Biochemical Pharmacology, Vol. 28, pp. 1023–1028.
Pergamon Press Ltd. 1979. Printed in Great Britain.

0006-2952/79/0401-1023 $02.00/0

VARIABILITY OF ANDROSTERONE METABOLISM IN MALE WISTAR RATS

MICHIO MATSUI and SHINOBU AOYAGI

Kyoritsu College of Pharmacy, Shibakōen, Minato-ku, Tokyo 105, Japan

(*Received* 3 *August* 1978; *accepted* 3 *October* 1978)

Abstract—[^{3}H]androsterone was administered intraperitoneally into male Wistar rats with biliary fistulas. The major portion of the radioactivity was recovered in the bile within 24 hr. There was marked diversity in androsterone metabolism. Half of the rats excreted large amounts of steroid monoglucuronides rapidly into bile, whereas the remaining rats eliminated various steroid conjugates at a much slower rate. In both groups, androsterone was metabolized to 2- and 16-oxygenated $C_{19}O_3$ and $C_{19}O_4$ steroids and probably polyoxygenated steroids. In contrast, the injected [^{3}H]androsterone glucuronide was recovered rapidly and quantitatively in the bile. These results indicate the extensive metabolism of androsterone prior to glucuronidation and probably the existence of variations of UDP-glucuronyltransferase toward the oxygenated metabolites of androsterone.

Some remarkable features characterize the metabolism of androsterone in female Wistar rats, i.e. discontinuous variations in biliary metabolites [1] and hepatic UDP-glucuronyltransferase activity [2]. These observations together with recent findings on the metabolism of androsterone glucuronide and androsterone sulfate [3] indicate that the hepatic UDP-glucuronyltransferase must be principally responsible for the diversity of the *in vivo* metabolism of androsterone in female rats. Experiments with male Wistar rat liver demonstrated the existence of variations in transferase activity toward androsterone similar to those of female rats [2]. These results indicate clearly the variability of androsterone metabolism in male rats. In the present paper, [^{3}H]androsterone and [^{3}H]androsterone glucuronide were administered intraperitoneally into male Wistar rats, and the biliary metabolites were isolated and identified by gas chromatography–mass spectrometry.

MATERIALS AND METHODS

Materials. [1,2-^{3}H]Androsterone (40.8 Ci/m-mole) was purchased from New England Nuclear, Boston, MA, U.S.A. [1,2-^{3}H]androsterone glucuronide (8.1 μCi/μmole) and unlabeled androsterone glucuronide were prepared as described previously [3]. The radiochemical purities of these labeled steroids were confirmed by thin-layer chromatography (t.l.c.) shortly before use, as described previously [1, 3], and were found to be more than 98 per cent. Androsterone was obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. 5α-Androstane-3α,16α,17β-triol was prepared as described by Lieberman *et al.* [4], and 5α-androstane-3β,16α,17β-triol and 3β,17β-dihydroxy-5α-androstan-16-one were prepared by the procedure of Leeds *et al.* [5]. Synthesis of 2α,3α-dihydroxy-5α-androstan-17-one has been described previously [6, 7].

2α,3α,16α-Trihydroxy-5α-androstan-17-one. The procedure described for the synthesis of 3β,16α-diacetoxy-5α-androstan-17-one [5] was employed with some modifications. A solution of 2α,3α-dihydroxy-5α-androstan-17-one (350 mg) [7] and *p*-toluenesulfonic acid monohydrate (110 mg) in isopropenyl acetate (100 ml) was distilled slowly for 24 hr to remove half the solvent. The product was then purified by chromatography on a column (700 × 25 mm i.d.) of silica gel GF (70 g, Merck) using chloroform as solvent, and crystallized from methanol to give 5α-androst-16-ene-2α,3α,17-triol triacetate (200 mg), m.p. 194–198° (Found: C, 69.51; H, 8.38. $C_{25}H_{36}O_6$ requires C, 69.42; H, 8.39). N.m.r. δ 0.88 (3H, s, 18-H_3 or 19-H_3), 0.90 (3H, s, 19-H_3 or 18-H_3), 1.98 (3H, s, 2-$OCOCH_3$), 2.09 (3H, s, 3-$OCOCH_3$), 2.15 (3H, s, 17-$OCOCH_3$), 5.02 (1H, m, 2-H), 5.30 (1H, m, 3-H), and 5.50 (1H, m, 16-H). 5α-Androst-16-ene-2α,3α,17-triol triacetate (196 mg) was treated with 1% perbenzoic acid in chloroform (20 ml) at 4° for 16 hr, and the product was crystallized from acetone-n-hexane to afford 16α,17α-oxido-5α-androstane-2α,3α,17β-triol triacetate (171 mg), m.p. 169–172° (Found: C, 66.98; H, 8.10. $C_{25}H_{36}O_7$ requires C, 66.94; H, 8.09). N.m.r. δ 0.88 (6H, s, 18-H_3 and 19-H_3), 1.97 (3H, s, 2-$OCOCH_3$), 2.08 (6H, s, 3-$OCOCH_3$ and 17-$OCOCH_3$), 3.86 (1H, s, 16-H), 5.00 (1H, m, 2-H), and 5.28 (1H, m, 3-H). The rearrangement and deacetylation of 16α,17α-oxido-5α-androstane-2α,3α,17β-triol triacetate (165 mg) were performed in methanol–6 N H_2SO_4 (40 ml, 2: 1, v/v) [5] at 25° for 30 hr, and the product was crystallized from acetone to give 2α,3α,-16α-trihydroxy-5α-androstan-17-one (98 mg), m.p. 178–185° (Found: C, 68.10; H, 9.49. $C_{19}H_{30}O_4 \cdot 3/4\ H_2O$ requires C, 67.93; H, 9.45). N.m.r. δ 0.82 (3H, s, 19-H_3), 0.94 (3H, s, 18-H_3), 3.80 (1H, m, 2-H), 3.96 (1H, m, 3-H), and 4.36 (1H, m, 16-H).

2α,3α,17β - Trihydroxy - 5α - androstan - 16 - one. 2α,3α,16α-Trihydroxy-5α-androstan-17-one (40 mg) was rearranged in 0.05 N NaOH in 80% aqueous methanol (25 ml) [5] at 25° for 20 hr, and the product was crystallized from acetone-*n*-hexane to afford 2α,-3α,17β-trihydroxy-5α-androstan-16-one (25 mg), m.p. 187–195° (Found: C, 70.78; H, 9.50. $C_{19}H_{30}O_4$ requires C, 70.77; H, 9.38). N.m.r. δ 0.72 (3H, s, 18-H_3), 0.84 (3H, s, 19-H_3), 3.78 (1H, s, 17-H), 3.82 (1H, m, 2-H), and 4.00 (1H, m, 3-H).

5α-Androstane-2α,3α,16α,17β-tetrol. 2α,3α,16α-Trihydroxy-5α-androstan-17-one (18 mg) was dis-

solved in methanol (15 ml) and reduced with sodium borohydride (100 mg). The reaction mixture was poured into water, and separated crystals were collected and crystallized from aqueous methanol to give 5α-androstane-2α,3α,16α,17β-tetrol (10 mg), m.p. 246–250° (Found: C, 69.24; H, 9.94. $C_{19}H_{32}O_4 \cdot 1/4\ H_2O$ requires C, 69.37; H, 9.96).

Analytical equipment. Melting points were determined with a Kofler hot-stage apparatus. ^1H n.m.r. spectra were determined 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. Gas chromatography (g.c.) 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°; detector and flash heater 240°) and 1.5% SE-30 (1.5 m × 3 mm; column 230°; detector and flash heater 250°) as the stationary phase. Nitrogen gas flow-rate was 40 ml/min. Gas chromatography–mass spectrometry (g.c.–m.s.) was carried out on a JEOL JMS-D100 spectrometer using 1.5% SE-30 column (2.0 m × 3 mm, column 255°, detector and flash heater 270°). The temperatures of the molecular separator and ion source were 260°. Mass spectra were recorded with a bombarding electron energy of 24 eV and a filament current of 300 μA. Radioactivity was counted in an Aloka LSC-502 liquid scintillation spectrometer in a toluene medium as described previously [8]. The efficiency of ^3H-counting was about 40 per cent. The results are expressed in dis./min. Radioactive zones on t.l.c. were detected with a Packard model 7201 autoscanner.

Animals and administration of labeled steroids. The common bile duct was cannulated in male rats of the Wistar strain (140–270 g: Matsumoto Experimental Animal Lab., Tokyo, Japan) as described previously [8]. After operation the rat was kept in a restraining cage with free access to water and food pellets. An ethanol solution of [^3H]androsterone (diluted with unlabeled androsterone: 0.15 ml, 0.73 μCi, 3.4 μmoles) or [^3H]androsterone glucuronide (diluted with unlabeled androsterone glucuronide: 0.15 ml, 0.73 μCi, 5.3 μmoles) was diluted with 0.15 ml saline and injected intraperitoneally 18–20 hr after operation into male rats. Bile was collected at 0–1, 1–2, 2–4, 4–6, 6–24 and 24–48 hr.

Extraction and analysis of biliary metabolites. The bile sample collected for 0–24 hr was processed separately for each rat. The bile was extracted with ethyl acetate and the ethyl acetate extract was washed with water, dried over Na_2SO_4, and evaporated *in vacuo* (free steroid fraction). The aqueous fraction was evaporated *in vacuo* to a volume of 20 ml and then passed through a column (700 × 20 mm i.d.) packed with Amberlite XAD-2 resin (100 g). The column was washed with 100 ml water, followed by elution with 400 ml methanol [9]. The methanol effluent was evaporated *in vacuo* to afford the conjugate fraction. The conjugate fraction was dissolved in 3 ml chloroform–methanol (1:1, v/v), containing 0.01 M NaCl, and applied on a Sephadex LH-20 column (20 g, 800 × 15 mm i.d.) [10]. The column was eluted with 200 ml of the same solvent system, followed by 200 ml methanol and afforded the monoglucuronide, monosulfate, and diconjugate fractions.

Hydrolysis of the conjugate fractions was performed as reported previously [8]. Briefly, the monoglucuronide fraction was hydrolyzed by incubation with β-glucuronidase (Ketodase), the monosulfate fraction was solvolyzed in acidified ethyl acetate, and the diconjugate fraction was hydrolyzed by solvolysis and by incubation with β-glucuronidase.

The liberated steroids were separated by t.l.c. on plates coated with silica gel GF (Merck), using chloroform–acetone (29:1, v/v) as solvent. Radioactive zones were scraped off and eluted with methanol. Polar steroid fractions which remained near the starting line on the t.l.c. plates were separated further with the solvent system cyclohexane–ethyl acetate (2:3, v/v), as described previously [7]. In general, t.l.c. plates were developed three or four times in the same solvent system.

The metabolites separated from t.l.c. plates were trimethylsilylated [11] and analyzed by g.c. and g.c.–m.s. Retention times were calculated relative to 5α-cholestane. Quantitation of metabolites was done by the peak-height measurement, using 5α-cholestane as an internal standard.

RESULTS

Excretion and distribution of radioactivity. The biliary excretion of the radioactivity following administration of [^3H]androsterone and [^3H]androsterone glucuronide in male rats is shown in Table 1. There were

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

Bile (hr)	[^3H]androsterone		[^3H]Androsterone glucuronide
	HE rat†	LE rat‡	
0–1	69.7 ± 15.5	21.9 ± 5.2	71.3 ± 4.1
1–2	15.7 ± 4.8	15.4 ± 2.7	17.1 ± 1.9
2–4	10.3 ± 4.8	18.1 ± 6.2	8.3 ± 1.1
4–6	2.8 ± 1.6	4.5 ± 0.7	1.5 ± 0.2
6–24	2.8 ± 1.6	6.4 ± 1.5	0.9 ± 0.3
Total	101.4 ± 3.9	66.2 ± 11.0	99.1 ± 2.1

* Dose: [^3H]androsterone (0.73 μCi, 3.4 μmoles); [^3H]androsterone glucuronide (0.73 μCi, 5.3 μmoles). Each value represents the mean ± S.D. of three animals.

† Rats with a high rate of biliary excretion of metabolites.

‡ Rats with a low rate of biliary excretion of metabolites.

Table 2. Distribution of the (0–24 hr) biliary radioactivity (% dose) in various conjugate fractions

Fraction	[^{3}H]Androsterone		[^{3}H]Androsterone glucuronide
	HE rat*	LE rat†	
Monoglucuronide	69.2 ± 14.1‡	19.9 ± 11.2	97.2 ± 2.0
	(69.6 ± 13.7)	(28.6 ± 13.3)	(98.0 ± 1.0)
Monosulfate	7.9 ± 3.7	25.8 ± 1.8	0
	(7.9 ± 3.8)	(39.9 ± 9.4)	(0)
Diconjugate	20.3 ± 10.0	20.1 ± 2.6	1.1 ± 0.1
	(20.5 ± 10.2)	(30.7 ± 3.1)	(1.1 ± 0.1)

* Rats with a high rate of biliary excretion of metabolites.
† Rats with a low rate of biliary excretion of metabolites.
‡ Each value represents the mean ± S.D. of three animals. Values in parentheses indicate per cent of the total radioactivity in each bile fraction.

large variations in the excretion of androsterone metabolites. No apparent relationship existed between the rates of biliary excretion of metabolites and the rates of bile flow or the animal weights. It was found that half of the rats (high excretion rate, HE rats) excreted about 70 per cent of the radioactivity during the first hr and that the radioactivity was recovered quantitatively in the bile within 24 hr. This excretion pattern was quite similar to that of [^{3}H]androsterone glucuronide. In contrast, the remaining rats (low excretion rate, LE rats) eliminated 22 per cent of the radioactivity in the first hr and about 66 per cent of the dose appeared in the bile during 24 hr. Less than 1 per cent of the dose was recovered in the 24–48 hr bile fraction.

The biliary radioactivity was found entirely in the conjugate fractions, as shown in Table 2. There were significant differences in the constitutions of the conjugates between the HE and LE rats. The monoglucuronides were the major conjugate in the HE rats, whereas the radioactivity was distributed in various conjugate fractions in the LE rats. In the rats dosed with [^{3}H]androsterone glucuronide, the monoglucuronide was almost the sole conjugate present in the bile.

Hydrolysis of conjugates. Table 3 lists the results of hydrolysis of each conjugate fraction with β-glucuronidase or by solvolysis. As for the monoglucuronide fraction from the rats injected with [^{3}H]androsterone glucuronide, it was quantitatively hydrolyzed with β-glucuronidase. Enzymic hydrolysis of the monoglucuronide fraction and solvolysis of the monosulfate fraction from the rats dosed with [^{3}H]androsterone demonstrated each to be largely hydrolyzable. In order to obtain information concerning the nature of conjugation, hydrolysis of the diconjugate fraction was done by two procedures. In the first one, the fraction was hydrolyzed first by solvolysis and subsequently with β-glucuronidase. In the second one, the sequence of the hydrolytic procedures was done in the reverse way. The results show that the diconjugates of the LE rats consisted of mainly disulfates and probably to a minor extent sulfoglucuronides, while the diconjugates of the HE rats seemed to be present predominantly as diglucuronides and disulfates.

Identification of steroids. The liberated metabolites were separated by t.l.c., trimethylsilylated, and analyzed by g.c. and g.c.–m.s. (Table 4). The identified metabolites provided relative retention times and mass spectra identical with those of the respective reference

Table 3. Hydrolysis (%) of various conjugate fractions*

Fraction	[^{3}H]androsterone		[^{3}H]androsterone glucuronide
	HE rat†	LE rat‡	
Monoglucuronide	80.5 ± 10.0§	67.4 ± 9.6	99.2 ± 0.2
Monosulfate	61.5 ± 5.4	75.1 ± 3.5	
Diconjugate (1) S	28.1 ± 2.4	65.0 ± 5.6	
G	48.0 ± 20.7	13.2 ± 3.4	
Diconjugate (2) G	63.5‖	1.5‖	
S	23.1	72.8	

* Monoglucuronide fraction was hydrolyzed with β-glucuronidase; the monosulfate fraction was solvolyzed; the diconjugate (1) fraction was first solvolyzed (S) and then hydrolyzed with β-glucuronidase (G), and the diconjugate (2) fraction was first hydrolyzed with β-glucuronidase (G), followed by solvolysis (S).
† Rats with a high rate of biliary excretion of metabolites.
‡ Rats with a low rate of biliary excretion of metabolites.
§ Each value represents the mean per cent ± S.D. of three animals.
‖ Results of one animal.

Table 4. Gas chromatographic and mass spectrometric data of trimethylsilyl derivatives of metabolites obtained by hydrolysis of biliary conjugates

Metabolite	Conjugate*	Gas chromatography† (RRT)		Gas chromatography–mass spectrometry‡ (*m/e*)						
		CHDMS	SE-30	M^+	BP	Other prominent ions				
Androsterone	G	0.74	0.39	362	272	271	347	215	257	155
2α, 3α-Dihydroxy-5α-androstan-17-one	G	1.01	0.65	450	271	435	253	143	129	142
3β, 17β-Dihydroxy-5α-androstan-16-one	G,DS	1.70	0.95	450	129	435	215	216	117	143
5α-Androstane-3α, 16α, 17β-triol	G,S	0.61	0.90	524	191	434	344	215	255	205
5α-Androstane-3β, 16α, 17β-triol	S,DS	0.83	1.19	524	191	434	344	215	255	217
2α, 3α,16α-Trihydroxy-5α-androstan-17-one	G	1.15	1.02	538	117	214	304	194	394	379
2α, 3α, 17β-Trihydroxy-5α-androstan-16-one	G	1.42	1.22	538	129	523	143	117	304	214
5α-Androstane-2α, 3α, 16α, 17β-tetrol	G	0.87	1.42	612	191	522	143	129	205	169

* G = monoglucuronide, S = monosulfate, DS = disulfate.
† Gas chromatographic conditions are described in Materials and Methods. RRT=relative retention time to 5α-cholestane.
‡ Gas chromatography–mass spectrometric conditions are described in Materials and Methods.
M^+ = molecular ion. BP = base peak.

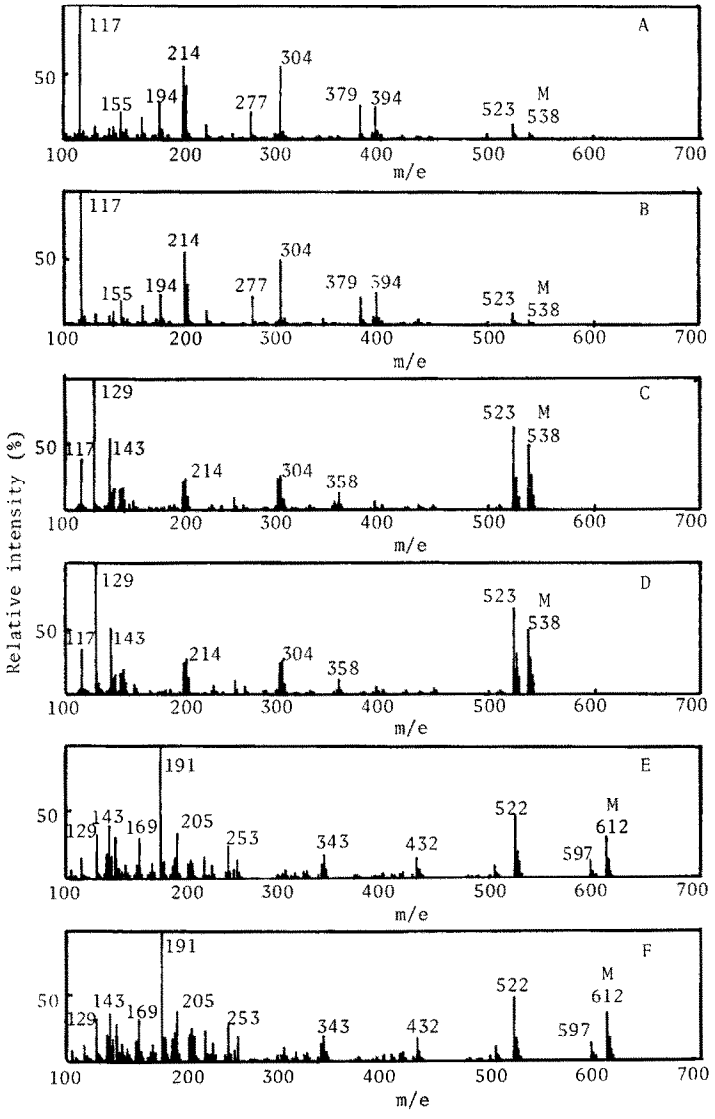

Fig. 1. Mass spectra of the trimethylsilyl derivatives of the metabolite identified as 2α, 3α, 16α-trihydroxy-5α-androstan-17-one (A), 2α, 3α, 16α-trihydroxy-5α-androstan-17-one (B), the metabolite identified as 2α, 3α, 17β-trihydroxy-5α-androstan-16-one (C), 2α, 3α, 17β-trihydroxy-5α-androstan-16-one (D), the metabolite identified as 5α-androstane-2α, 3α, 16α, 17β-tetrol (E), and 5α-androstane-2α, 3α, 16α, 17β-tetrol (F).

Table 5. Metabolites (% dose) present in the (0–24 hr) bile following intraperitoneal injection of [^{3}H]androsterone and [^{3}H]androsterone glucuronide in male Wistar rats

Fraction	Metabolite	[^{3}H]Androsterone		[^{3}H]Androsterone glucuronide
		HE rat*	LE rat†	
Monoglucuronide	Androsterone	3.5 ± 1.5‡		85.4 ± 1.4
	2α, 3α-Dihydroxy-5α-androstan-17-one	16.2 + 1.6		
	3β, 17β-Dihydroxy-5α-androstan-16-one	Trace		
	5α-Androstane-3α, 16α, 17β-triol	Trace		
	2α, 3α, 16α-Trihydroxy-5α-androstan-17-one	Trace		
	2α, 3α, 17β-Trihydroxy-5α-androstan-16-one	Trace		
	5α-Androstane-2α, 3α, 16α, 17β-tetrol	Trace		
Monosulfate	5α-Androstane-3α, 16α, 17β-triol		Trace	
	5α-Androstane-3β, 16α, 17β-triol		Trace	
Disulfate	3β, 17β-Dihydroxy-5α-androstan-16-one		Trace	
	5α-Androstane-3β, 16α, 17β-triol		Trace	

* Rats with a high rate of biliary excretion of metabolites.
† Rats with a low rate of biliary excretion of metabolites.
‡ Each value represents the mean ± S.D. of three animals. Trace means the metabolite was less than 1 per cent of the injected dose.

steroids. Androsterone was metabolized extensively to oxygenated steroids. Gas chromatography–mass spectrometry analysis of the trimethylsilyl derivatives of androsterone metabolites revealed the occurrence of several monooxo-trihydroxy (molecular ion at *m/e* 538) and tetrahydroxy (molecular ion at *m/e* 612) steroids. Since we identified 2- and 16-oxygenated $C_{19}O_3$ steroids as androsterone metabolites, we synthesized three 2,3,16,17-tetraoxygenated steroids by introduction of a 16-hydroxy group into 2α,3α-dihydroxy-5α-androstan-17-one, following the method described for the preparation of 3,16,17-oxygenated steroids [5]. Figure 1 shows the mass spectra of the identified $C_{19}O_4$ steroids together with newly prepared reference steroids. Eriksson *et al.* [12] recorded two 5ξ-androstane-2ξ,3ξ,16ξ,17ξ-tetrols (relative retention times 1.42 and 1.53 on SE-30 column) as metabolites of 4-androstene-3,17-dione in perfused male rat liver. We can now identify one of them (relative retention time 1.42) as 5α-androstane-2α,3α 16α,17β-tetrol by comparison of the mass spectra and relative retention times on SE-30.

Table 5 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. Androsterone glucuronide was almost quantitatively recovered in the bile. In the HE rats, androsterone was metabolized to the monoglucuronides of androsterone, 2α,3α-dihydroxy-5α-androstan-17-one, 3β,17β-dihydroxy-5α-androstan-16-one, 5α-androstane-3α,16α,17β-triol, 2α,3α,16α-trihydroxy-5α-androstan-17-one, 2α,3α,17β-trihydroxy-5α-androstan-16-one, and 5α-androstane-2α,3α,16α,17β-tetrol. In the LE rats, 5α-androstane-3α,16α,17β-triol and 3β,17β-dihydroxy-5α-androstan-16-one were isolated as the monosulfate and the disulfate respectively. In addition, 5α-androstane-3β,16α,17β-triol was identified in the mono- and disulfate fractions. The 17β-hydroxy-16-oxosteroids identified above might be artifacts formed by rearrangement of the 16β-hydroxy-17-oxosteroids during the isolation procedure, because 16β-hydroxyandrosterone was entirely converted into 3α,17β-dihydroxy-5α-androstan-16-one by t.l.c. on silica gel plates [13]. Epimerization of the 3α-hydroxy group of the administered androsterone into the 3β-hydroxy group, probably via 3-oxosteroid, was characteristic of the male rats. In this study, however, comparatively small portions of androsterone metabolites were characterized. The remaining metabolites were probably in the form of polyoxygenated steroids, because the major portions of the radioactive steroids were much more polar than 5α-androstane-2α,3α,16α,17β-tetrol and appeared near the starting line on t.l.c. plates with the solvent system cyclohexane–ethyl acetate (2:3, v/v). Eriksson *et al.* [12] reported that most C_{19} steroids excreted by male rats were substituted by five or more oxygens, which have not been identified.

DISCUSSION

The results of the present study demonstrate that androsterone was metabolized extensively to oxygenated steroids in male Wistar rats. Furthermore, wide individual differences were observed in biotransformation and biliary excretion. It was found that half of the rats (HE rats) excreted large amounts of steroid monoglucuronides rapidly into bile, whereas the remaining rats (LE rats) excreted various steroid conjugates slowly into bile. In marked contrast, the injected androsterone glucuronide was excreted rapidly into bile and behaved like a metabolic end-product, as was found in female Wistar rats [1] and humans [14]. There has been accumulating evidence that steroid glucuronides are excreted in rat bile more rapidly than steroid sulfates [3, 15, 16].

In a previous paper, we described the marked variations in biliary metabolites of androsterone in female Wistar rats [1]. About half of the rats excreted mainly androsterone glucuronide into bile, while the remaining rats excreted predominantly the monosulfates of $C_{19}O_2$ and $C_{19}O_3$ steroids in the bile. Subsequent *in vitro* studies with male and female Wistar rats revealed the discontinuous variations in hepatic microsomal UDP-glucuronyltransferase activities toward androsterone but not toward testosterone, females showing greater

enzyme activity than males [2]. The rats with a high level of the transferase activity (HG) and the rats with a low level of the transferase activity (LG) were found approximately in the ratio 5:4 and the HG to LG specific activity ratio was about 9, irrespective of the sex. Thus, the variability of the UDP-glucuronyltransferase activity should be principally responsible for the large variations in the *in vivo* metabolism of androsterone in female rats. On the other hand, androsterone was metabolized mainly to polyoxygenated steroid monoglucuronides in male HE rats. Low recovery of androsterone in the monoglucuronide fraction might reflect the increased hydroxylase activities in male rats [17], leading to a type of metabolite different from that in female rats. However, these results imply that the variation of UDP-glucuronyltransferase should exist not only for androsterone but for the oxygenated metabolites of androsterone.

The relationship between the UDP-glucuronyltransferase activity *in vitro* and the glucuronidation *in vivo* is not clear. The glucuronidation *in vivo* can be affected by several factors such as UDP-glucuronic acid, β-glucuronidase, and other metabolizing enzymes besides UDP-glucuronyltransferase. The situation is complicated by the fact that UDP-glucuronyltransferase is latent and probably inside the microsomal vesicle. The enzyme can be activated by physical, chemical, or enzymatic perturbation of the membrane structure [18, 19]. Comparative studies indicate that UDP-glucuronyltransferase may be operating largely in a constrained form *in vivo* [19]. From comparative studies on the glucuronidation of 1-naphthol in perfused liver and hepatic UDP-glucuronyltransferase, Bock *et al.* [20] reported that the markedly decreased UDP-glucuronyltransferase in Gunn rats does not lead to impaired glucuronide formation in perfused livers, due probably to compensatory activation of the latent enzyme. In contrast to this, our female Wistar rats showed good correlation between glucuronidations *in vivo* and *in vitro*. This may be ascribable to the striking differences in UDP-glucuronyltransferase activities toward androsterone of HE and LE rats. In fact, activation of the latent enzyme with Triton X-100 amplified the diversity between HG and LG microsomes [2]. To extend our findings, it is of interest to investigate the specificity of "androsterone UDP-glucuronyltransferase" in male rats. Work is now under progress in our laboratory to study the nature and extent of the variability of hepatic UDP-glucuronyltransferase in rats of the Wistar and other strains.

REFERENCES

1. M. Matsui and M. Hakozaki, *J. Steroid Biochem.* **8**, 319 (1977).
2. M. Matsui and M. Hakozaki, *Biochem. Pharmac.*, in press.
3. M. Matsui and M. Hakozaki, *J. Steroid Biochem.* **8**, 1243 (1977).
4. S. Lieberman, B. Praetz, P. Humphries and K. Dobriner, *J. biol. Chem.* **204**, 491 (1953).
5. N. S. Leeds, D. K. Fukushima and T. F. Gallagher, *J. Am. chem. Soc.* **76**, 2943 (1954).
6. J. Edwards, U.S. 3066138 (1962); *Chem. Abstr.* **60**, 608 (1964).
7. M. Matsui and Y. Kinuyama, *J. Steroid Biochem.* **8**, 323 (1977).
8. M. Matsui, Y. Kinuyama and M. Hakozaki, *Steroids* **24**, 557 (1974).
9. M. Matsui, M. Hakozaki and Y. Kinuyama, *J. Chromat.* **115**, 625 (1975).
10. T. Laatikainen and R. Vihko, *Steroids* **14**, 119 (1969).
11. B. P. Lisboa, J-Å. Gustafsson and J. Sjövall, *Eur J. Biochem.* **4**, 496 (1968).
12. H. Eriksson, J.-Å. Gustafsson and Å. Pousette, *Eur. J. Biochem.* **27**, 327 (1972).
13. M. Matsui and M. Hakozaki, *Steroids* **31**, 219 (1978).
14. P. K. Siiteri and S. Lieberman, *Biochemistry* **2**, 1171 (1963).
15. S. Honma and T. Nambara, *Chem. pharm. Bull., Tokyo* **22**, 687 (1974).
16. T. A. Baillie, H. Eriksson, J. E. Herz and J. Sjövall, *Eur. J. Biochem.* **55**, 157 (1975).
17. H. Schriefers, in *Vitamins and Hormones* (Eds. R. S. Harris and P. L. Muson), Vol. 25, p. 271. Academic Press, New York (1967).
18. D. Zakim and D. A. Vessey, *Biochem. Soc. Trans.* **2**, 1165 (1974).
19. G. J. Dutton and B. Burchell, in *Progress in Drug Metabolism* (Eds. J. W. Bridges and L. F. Chasseaud), Vol. 2, p. 1. John Wiley, London (1977).
20. K. W. Bock, U. C. V. Clausbruch and H. Ottenwälder, *Biochem. Pharmac.* **27**, 369 (1978).