Biochemical Pharmacology, Vol. 40, No. 3, pp. 595–600, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

CHARACTERIZATION OF PARACETAMOL UDP-GLUCURONOSYLTRANSFERASE ACTIVITY IN HUMAN LIVER MICROSOMES

J. O. MINERS,*† K. J. LILLYWHITE,* K. YOOVATHAWORN,‡ M. PONGMARUTAI,§ and D. J. BIRKETT*

*Department of Clinical Pharmacology, Flinders Medical Centre, Bedford Park, Adelaide, South Australia 5042; ‡Department of Pharmacology, Mahidol University, Bangkok, Thailand; and §Department of Pharmacology, Prince of Songkla University, Had Yai, Thailand

(*Received* 25 *January* 1990; *accepted* 13 *March* 1990)

Abstract—A specific high performance liquid chromatographic assay has been developed for the measurement of paracetamol glucuronide formation by the microsomal fraction of human liver. The procedure has been used to characterize paracetamol glucuronidation kinetics in human liver microsomes and to assess the substrate specificity of the paracetamol UDP-glucuronosyltransferase (UDPGT) activity. Paracetamol glucuronidation followed Michaelis–Menten kinetics, suggesting the involvement of a single form of UDPGT, or possibly two or more forms of UDPGT with similar affinities for paracetamol, in this reaction. Mean apparent K_m and V_{max} values were 7.37 ± 0.99 mM and 4.76 ± 1.35 nmol/min/mg, respectively. Addition of the non-ionic detergent Brij 58 to microsomal incubations resulted in approximately 50% activation of microsomal paracetamol UDPGT-activity. This contrasts to the approximately three-fold activation of 4-methylumbelliferone, morphine and 4-nitrophenol glucuronidation observed following Brij 58 treatment of human liver microsomes. The glucuronidated xenobiotics chloramphenicol, digitoxigenin monodigitoxoside, 4-hydroxybiphenyl, 4-methylumbelliferone, morphine, 1-naphthol and 4-nitrophenol were screened for inhibitory effects on paracetamol glucuronidation. Of these compounds, only digitoxigenin monodigitoxoside and 1-naphthol were found to cause significant inhibition of paracetamol UDPGT activity. Along with the results of previous studies of the kinetics and inhibitor profile of human liver glucuronidation reactions (Miners *et al., Biochem Pharmacol* **37**: 665–671, 1988 and **37**: 2839–2845, 1988), these data indicate that the model glucuronidated substrates paracetamol, morphine and 4-methyllumbelliferone may be used to differentiate at least four human liver UDPGT isozyme activities.

Conjugation with glucuronic acid is a major pathway for the deactivation and elimination of a large number of lipophilic xenobiotics and endogenous compounds in humans and other mammalian species. Glucuronidation reactions are catalysed by the UDP-glucuronosyltransferase (UDPGT) family of isozymes. The total number of isozymes in this family remains unknown but accumulating evidence suggests the existence of at least eleven forms of UDPGT in rat liver [1–3]. Similarly, recent kinetic and inhibitor studies in human liver microsomes [4–9], the cloning of UDPGT cDNAs [10–12], enzyme purification [3, 13], immunochemical analysis [12] and developmental studies [14] have provided overwhelming evidence for the heterogeneity of UDPGT in man.

The widely used non-prescription analgesic/antipyretic agent paracetamol (acetaminophen, *N*-acetyl-4-aminophenol) is eliminated in humans primarily by glucuronidation, with sulphation and oxidation by cytochrome P450 to a reactive electrophilic species which is subsequently conjugated with glutathione accounting for the remainder of the drug's metabolic clearance [15]. Paracetamol is extremely safe at usual therapeutic doses and this has led to its widespread use as a model drug for investigating the regulation of xenobiotic glucuronidation in humans *in vivo*. Paracetamol has been used as a probe for the study of the effects of age [16, 17], hormonal factors [18–20], environmental factors [21–23] and enzyme induction or inhibition by concomitant drug administration [21, 22, 24] on drug glucuronidation in man.

Despite the widespread use of paracetamol as a model glucuronidated drug in humans, little is known of the relationship between the form(s) of UDPGT responsible for paracetamol glucuronidation and other xenobiotic metabolizing UDPGT isozymes or isozyme activities in human liver. This communication describes the development of an assay for the measurement of paracetamol UDPGT activity and its use to characterize paracetamol glucuronidation kinetics in human liver microsomes. In addition, the extent of inhibition of paracetamol glucuronidation by certain other xenobiotics has been investigated to determine relationships between paracetamol UDPGT activity and other xenobiotic glucuronidating activities characterised in human liver to date.

MATERIALS AND METHODS

Chemicals and reagents. Brij 58 (polyoxyethylene 20-cetyl ether), choramphenicol, 4-hydroxy-bi-

† To whom correspondence should be addressed.

phenyl, 4-methylumbelliferone, 1-naphthol, 4-nitrophenyl-beta-D-glucuronide, paracetamol and UDP-glucuronic acid (sodium salt) were purchased from the Sigma Chemical Co. (St Louis, MO). Digitoxigenin monodigitoxoside and 4-nitrophenol were purchased from Atomergic Chemicals (Plainview, NY) and from the Aldrich Chemical Co. (Milwaukee, WI), respectively. Morphine HCl was obtained from Glaxo Australia (Melbourne, Australia). All other reagents and solvents were of analytical reagent grade.

Human liver samples. Human liver samples were obtained with the consent of next-of-kin from renal transplant donors. The approval of the Flinders Medical Centre Ethical Review Committee was obtained to use such material for xenobiotic metabolism studies. Relevant details of the donors of livers used in this study (F5, F8, F9 and F10) have been published elsewhere [8, 25]. Liver samples were stored at −80° until used; experience in this laboratory has shown that UDPGT activities towards paracetamol and other xenobiotic substrates such as 4-methylumbelliferone [8] and morphine [6] are stable in liver stored at this temperature for at least 2.5 years. Microsomes were prepared by differential centrifugation as previously described [25].

Measurement of paracetamol glucuronidation by human liver microsomes. A standard microsomal incubation contained UDP-glucuronic acid (UDPGA; 20 mM), $MgCl_2$ (5 mM), Tris–HCl (0.1 M, pH 7.4), microsomal protein (0.5 mg), Brij 58 (detergent–protein ratio 0.15; i.e. final concentration 0.03% w/v), and paracetamol (25–20,000 μM) in a final volume of 0.25 mL. Incubations were performed at 37° for 1 hr. Reaction times were shown to be linear for incubation times to 2.5 hr and for microsomal protein concentrations up to 4 mg/mL. In addition, reaction rates were optimized for pH, Mg^{2+} and detergent concentrations. While the concentrations of $MgCl_2$ and Brij 58 used in the microsomal incubations were shown to result in maximal paracetamol UDPGT activity, reaction rate was relatively unaffected by pH in the range 7.4 to 8.2. The reaction was stopped by the addition of 70% perchloric acid (0.02 mL) and cooling on ice. 4-Nitrophenol glucuronide (0.05 mL of a 1 mM aqueous solution) was also added to enable the determination of analyte recovery in each experiment (see below). The contents of each incubation tube were loaded onto a C-18 Sep-pak minicolumn (Waters Millipore) which was eluted sequentially with phosphate buffer (5 mM, pH 3.0; 5 mL) and methanol (8 mL). The methanol fraction was collected and evaporated to dryness under a stream of N_2. The residue was reconstituted in 0.2 mL of mobile phase and the entire sample injected into the chromatograph.

The high performance liquid chromatograph used comprised a U6K injector, model 6000 solvent delivery system, model 760 data module (all Waters Millipore) and a model 110 fixed wavelength detector (Altex) operating at 254 nm. The chromatograph was fitted with a μ-Bondapak 10 micron C-18 column (30 cm × 3.9 mm i.d.; Waters Millipore) and operated at ambient temperature. The mobile phase was 3.75% acetonitrile/96.25% 10 mM orthophosphoric acid (pH 2.7) at a flow rate of 2 mL/min. Under these conditions the retention times for paracetamol glucuronide, paracetamol and 4-nitrophenol glucuronide were 4.5, 7.0 and 10.9 min, respectively (Fig. 1). The retention time for paracetamol glucuronide was confirmed by comparison to an authentic standard [26] and by hydrolysis of the incubation product with β-glucuronidase. Owing to the limited availability of pure paracetamol glucuronide, this metabolite was routinely quantitated by comparison of measured peak heights to those of a standard curve generated for unchanged paracetamol (in the concentration range 2.5 to 250 μM) and application of the known relative response factor for paracetamol glucuronide [26]. The use of paracetamol metabolite response factors has been previously widely applied to the quantitation of paracetamol metabolites in urine [17–19, 22, 26]. Recoveries of paracetamol glucuronide and 4-nitrophenol glucuronide from microsomal incubations using the technique outlined above were shown to be 93.2 ± 3.4% and 95.0 ± 3.6% (both N = 6), respectively. Thus, to confirm adequate metabolite recovery in each experiment, 4-nitrophenol glucuronide was routinely added to incubation tubes prior to extraction (see above) and peak heights were compared to those of a directly injected authentic standard. Along with the use of the relative response

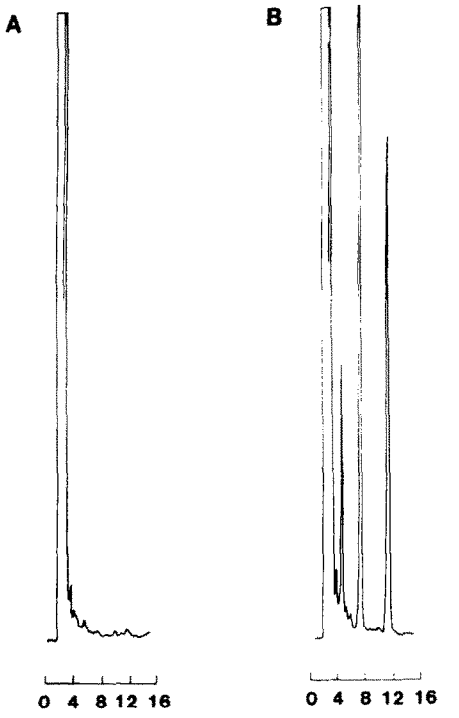

Fig 1. Representative chromatograms of extracts of microsomal incubations: (A) blank chromatogram: microsomal incubation performed without paracetamol; (B) microsomal incubation performed as described in Materials and Methods (paracetamol concentration 0.25 mM). Retention times: paracetamol glucuronide 4.5 min; paracetamol 7.0 min; 4-nitrophenyl glucuronide 10.9 min.

factor to determine paracetamol glucuronide concentrations, this approach obviates the need to use paracetamol glucuronide standards routinely. The within-day coefficient of variation of the assay, determined by measuring paracetamol glucuronidation formation (at a substrate concentration of 250 μM) in ten separate incubations of the same batch of hepatic microsomes, was 5.6%.

Kinetic and inhibitor studies. In the experiments performed to determine the apparent K_m and V_{max} for paracetamol glucuronidation, the UDPGA concentration was held constant (20 mM) and activity was measured for 13 paracetamol concentrations over the range 25 to 20,000 μM. It should be noted that the UDPGA concentration used in these experiments was approximately 30-fold higher than the apparent K_m determined for UDPGA (see Results section). To determine the apparent K_m for UDPGA the concentration of paracetamol was kept constant (5 mM) and activity was measured for twelve UDPGA concentrations over the range 0.25 to 20 mM. The paracetamol glucuronidation and UDPGA kinetics experiments were performed using microsomes from four livers (F5, F8, F9, F10).

The extent of inhibition of paracetamol glucuronidation by chloramphenical, digitoxigenin monodigitoxoside, 4-hydroxybiphenyl, 4-methylumbelliferone, morphine, 1-naphthol and 4-nitrophenol was assessed in microsomes from livers F8–10. The concentrations of paracetamol and inhibitor used in these experiments were 0.5 mM and 1.0 mM, respectively. In the experiment investigating 4-nitrophenol as an inhibitor, 4-nitrophenol glucuronide was not added to the incubation tubes in the usual way prior to extraction.

Other assays. Microsomal protein concentration was measured by the procedure of Lowry *et al.* [27] using crystalline bovine serum albumin as standard.

Analysis of results. Results are presented as mean ± SD. The Michaelis–Menten parameters V_{max} and apparent K_m were determined as previously described [8] using an iterative programme based on non-linear least squares regression analysis to fit experimental data to the Michaelis–Menten equation. Initial estimates of these parameters were obtained by graphical analysis of Eadie–Hofstee plots.

RESULTS

Maximal activation of paracetamol UDPGT-activity occurred at a Brij 58 to microsomal protein ratio of 0.15 (Fig. 2). The observed mean increase in enzyme activity in the four livers studied (F5, F8–9) was 52 ± 11%. Brij 58 to protein ratios above 1 resulted in decreased enzyme activity. Triton X-100 and digitonin did not increase the extent of activation over that observed with Brij 58 (results not shown). The Brij 58 activation curves for 4-methylumbelliferone glucuronidation and the low affinity component of morphine glucuronidation are also shown in Fig. 2 for comparison.

Paracetamol glucuronide formation followed Michaelis–Menten kinetics in all four livers studied (Fig. 3). Values of apparent K_m and V_{max} are shown in Table 1. The mean apparent K_m and V_{max} values

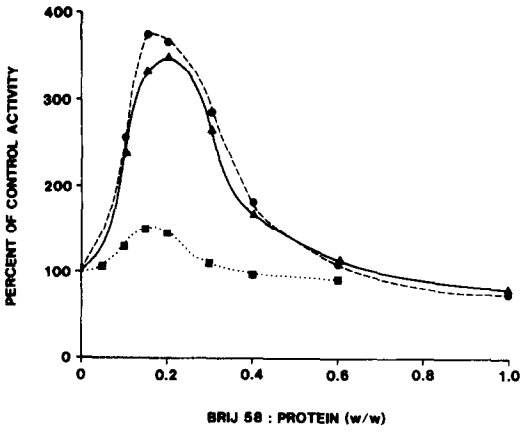

Fig. 2. Activation of paracetamol (■ . . . . ■), morphine (low affinity morphine UDPGT activity; (●– – –●) and 4-methylumbelliferone (▲——▲) glucuronidation in human liver microsomes by Brij 58. Experiment performed in pooled microsomes from livers F5, F8, F9 and F10.

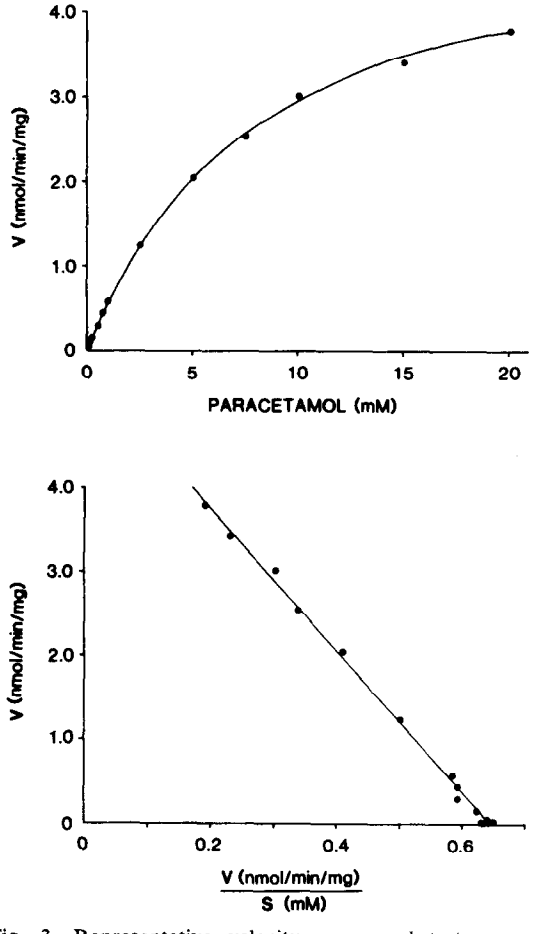

Fig. 3. Representative velocity versus substrate concentration curve (upper panel) and Eadie–Hofstee plot (lower panel) for paracetamol glucuronidation in human liver microsomes (liver F9).

Table 1. Computer derived Michaelis–Menten parameters for paracetamol glucuronidation in human liver microsomes

Liver	K_m (mM)	V_{max} (nmol/min/mg)
F5	8.24	5.79
F8	5.96	2.98
F9	7.45	5.84
F10	7.82	4.46
Mean (± SD)	7.37 ± 0.99	4.76 ± 1.35

K_m and V_{max} calculated by fitting experimental data to Michaelis–Menten equation for a single enzyme system. See the Analysis of results section of Materials and Methods for details.

Table 2. Effect of various xenobiotics on paracetamol glucuronidation in human liver microsomes

Xenobiotic	Percent control activity remaining
Chloramphenicol	103.7 ± 6.0
Digitoxigenin monodigitoxoside	63.1 ± 5.1
4-Hydroxybiphenyl	98.0 ± 7.6
4-Methylumbelliferone	94.4 ± 6.2
Morphine	91.1 ± 1.6
1-Naphthol	59.3 ± 3.0
4-Nitrophenol	92.0 ± 4.7

Values are mean ± SD from three livers. The concentrations of paracetamol and inhibitor were 0.5 mM and 1.0 mM, respectively.

were 7.37 ± 0.99 mM and 4.76 ± 1.35 nmol/min/mg protein, respectively. Variation in apparent K_m was relatively small (1.38-fold), while V_{max} varied approximately 2-fold in the four livers studied. Using a fixed concentration of paracetamol and varying UDPGA concentration also resulted in linear Eadie–Hofstee plots (data not shown). The mean apparent K_m for UDPGA in the four livers was 679 ± 93 μM.

The effects of chloramphenicol, digitoxigenin monodigitoxoside, 4-hydroxybiphenyl, 4-methylumbelliferone, morphine, 1-naphthol and 4-nitrophenol on paracetamol glucuronidation in microsomes from livers 8–10 are summarized in Table 2. Of these compounds, only digitoxigenin monodigitoxoside and 1-naphthol caused >10% inhibition of paracetamol UDPGT-activity.

DISCUSSION

Paracetamol glucuronidation by human liver microsomes has been characterized using a newly developed high performance liquid chromatographic procedure for the quantitation of the glucuronide conjugate of paracetamol extracted from microsomal incubations. Pacifici *et al.* [28] recently described a procedure for the measurement of paracetamol glucuronidation and sulphation in subcellular fractions. Their procedure utilized tritiated substrate and determined conjugate formation as radioactivity remaining in ethylacetate-extracted incubates. The method described in this paper allows direct quantitation of paracetamol glucuronide and therefore has major advantages in terms of specificity.

Paracetamol glucuronidation in human liver microsomes followed Michaelis–Menten kinetics. This is consistent with the involvement of a single isozyme of UDPGT in paracetamol glucuronidation, although it is acknowledged that the existence of two or more forms of UDPGT with similar affinities for paracetamol would not be distinguished by the kinetic studies described here. The mean apparent K_m (7.37 mM) for paracetamol in the four livers studied is somewhat higher than those determined previously for other glucuronidated xenobiotics, such as 4-methylumbelliferone (91.8 μM) [8], morphine (high affinity component 5.3 μM; low affinity component 1203 μM) [6], and 1-naphthol (high affinity component 1.8 μM; low affinity component 87.2 μM) [6]. It should be noted that in the previous studies with morphine [6] and 1-naphthol [8], where biphasic glucuronidation kinetics were observed, non-linearity of Eadie–Hofstee plots was apparent at concentrations near the lower limit of the range used in the present study. Hence, if a high affinity paracetamol UDPGT activity was to exist, it is anticipated that its apparent K_m and V_{max} values would both need to be very low. Based on a kinetic model for paracetamol elimination, it has been suggested that paracetamol glucuronidation may be saturable in humans [29]. However, the high K_m for paracetamol glucuronidation determined here would indicate that marked non-linearity for glucuronidation pathway is likely only following massive overdose.

The extent of activation of paracetamol UDPGT activity in human liver microsomes by Brij 58 and other non-ionic detergents differs to that reported previously for 4-methylumbelliferone [8], morphine [6], 1-naphthol [8] and 4-nitrophenol [30]. Although maximum activation of all activities occurred at the same detergent to protein ratio (0.15–0.20), paracetamol UDPGT activity was increased by only approximately 50% whereas the other UDPGT activities were activated approximately 3-fold. The different activation characteristics of paracetamol glucuronidation may indicate that the paracetamol UDPGT activity of human liver microsomes is distinct to those associated with 4-methylumbelliferone, morphine and 4-nitrophenol glucuronidation. However, since activation of UDPGT by detergents can reflect properties of both the enzyme and a transport mechanism for UDP-glucuronic acid [31], other explanations for the aberrant Brij 58 activation characteristics observed in this study are possible.

On the basis of our previous [6–9] kinetic and inhibitor studies of xenobiotic glucuronidation in human liver microsomes, we have argued that morphine and 4-methylumbelliferone may be used as probe substrates for at least three UDPGT isozyme activities in human liver microsomes. In the present study neither of these substrates were found to inhibit paracetamol glucuronidation. This observation strongly suggests that the paracetamol UDPGT is

distinct to the 4-methylumbelliferone and morphine UDPGT activities characterized earlier and that paracetamol, 4-methylumbelliferone and morphine may be used as model substrates to differentiate at least four different UDPGT isozyme activities in human liver. Furthermore, paracetamol glucuronidation was unaffected by chloramphenicol and 4-hydroxybiphenyl, indicating that these compounds are not substrates for the paracetamol UDPGT activity. In contrast, both 1-naphthol and digitoxigen monodigitoxoside inhibited paracetamol glucuronidation. Available evidence suggests 1-naphthol is a promiscuous substrate for UDPGTs in both human [6, 8] and rat [32] liver. It is possible that digitoxigenin monodigitoxoside, which appears to be glucuronidated by a single isozyme of UDPGT in rat liver [33], may be an alternate substrate for the paracetamol UDPGT activity of human liver but confirmation of this requires further investigation.

Acknowledgements—This work was supported by a grant from the National Health and Medical Research Council of Australia.

REFERENCES

1. Siest G, Antoine B, Fournel S, Magdalou J and Thomassin J, The glucuronosyltransferases: what progress can pharmacologists expect from molecular biology and cellular enzymology? *Biochem Pharmacol* **36**: 983–987, 1987.
2. Mackenzie PI and Haque SJ, Multiplicity and structure of UDP-glucuronosyltransferases as revealed by gene cloning. In: *Microsomes and Drug Oxidations* (Eds. Miners JO, Birkett DJ, Drew R, May BK and McManus ME), pp. 271–278. Taylor & Francis, London, 1988.
3. Tephly TR, Coffman BL, Falany CN, Green MD, Irshaid Y, Puig JF, Knapp SA and Baron J, Purification and characterisation of mammalian UDP-glucuronosyltransferases. In: *Microsomes and Drug Oxidations* (Eds. Miners JO, Birkett DJ, Drew R, May BK and McManus ME), pp. 263–270. Taylor & Francis, London, 1988.
4. Bock KW, Brunner G, Hoensch H, Huber E and Josting D, Determination of UDP-glucuronosyltransferase in needle biopsy specimens of human liver. *Eur J Clin Pharmacol* **14**: 367–373, 1978.
5. Bock KW, Lilienblum W and von Bahr C, Studies of UDP-glucuronyltransferase activities in human liver microsomes. *Drug Metab Dispos* **12**: 93–97, 1984.
6. Miners JO, Lillywhite KJ and Birkett DJ, *In vitro* evidence for the involvement of at least two forms of human liver UDP-glucuronyltransferase in morphine-3-glucuronidation. *Biochem Pharmacol* **37**: 2839–2845, 1988.
7. Miners JO, Lillywhite KJ, Matthews AP and Birkett DJ, *In vitro* assessment of UDP-glucuronosyltransferase multiplicity and substrate specificity in human liver. In: *Microsomes and Drug Oxidations* (Eds. Miners JO, Birkett DJ, Drew R, May B and McManus ME), pp. 279–286. Taylor & Francis, London, 1988.
8. Miners JO, Lillywhite KJ, Matthews AP, Jones ME and Birkett DJ, Kinetic and inhibitor studies of 4-methylumbelliferone and 1-naphthol glucuronidation in human liver microsomes. *Biochem Pharmacol* **37**: 665–671, 1988.
9. Miners JO, Lillywhite KJ, Matthews AP, Yoovathaworn K, Pongmuratai M, Mackenzie PI and Birkett DJ, Human liver UDP-glucuronosyltransferase: *in vitro* assessment of multiplicity and substrate specificity. In: *Xenobiotic Metabolism and Disposition* (Eds. Kato R, Estabrook RW and Cayen MN), pp. 89–95. Taylor & Francis, London, 1989.
10. Fournel-Gigleux S, Jackson MR, Wooster R and Burchell B, Expression of a human liver cDNA encoding a UDP-glucuronosyltransferase catalysing the glucuronidation of hyodeoxycholic acid in cell culture. *FEBS Lett* **243**: 119–122, 1989.
11. Harding D, Fournel-Gigleux S, Jackson MR and Burchell B, Cloning and substrate specificity of a human phenol UDP-glucuronosyltransferase expressed in COS-7 cells. *Proc Natl Acad Sci USA* **85**: 8381–8385, 1988.
12. Jackson MR, McCarthy LR, Harding D, Wilson S, Coughtrie MWH and Burchell B, Cloning of a human liver microsomal UDP-glucuronosyltransferase cDNA. *Biochem J* **242**: 581–588, 1987.
13. Irshaid YM and Tephly TR, Isolation and purification of two human liver UDP-glucuronosyltransferases. *Mol Pharmacol* **31**: 27–34, 1987.
14. Leakey JEA, Hume R and Burchell B, Development of multiple activities of UDP-glucuronosyltransferase in human liver. *Biochem J* **243**: 859–861, 1987.
15. Hinson JA, Biochemical toxicology of acetaminophen. In: *Reviews in Biochemical Toxicology* (Eds. Hodgson E, Bend JR and Philpot RM), pp. 103–129. Elsevier, New York, 1980.
16. Miller RP, Roberts RJ and Fischer LJ, Acetaminophen elimination kinetics in neonates, children and adults. *Clin Pharmacol Ther* **19**: 284–294, 1976.
17. Miners JO, Penhall R, Robson RA and Birkett DJ, Comparison of paracetamol metabolism in young adult and elderly males. *Eur J Clin Pharmacol* **35**: 157–160, 1988.
18. Miners JO, Attwood J and Birkett DJ, Influence of sex and oral contraceptive steroids on paracetamol metabolism. *Br J Clin Pharmacol* **16**: 503–509, 1983.
19. Miners JO, Robson RA and Birkett DJ, Paracetamol metabolism in pregnancy. *Br J Clin Pharmacol* **22**: 359–362, 1986.
20. Wojcicki J, Gawronska-Szklarz B, Kazimierczyk J, Baskiewicz Z and Raczynski A, Comparative pharmacokinetics of paracetamol in men and women considering follicular and luteal phases. *Arzneim-Forsch* **29**: 350–354, 1979.
21. Bock KW, Wiltfang J, Blume R, Ullrich D and Bircher J, Paracetamol as a test drug to determine glucuronide formation in man. Effects of inducers and of smoking. *Eur J Clin Pharmacol* **31**: 677–683, 1987.
22. Miners JO, Attwood J and Birkett DJ, Determinants of acetaminophen metabolism: effects of inducers and inhibitors of drug metabolism on acetaminophen's metabolic pathways. *Clin Pharmacol Ther* **35**: 480–486, 1984.
23. Mucklow JC, Fraser HS, Bulpitt CJ, Kahn C, Mould G and Dollery CT, Environmental factors affecting paracetamol metabolism in London factory and office workers. *Br J Clin Pharamcol* **10**: 67–74, 1980.
24. Prescott LF, Critchley JAJH, Balali-Mood M and Pentland B, Effects of microsomal enzyme induction on paracetamol metabolism in man. *Br J Clin Pharmacol* **12**: 149–153, 1981.
25. Robson RA, Matthews AP, Miners JO, McManus ME, Meyer UA, Hall de la M and Birkett DJ, Characterisation of theophylline metabolism in human liver microsomes. *Br J Clin Pharmacol* **24**: 293–300, 1987.
26. Miners JO, Adams JF and Birkett DJ, A simple hplc assay for urinary paracetamol metabolites and its use to characterise the C3H mouse as a model for paracetamol metabolism studies. *Clin Exp Physiol Pharmacol* **11**: 209–217, 1984.
27. Lowry OH, Rosebrough MJ, Farr AL and Randall AJ,

Protein measurements with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
28. Pacifici GM, Back DJ and Orme ML'E, Sulphation and glucuronidation of paracetamol in human liver: assay conditions. *Biochem Pharmacol* **37**: 4405–4407, 1988.
29. Slattery JT and Levy G, Acetaminophen kinetics in acutely poisoned patients. *Clin Pharmacol Ther* **25**: 184–195, 1979.
30. Dragacci S, Thomassin J, Magdalou J, Souhaili El Amiri H, Boissel P and Siest G, Properties of human hepatic UDP-glucuronosyltransferases. Relationship to other inducible enzymes in patients with cholestasis. *Eur J Clin Pharmacol* **32**: 485–491, 1987.
31. Hallinan T, Comparison of compartmented and of conformational phospholipid-constraint models for the intramembranous arrangement of UDP-glucuronosyltransferase. In: *Conjugation Reactions in Drug Biotransformation* (Ed. Aitio A), pp. 257–267. Elsevier/North Holland, Amsterdam, 1978.
32. Roy Chowdbury J, Roy Chowdbury N, Falany CN, Tephly TR and Arias IM, Isolation and characterisation of multiple forms of rat liver UDP-glucuronate glucuronosyltransferase. *Biochem J* **233**: 827–837, 1986.
33. von Meyernick L, Coffman BL, Green MD, Kirkpatrick RB, Schmoldt A and Tephly TR, Separation, purification and characterisation of digitoxigenin monodigitoxoside UDP-glucuronosyltransferase activity. *Drug Metab Dispos* **13**: 700–704, 1985.