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GENDER-RELATED DIFFERENCES IN THE AMOUNT AND FUNCTIONAL STATE OF RAT LIVER UDP-GLUCURONOSYLTRANSFERASE

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Abstract—The basis for gender-dependent differences in rates of glucuronidation of xenobiotics is uncertain. To clarify this issue, the glucuronidation of *p*-nitrophenol was compared in liver microsomes from adult male and female rats. The activity of native UDP-glucuronosyltransferase was 47% higher in microsomes from male than from female rats. Immunoblotting of microsomal protein with anti-UDP-glucuronosyltransferase antiserum revealed 66% more immunoreactive protein in male microsomes. A kinetic method for measuring glucuronidating enzyme content confirmed the result of the immunoblot. Responses of UDP-glucuronosyltransferase to activation by palmitoyl-lysophosphatidylcholine or high pressure indicated that the activity of the enzyme was more latent in male than in female microsomes. Differences in enzyme latency could be due to differences in membrane structure. A comparison of microsomal fatty acid composition revealed significantly higher levels of oleic and linoleic acids and lower levels of stearic and docosahexaenoic acids in male than in female microsomes. The phospholipid composition, ratio of cholesterol : phospholipid, and membrane fluidity were similar in male and female microsomes. These results indicate that gender-dependent differences in UDP-glucuronosyltransferase activity are due to differences in both the amount and functional state of the enzyme.

Key words: glucuronidation; *p*-nitrophenol; gender; rat; liver

The microsomal UGTs§ are a family of enzymes that conjugate a broad variety of endogenous and exogenous molecules [1]. Gender-dependent differences in rates of glucuronidation are known to exist [2]. More specifically, glucuronidation reactions have been reported to be faster in male than in female animals due to increased UGT activity [3–7]. However, the basis for the increased enzyme activity in male animals remains to be explained.

The UGTs are known to have complex regulatory properties that depend, in part, on interactions between the enzyme and its membrane environment [8–10]. It is known, for example, that the activity of UGT can vary more than 100-fold as a function of the lipid used to reconstitute pure enzyme [11]. Thus, gender-dependent differences in enzyme activity could reflect differences in functional state (activity per molecule). Alternatively, gonadal hormones modulate the expression of genes coding for other xenobiotic metabolizing enzymes such as the cytochromes P450 and glutathione *S*-transferases [12, 13]. It is possible, therefore, that gender-dependent differences in UGT activity could be secondary to differences in amounts of enzyme.

Given the significance of glucuronidation reactions for protection against xenobiotic-induced toxicity, it is important to understand the factor(s) contributing to differences in enzyme activity. This information could lead, for example, to strategies for increasing the capacity for glucuronidation. We examined, in the present work, which of these factors account for differences in the activity of UGT in male versus female liver microsomes. The data presented show that the activity of UGT is greater in microsomes from male rats because the amount of enzyme is greater. An apparent difference in the functional state of the enzyme was also detected.

MATERIALS AND METHODS

Chemicals. UDPGA (ammonium salt), *p*-nitrophenol, D-saccharic acid 1,4-lactone, PLPC and DPH were obtained from the Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest grade commercially available.

Animals. Male and female Wistar rats (15 to 17-weeks-old) were used for all experiments. They were fed a standard laboratory pellet diet *ad lib.* and allowed free access to water. Female animals were

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§ Abbreviations: UGT, uridine diphosphate-glucuronosyltransferase; UDPGA, uridine diphosphoglucuronic acid; PLPC, palmitoyl-lysophosphatidylcholine; and DPH, 1,6-diphenyl-1,3,5-hexatriene.

killed irrespective of the day of estrus, since in previous work [14] we found no differences in either native UGT activity or percent activation in female rats stratified for stages in the estrus cycle, in concordance with others [6].

Isolation of microsomes. All animals were deprived of food 18 hr before being killed. They were exsanguinated by cardiac puncture under pentobarbital anesthesia (50 mg/kg body weight) between 10:00 and 11:00 a.m., to avoid possible effects of diurnal variations. Livers were perfused *in situ* with ice-cold 0.9% NaCl solution through the portal vein, and then they were promptly removed and weighed. The liver–body weight ratio was calculated. Microsomal pellets were obtained [15] and resuspended in 0.15 M Tris–HCl buffer (pH 7.40). Protein content of microsomal preparations was determined by the Biuret method [16] and then adjusted to about 12 mg/mL with the same buffer before enzyme assays.

Activation of microsomal UGT with PLPC. An excess of PLPC was incorporated in the incubation medium with respect to the normal lipid components of microsomes to displace endogenous lipids from the enzyme microenvironment. This methodology serves to abolish possible differences in membrane lipid composition between groups and was used both to estimate the influence of the enzyme environment on glucuronidating activity and to estimate the amount of catalytic units [17]. For the last purpose, V_{max} toward UDPGA was calculated in both experimental groups. The concentration of PLPC required to maximally activate UGT was determined by adding variable quantities of this detergent to microsomes (0.05 to 1.00 mg/mg microsomal protein) before determining enzyme activity. The optimal detergent:protein ratio was about 0.15 (w/w) for both groups and was used systematically in the experiments.

Enzyme assay. Glucuronidation of *p*-nitrophenol was determined by measuring the rate of disappearance of substrate using a spectrophotometric method [18]. D-Saccharic acid, 1,4-lactone (2 mM) was included systematically in the incubation medium to inhibit enzymatic hydrolysis of *p*-nitrophenol glucuronides. The assay contained: 0.8 mM *p*-nitrophenol, 9 mM $MgCl_2$ and native or activated microsomes (about 0.6 and 0.3 mg of microsomal protein, respectively); reactions were initiated with the addition of UDPGA (3.6 mM). For kinetic studies performed using PLPC-activated preparations, enzyme activity was measured at different concentrations of UDPGA (0.6, 1.0, 1.8, 3.6, 6.0 and 12.0 mM) to estimate the apparent V_{max} value toward UDPGA. This value was calculated with the aid of a non-linear square-fitting program [19].

Immunoblot analysis of microsomes. SDS–PAGE (10%) was performed under reducing conditions according to the method of Laemmli [20]. Proteins were transferred onto nitrocellulose sheets (Sigma) by the method of Towbin *et al.* [21]. The nitrocellulose sheet was then incubated with a rabbit anti-UGT antiserum [22] for 2 hr. Subsequently, the nitrocellulose membrane was probed with goat anti-rabbit antibody conjugated to alkaline phosphatase (Sigma). The UGTs were then detected by the alkaline phosphatase color reaction using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium. Immunoreactive protein bands were quantified by densitometry (Shimadzu, CS-9000).

High pressure studies. Microsomal samples were subjected to hydrostatic pressure at 10° since maximal activation has been shown to occur at this temperature [23, 24]. The activity of UGT was determined immediately after pressure treatment.

Lipid analysis. Aliquots of the suspended microsomes were extracted by the procedure of Folch *et al.* [25]. Phospholipids were separated by TLC with a double development [26] using the solvents $Cl_3CH/CH_3OH/NH_3/H_2O$ (70:25:3.5:1.5) in the first run and Cl_3CH/CH_3OH/acetic acid/H_2O (80:10:2:0.75) in the second run. Total cholesterol was determined by the cholesterol oxidase method [27]. Lipid phosphate was measured by the method of Chen *et al.* [28].

Fatty acid composition was determined by gas–liquid chromatography of the methyl esters [29] in a GOW-MAC chromatograph model 580. A Krompex column filled with 15% diethylene glycol succinate (DEGS) on 100–120 chromosorb W-HP was used.

Polarization studies. Steady-state fluorescence polarization measurements of DPH were made at 37° using a Perkin–Elmer spectrofluorometer equipped with two polarizers as previously described [30]. The rotational motion of the probe molecule is characterized by the steady-state fluorescence anisotropy defined by:

$$r = \frac{I_{vv} - G\,I_{vh}}{I_{vv} + 2\,G\,I_{vh}}$$

where I_{vv} and I_{vh} are the vertical and horizontal polarized fluorescence intensities, respectively, when the excitation beam is vertically polarized. G is a "grating factor" that corrects for the artifacts of the instruments and is equal to I_{hv}/I_{hh}, with I_{hv} and I_{hh} being the vertical and horizontal polarized fluorescence intensities when the excitation beam is horizontally polarized.

Statistical analysis. Results are presented as means ± SD for 5 rats in each group. Comparisons between groups were made by Student's *t*-test. A difference between groups of $P < 0.05$ was considered significant.

RESULTS

The relative liver weights (percentage of body weight) were similar in male and female rats (3.5 ± 0.3 and 3.7 ± 0.1%, respectively). Recovery of microsomal protein was also similar in both groups (14.7 ± 1.0 mg/g liver weight for males and 13.5 ± 1.7 mg/g liver weight for females).

Effect of gender on the activity of UGT. The activity of UGT was determined in both untreated and PLPC-treated liver microsomes. As shown in Table 1, enzyme activity was 47% higher in untreated male than female microsomes. After treatment with PLPC, this difference increased. Thus, the activity of UGT was 84% higher in male than female microsomes after treatment with detergent.

Table 1. Gender-dependent differences in the glucuronidation of *p*-nitrophenol

	p-Nitrophenol glucuronidation (nmol/min/mg microsomal protein)		
	Native	PLPC	% Activation
Male	6.3 ± 0.5*	53.0 ± 6.8*	741 ± 97*
Female	4.3 ± 0.4	28.8 ± 4.4	570 ± 80

Hepatic glucuronidation of *p*-nitrophenol was measured in native and activated microsomes. To activate UGT, microsomes were incubated with PLPC at a ratio of detergent:protein of 0.15 (w/w). Enzyme assays were performed as described in Materials and Methods using 0.8 mM *p*-nitrophenol and 3.6 mM UDPGA. Values are means ± SD, N = 5.

* Significantly different from female rats ($P < 0.05$).

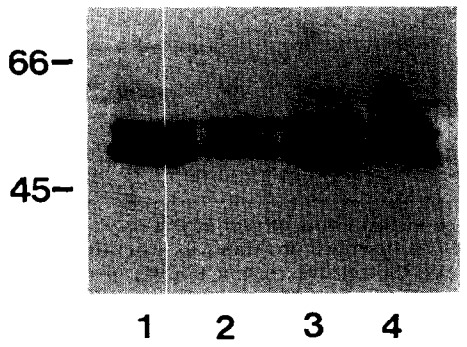

Fig. 1. Gender-dependent differences in the amount of UGT detected in microsomes by western blotting. Lanes 1 and 3 represent microsomal UGT from male rats and lanes 2 and 4 that from females. Equal amounts of total microsomal protein (10 μg) were loaded in each lane. Densitometry was performed on total UGT as described in Materials and Methods. Mean values of the corresponding areas are: male rats, 1433 arbitrary units; and female rats, 863 arbitrary units.

Gender-dependent differences in the amount of UGT in liver microsomes. Western blotting was done to investigate the possibility that the activity of UGT was higher in male microsomes because of gender-dependent differences in amounts of enzyme. As shown in Fig. 1, an immunoblot with antiserum raised against a phenol-conjugating isoform of UGT revealed more immunoreactive protein in male than female microsomes. By densitometry, the content of UGT was about 66% higher in male than in female liver microsomes.

Our antiserum cross-reacts with multiple isoforms of UGT, which is also true for monoclonal antibodies [31]. Since we were interested specifically in the isoforms of UGT that conjugate *p*-nitrophenol, it was necessary to use a second approach to compare the amounts of enzyme in male and female microsomes. In previous reports, we showed that a kinetic analysis could be used to confirm differences in amounts of enzyme suggested by western blot analysis [17, 22]. Lysophosphatidylcholines are excellent lipids for this purpose because they are activators of pure, delipidated UGT [11] as well as enzyme in microsomes [14, 17, 22]. The estimation of V_{max} toward UDPGA, using microsomes pretreated with PLPC, confirmed the results of the immunoblot. Thus, as shown in Table 2, V_{max} was about 78% higher in male than in female rat liver microsomes K_m was unaffected by gender.

Table 2. Apparent kinetic constants toward UDPGA

	V_{max} (nmol/min/mg microsomal protein)	K_m (mM)
Male	64.0 ± 13.8*	0.9 ± 0.2
Female	36.0 ± 7.4	1.1 ± 0.2

Kinetic parameters for UDPGA were determined using variable concentrations of this substrate (0.6 to 12.0 mM) and a fixed concentration of *p*-nitrophenol (0.8 mM). Microsomes were incubated with PLPC at a ratio of detergent:protein of 0.15 (w/w). Apparent kinetic constants were calculated with a non-linear regression program. Data represent mean values ± SD, N = 5.

* Significantly different from females ($P < 0.05$).

Effect of gender on the latency of UGT in microsomes. The activity of UGT in untreated microsomes is constrained because a variety of chemical and physical treatments are associated with a large increase in the activity of the enzyme [23, 32, 33]. The basis for this constraint is controversial [10, 33–35]. Nevertheless, it is clear that UGT can exist in microsomes in a native or constrained state and in an activated state. We examined the possibility that there could be gender-dependent differences in the latency of UGT. As shown in Table 1, pretreatment with PLPC resulted in greater activation of UGT in male (741 ± 97%) than in female (570 ± 80%) microsomes, suggesting that the average degree of restriction of all native *p*-nitrophenol glucuronidating isoforms in expressing their activity was higher in males, thus exhibiting a lower activity per molecule (a higher latency).

High pressure was used to confirm the existence of more constrained enzyme in microsomes from male versus female rats. The data in Fig. 2 show the effect of high pressure on the activity of UGT, which was assayed after release of pressure. The extent of activation increased smoothly at pressures above 1.5 kbar in both male and female microsomes. However, the extent of activation was greater in microsomes prepared from male than female rats. For example, at 2.25 kbar, enzyme activity was stimulated 4.6-fold in microsomes from male rats but only 2.3-fold in microsomes from female rats. These data are consistent with the idea that the activity of UGT is more constrained in male than female microsomes.

Effect of gender on the lipid composition and viscosity of the microsomal membrane. Gender-dependent differences in membrane structure could

Table 3. Cholesterol and phospholipid composition of microsomal membranes

	Male	Female
Phospholipid composition (%)		
Phosphatidylcholine	51.2 ± 2.6	52.5 ± 3.3
Sphingomyelin	4.5 ± 0.7	3.8 ± 0.4
Phosphatidylethanolamine	29.2 ± 3.9	29.1 ± 1.9
Phosphatidylserine + phosphatidylinositol	15.1 ± 3.2	14.6 ± 2.0
Phosphatidylcholine : sphingomyelin ratio (mol/mol)	11.5 ± 3.0	13.7 ± 3.1
Cholesterol : phospholipid ratio (mol/mol)	0.15 ± 0.01	0.14 ± 0.03

Values are means ± SD, N = 5.

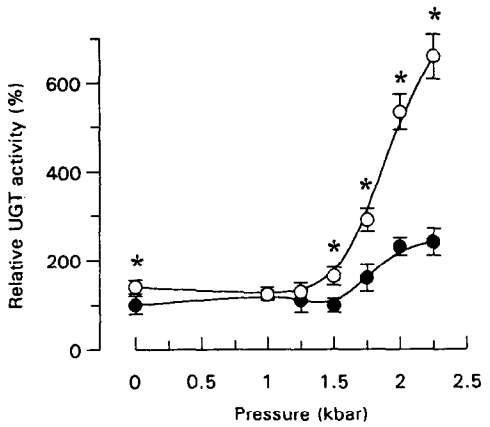

Fig. 2. Effect of high pressure treatment on the activity of UGT. Microsomes (4 mg/mL) from male (○) and female rats (●) were treated at the indicated pressure for 10 min at 10°. The reaction mixture contained 10.0 mM UDPGA and 0.05 mM *p*-nitrophenol. Assays were performed with 40 μg microsomal protein. Activities were measured after release of pressure and were expressed as a percentage of female native activity (1.1 ± 0.2 nmol/min/mg microsomal protein). Values are means ± SD, N = 5. Key: (*)significantly different from the corresponding female activity ($P < 0.05$).

Table 4. Fatty acid composition of liver microsomes

Fatty acid	Male	Female
16:0	22.9 ± 1.6	20.5 ± 1.5
16:1	0.9 ± 0.3	0.9 ± 0.1
16:2	0.5 ± 0.2	0.6 ± 0.2
18:0	30.8 ± 1.7	34.2 ± 2.9*
18:1	6.8 ± 0.7	4.5 ± 0.7*
18:2	11.4 ± 1.2	9.4 ± 0.8*
18:3	0.9 ± 0.4	1.8 ± 0.9
20:3	0.5 ± 0.3	0.4 ± 0.1
20:4	22.5 ± 3.1	21.5 ± 0.7
22:6	2.8 ± 1.7	6.2 ± 2.0*
UI†	1.35 ± 0.04	1.47 ± 0.08*

The methyl esters of the fatty acids were analyzed by GLC as described in Materials and Methods. Values indicate the relative content (% wt) of each individual fatty acid and are expressed as means ± SD, N = 5.

* Significantly different from female rats ($P < 0.05$).

† Unsaturation index (UI) = $\Sigma n_i x_i / FA$, where n_i = number of double bonds in each fatty acid, x_i = moles of each fatty acid, and FA = total moles of fatty acid.

potentially contribute to the differences in enzyme constraint described above. Lipid–protein interactions are known, for example, to be an important determinant of the functional state of UGT [11, 36]. Changes in the ratio of cholesterol : phospholipid have also been reported to alter the activity of UGT [37, 38]. As shown in Table 3, the phospholipid composition and ratio of cholesterol : phospholipid were identical in male and female microsomes. In contrast, the fatty acid composition differed in microsomes from male and female rats. As shown in Table 4, male microsomes contained more oleic (18:1) and linoleic (18:2) acids but less stearic (18:0) and docosahexaenoic (22:6) acids than female microsomes. The unsaturation index was greater in female than male microsomes. Finally, we detected no difference in the fluidity of male and female microsomes. Thus, the fluorescence anisotropy (*r*) for DPH was the same in microsomes from male (0.116 ± 0.004) and female (0.113 ± 0.006) rats.

DISCUSSION

Gender-dependent differences in xenobiotic metabolism are known to exist in both humans and experimental animals. In general, males demonstrate greater metabolic activity than females. Although considerable progress has been made in understanding the importance of gender in regulating the levels of specific isoforms of cytochrome P450, much less is known about the UGTs [12, 39, 40]. It does appear, however, that females tend to have higher plasma concentrations as well as longer half-lives for drugs metabolized primarily by glucuronidation [41–43]. The current experiments help to clarify the enzymatic basis for gender-dependent differences in rates of glucuronidation by liver microsomes. The data show that the activity of UGT is higher in liver microsomes from male than female rats because there is more enzyme. We also detected gender-dependent differences in the functional state of UGT.

The cellular level at which hormones or other effectors modulate the amount of UGT is unknown. Higher levels of enzyme could be present in male rats due to differences in rates of enzyme synthesis

or degradation which, in turn, could reflect differences in rates of gene transcription, mRNA stability or protein stability. Measurements of mRNA levels should provide some insight into this question. Studies in rats have shown that the expression of several sexually differentiated proteins including cytochrome P450 2C11 is regulated by plasma growth hormone profiles [40]. Gonadal hormones confer gender-specific patterns of liver gene expression indirectly through their effects on the hypothalamic-pituitary axis and its control of growth hormone secretion [39, 40]. It is reasonable to speculate that similar mechanisms may be responsible for gender-specific expression of UGT.

Another interesting and potentially important finding is the difference in functional state in UGT in male and female microsomes. Treatment of microsomes with detergent or high pressure showed that the activity of UGT was more constrained, i.e. latent, in male than female microsomes. Lipid–protein and protein–protein interactions are important determinants of functional state [11, 36, 44]. Changes in either of these interactions are known to result in marked increases in enzyme activity. The basis for the observed gender-dependent difference in functional state is unclear. Differences in protein–protein interactions may occur since there is evidence that UGT exists as an oligomer [44, 45]. The fact that the concentration of UGT is higher in male microsomes might alter the number of subunits comprising an oligomer and hence intermolecular interactions and functional state. This possibility is attractive because it links the observed differences in enzyme concentration and functional state. Another possibility is that the composition of lipid comprising the microenvironment of UGT differs in male and female microsomes, accounting for the difference in functional state. In fact, the data in Table 4 suggest that differences in fatty acid composition may contribute to gender-specific differences in functional state. This is a reasonable explanation since the secretory pattern of growth hormone is important for the regulation of gender-specific differences in the fatty acid composition of liver phosphatidylcholine in addition to levels of xenobiotic metabolizing enzymes [46].

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REFERENCES

1. Burchell B, Nebert DW, Nelson DR, Bock KW, Iyanagi T, Jansen PLM, Lancet D, Mulder GJ, Roy Chowdhury J, Siest G, Tephly TR and Mackenzie PI, The UDP-glucuronosyltransferase gene superfamily: Suggested nomenclature based on evolutionary divergence. *DNA Cell Biol* **10**: 487–494, 1991.
2. Mulder GJ, Sex differences in drug conjugations and their consequences for drug toxicity. Sulfation, glucuronidation and glutathione conjugation. *Chem Biol Interact* **57**: 1–15, 1986.
3. Lamartiniere CA, Dieringer CS, Kita E and Lucier GW, Altered sexual differentiation of hepatic uridine diphosphate glucuronyltransferase by neonatal hormone treatment in rats. *Biochem J* **180**: 313–318, 1979.
4. Matsui M and Watanabe HK, Developmental alteration of hepatic UDP-glucuronosyltransferase and sulphotransferase towards androsterone and 4-nitrophenol in Wistar rats. *Biochem J* **204**: 441–448, 1982.
5. Santa María C and Machado A, Changes of some hepatic enzyme activities related to phase II drug metabolism in male and female rats as a function of age. *Mech Ageing Dev* **44**: 115–125, 1988.
6. Zanninelli G, Watson K, Patton G, Robins S and Gollan JL, Sex differences in function, structure and phospholipid composition of hepatocellular endoplasmic reticulum. In: *Biotechnology of Cell Regulation* (Eds. Verna R and Nishizuka Y), Vol. 4, pp. 385–388. Raven Press, New York, 1991.
7. Catania VA, Luquita MG, Sánchez Pozzi EJ, Ferri AM and Mottino AD, Absence of hepatic *p*-nitrophenol UDP-glucuronosyltransferase induction by spironolactone in male rats: Possible involvement of testosterone. *Can J Physiol Pharmacol* **70**: 1502–1507, 1992.
8. Zakim D, Goldenberg J and Vessey DA, Influence of membrane lipid on the regulatory properties of UDP-glucuronyltransferase. *Eur J Biochem* **38**: 59–63, 1973.
9. Singh OMP, Graham AB and Wood GC, The phospholipid-dependence of UDP-glucuronosyltransferase. Purification, delipidation, and reconstitution of microsomal enzyme from guinea-pig liver. *Eur J Biochem* **116**: 311–316, 1981.
10. Zakim D and Dannenberg AJ, How does the microsomal membrane regulate UDP-glucuronosyltransferases? *Biochem Pharmacol* **43**: 1385–1393, 1992.
11. Magdalou J, Hochman Y and Zakim D, Factors modulating the catalytic specificity of a pure form of UDP-glucuronosyltransferase. *J Biol Chem* **257**: 13624–13629, 1982.
12. Kobliakov V, Popova N and Rossi L, Regulation of the expression of the sex-specific isoforms of cytochrome P-450 in rat liver. *Eur J Biochem* **195**: 585–591, 1991.
13. Singhal SS, Saxena M, Ahmad H and Awasthi YC, Glutathione *S*-transferases of mouse liver: Sex-related differences in the expression of various isozymes. *Biochim Biophys Acta* **1116**: 137–146, 1992.
14. Luquita MG, Sánchez Pozzi EJ, Catania VA and Mottino AD, Analysis of *p*-nitrophenol glucuronidation in hepatic microsomes from lactating rats. *Biochem Pharmacol* **47**: 1179–1185, 1994.
15. Siekevitz P, Preparation of microsomes and submicrosomal fractions. *Methods Enzymol* **5**: 61–68, 1962.
16. Gornall AG, Bardawill CJ and David MM, Determination of serum proteins by means of the biuret reaction. *J Biol Chem* **177**: 751–766, 1949.
17. Dannenberg AJ, Worman HJ and Scarlata S, Developmental changes in the amount and functional state of UDP-glucuronosyltransferase. *Biochim Biophys Acta* **1116**: 250–255, 1992.
18. Lucier GW, Sonowane BR and McDaniel OS, Glucuronidation and deglucuronidation reactions in hepatic and extrahepatic tissues during perinatal development. *Drug Metab Dispos* **5**: 279–287, 1977.
19. Yamaoka K, Tanigawara Y, Nakagawa T and Uno T, A pharmacokinetic analysis program (MULTI) for microcomputer. *J Pharmacobiodyn* **4**: 879–885, 1981.
20. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685, 1970.
21. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedures and some applications. *Proc Natl Acad Sci USA* **76**: 4350–4354, 1979.
22. Dannenberg AJ and Zakim D, Dietary lipid regulates

the amount and functional state of UDP-glucuronosyltransferase in rat liver. *J Nutr* **122**: 1607–1613, 1992.
23. Dannenberg AJ, Kavecansky J, Scarlata S and Zakim D, Organization of microsomal UDP-glucuronosyltransferase. Activation by treatment at high pressure. *Biochemistry* **29**: 5961–5967, 1990.
24. Kavecansky J, Dannenberg AJ and Zakim D, Effects of high pressure on the catalytic and regulatory properties of UDP-glucuronosyltransferase in intact microsomes. *Biochemistry* **31**: 162–168, 1992.
25. Folch J, Less M and Sloane-Stanley GH, A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**: 497–509, 1957.
26. Neskovic N and Kostic D, Quantitative analysis of rat liver phospholipids by a two-step thin-layer chromatographic procedure. *J Chromatogr* **35**: 297–300, 1968.
27. Omodeo-Salé F, Marchesini S, Fichman PH and Berra B, A sensitive enzymatic assay for determination of cholesterol in lipid extracts. *Anal Biochem* **142**: 347–350, 1984.
28. Chen PS, Toribara TY and Warner H, Microdetermination of phosphorus. *Anal Chem* **28**: 1756–1758, 1956.
29. Metcalfe LD, Schmitz AA and Pelka JR, Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal Chem* **38**: 514–515, 1966.
30. Sánchez Pozzi EJ, Rodríguez Garay EA and Mottino AD, Analysis of the interaction uridin 5′-diphosphoglucuronic acid with intestinal bilirubin UDP-glucuronyltransferase. *Int J Biochem* **24**: 1429–1434, 1992.
31. van Es HHG, Goldhoorn BG, Paul-Abrahamse M, Oude Elferink RPJ and Jansen PLM, Immunochemical analysis of uridine diphosphate-glucuronosyltransferase in four patients with the Crigler-Najjar syndrome type I. *J Clin Invest* **85**: 1199–1205, 1990.
32. Dannenberg AJ, Wong T and Zakim D, Effect of brief treatment at alkaline pH on the properties of UDP-glucuronosyltransferase. *Arch Biochem Biophys* **277**: 312–317, 1990.
33. Dannenberg A and Zakim D, Effects of prochlorperazine on the function of integral membrane proteins. *Biochem Pharmacol* **37**: 1259–1262, 1988.
34. Berry C and Hallinan T, Summary of a novel, three-component regulatory model for uridine diphosphate glucuronyltransferase. *Biochem Soc Trans* **4**: 650–652, 1976.
35. Vanstapel F and Blanckaert N, Topology and regulation of bilirubin UDP-glucuronyltransferase in sealed native microsomes from rat liver. *Arch Biochem Biophys* **263**: 216–225, 1988.
36. Dannenberg AJ, Rotenberg M and Zakim D, Regulation of UDP-glucuronosyltransferase by lipid–protein interactions. *J Biol Chem* **264**: 238–242, 1989.
37. Castuma CE and Brenner RR, Cholesterol-dependent modification of microsomal dynamics and UDP-glucuronyltransferase kinetics. *Biochemistry* **25**: 4733–4738, 1986.
38. Rotenberg M and Zakim D, Effects of cholesterol on the function and thermotropic properties of pure UDP-glucuronosyltransferase. *J Biol Chem* **266**: 4159–4161, 1991.
39. Kamataki T, Shimada M, Maeda K and Kato R, Pituitary regulation of sex-specific forms of cytochrome P-450 in liver microsomes of rats. *Biochem Biophys Res Commun* **130**: 1247–1253, 1985.
40. Waxman DJ, Pampori NA, Ram PA, Agrawal AK and Shapiro BH, Interpulse interval in circulating growth hormone patterns regulates sexually dimorphic expression of hepatic cytochrome P450. *Proc Natl Acad Sci USA* **88**: 6868–6872, 1991.
41. Greenblatt DJ, Divoll M, Harmatz JS and Shader RI, Oxazepam kinetics: Effects of age and sex. *J Pharmacol Exp Ther* **215**: 86–91, 1980.
42. Divoll M, Greenblatt DJ, Harmatz JS and Shader RI, Effects of age and gender on disposition of temazepam. *J Pharm Sci* **70**: 1104–1107, 1981.
43. Bonate PL, Gender-related differences in xenobiotic metabolism. *J Clin Pharmacol* **31**: 684–690, 1991.
44. Vessey DA and Kempner ES, *In situ* structural analysis of microsomal UDP-glucuronosyltransferase by radiation inactivation. *J Biol Chem* **264**: 6334–6338, 1989.
45. Peters WHM, Jansen PLM and Nauta H, The molecular weights of UDP-glucuronosyltransferase determined with radiation-inactivation analysis. *J Biol Chem* **259**: 11701–11705, 1984.
46. Oscarsson J and Edén S, Sex differences in fatty acid composition of rat liver phosphatidylcholine are regulated by the plasma pattern of growth hormone. *Biochim Biophys Acta* **959**: 280–287, 1988.