Strain differences in purified rat hepatic 3 α -hydroxysteroid UDP-glucuronosyltransferase

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Qualitative and quantitative differences of purified hepatic 3 α -hydroxysteroid UDP-glucuronosyltransferase were investigated in Wistar and Sprague–Dawley rats. Individual differences in the glucuronidation rate of androsterone and chenodeoxycholic acid were observed in hepatic microsomal fractions from Wistar but not Sprague–Dawley rats. No individual variation was observed in the glucuronidation of testosterone, *p*-nitrophenol or oestrone. The 3 α -hydroxysteroid UDP-glucuronosyltransferases from livers of Wistar and Sprague–Dawley rats were isolated and highly purified by using Chromatofocusing and affinity chromatography. The amount of 3 α -hydroxysteroid UDP-glucuronosyltransferase in the liver of Wistar rats exhibiting low rates for androsterone glucuronidation is about 10% or less than that found in hepatic microsomal fractions obtained from Wistar rats having high rates for androsterone glucuronidation. The apparent K_m for androsterone with purified 3 α -hydroxysteroid UDP-glucuronosyltransferase from Wistar rats with high glucuronidation activity (6 μ M) was not different from that observed for the enzyme purified from Sprague–Dawley animals, whereas that for the enzyme purified from Wistar rats with low glucuronidation activity was substantially higher (120 μ M). Despite the differences in apparent K_m values for androsterone, the apparent K_m for UDP-glucuronic acid (0.3 mM) was not different in the different populations of rats.

Hepatic UDP-glucuronosyltransferases (EC 2.4.1.17) catalyse the transfer of glucuronic acid from UDP-glucuronic acid to various endogenous and exogenous compounds (Dutton, 1980). The heterogeneity of this family of isoenzymes has been well documented by enzyme purification studies in rabbits (del Villar *et al.*, 1977; Tukey & Tephly, 1981; Tukey *et al.*, 1982), rats (Gorski & Kasper, 1977; Burchell, 1978; Falany & Tephly, 1983) and mice (Mackenzie & Owens, 1983; Mackenzie *et al.*, 1984).

Genetic variation in the rate of androsterone glucuronidation has been demonstrated in certain strains of rats. Matsui & Aoyagi (1979) showed

that Wistar rats displayed individual variations in the biliary excretion of exogenously administered androsterone. The animals could be divided into two groups on the basis of hepatic microsomal glucuronidation rates for androsterone: those with high activity (HA) and those with low activity (LA) towards this substrate (Matsui & Hakozaiki, 1979). This variation was also observed in Domyo and Wistar–King rat strains but not in Long Evans or Sprague–Dawley strains (Matsui *et al.*, 1979). No individual variation was observed in the glucuronidation of testosterone, *p*-nitrophenol, bilirubin or phenolphthalein within the different strains (Matsui *et al.*, 1979). Individual variability in the glucuronidation of androsterone was subsequently shown to be age-related, with differences observed only after 30 days of age (Matsui & Watanabe, 1982b). It is not known, however, whether the low rate of glucuronidation of androsterone in certain rats of the Wistar strain is due to

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decreased amounts of the UDP-glucuronosyltransferase protein or to the presence of a less active form of the enzyme.

Our laboratory has previously purified two steroid UDP-glucuronosyltransferases from microsomal fractions of livers of Sprague-Dawley rats (Falany & Tephly, 1983; Falany *et al.*, 1985): a 17β -hydroxysteroid UDP-glucuronosyltransferase that glucuronidates the 17β -hydroxy position of testosterone and other 17β -hydroxysteroids, and a 3α -hydroxysteroid UDP-glucuronosyltransferase that conjugates the 3α -hydroxy position of androsterone and aetiocholanolone. The 3α -hydroxysteroid UDP-glucuronosyltransferase also glucuronidates the 3α -hydroxy position of bile acids (Kirkpatrick *et al.*, 1984). Neither enzyme catalyses the glucuronidation of oestrone. The purpose of the present study was to investigate the 3α -hydroxysteroid UDP-glucuronosyltransferase of HA and LA Wistar rats. Sprague-Dawley rats were also examined. Since individual differences were not observed in the glucuronidation of testosterone within these rat strains (Matsui *et al.*, 1979), the 17β -hydroxysteroid UDP-glucuronosyltransferase was included as a basis of comparison.

Experimental

Materials

[4- ^{14} C]Oestrone (57 mCi/mmol), [4- ^{14} C]testosterone (50 mCi/mmol), [1,2- ^{3} H]androsterone (40–60 Ci/mmol), *p*-nitro[U- ^{14} C]phenol (56 mCi/mmol) and UDP-[U- ^{14} C]glucuronic acid (180 mCi/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). Chenodeoxycholic acid ($3\alpha,7\alpha$ -dihydroxy-5 β -cholic acid) was purchased from Calbiochem/Behring (La Jolla, CA, U.S.A.). UDP-glucuronic acid, L- α -phosphatidylcholine (egg-yolk type XI-E), oestrone, *p*-nitrophenol, testosterone and androsterone were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Chromatofocusing materials were supplied by Pharmacia Fine Chemicals (Piscataway, NJ, U.S.A.). Emulgen 911 was obtained from Kao Atlas Ltd. (Tokyo, Japan). Protein concentrations were determined with the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA, U.S.A.). UDP-hexanolamine-Sepharose 4B affinity resin was prepared as previously described (Tukey & Tephly, 1981).

Animals

Female Sprague-Dawley rats (180–220 g) used in this study were obtained from Bio-Labs (St. Paul, MN, U.S.A.), and male Wistar rats (200–225 g) were purchased from Charles River (Wilmington, MA, U.S.A.). The animals were housed over wire mesh in stainless-steel cages and were

allowed food (Purina Rodent Chow; Purina, St. Louis, MO, U.S.A.) and water *ad libitum*. The light-dark cycle was 6:00 a.m. to 6:00 p.m. All rats were deprived of food overnight before preparation of hepatic microsomal fractions (referred to below simply as 'microsomes').

Preparation of microsomes

The rats were killed by cerebral concussion and their livers perfused *in situ* with ice-cold 1.15% (w/v) KCl. Livers were then removed and homogenized in 4 vol. of 1.15% KCl with a motor-driven Teflon pestle. Microsomes were then isolated by centrifugation (Tukey *et al.*, 1978) and stored at -70°C with a 1.15% KCl overlay until used. The microsomal pellets could be stored for about 3 months with no apparent loss of glucuronidation activity.

Enzyme assays

The rate of glucuronidation of chenodeoxycholic acid was assayed by using a radiometric method utilizing UDP-[^{14} C]glucuronic acid as described previously (Kirkpatrick *et al.*, 1984). *p*-Nitrophenol-glucuronidation activity was determined as previously described by Tukey *et al.* (1978), except that a final concentration of 4 mM for the substrate was used. Testosterone, androsterone and oestrone glucuronidation were assayed as previously described (Tukey *et al.*, 1978), with final substrate concentrations of 50 μM , 56.25 μM and 37.5 μM respectively. All reactions were performed at 37°C with 5 mM-UDP-glucuronic acid in the presence of 100 μg of added phosphatidylcholine. Kinetic studies with various concentrations of aglycone substrates were performed at a constant concentration of UDP-glucuronic acid (5 mM). Kinetic parameters were determined by regression analysis of double-reciprocal plots of velocity versus substrate concentration. All reaction rates were linear with respect to time and protein concentration.

Separation and purification of rat liver 17β -hydroxysteroid UDP-glucuronosyltransferases and 3α -hydroxysteroid UDP-glucuronosyltransferases

Frozen pellets of microsomes were thawed, the KCl overlay was removed and a 10% (w/v) suspension was formed by gentle homogenization in 25 mM-ethanolamine/HCl buffer, pH 9.4. All buffers utilized in the purification procedures also contained 20% (v/v) glycerol, 0.1 mM-dithiothreitol and 0.05% Emulgen 911. The microsomal suspension was then solubilized by the further addition of 0.5 mg of Emulgen 911/mg of protein, stirred for 20 min on ice and then centrifuged at 100 000 *g* for 30 min. Chromatofocusing of solubilized microsomal protein was conducted as previously described with a pH 9–pH 7 gradient (Falany & Tephly,

1983; Kirkpatrick *et al.*, 1984). Column fractions were assayed for 3 α - and 17 β -hydroxysteroid UDP-glucuronosyltransferase activities, with androsterone and testosterone respectively as substrates. After Chromatofocusing, fractions containing testosterone- or androsterone-glucuronidation activities were pooled and further purified by using affinity chromatography on UDP-hexanolamine-Sepharose 4B (Falany & Tephly, 1983; Kirkpatrick *et al.*, 1984).

Gel electrophoresis

Polyacrylamide-gel electrophoresis was performed in the presence of sodium dodecyl sulphate in a Hoefer Scientific model 6000 apparatus (Laemmli, 1970). Samples were pretreated by the addition of 2-mercaptoethanol and sodium dodecyl sulphate to final concentrations of 5% and 1% respectively, and then placed in a boiling-water bath for 10 min. After electrophoresis, the slabs were stained for 2 h in propan-2-ol/acetic acid/water (5:2:13, by vol.) mixture containing 0.1% Coomassie Blue G-250. The slabs were destained with propan-2-ol/acetic acid/water (1:1:8, by vol.). Minimum subunit M_r values were estimated by comparison with commercial standards (Pharmacia).

Results

Strain differences in androsterone glucuronidation

A study comparing glucuronidation rates in hepatic microsomes prepared from Sprague-Dawley and Wistar rats was conducted (Table 1). Liver microsomes from Wistar rats exhibited slightly lower rates (approx. 20%) for *p*-nitrophenol conjugation compared with those from Sprague-Dawley animals, but there were no apparent differences in the rates of testosterone or oestrone glucuronidation between these two strains of rats. In addition, no individual variability in the glucuronidation rates of these three substrates were observed for either strain. Individual differences in the glucuronidation rates of androsterone

and chenodeoxycholic acid, substrates previously shown to be glucuronidated by the 3 α -hydroxysteroid UDP-glucuronosyltransferase (Kirkpatrick *et al.*, 1984), were observed in Wistar rats but not with Sprague-Dawley rats. A number (50%) of Wistar rats (LA) were identified as having significantly lower rates of androsterone and chenodeoxycholic acid glucuronidation. These results agree with those obtained by Matsui *et al.* (1979). A second population of Wistar rats (HA) had rates of androsterone and chenodeoxycholic acid glucuronidation in liver microsomes that were similar to those obtained in liver microsomes of Sprague-Dawley rats. No individual variabilities were observed in the hepatic microsomal glucuronidation rates for these substrates in the Sprague-Dawley strain. These results are similar to those reported by Matsui *et al.* (1979) and Matsui & Watanabe (1982a).

The apparent K_m for androsterone in Sprague-Dawley rat liver microsomes was 5 μ M. The apparent K_m values for androsterone in HA and LA Wistar rats were 5 and 120 μ M respectively; the V_{max} values were 12.9 and 2.8 nmol/min per mg respectively. These results are similar to those obtained by Matsui & Hakozaki (1979), who reported apparent K_m values for androsterone of 20 and 100 μ M in hepatic microsomes prepared from HA and LA Wistar rats respectively. The apparent K_m for UDP-glucuronic acid (0.3 mM) was not different in hepatic microsomal preparations from either Wistar rats (LA or HA) or Sprague-Dawley rats. Thus LA Wistar rats have lower hepatic androsterone-glucuronidation rates and a higher apparent K_m for androsterone compared with HA Wistar or Sprague-Dawley animals.

Separation of 17 β -hydroxysteroid UDP-glucuronosyltransferases and 3 α -hydroxysteroid UDP-glucuronosyltransferases from Wistar (HA and LA) and Sprague-Dawley rats

Fig. 1 illustrates typical profiles of 17 β - and 3 α -hydroxysteroid UDP-glucuronosyltransferases eluted from Chromatofocusing columns when a

Table 1. Comparison of UDP-glucuronosyltransferase activities with various substrates in solubilized rat liver microsomes
For full experimental details see the text. Each value represents the mean $\pm$ S.E.M. for at least four animals.

Substrate	UDP-glucuronosyltransferase activity (nmol of substrate conjugated/min per mg of protein)		
	Sprague-Dawley rats	Wistar rats	
		HA	LA
Androsterone	11.0 $\pm$ 0.7	9.5 $\pm$ 0.6	1.1 $\pm$ 0.1
Chenodeoxycholic acid	0.8 $\pm$ 0.2	0.6 $\pm$ 0.1	0.14 $\pm$ 0.04
Testosterone	5.4 $\pm$ 0.4	5.9 $\pm$ 0.2	5.7 $\pm$ 0.2
Oestrone	0.9 $\pm$ 0.2	0.8 $\pm$ 0.2	0.9 $\pm$ 0.2
<i>p</i> -Nitrophenol	89.6 $\pm$ 3.7	69.5 $\pm$ 4.7	70.0 $\pm$ 3.0

Fig. 1. Chromatofocusing of Sprague-Dawley-rat and Wistar-rat liver microsomes

Solubilized microsomal protein (120 mg) from Sprague-Dawley (a) and Wistar rats with high (b) and low (c) androsterone-glucuronidation activity were applied to a Chromatofocusing column previously equilibrated with 25 mM-ethanolamine/HCl buffer, pH 9.4, and eluted with Polybuffer 96/HCl, pH 7.0, to generate a pH 9–pH 7 gradient (■). Fractions (5 ml) were collected and assayed for testosterone (●) and androsterone (○) glucuronidation. One unit of activity represents 1 nmol conjugated/min.

pH 9–pH 7 gradient is employed. Separation of two peaks of 17β -hydroxysteroid UDP-glucuronosyltransferase activity (pH 8.5 and 8.0) and two peaks of 3α -hydroxysteroid UDP-glucuronosyltransferase activity (pH 8.0 and 7.6) were observed

when activities were monitored from a column loaded with solubilized liver microsomes from either Sprague-Dawley rats (Fig. 1a) or HA Wistar rats (Fig. 1b). These results are similar to those reported previously for the separation of these isoenzymes from Sprague-Dawley-rat liver (Falany & Tephly, 1983).

The Chromatofocusing elution profile of 3α - and 17β -hydroxysteroid UDP-glucuronosyltransferases was different when solubilized liver microsomes from LA Wistar rats were used. One peak of 17β -hydroxysteroid UDP-glucuronosyltransferase was eluted at pH 8.5 and one small peak of 3α -hydroxysteroid UDP-glucuronosyltransferase was eluted at about pH 7.9 (Fig. 1c). Thus, when the activity of 3α -hydroxysteroid UDP-glucuronosyltransferase is low, only one major peak of 17β -hydroxysteroid-glucuronidation activity is observed. The amount of 3α -hydroxysteroid UDP-glucuronosyltransferase found within the peak at pH 7.9 represents about 10% of the activity seen in Fig. 1(a) (HA Wistar rats). Interestingly, only a small amount of 17β -hydroxysteroid UDP-glucuronosyltransferase is found within this peak. The recoveries of androsterone UDP-glucuronosyltransferase activity from the Chromatofocusing columns were similar for LA and HA rat liver (about 30%).

Fractions containing either 17β - or 3α -hydroxysteroid UDP-glucuronosyltransferases were pooled and applied to UDP-hexanolamine-Sepharose 4B affinity columns. The columns were then treated with the equilibration buffer containing 40 mM-KCl and 50 μ g of phosphatidylcholine/ml (Falany & Tephly, 1983). 17β -Hydroxysteroid UDP-glucuronosyltransferases and 3α -hydroxysteroid UDP-glucuronosyltransferases from both strains of rats were eluted from the affinity columns with 0.1 mM-UDP-glucuronic acid. These results indicated that there were no strain differences in the elution characteristics of these transferases from affinity columns. In addition, the recoveries of activity applied to the affinity columns (75–80%) were not different between the various samples.

The 17β -hydroxysteroid UDP-glucuronosyltransferase displayed a subunit M_r of 50000 from LA and HA Wistar rats as well as from Sprague-Dawley rats (Fig. 2). These results suggest that, qualitatively, the 17β -hydroxysteroid UDP-glucuronosyltransferases are similar in Wistar and Sprague-Dawley rats. The subunit M_r values (52000) of the purified 3α -hydroxysteroid UDP-glucuronosyltransferases from HA Wistar rats and Sprague-Dawley rats were similar (Fig. 3). No detectable 3α -hydroxysteroid UDP-glucuronosyltransferase activity was eluted at pH 7.6 when microsomes from LA Wistar rats were resolved by using Chromatofocusing. Sodium dodecyl sulph-

Fig. 2. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of purified 17 β -hydroxysteroid UDP-glucuronosyltransferase isoenzymes

Electrophoresis was performed by the method of Laemmli (1970) in 10% polyacrylamide gels. Lane A contains commercial standards with minimum M_r values of 94 000, 67 000, 43 000, 30 000 and 21 000. Affinity-purified 17 β -hydroxysteroid UDP-glucuronosyltransferases were from Sprague-Dawley (lane B) and HA (lane C) and LA (lane D) Wistar rats. Electrophoresis was run from top to bottom, and the gels were stained with Coomassie Blue G-250.

Fig. 3. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of purified 3 α -hydroxysteroid UDP-glucuronosyltransferase isoenzymes

Electrophoresis and standards with minimum M_r values were the same as those given in the Fig. 2 legend. Lane B contains affinity-purified 3 α -hydroxysteroid UDP-glucuronosyltransferase from Sprague-Dawley rats. Lane C and lane D (20-fold concentrated) contain this transferase from HA and LA Wistar rats respectively.

ate/polyacrylamide-gel electrophoresis was performed with fractions, obtained at pH 7.9 from LA Wistar rats, that had been further purified by affinity chromatography and that were concentrated 10-fold. It was necessary to concentrate these affinity-purified fractions in order to detect the protein bands on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 3, lane D). A major protein monomer was observed at M_r 52 000, corresponding to the mobility of the 3 α -hydroxysteroid UDP-glucuronosyltransferase. A very

small amount of 17 β -hydroxysteroid UDP-glucuronosyltransferase (M_r 50 000) may also be present. Thus livers of LA Wistar rats contain about 10% or less 3 α -hydroxysteroid UDP-glucuronosyltransferase protein than do livers of HA Wistar rats.

Comparison of kinetic parameters and substrate specificities of purified 3 α -hydroxysteroid UDP-glucuronosyltransferase and 17 β -hydroxysteroid UDP-glucuronosyltransferase in LA Wistar, HA Wistar and Sprague-Dawley rats

The apparent K_m values for testosterone with purified 17 β -hydroxysteroid UDP-glucuronosyltransferases from HA and LA Wistar rats and Sprague-Dawley rats are similar (Table 2). In

Table 2. Kinetic parameters for purified 3 α -hydroxysteroid UDP-glucuronosyltransferase and 17 β -hydroxysteroid UDP-glucuronosyltransferases from Sprague-Dawley and Wistar rats

	K_m (μ M)		
	Sprague-Dawley rats	Wistar rats	
		HA	LA
3 α -Hydroxysteroid UDP-glucuronosyltransferase			
Androsterone	9	6	120
17 β -Hydroxysteroid UDP-glucuronosyltransferase			
Testosterone	13	12	20
<i>p</i> -Nitrophenol	2000	2000	2000

addition to testosterone, this isoenzyme glucuronidated *p*-nitrophenol and α -naphthol, but not 4-methylumbelliferone, oestrone or androsterone. Therefore the 17 β -hydroxysteroid UDP-glucuronosyltransferases in LA and HA Wistar rats and Sprague-Dawley rats appear to be qualitatively similar. In addition, the apparent K_m for *p*-nitrophenol glucuronidation catalysed by the 17 β -hydroxysteroid transferase enzyme was similar in both strains of rats.

The kinetic parameters for androsterone with the purified 3 α -hydroxysteroid UDP-glucuronosyltransferase were similar when HA Wistar rats and Sprague-Dawley rats were compared (Table 2). However, consistent with results obtained with microsomal preparations, the apparent K_m value for androsterone was significantly higher with the enzyme purified from LA Wistar rats.

Discussion

The Wistar strain of rats has been used extensively for the study of glucuronidation and for the purification of individual isoenzymes of UDP-glucuronosyltransferase (Bock *et al.*, 1979; Burchell, 1978, 1980; Ullrich & Bock, 1984). Our study confirms the work of Matsui & Hakozaiki (1979) and Matsui *et al.* (1979), who demonstrated individual variability in the glucuronidation of androsterone, but not for other substrates, such as testosterone or *p*-nitrophenol, within a population of Wistar rats. Work in our laboratory has previously shown that androsterone is glucuronidated by a specific isoform (3 α -hydroxysteroid) of UDP-glucuronosyltransferase (Falany & Tephly, 1983; Falany *et al.*, 1985). The present study has shown that, in addition to androsterone, LA Wistar rats also are deficient in their ability to glucuronidate the bile acid chenodeoxycholic acid, another important substrate for the 3 α -hydroxysteroid UDP-glucuronosyltransferase enzyme (Kirkpatrick *et al.*, 1984).

Three possible explanations could account for the observed low activity of the 3 α -hydroxysteroid

UDP-glucuronosyltransferase in LA Wistar rats. First, it is possible that a smaller amount of the protein is present in the livers of these animals. Secondly, it is possible that the same amount of protein is present in the animals with low 3 α -hydroxysteroid-glucuronidation activity but that this protein is somewhat modified, resulting in lower catalytic activity. Thirdly, a combination of the two preceding possibilities could exist. Our results suggest that there are both qualitative as well as quantitative differences in the 3 α -hydroxysteroid UDP-glucuronosyltransferase of LA Wistar rats. Quantitative differences in the amount of this enzyme are suggested by the fact that a 10-fold concentration of the affinity-purified enzymes was necessary before a protein band was observed after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis when enzyme from LA Wistar rats was used. Since similar recoveries of activity were observed after the Chromatofocusing and affinity-chromatography procedures, the amount of enzyme present in hepatic microsomes is less in LA Wistar rats.

The qualitative differences in the 3 α -hydroxysteroid UDP-glucuronosyltransferases in LA and HA Wistar rat liver appear to be at the 3 α -hydroxysteroid-binding site. We in the present study and others (Matsui & Hakozaiki, 1979) have reported that LA Wistar rats have a high apparent K_m for androsterone, whereas the HA-Wistar-rat liver transferase exhibited a low apparent K_m for this substrate. These data indicate that the form of the enzyme present in animals with low androsterone-glucuronidation activity has a lower affinity for this substrate. In contrast, no difference in the apparent K_m for UDP-glucuronic acid was observed. Assuming that the UDP-glucuronosyltransferases bind to the UDP-hexanolamine-Sepharose 4B affinity column via the UDP-glucuronic acid-binding site, the fact that the 3 α -hydroxysteroid UDP-glucuronosyltransferase from LA Wistar rats is specifically eluted from the affinity column under the same conditions as that from HA Wistar and Sprague-Dawley rats sup-

ports the observation that the UDP-glucuronic acid-binding site was similar in enzymes obtained from livers of these three groups of animals. The observation that the apparent K_m for androsterone with the 3 α -hydroxysteroid UDP-glucuronosyltransferase from LA Wistar rats remained high after extensive purification of the enzyme suggests that the defect in this isoenzyme is not due to an abnormal membrane microenvironment, as postulated by Matsui & Watanabe (1982a).

That more than 95% of the testosterone-glucuronidation activity is eluted in a single peak (pH 8.5) when solubilized microsomes from LA Wistar rats are applied to a Chromatofocusing column indicates that larger amounts of the 17 β -hydroxysteroid UDP-glucuronosyltransferase enzyme uncontaminated with androsterone-glucuronidation activity can be isolated from these animals compared with Sprague-Dawley-rat liver microsomes. The fact that some of the Wistar rats have small amounts of 3 α -hydroxysteroid UDP-glucuronosyltransferase also has implications for the purification of other UDP-glucuronosyltransferase isoenzymes in this strain of rats. Chowdhury *et al.* (1984, 1985) have suggested, from experiments with microsomes from Wistar rats, that isoenzymes of UDP-glucuronosyltransferase that conjugate bilirubin and oestrone are eluted from a Chromatofocusing column in the pH 8–pH 7 range. Since some Wistar rats are low in 3 α -hydroxysteroid UDP-glucuronosyltransferase, it should be possible to purify these other UDP-glucuronosyltransferases without substantial contamination with the 3 α -hydroxysteroid enzyme.

Matsui & Watanabe (1982a) have reported that the genetic expression of high UDP-glucuronosyltransferase activity towards androsterone in the Wistar rat is inherited in an autosomal dominant fashion. The data presented in the present study suggest that the low androsterone- and bile acid-glucuronidation activity in LA Wistar rats is a result of a small amount of a modified 3 α -hydroxysteroid UDP-glucuronosyltransferase protein. The possibility also exists that there is a second enzyme that glucuronidates androsterone in these animals but has a lower affinity for this substrate. Matsui & Watanabe (1982b) showed that androsterone glucuronidation was markedly enhanced after 30 days of age in the HA group, whereas the activity remained low in the LA group. However, age-related changes in the apparent K_m for androsterone were not reported. Therefore it is not known whether the increase in androsterone-glucuronidation activity in HA Wistar rats is accompanied by a decrease in the

apparent K_m for substrate or whether the increase is due primarily to an increase in the amount of protein. Further studies, with specific antibodies to the 3 α -hydroxysteroid UDP-glucuronosyltransferase, are needed to help resolve these issues.

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