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Conjugation pathways in liver disease

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1 The activities of microsomal glucuronyltransferase and thiomethyltransferase, and those of cytosolic sulphotransferase, acetyltransferase, glutathione transferase and thiomethyltransferase were measured in abnormal (cirrhosis and chronic hepatitis) and normal livers.

2 Glucuronyltransferase and sulphotransferase were investigated with 2-naphthol and ethinyloestradiol as substrates. *p*-Aminobenzoic acid, benzo(a)pyrene-4,5-epoxide and 2-mercaptoethanol were the substrates of acetyltransferase, glutathione transferase and thiomethyltransferase, respectively.

3 Enzyme activities are expressed as nmol min^{-1} incubation mg^{-1} protein and the averages (± s.d.) are given. With 2-naphthol as substrate, the glucuronyltransferase activity was 6.55 ± 4.10 (abnormal liver, $n = 33$) and 7.81 ± 4.02 (normal liver, $n = 26$) (NS); whereas sulphotransferase activity was 0.28 ± 0.18 (abnormal liver, $n = 35$) and 0.68 ± 0.43 (normal liver, $n = 26$) ($P < 0.01$). Glucuronyltransferase activity towards ethinyloestradiol was 102.5 ± 56.9 (abnormal liver, $n = 30$) and 107 ± 59.9 (normal liver, $n = 26$) (NS), whereas sulphotransferase activity was 57.2 ± 36.0 (abnormal liver, $n = 35$) and 122 ± 67.6 (normal liver, $n = 28$) ($P < 0.01$). Acetyltransferase activity was 0.84 ± 0.83 (abnormal liver, $n = 35$) and 3.84 ± 1.65 (normal liver, $n = 26$) ($P < 0.01$). Glutathione transferase activity was 0.83 ± 0.68 (abnormal liver, $n = 35$) and 2.90 ± 1.59 (normal liver, $n = 25$) ($P < 0.01$) and thiomethyltransferase activity was 1.00 ± 0.69 (abnormal liver, $n = 34$) and 3.99 ± 1.49 (normal liver, $n = 25$) ($P < 0.01$).

4 Liver disease lowers the activities towards the substrates studied of sulphotransferase, acetyltransferase, glutathionetransferase and thiomethyltransferase but not that of glucuronyltransferase. Thus, overall hepatic conjugating capacity is decreased in liver injury. However, enzyme activity is substrate dependent and it is not possible to extrapolate the results for other compounds.

Keywords liver disease glucuronyltransferase sulphotransferase acetyltransferase glutathione transferase thiomethyltransferase

Introduction

In vivo, the hepatic metabolism of drugs is influenced by a multitude of factors such as the activity of the various drug metabolizing enzymes and the availability of substrates and cofactors at the metabolic site as well as by the mass of liver parenchyma. Liver disease may affect one or more of these factors and the prediction of drug metabolising activity in patients with liver

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damage is difficult. Pharmacokinetic studies have been performed in patients with liver disease (Bass & Williams, 1988; Branch & Shand, 1976; Howden *et al.*, 1989; Williams & Mamelock, 1980) and although conflicting data have been reported the impression prevails that hepatic metabolic capacity is decreased. Metabolism *in vitro* may not predict accurately the rate *in vivo*, but it is a suitable means of establishing the activity of an enzyme. Farrell *et al.* (1979), Boobis *et al.* (1980) and Brodie *et al.* (1981) have observed that cytochrome P-450 content and the activity of aryl hydrocarbon hydroxylase are decreased in liver disease. Lower activities of aldrin epoxidase (Woodhouse *et al.*, 1983), 7-ethoxycoumarin *O*-deethylase (Woodhouse *et al.*, 1983, 1984) and 7-ethoxyresorufin-*O*-deethylase (Woodhouse *et al.*, 1987) have been reported in alcoholic cirrhosis and both the high and low affinity components of ethoxycoumarin *O*-deethylase are impaired (Woodhouse *et al.*, 1987). Thus, the oxidative capacity of the injured liver is lowered. Bock *et al.* (1978) have no difference in the activity of glucuronyltransferase, a microsomal conjugation enzyme, between livers with alcoholic injury and normal livers. To our knowledge, this has been the only study of the effect of liver disease on phase II enzymes *in vitro*.

Conjugation pathways play an important role in detoxication and the paucity of data on conjugation reactions in liver disease prompted us to study conjugation enzymes in livers with impaired function. The investigation was extended to two microsomal enzymes, glucuronyltransferase and thiomethyltransferase, and three cytosolic enzymes, glutathione transferase, acetyltransferase and sulphotransferase. Glucuronyltransferase and sulphotransferase share substrates and they account for most of the metabolism of molecules bearing hydroxyl groups. Glutathione transferase is a key enzyme in the inactivation of toxic molecules such as epoxides. Acetyltransferase is an important enzyme in the metabolism of amines whereas thiomethyltransferase is active on thiol groups.

Methods

Chemicals

The radioactive compounds 2-(1,4,5,8-[^{14}C])-naphthol (51 mCi mmol^{-1}). (1-[^{14}C])acetyl coenzyme A (55.5 mCi mmol^{-1}) and S-adenosyl-L-(methyl-[^{14}C])methionine (60 mCi mmol^{-1}) were obtained from the Radiochemical Centre (Amersham, England). Ethinyloestradiol (59.2 Ci mmol^{-1}) was from New England Nuclear (Florence, Italy). Unlabelled and radioactive (G-[^{3}H])4,5-dihydro-benzo(a)pyrene-4,5-oxide (288 mCi mmol^{-1}) were obtained from the NCI chemical Carcinogen Reference Standard Repository, NIH (Bethesda, MA, USA). Labelled 2-naphthol and ethinyloestradiol were purified by t.l.c. to a final purity of greater than 99%. The final radiochemical purity of the other compounds was greater than 98%, as declared by the producers.

Unlabelled 2-naphthol, ethinyloestradiol, *p*-aminobenzoic acid, 2-mercaptoethanol, reduced glutathione, uridine 5'-diphosphoglucuronic acid, S-adenosyl methionine, 5'-adenosine 3'-phosphosulphate, acetyl coenzyme A and Tris-(hydroxymethylaminemethane) were purchased from Sigma (St Louis, MO, USA).

Biological material

Liver specimens were obtained by two different procedures. Percutaneous needle biopsies of human liver were obtained by means of the Menghini technique for diagnostic histology in patients with suspected liver disease. All needle biopsies had abnormal cell architecture. In the text, they are referred to as abnormal livers. Classification of the patients into three groups, namely 'chronic persistent hepatitis', 'chronic active hepatitis' and 'cirrhosis' was made on the basis of liver histology. Ascites was present only in two cirrhotics whereas no patients were suffering from encephalopathy. The relevant clinical data for the patients are summarized in Table 1. Wedge biopsies of liver were obtained for diagnostic purposes at laparotomy from patients undergoing cholecystectomy. Only those liver specimens with normal cell architecture were included in the study and they served as control samples. In the text, they are referred to as normal livers. Donors of normal livers were aged between 23 and 69 years (average 52 years), two thirds of the donors were women. The surplus tissue from needle and wedge biopsies after portions were taken for histological analysis was made available for our study. Hepatic tissue was stored at −80° C.

Needle biopsies (15–25 mg wet tissue) were homogenized in 0.3 ml of 0.25 M sucrose (pH 7.4 with Tris base) using a glass-Teflon homogenizer ('size O', Thomas, Philadelphia, PA, USA). The homogenates were aspirated using a Pasteur-pipette and transferred into centrifuge tubes. The grinding chamber and the pestle were rinsed with 0.2 ml of sucrose and the washings were combined with the homogenate. Wedge biopsies (0.3–0.5 g wet tissue) were homogenized in 10

Table 1 Relevant clinical data of donors of abnormal liver samples

	Persistent chronic hepatitis	*Active chronic hepatitis*	*Cirrhosis*
Number of patients	8	20	7
Women	4	10	2
Men	4	10	5
Age (years)	41 ± 18 (20–67)	52 ± 10 (27–66)	50 ± 13 (28–64)
Albumin concentration ($g\ l^{-1}$)	40.5 ± 5.5	40.4 ± 5.6	37.4 ± 6.5
PTA[1]	75.1 ± 16.4	75.1 ± 12.6	71.1 ± 12.2
GOT ($u\ l^{-1}$)	56.7 ± 30.0	100.2 ± 62.2	65.0 ± 24.7
GPT ($u\ l^{-1}$)	97.0 ± 72.8	153.0 ± 111.6	54.0 ± 24.6
Serum bilirubin concentration ($mg\ 100\ ml^{-1}$)	0.66 ± 0.33	0.63 ± 0.39	1.25 ± 1.2

[1]Prothrombin activity. Values are expressed as a percentage of a standard unit. Normal values are between 70% and 100%.

volumes of 0.25 M sucrose using a glass-Teflon homogenizer ('size A', Thomas, Philadelphia, PA, USA). Homogenates were centrifuged at 12,000 *g* for 15 min. The supernatants were centrifuged again at 105.00 *g* for 1 h. The final supernatants were used as the cytosolic fractions. The high speed pellets were resuspended in 0.1 M Tris-HCl (pH 7.4) containing 30% (v/v) glycerol. Recovery of microsomal protein was measured in wedge and needle biopsies.

Enzyme assays

Details of the enzyme assays are summarized in Table 2. Thiomethyltransferase activity was measured as described by Otterness *et al.* (1986) for mouse liver microsomes. Optimal conditions for the human liver enzyme were established first and the rate of 2-mercaptoethanol methylation was linear up to 1 $mg\ ml^{-1}$ protein concentration and up to at least 15 min of incubation; optimum pH was 9. All enzyme activities quoted were obtained under conditions that were linear with incubation time and protein concentration. Each sample was assayed in duplicate and provided with two blanks. Enzyme activity was computed after correction for the blanks. When the radioactivity in the test samples was less than twice that in the blanks, enzyme activity was considered equal to the limit of detection of the assay in the calculation of mean and in group comparison.

The protein concentration was measured as described by Lowry *et al.* (1951). Final dilution of the subcellular fraction for protein determination was 1:50 or greater. At such a dilution Tris and glycerol did not interfere in the assay.

Statistical analysis

The level of significance of the difference between 'normal' and 'abnormal' livers was tested by the Kruskal-Wallis method (Colquhoun, 1971), a non-parametric test. The same test was used to determine the level of difference between samples from women and men. The level of significance of the difference between 'normal' livers and 'cirrhotic' livers, or livers with 'chronic active' or 'chronic persistent' hepatitis, was determined by Dunnett's test (Dunnett, 1955).

Results

The recovery of microsomal protein was measured in abnormal and normal livers. In abnormal livers, it ranged between 8.6 and 39.0 $mg\ g^{-1}$ liver (average (± s.d.) 23.3 ± 10.9 $mg\ g^{-1}$ liver) whereas, in normal livers, it ranged between 18.7 and 40.0 $mg\ g^{-1}$ liver (average (± s.d.) 28.0 ± 3.9 $mg\ g^{-1}$ liver). The recovery of microsomal protein was significantly ($P < 0.05$) lower in abnormal than in normal livers.

The activities of glucuronyltransferase and sulphotransferase towards 2-naphthol and the activities of acetyltransferase, glutathione transferase and thiomethyltransferase towards their respective substrates are shown in Figure 1 and Table 3. Glucuronyltransferase activity was not different in abnormal compared with normal livers. Sulphotransferase, acetyltransferase, glutathione transferase and thiomethyltransferase activities were one third in abnormal compared with normal livers and the difference was statistically significant ($P < 0.01$). No significant differ-

Table 2 Details of the enzyme assays

Enzyme	*Fraction*	*Substrate*	*pH*	*Sensitivity*	*Reference*
Glucuronyl transferase	Microsomal	0.5 mmol l^{-1} [^{14}C]-1-naphthol 5.0 mmol l^{-1} UDPGA	7.4	1.0 pmol min^{-1}	1
Glucuronyl transferase	Microsomal	0.2 mmol l^{-1} [^{3}H]-ethinyloestradiol 5.0 mmol l^{-1} UDPGA	7.4	4.4 pmol min^{-1}	2
Sulphotransferase	Cytosolic	0.1 mmol l^{-1} [^{14}C]-2-naphthol 0.2 mmol l^{-1} PAPS	5.5	2.0 pmol min^{-1}	3
Sulphotransferase	Cytosolic	0.5 mmol l^{-1} [^{3}H]-ethinyloestradiol 0.2 mmol l^{-1} PAPS	7.4	0.5 pmol min^{-1}	4
Glutathione transferase	Cytosolic	0.1 mmol l^{-1} [^{3}H]BPO[a] 4.1 mmol l^{-1} reduced glutathione	7.8	0.05 nmol min^{-1}	5
Acetyltransferase	Cytosolic	0.5 mmol l^{-1} *p*-aminobenzoic acid 2.0 mmol l^{-1} [^{14}C]ACoA	7.4	0.11 pmol min^{-1}	6
Thiomethyl transferase	Microsomal	75 mmol l^{-1} 2-mercaptoethanol 0.2 mmol l^{-1} SAM	9.5	2.2 pmol min^{-1}	7

[a]4,5-dihydro-benzo(a)-pyrene-4,5-oxide

1 Bock *et al.* (1978); 2 and 4 Pacifici & Back (1988); 3 Pacifici *et al.* (1988d); 5 Pacifici *et al.* (1988c); 6 Pacifici *et al.* (1986); 7 Otterness *et al.* (1986).

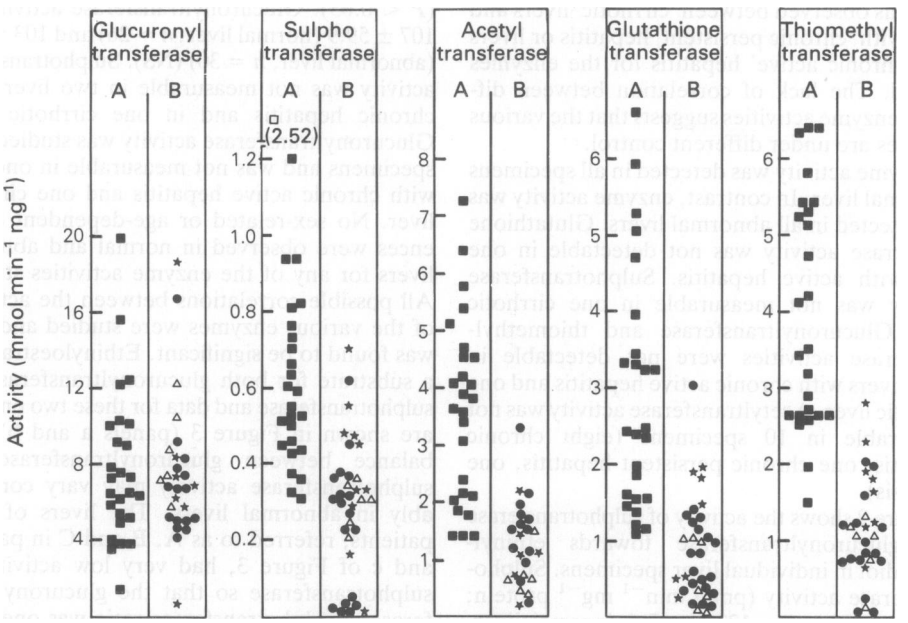

Figure 1 Activities of glucuronyltransferase, sulphotransferase, acetyltransferase, glutathione transferase and thiomethyltransferase in normal livers (A) and abnormal livers (B). Stars, circles and triangles refer to chronic persistent hepatitis, chronic active hepatitis and cirrhosis samples, respectively. The average activities of the different enzymes are shown in Table 2. 2-Naphthol was the substrate for glucuronyltransferase and sulphotransferase whereas p-aminobenzoic acid, benzo(a)pyrene-4,5-oxide and 2-mercaptoethanol were the substrates for acetyltransferase, glutathione transferase and thiomethyltransferase, respectively.

Table 3 Activities (mean ± s.d.) of the various enzymes in normal and abnormal livers

	Activity (nmol min^{-1} mg^{-1})				
	Glucuronyl transferase	*Sulpho transferase*	*Acetyl transferase*	*Glutathione transferase*	*Methyl transferase*
Normal livers (A)	7.81 ± 4.02	0.68 ± 0.43	3.84 ± 1.65	2.90 ± 1.59	3.99 ± 1.49
% C.V.	51	63	43	55	37
n	26	26	26	25	25
Abnormal livers (B)	6.55 ± 4.10	0.28 ± 0.18	0.84 ± 0.83	0.83 ± 0.68	1.00 ± 0.69
% C.V.	48	64	99	82	69
n	33	35	35	35	34
p	NS	< 0.01	< 0.01	< 0.01	< 0.01

n refers to the number of liver specimens investigated.
% C.V. coefficient of variation; s.d. expressed as % of mean.

ence was observed between 'cirrhotic' livers and livers with 'chronic persistent' hepatitis or livers with 'chronic active' hepatitis for the enzymes studied. The lack of correlation between different enzyme activities suggests that the various enzymes are under different control.

Enzyme activity was detected in all specimens of normal liver. In contrast, enzyme activity was not detected in all abnormal livers. Glutathione transferase activity was not detectable in one liver with active hepatitis. Sulphotransferase activity was not measurable in one cirrhotic liver. Glucuronyltransferase and thiomethyltransferase activities were not detectable in three livers with chronic active hepatitis and one cirrhotic liver. Acetyltransferase activity was not measurable in 10 specimens (eight chronic hepatitis, one chronic persistent hepatitis, one cirrhosis).

Figure 2 shows the activity of sulphotransferase and glucuronyltransferase towards ethinyloestradiol in individual liver specimens. Sulphotransferase activity (pmol min^{-1} mg^{-1} protein; mean ± s.d.) was 122 ± 67.6 (normal liver, $n = 28$) and 57.2 ± 36.0 (abnormal liver, $n = 35$) ($P < 0.05$). Glucuronyltransferase activity was 107 ± 59.9 (normal liver, $n = 26$) and 103 ± 56.9 (abnormal liver, $n = 30$) (NS). Sulphotransferase activity was not measurable in two livers with chronic hepatitis and in one cirrhotic liver. Glucuronyltransferase activity was studied in 30 specimens and was not measurable in one liver with chronic active hepatitis and one cirrhotic liver. No sex-related or age-dependent differences were observed in normal and abnormal livers for any of the enzyme activities studied. All possible correlations between the activities of the various enzymes were studied and none was found to be significant. Ethinyloestradiol is a substrate for both glucuronyltransferase and sulphotransferase and data for these two enzymes are shown in Figure 3 (panels a and b). The balance between glucuronyltransferase and sulphotransferase actively may vary considerably in abnormal livers. The livers of three patients, referred to as A, B and C in panels a and c of Figure 3, had very low activities of sulphotransferase so that the glucuronyltransferase to sulphotransferase ratio was one order of magnitude greater than the mean value.

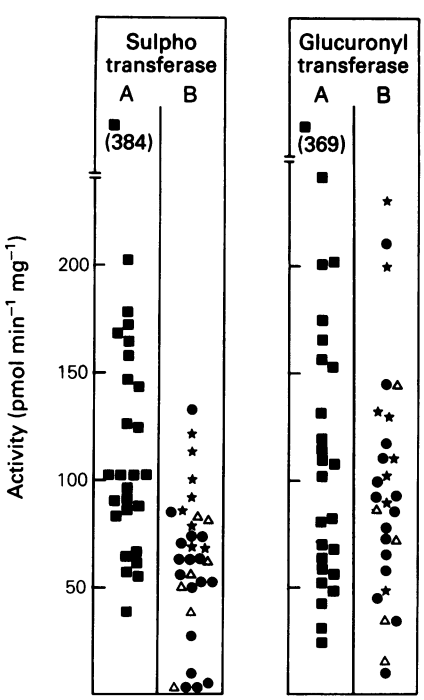

Figure 2 Activities of sulphotransferase and glucuronyltransferase towards ethinyloestradiol in normal (A) and abnormal (B) livers. Stars, circles and triangles refer to chronic persistent hepatitis, chronic active hepatitis and cirrhosis samples, respectively.

Discussion

The present paper shows that liver disease is accompanied by a decrease in the activities of sulphotransferase, acetyltransferase, glutathione transferase, all cytosolic enzymes, and thiomethyltransferase, a microsomal enzyme, but not in that of glucuronyltransferase, a microsomal enzyme. The lack of any effect of liver disease on glucuronyltransferase activity was observed with both 2-naphthol and ethinyloestradiol as substrates. However, it should be noted that enzyme activity is substrate dependent, and with other substrates, the picture could be different.

Whether the procedure used for liver sampling influences the activity of conjugation enzymes is not known. Boobis *et al.* (1980) have observed that the content of microsomal protein g^{-1} liver is slightly but significantly lower in needle than in wedge biopsies of normal liver. However, cytochrome P-450 content and the activities of cytochrome c reductase and aryl hydrocarbon hydroxylase expressed per mg protein are not different in needle and wedge biopsies of normal liver (Boobis *et al.*, 1980). With the exception of glucuronyltransferase, the activities of the enzymes described in this study were one third in abnormal compared to normal liver samples. It is likely that this difference reflects the effect of liver disease rather than the sampling procedure.

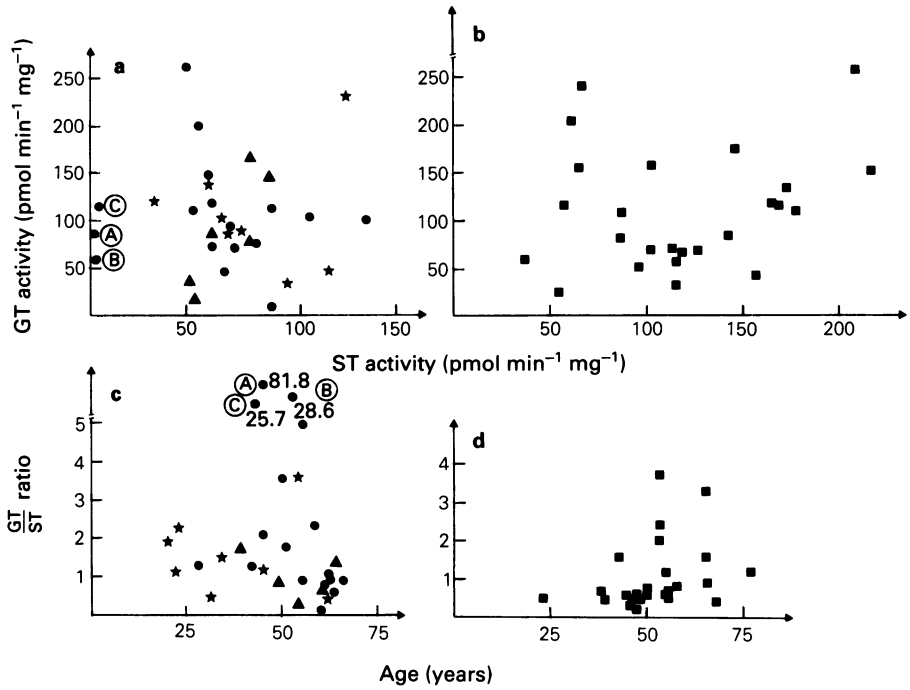

Figure 3 Panels a and b. Relationship between the activities of glucuronyltransferase (GT) and sulphotransferase (ST) in abnormal (panel a) and normal (panel b) livers. Panels c and d. Ratio of glucuronyltransferase to sulphotransferase activities against the liver donor's age in abnormal (panel c) and normal (panel d) livers. Stars, circles and triangles refer to chronic persistent hepatitis, chronic active hepatitis and cirrhosis samples, respectively. The circled letters A, B and C refer to samples from three male subjects, 43–45 years old, with particularly low sulphotransferase activity.

On the basis of the present results, we would expect a lowered conjugation capacity in patients with liver disease. Thus both phase I and phase II enzymes are influenced by liver damage.

The activities reported previously of sulphotransferase (Pacifici *et al.*, 1988b) and glucuronyltransferase (Pacifici *et al.*, 1988a), towards 2-naphthol, acetyltransferase (Pacifici *et al.*, 1986) and glutathione transferase (Pacifici *et al.*, 1988c) in normal liver are in accord with those reported here. There are no previous data on thiomethyltransferase activity. From the limited material studied, it is difficult to assess the possible contribution of differences in sex, age, diet or environment on interindividual variability in the enzyme activities. The activities of phase I enzymes such as aryl hydrocarbon hydroxylase (Boobis *et al.*, 1980; Brodie *et al.*, 1981; Farrell *et al.*, 1979), ethoxycoumarin *O*-deethylase (Woodhouse *et al.*, 1984), ethoxyresorufin *O*-deethylase (Woodhouse, 1987), microsomal epoxide hydrolase (Woodhouse *et al.*, 1983) and cytosolic epoxide hydrolase (Pacifici *et al.*, 1988d) show considerable variability in normal liver.

The sensitivity of the various assays expressed as the 'signal to noise' ratio ranged between 20 and 50 for glucuronyltransferase, sulphotransferase, glutathione transferase and thiomethyltransferase whereas it varied between 4 and 6 for acetyltransferase. Thus, the acetyltransferase assay is less sensitive than those for the other enzymes studied. This may, at least in part, explain why acetyltransferase activity was not detectable in ten abnormal livers. The average activity of this enzyme quoted may thus be overestimated in abnormal livers. The lack of detectable activity for the different conjugating enzymes gives some indication as to what extent the synthetic reactions can be depressed in liver disease. A detoxication role is often attributed to phase II enzymes. Decreased conjugating enzyme activity may reduce hepatic detoxication and it may be of relevance that patients with liver cirrhosis have a higher incidence of primary liver cancer (Johnson *et al.*, 1978), a disease in which environmental carcinogens may play an important role.

Ethinyloestradiol, a widely used drug (Orme

et al., 1983), undergoes extensive metabolism (Bolt, 1979), including conjugation with glucuronic acid and sulphate (Pacifici & Back, 1988 and present study). Orme (1982) has observed a 20–30-fold variation in the steady state plasma concentrations of ethinyloestradiol. Such a variation may reflect, at least in part, inter-individual variability in its metabolism. It is of relevance that the rates of sulphation and glucuronidation of ethinyloestradiol vary considerably in normal livers. In abnormal liver, the balance between sulphation and glucuronidation may be remarkably altered in some patients, because of severe depression of sulphotransferase activity.

The glucuronidation of 2-naphthol *in vitro* is not inhibited by ethinyloestradiol (Pacifici *et al.*, 1988a), suggesting that 2-naphthol and ethinyloestradiol are glucuronidated by two different isoenzymes. Thus, the lack of any difference in the rate of 2-naphthol and ethinyloestradiol glucuronidation between normal and abnormal livers suggests that different glucuronyltransferase isoenzymes are unaffected by liver disease and this finding is corroborated by data from Bock *et al.* (1978) on the glucuronidation of morphine. These findings also suggest that the glucuronidating capacity of patients with liver disease should be similar to that of healthy subjects. However, the five-fold variability in the recovery of microsomal protein from abnormal liver might be linked with variability in the mass of liver parenchyma. Thus, the *in vivo* glucuronidating capacity of those patients whose functional hepatic parenchyma is much less may be limited. The recovery of microsomal protein from normal liver reported in this study accords with that described previously (Pacifici *et al.*, 1988e).

The metabolism of drugs *in vivo* is complex and liver disease may differentially influence metabolic pathways. The increase in the half-life of a drug which often occurs in liver injury may be due to alterations of either phase I or phase II enzymes. However, it is of interest that in cirrhotic patients the half-lives of morphine and oxazepam, drugs that are essentially glucuronidated, are increased less than that of paracetamol and chloramphenicol which undergo more complex metabolism, including glucuronidation (Howden *et al.*, 1989; Williams & Mamelock, 1980). These data support our finding that liver disease has no effect on glucuronyltransferase activity. More work is required to ascertain whether the metabolism of drugs that are primarily acetylated, sulphated or methylated is decreased *in vivo* in patients with impaired liver function.

References

Bass, N. M. & Williams, R. L. (1988). Guide to drug dosage in hepatic disease. *Clin. Pharmacokin.*, **15**, 396–420.

Bock, K. W., Brunner, G., Hoensch, H., Huber, E. & Josting, D. (1978). Determination of microsomal UDP-glucuronyltransferase in needle-biopsy specimens of human liver. *Eur. J. clin. Pharmac.*, **14**, 367–373.

Bolt, H. M. (1979). Metabolism of estrogens–natural and synthetic. *Pharmac. Ther.*, **4**, 19–48.

Boobis, A. R., Brodie, M. J., Kahn, G. C., Fletcher, D. R., Saunders, J. H. & Davies, D. S. (1980). Monooxygenase activity of human liver in microsomal fractions of needle biopsy specimens. *Br. J. clin. Pharmac.*, **9**, 11–19.

Branch, R. A. & Shand, D. G. (1976). Hepatic drug clearance in chronic liver disease. In *The effect of disease states on drug pharmacokinetics*, ed. Benet, L. Z., pp 77–86. New York: American Pharmaceutical Association Academy of Pharmaceutical Sciences.

Brodie, M. J., Boobis, A. R., Bulpitt, C. J. & Davies, D. S. (1981). Influence of liver disease and environmental factors on hepatic monooxygenase activity *in vitro*. *Eur. J. clin. Pharmac.*, **20**, 39–46.

Colquhoun, D. (1971). *Lectures in biostatistics*, ed. Colquhoun, D., pp 191–204. Oxford: Clarendon Press.

Dunnett, C. W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Statist. Ass.*, **50**, 1096–1121.

Farrell, G. C., Cooksley, W. G. E. & Powell, L. W. (1979). Drug metabolism in liver disease, activity of hepatic microsomal metabolizing enzymes. *Clin. Pharmac. Ther.*, **26**, 483–492.

Howden, C. W., Birnie, G. G. & Brodie, M. J. (1989). Drug metabolism in liver disease. *Pharmac. Ther.*, **40**, 439–474.

Johnson, P. J., Kraser, B., Portman, B., Eddleston, A. L. W. P. & Willias, R. (1978). Hepatocellular carcinoma in Great Britain, influence of age, sex, HBsAg status and aetiology of underlying cirrhosis. *Gut*, **19**, 1022–1026.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Proteins measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

Orme, M. L'E. (1982). The clinical pharmacology of oral contraceptive steroids. *Br. J. clin. Pharmac.*, **14**, 31–42.

Orme, M. L'E., Back, D. J. & Breckenridge, A. M. (1983). Clinical pharmacokinetics of oral contraceptive steroids. *Clin. Pharmacokin.*, **8**, 95–136.

Otterness, D. M., Keith, R. A., Kerremans, A. L. & Weinshilboum, R. M. (1986). Mouse liver thiol-methyltransferase. *Drug Metab. Dispos.*, **14**, 680–688.

Pacifici, G. M. & Back, D. J. (1988). Sulphation and glucuronidation of ethinyloestradiol in human liver *in vitro*. *J. steroid Biochem.*, **31**, 345–349.

Pacifici, G. M., Bencini, M. & Rane, A. (1986). Acetyltransferase in humans, development and tissue distribution. *Pharmacology*, **32**, 283–291.

Pacifici, G. M., Franchi, M., Bencini, C., Repetti, F., Di Lascio, N. & Muraro, G. B. (1988e). Tissue distribution of drug-metabolizing enzyme in humans. *Xenobiotica*, **18**, 849–856.

Pacifici, G. M., Franchi, M., Colizzi, C., Giuliani, L. & Rane, A. (1988b). Sulphotransferase in humans, development and tissue distribution. *Pharmacology*, **36**, 411–419.

Pacifici, G. M., Franchi, M., Colizzi, C., Giuliani, L. & Rane, A. (1988c). Glutathione S-transferase in humans, development and tissue distribution. *Arch. Tox.*, **61**, 265–269.

Pacifici, G. M., Franchi, M. & Giuliani, L. (1988a). Is there cytosolic glucuronyltransferase in human liver? In *Cellular and molecular aspects of glucuronidation*, eds Bock, K. W., Burchell, B., Caldwell, J., Magdalou, J., Mulder, G. J. & Siest, G. Colloque INSERM John Libbey Eurotext Ltd, **173**, 93–101.

Pacifici, G. M., Temellini, A., Giuliani, L., Rane, A., Thomas, H. & Oesch, F. (1988d). Cytosolic epoxide hydrolase in humans, development and tissue distribution. *Arch. Tox.*, **62**, 254–257.

Rowland, M., Blaschke, T. F., Meffin, P. J. & Williams, R. L. (1976). Pharmacokinetics in disease states modifying hepatic and metabolic function. In *The effect of disease states on drug pharmacokinetics*, ed Benet, L. Z., pp 53–75. New York: American Pharmaceutical Association Academy of Pharmaceutical Sciences.

Williams, R. L. & Mamelock, R. D. (1980). Hepatic disease and drug pharmacokinetics. *Clin. Pharmacokin.*, **5**, 528–547.

Woodhouse, K. W., Williams, F. M., Mutch, E., Wright, P., James, O. F. W. & Rawlins, M. D. (1983). The effect of alcoholic cirrhosis on the activities of microsomal aldrin epoxidase, 7-ethoxycoumarin *O*-deethylase and epoxide hydrolase, and on the concentrations of reduced glutathione in human liver. *Br. J. clin. Pharmac.*, **15**, 667–672.

Woodhouse, K. W., Williams, F. M., Mutch, E., Wright, P., James, O. F. W. & Rawlins, M. D. (1984). The effect of alcoholic cirrhosis on the two kinetic components (high and low affinity) of the microsomal 0-deethylation of 7-ethoxycoumarin in human liver. *Eur. J. clin. Pharmac.*, **26**, 61–64.

Woodhouse, K. W., Mutch, E., Williams, F. M., James, O. F. W. & Rawlins, M. D. (1987). 7-Ethoxyresorufin deethylase (EROD) in human liver – the effect of alcoholic liver disease. *Eur. J. clin. Pharmac.*, **32**, 559–562.

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