterone sulphate (0.83 μCi, 5.24 μmol).
† Mean ± S.D. (n = 3).

Table 2. Distribution of the [0–24 h] biliary radioactivity (% dose) in various fractions

Fraction	[^{3}H]-Androsterone glucuronide	[^{3}H]-Androsterone sulphate
Monoglucuronide	94.5 ± 1.6*	0
	(95.2 ± 1.5)†	(0)
Monosulphate	0	33.0 ± 3.4
	(0)	(45.7 ± 5.2)
Diconjugate	3.2 ± 0.7	39.6 ± 9.2
	(3.2 ± 0.6)	(53.9 ± 5.3)

* Mean ± S.D. (n = 3). † Figures in parentheses indicate % of the total radioactivity in each bile fraction.

Table 3. Gas chromatographic and mass spectrometric data of trimethylsilyl derivatives of metabolites obtained by hydrolysis of biliary conjugates

Metabolite	Conjugate*	GC(RRT)†		GC–MS (m/e)‡							
		CHDMS	SE-30	M$^+$	BP	Other prominent ions					
androsterone	G,S	0.74	0.39	362	272	271	347	215	257	129	155
5α-androstane-3α,17β-diol	G,D	0.31	0.50	436	241	256	129	346	215	331	148
3α,11β-dihydroxy-5α-androstan-17-one	S	1.13	0.71	450	156	199	184	186	157	360	394
3α,17β-dihydroxy-5α-androstan-16-one	D	1.03	0.76	450	129	215	216	435	117	173	
5α-androstane-3α,15α,17β-triol	S,D	0.49	0.71	524	217	191	218	219	434	169	
5α-androstane-3α,16α,17β-triol	S,D	0.61	0.93	524	191	434	344	169	215	254	

* G = monoglucuronide, S = monosulphate, D = disulphate. † Gas chromatographic conditions are described in "EXPERIMENTAL". RRT = relative retention time to 5α-cholestane. ‡ Gas chromatography-mass spectrometric conditions are described in "EXPERIMENTAL". M$^+$ = molecular ion. BP = base peak.

bolites was about 73% in the rats dosed with androsterone glucuronide. In contrast, only 32% of individual metabolites was recovered in the rats treated with androsterone sulphate, the remaining metabolites being probably in the form of polyhydroxylated steroids ($C_{19}O_4$, etc.), which could not be identified. In the rats dosed with [^{3}H]-androsterone glucuronide, this conjugate was recovered almost exclusively in the bile. In addition to this, small amounts of 5α-androstane-3α,17β-diol were identified in the monoglucuronide fraction. In marked contrast to this, the major biliary metabolites after [^{3}H]-androsterone sulphate administration were monosulphates of androsterone and 3α,11β-dihydroxy-5α-androstan-17-one, and disulphates of 5α-androstane-3α,17β-diol and 3α,17β-dihydroxy-5α-androstan-16-one. As minor metabolites, 5α-androstane-3α,15α,17β-triol and -3α,16α,17β-triol were identified in both mono- and disulphate fractions.

DISCUSSION

The objective of the present paper was to study biliary metabolites of androsterone glucuronide and androsterone sulphate in female rats and to compare these metabolites with those of androsterone [1]. The present study reveals that the metabolism of androsterone glucuronide differs markedly from that of androsterone sulphate.

The injected androsterone glucuronide was excreted rapidly and almost quantitatively in the bile. These results imply that androsterone glucuronide

Table 4. Metabolites (% dose) present in the bile collected for 24 h following intraperitoneal administration of [^{3}H]-androsterone glucuronide and [^{3}H]-androsterone sulphate in female rats

Fraction	Metabolite	[^{3}H]-Androsterone glucuronide	[^{3}H]-Androsterone sulphate
Monoglucuronide	androsterone	72.0 ± 6.2*	
	5α-androstane-3α,17β-diol	1.0 ± 0.7	
Monosulphate	androsterone		7.0 ± 0.5
	3α,11β-dihydroxy-5α-androstan-17-one		8.7 ± 4.4
	5α-androstane-3α,15α,17β-triol		trace†
	5α-androstane-3α,16α,17β-triol		trace
Disulphate	5α-androstane-3α,17β-diol		11.4 ± 0.9
	3α,17β-dihydroxy-5α-androstan-16-one		5.2 ± 1.9
	5α-androstane-3α,15α,17β-triol		trace
	5α-androstane-3α,16α,17β-triol		trace

* Mean ± S.D. (n = 3). † Metabolites less than 1% of the injected dose.

behaves like a metabolic end-product in female rats, as was found in humans [2]. Only 1% of the injected dose was converted into 5α-androstane-3α,17β-diol monoglucuronide. This conjugate seems to be biotransformed directly from androsterone glucuronide. Androsterone glucuronide was metabolized without hydrolysis of the glucuronic acid moiety to the 3-glucuronide of 5α-androstane-3α,17β-diol by incubation with female rat liver microsomal enzymes fortified with NADPH regenerating system [17].

Metabolites of androsterone sulphate were much more slowly excreted in the bile than those of androsterone glucuronide. Approximately 7% of the injected androsterone sulphate was recovered in the bile as androsterone and the major portion was metabolized to the monosulphate of 3α,11β-dihydroxy-5α-androstan-17-one and disulphates of 5α-androstane-3α,17β-diol and 3α,17β-dihydroxy-5α-androstan-16-one. There has been accumulating evidence that steroid glucuronides are excreted in rat bile more rapidly than steroid sulphates [18, 19]. Evidence was obtained for the direct conversion of androsterone sulphate to 5α-androstane-3α,17β-diol 3-sulphate by incubation with female rat liver microsomal enzymes fortified with NADPH regenerating system [17]. Milgrom *et al.* [20] described that 3-sulphates and 3-glucuronides of androst-5-ene-3β,17β-diol and dehydroepiandrosterone were the substrates for the 17β-hydroxysteroid-oxido-reductases derived from guinea pig liver supernatant and microsomes and that both oxidation and reduction of 17β-hydroxy and 17-oxo groups took place at pH 7.4. Therefore, androsterone sulphate (and glucuronide) and 5α-androstane-3α,17β-diol 3-sulphate (and 3-glucuronide) might be interconvertible *in vivo*. Recently, Gustafsson *et al.*[21] described specific 15β-hydroxylating enzymes active on C_{19}-steroid 17β-sulphates and 3,17β-disulphates in female rat liver microsomes. C_{19}-steroid 3-sulphates were not hydroxylated by the enzymes. In a previous paper, we demonstrated that testosterone sulphate was metabolized *in vivo* to mono- and disulphates of 5α-androstane-3α,17β-diol and the disulphate of 5α-androstane-3α,15β,17β-triol in female rats [22]. Thus, mono- and disulphates of 5α-androstane-3α,17β-diol must be converted into the 15β-hydroxylated metabolite. In contrast to this, we were unable to isolate the 15β-hydroxysteroid as a metabolite of androsterone sulphate, though considerable amounts of 5α-androstane-3α,17β-diol 3,17-disulphate were present in the bile. 5α-Androstane-3α,17β-diol 3,17-disulphate should be produced by different pathways from testosterone sulphate (via 5α-androstane-3α,17β-diol 17-sulphate) and androsterone sulphate (via 5α-androstane-3α,17β-diol 3-sulphate). These results suggest that the 17-sulphate might be hydroxylated more readily than the 3,17-disulphate *in vivo*. 3α,17β-Dihydroxy-5α-androstan-16-one was found in the disulphate fraction. This metabolite should be produced via formation of the 16-hydroxysteroids. It is well documented that the 16β-hydroxy-17-oxosteroid is readily rearranged to the stable epimer, the 17β-hydroxy-16-oxosteroid, by means of alkali or acid [6, 8], and by isolation procedure from urine [23]. In order to examine the artifact formation during the isolation procedures, 3β,16β-dihydroxyandrost-5-en-17-one, a model compound, was treated similarly to the bile and analyzed by GC. 3β,16β-Dihydroxyandrost-5-en-17-one was entirely recovered by the procedures involving Amberlite XAD-2, Sephadex LH-20 chromatography and solvolysis. However, separation by t.l.c. revealed the complete conversion of the 16β-hydroxy-17-oxosteroid to the 17β-hydroxy-16-oxosteroid. 3β,16α-Dihydroxyandrost-5-en-17-one was not produced by these procedures. Furthermore, t.l.c. separation of 3α,16α-dihydroxy-5α-androstan-17-one showed partial epimerization to 3α,17β-dihydroxy-5α-androstan-16-one. At present, we are unable to eliminate the possibility that 3α,16β-dihydroxy-5α-androstan-17-one (and 16α-epimer) might be rearranged to the stable epimer during the isolation procedure. We hope that an investigation under way in this laboratory may clarify this problem.

In a previous paper [1], we reported large variations in biliary metabolites of androsterone in female rats. In rats with a high rate of biliary excretion of metabolites (the HE rat), androsterone glucuronide was the predominant metabolite present in the bile. The results of the present study show that androsterone glucuronide was eliminated unchanged rapidly and exclusively in the bile. Thus, the injected androsterone must be predominantly conjugated with glucuronic acid in the HE rat probably due to high activity of the UDP-glucuronyltransferase system and/or low activity of sulphotransferase or hydroxylase enzymes and excreted in the bile. In rats with a low rate of biliary excretion of androsterone metabolites (the LE rat), monosulphates of 3α,7α- and 3α,11β-dihydroxy-5α-androstan-17-ones were the major biliary metabolites, which were previously isolated from the pooled faeces of germfree rats [24]. The present study demonstrates that androsterone sulphate was metabolized mainly to the monosulphate of 3α,11β-dihydroxy-5α-androstan-17-one and disulphates of 5α-androstane-3α,17β-diol and 3α,17β-dihydroxy-5α-androstan-16-one. No production of 3α,7α-dihydroxy-5α-androstan-17-one monosulphate from androsterone sulphate should indicate that 7α-hydroxylase enzyme might be active only on the free steroid. The contribution of the catabolic pathway of androsterone involving initial conjugation with sulphuric acid in the LE rat was not clarified in this study. However, considerable difference in the metabolic pattern between androsterone and its sulphate suggests that this pathway is a minor one.

An investigation of the levels and activities of various enzymes involved in androsterone metabolism may be of interest for obtaining further insight into the regulatory mechanism responsible for large variations in androsterone metabolites in female rats.

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