murine mammary carcinoma cells. *Radiat Res* **114**: 215–224, 1988.

29. Ford JM and Hait WN, Buthionine sulfoximine supersensitizes multidrug resistant cells to verapamil. *Proc Am Assoc Cancer Res* **30**: 570, 1989.

Biochemical Pharmacology, Vol. 41, No. 4, pp. 635–638, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

A comparison of levels of glutathione transferases, cytochromes P450 and acetyltransferases in human livers

(*Received* 2 *July* 1990; *accepted* 2 *October* 1990)

Soluble glutathione (GSH) transferases (GSTs) (EC 2.5.1.18) catalyse a number of GSH-dependent reactions including the detoxification of electrophilic metabolites of xenobiotics. In rat, mouse and human, GSTs have been clearly demonstrated to consist of a supergene family including three multigene families referred to as alpha, mu and pi, with considerable homology in each family across the species. The hepatocyte is the richest source of GSTs in all these species. In the human liver, alpha enzymes predominate; mu enzymes are present in 30–40% of the population; and the pi enzyme is restricted to the biliary epithelial cell [1, 2]. In the alpha family, two subunits, referred to variously as Y1 and Y4 [3], B1 and B2 [4] or αx and αy, [5] have been identified. In the mu family there are two expressing alleles: one referred to as GST μ [6] and the other as GST ψ [7] and a null allele, the inheritance of the last named giving rise to mu-deficient individuals in the population [8].

Cytochrome P450 monooxygenases (P450) ("unspecific monooxygenase", EC 1.14.14.1) are the major enzymes involved in the oxidation of drugs, carcinogens and steroids. As in the case of the GSTs, a supergene family of these enzymes exists and the individual proteins are characterized by distinct catalytic specificities [9]. In recent years, considerable insight has been gained regarding the nature of the human P450s [10, 11] and their wide variation among individuals. In some cases, the nature of the variation is genetic polymorphism (e.g. debrisoquine 4-hydroxylation); while in other cases the variation seems to be due to induction of a specific P450 (e.g. induction of phenacetin O-deethylation by cigarette smoking or consumption of char-broiled meat [12, 13]).

N-Acetylation of arylamines is a major route in their biotransformation and, by affecting their disposition, may play a modulatory role in the metabolic activation of these compounds. O-Acetylation and N-O-transacetylation of *N*-hydroxy metabolites also occur and lead directly to activation and DNA binding [14]. The enzymes involved are controlled by two genetic loci designated "monomorphic" and "polymorphic", N-acetylation reactions in humans being controlled principally by the polymorphic locus [15]. This has two major alleles designated as "*r*" and "*s*", giving rise to rapid acetylator (*rr* and *rs*) and slow acetylator (*ss*) phenotypes [16]. In addition, these same polymorphic isozymes probably participate in O-acetylation and N,O-acetylation reactions [17, 18]. In Caucasian populations rapid and slow metabolizers occur with similar frequency [15].

Obviously, all three of these enzymes, the GSTs, the P450s and acetyltransferases, can be involved in the overall metabolism of drugs and carcinogens and the relative levels of catalytic activity towards certain substrates and their products may be expected to influence the balance between bioactivation and detoxification [19]. Thus, it would be of interest to compare levels of some of these enzymes in human tissues to determine if levels of some of the enzymes might vary in concert, particularly since certain chemicals such as phenobarbital and polycyclic hydrocarbons induce several GSTs and P450s in experimental animal models.

In the present paper, we analysed 22 human liver samples for GST subunits by reverse phase HPLC. Fifteen of these liver samples were also assayed for activities of the cytochrome P450 mixed function oxygenase family and for N- and O-acetyltransferases; and values obtained compared to determine to what extent levels of any one of these drug metabolizing enzymes correlated with another.

Materials and Methods

Human livers were obtained from organ donors through Tennessee Donor Services (Nashville, TN, U.S.A.) and CHU Necker (Paris, France). Analysis of GSTs in homogenates was determined by the method in Ref. 5. Total cytochrome P450 was determined spectrally according to the method in Ref. 20, debrisoquine 4-hydroxylase and phenacetin *O*-deethylase activity according to the methods in Ref. 21 (gas chromatography and mass spectrometry) and in Ref. 22 (thin-layer radiochromatography), respectively and methods for *N*-acetyltransferases and *O*-acetyltransferase were as described in Ref. 23.

Results and Discussion

Table 1 shows the results of all the assays involved. Correlations between sulphamethazine and *N*-acetyl-2-aminofluorene *N*-acetyltransferase activity ($r = 0.94$) and *N*-hydroxy-2-aminofluorene-*O*-acetyltransferase activity ($r = 0.88$), have already been published [23]. However, no correlations were seen in comparisons made between levels of activity in any of the other drug metabolizing enzymes examined (see Table 2).

All samples contain both αx and αy but the levels of these two subunits are not related to each other (see Table 2). Thirty to forty per cent of the samples contained mu family enzymes. GST π was never detected in these assays.

Since all members of this sample contain GST αx and αy, each expressed at a level independent of the other, it appears that GST αx and αy are not allelic but products of different gene loci. A smaller sample of eight individuals has been analysed previously and both GSTs αx and αy were present in all individuals [24].

A correlation between *N*- and *O*-acetyltransferase levels has already been reported [23] and while it was of interest to see to what extent acetyltransferase levels might correlate with other drug metabolizing enzymes, it was not surprising

Table 1. Enzymes of drug metabolism in human liver: comparisons of GSH transferases, some cytochrome P450s and acetyltransferases

	GST subunits (μg/g liver)			P450 isoenzymes (nmol/mg microsomal protein			Acetyltransferases (nmol formed/min/mg protein)		(pmol bound/min/mg protein/mL DNA)
Sample	αx	αy	μ	P450	DB	PA	SMZ	AAF	AF
HL15	94	32		0.85	0.19	0.32	0.82	2.97	39.0
HL16	76	33		0.35	0.06	0.25	0.26	0.71	10.2
HL19	252	37	19	0.11			0.04	0.05	8.2
HL23	187	25		0.28	0.09	0.10	0.42	1.75	26.0
FH39	300	300		1.76	0.01	0.74	0.40	1.25	21.2
FH41	118	99		0.85	0.08	0.54	0.17	0.88	17.4
FH47	32	150	14	0.52	0.05	0.17	0.48	0.17	13.0
FH50	149	191	3	0.37	0.07	0.10	0.11	0.81	9.4
FH56	102	125	42	0.47	0.05	0.21	0.07	0.34	7.2
FH61	85	385		1.04	0.03	0.31	0.14	0.33	4.0
FH77	404	81		0.62	0.08	0.32	0.43	1.42	25.2
FH80	156	289		0.34	0.15	0.07	0.10	0.41	13.2
FH81	266	277		0.17	0.10	0.26	0.80	0.32	6.0
FH82	93	37		0.59	0.08	0.57	0.08	0.12	10.0
FH83	551	146		1.03	0.09	0.33	0.05	0.28	9.4
FH55	16	47	28	0.27	0.01	0.09			
HL49	99	83	6						
FH70	76	92	21						
HL25	125	53							
HL62	131	130	14						
FH84	169	94							
HL91	364	333							

P450 is total P450 determined spectrally. DB, PA, SMZ, AAF and AF are debrisoquine 4-hydroxylase, phenacetin *O*-deethylase, sulphomethazine *N*-acetyltransferase and *N*-acetyl aminofluorene *N*-acetyltransferase and aminofluorene *O*-acetyltransferase, respectively.

to find that they did not. However, a correlation between P450 and GSTs was thought to be a possibility, since subunits from both the alpha and mu GST families in the rat are induced by the same compounds, which induce total cytochrome P450 [25].

Total P450 (measured spectrally) and the activities of two specific P450s were examined and compared. The two P450 activities measured were debrisoquine 4-hydroxylation, catalysed by P450 IID6 and regulated by genetic polymorphism, and phenacetin *O*-deethylation, catalysed by P450 IA2 and inducible by cigarette smoking and ingestion of char-broiled meat [26, 27]. The total level of P450 can be elevated by treatment of individuals with barbiturates and other compounds (for review see Ref. 9). Initial work suggested that phenacetin O-deethylation and debrisoquine 4-hydroxylation are correlated to each other [28] but this is not the case in the present work or other later work [26]—the activities are catalysed by the distinct enzymes, P450 IA2 and P450 IID6 [29, 30], which are encoded by genes on separate chromosomes [31]. Neither of these two P450-mediated catalytic activities nor total P450 were correlated with each other or with levels of any of the GST or *N*-acetyltransferase activities.

The overall conclusion is that few of the enzymes which

Table 2. Correlation coefficients

			AAF	AF	P450	DB	PA	αx	αy	μ
1	SMZ	(15)	0.97	0.92	0.31	0.42	0.16	0.29	−0.29	0.47
2	AAF	(15)		0.94	0.25	0.49	0.05	0.00	−0.31	−0.53
3	AF	(15)			0.26	0.49	0.13	0.03	0.40	0.58
4	P450	(16)				−0.25	0.78	0.29	0.40	−0.28
5	DB	(16)					−0.21	0.13	−0.21	−0.57
6	PA	(16)						0.25	0.09	0.26
7	αx	(22)							0.27	0.21
8	αy	(22)								0.30
9	μ	(8)								

Figures in brackets refer to the numbers used in the comparisons. P450 is total P450 determined spectrally. SMZ, AAF, AF, DB, PA are sulphomethazine *N*-acetyltransferase, *N*-acetylaminofluorene *N*-acetyltransferase, aminofluorene *O*-acetyltransferase, debrisoquine 4-hydroxylase, phenacetin *O*-deethylase and αx, αy and μ are glutathione transferase subunits.

are involved in the metabolism of xenobiotics show any type of coordinate regulation with each other in man. Thus, in pathways that involve catalysis by several different enzymes, we would expect to see several independent variations in the human population interacting with each other.

B. KETTERER*†
D. J. MEYER*
E. LALOR*
P. JOHNSON‡
F. P. GUENGERICH§
L. M. DISTLERATH§
P. E. B. REILLY§
F. F. KADLUBAR‖
T. J. FLAMMANG‖
Y. YAMAZOE¶
P. H. BEAUNE**

* *Cancer Research Campaign Molecular Toxicology Research Group Department of Biochemistry University College and Middlesex School of Medicine London W1P 6DB, U.K.*
‡ *Liver Unit King's College School of Medicine and Dentistry London SE5 8RX, U.K.*
§ *Center in Molecular Toxicology and Department of Biochemistry School of Medicine Vanderbilt University Nashville TN 37232, U.S.A.*
‖ *National Center for Toxicological Research Jefferson AR 72079, U.S.A.*
¶ *Department of Pharmacology School of Medicine Keio University Tokyo 160 Japan*
** *Biochimie Pharmacologique et Métabolique Faculté de Médecine Necker—Enfants Malades 75730 Paris, France*

REFERENCES

1. Mannervik B and Danielson UH, Glutathione transferases—structure and catalytic activity. *CRC Crit Rev Biochem* **23**: 283–337, 1988.
2. Ketterer B, Meyer DJ and Clark AG, Soluble glutathione transferase isozymes. In: *Glutathione Conjugation—Mechanisms and Biological Significance* (Eds. Sies H and Ketterer B), pp. 73–135. Academic Press, London, 1988.
3. Soma Y, Satoh K and Sato K, Purification and subunit-structural and immunological characterization of five glutathione *S*-transferases in human liver, and the acidic form as a hepatic tumour marker. *Biochim Biophys Acta* **869**: 247–258, 1986.
4. Stockman PK, McLellan LI and Hayes JD, Characterization of the basic glutathione *S*-transferase B_1 and B_2 subunits from human liver. *Biochem J* **244**: 55–61, 1987.
5. Ostlund Farrants A-K, Meyer DJ, Coles B, Southan C, Aitken A, Johnson PJ and Ketterer B, The separation of glutathione transferase subunits by using reverse-phase high-pressure liquid chromatography. *Biochem J* **245**: 423–428, 1987.
6. Warholm M, Guthenberg C and Mannervik B, Molecular and catalytic properties of glutathione transferase μ from human liver: an enzyme efficiently conjugating epoxides. *Biochemistry* **22**: 3611–3617, 1983.
7. Board P, Genetic polymorphism of glutathione *S*-transferase in man. In: *Glutathione S-Transferases and Drug Resistance* (Eds. Hayes JD, Pickett CB and Mantle TJ), pp. 232–241. Taylor and Francis, London, 1990.
8. Board PG, Biochemical Genetics of glutathione *S*-transferase in man. *Am J Hum Genet* **33**: 36–43, 1981.
9. Distlerath LM and Guengerich FP, Enzymology of human liver cytochromes P-450. In: *Mammalian Cytochromes P*-450 (Ed. Guengerich FP), Vol. 1, pp. 133–198. CRC Press, Boca Raton, FL, 1987.
10. Guengerich FP, Polymorphisms of human cytochrome P-450 enzymes. *Trends Pharmacol Sci* **10**: 107–109, 1989.
11. Guengerich FP, Characterization of human microsomal cytochrome P-450 enzymes. *Annu Rev Pharmacol Toxicol* **19**: 241–264, 1989.
12. Pantuck EJ, Hsiao K-C, Maggio A, Nakamura K, Kuntzman R and Conney AH, Effect of cigarette smoking on phenacetin metabolism. *Clin Pharmacol Ther* **15**: 9–17, 1974.
13. Pantuck EJ, Kuntzman R and Conney AH, Decreased concentration of phenacetin in plasma of cigarette smokers. *Science* **175**: 1248–1250, 1972.
14. Weber WW, Levy GN and Hein DW, Acetylation. In: *Conjugation Reactions in Drug Metabolism* (Ed. Mulder GJ), pp. 163–192. Taylor and Francis, London, 1990.
15. Ohsako S and Deguchi T, Cloning and expression of cDNAs for polymorphic and monomorphic arylamine *N*-acetyltransferases from human liver. *J Biol Chem* **265**: 4630–4634, 1990.
16. Weber WW and Hein DW, N-Acetylation pharmacogenetics. *Pharmacol Rev* **37**: 25–79, 1985.
17. Flammang TJ, Yamazoe Y, Guengerich FP and Kadlubar FF, The *S*-acetyl-coenzyme A-dependent metabolic activation of the carcinogen *N*-hydroxy-2-aminofluorene by human liver cytosol and its relationship to the aromatic amine *N*-acetyltransferase phenotype. *Carcinogenesis* **8**: 1967–1970, 1987.
18. Land SJ, Zukowski K, Lee M-S, Debiec-Rychter M, King CM and Wang C-Y, Metabolism of aromatic amines: relationship of N-acetylation, O-acetylation, *N,O*-acetyltransfer and deacetylation in human liver and urinary bladder. *Carcinogenesis* **10**: 727–731, 1989.
19. Guengerich FP, Inter-individual variation in biotransformation of carcinogens: basis and relevance. In: *Monitoring People Exposed to Carcinogens: Analytical Epidemiological and Ethical Issues* (Eds. Koschier F, Skipper O and Groopman JD). Telford Press, Caldwell, New Jersey, in press.
20. Omura T and Sato R, The carbon monoxide-binding of pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
21. Kahn GC, Boobis AR, Murray S, Brodie MJ and Davies DS, Assay and characterisation of debrisoquine 4-hydroxylase activity of microsomal fractions of human liver. *Br J Clin Pharmacol* **13**: 637–645, 1982.
22. Larrey D, Distlerath LM, Danna GA, Wilkinson GR and Guengerich FP, Purification and characterization of the rat liver microsomal cytochrome P-450 involved in the 4-hydroxylation of debrisoquine, a prototype for genetic variation in oxidative drug metabolism. *Biochemistry* **23**: 2787–2795, 1984.
23. Flammang TJ, Hein DW, Talaska G and Kadlubar FF, *N*-Hydroxy-arylamine *O*-acetyltransferase and its relation to aromatic amine *N*-acetyltransferase polymorphism in the inbred hamster and in human tissue cytosol. In: *Carcinogenic and Mutagenic Responses to Aromatic Amines and Nitroarenes* (Eds.

† To whom correspondence should be addressed.

King CM, Romano LJ and Schuetzle D), pp. 137–148. Elsevier, New York 1988.
24. Hayes JD, Kerr LA and Cronshaw AD, Evidence that glutathione *S*-transferases B_1B_1 and B_2B_2 are the products of separate genes and that their expression in human liver is subject to inter-individual variation. *Biochem J* **264**: 437–445, 1989.
25. Peterson LA and Guengerich FP, Comparison of and relationship between glutathione S-transferase and cytochrome P-450 systems. In: *Glutathione Conjugation—Mechanisms and Biological Significance* (Eds. Sies H and Ketterer B), pp. 192–233. Academic Press, London, 1988.
26. Sesardic D, Boobis AR, Edwards RJ and Davies DS, A form of cytochrome P450 in man, orthologous form d in rat, catalyses the O-deethylation of phenacetin and is inducible by cigarette smoking. *Br J Clin Pharmacol* **26**: 363–372, 1988.
27. Butler MA, Guengerich FP and Kadlubar FF, Metabolic oxidation of the carcinogens 4-aminobiphenyl and 4,4′-methylene-bis(2-chloroaniline) by human hepatic microsomes and by purified rat cytochrome P-450 monooxygenases. *Cancer Res* **49**: 25–31, 1989.
28. Boobis AR, Kahn GC, Murray S, Brodie MJ and Davies DS, Evidence for a common defect in the impaired oxidation of substrates on the debrisoquine oxidation polymorphism. *Br J Clin Pharmacol* **14**: 602P–603P, 1982.
29. Distlerath LM, Reilly PEB, Martin MV, Davis GG, Wilkinson GR and Guengerich FP, Purification and characterization of the human liver cytochrome P-450 involved in debirsoquine 4-hydroxylation and phenacetin O-deethylation, two prototypes for genetic polymorphism in oxidative drug metabolism. *J Biol Chem* **260**: 9057–9067, 1985.
30. Butler MA, Iwasaki M, Guengerich FP and Kadlubar FF, Human cytochrome $P\text{-}450_{PA}$ (IA2), the phenacetin *O*-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc Natl Acad Sci USA* **86**: 7696–7700, 1989.
31. Nebert DW, Nelson DR, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R and Waterman MR, The P450 superfamily: update on listing of all genes and recommended nomenclature of the chromosomal loci. *DNA* **8**: 1–13, 1989.

Biochemical Pharmacology, Vol. 41, No. 4, pp. 638–641, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Effects of various tetracycline derivatives on *in vitro* and *in vivo* β-oxidation of fatty acids, egress of triglycerides from the liver, accumulation of hepatic triglycerides, and mortality in mice

(*Received* 19 *June* 1990; *accepted* 23 *September* 1990)

Severe, often fatal, microvesicular steatosis of the liver has been observed in numerous subjects who received high intravenous doses of tetracycline [1]. Two mechanisms contribute to the accumulation of hepatic triglycerides after administration of such high doses of tetracycline [2–5]. First, tetracycline inhibits the mitochondrial β-oxidation of fatty acids in mice and in humans [2]. Second, tetracycline decreases the egress of lipoproteins from the liver [3–5], mainly by inhibiting the association between the apoproteins and triglycerides [5].

In addition to tetracycline itself, several other tetracycline derivatives are available for human therapeutics. Some of these derivatives, including chlortetracycline [1], minocycline [6], rolitetracycline [7] and oxytetracycline [1], have been shown to also produce microvesicular steatosis of the liver in humans [1, 6] or animals [1, 7]. To our knowledge, however, no information is available on the effects of the various tetracycline derivatives on the oxidation of fatty acids, and the egress of triglycerides from the liver has been studied only with rolitetracycline [8].

Materials and Methods

Materials. Male Crl:CD-1(ICR)BR Swiss mice were purchased from Charles River (Saint-Aubin-les-Elbeuf, France). Some mice were fed *ad lib.* on a standard diet (M 25 biscuits, Extra Labo, Pietrement, Provins, France). Other mice were fasted for 24 or 48 hr before being used.

Lymecycline and rolitetracycline were generous gifts from, respectively, Farmitalia Carlo Erba (La Défense, France) and Hoechst (La Défense, France). The other tetracycline derivatives (in the form of the hydrochloride) and Triton WR 1339 were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). The tetracycline derivatives were dissolved in water, followed by the addition of NaOH to bring the pH back to 7.4. Mice received 0.5 mL i.p. of the tetracycline derivative solution (0.25 or 0.5 mmol/kg i.p.).

[U-^{14}C]Palmitic acid (928 mCi/mmol), [1-^{14}C]palmitic acid (54 mCi/mmol), and [1-^{14}C]butyric acid (54 mCi/mmol) were purchased from Amersham (Bucks, U.K.). [1-^{14}C]Octanoic acid (54 mCi/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.).

In vitro *β-oxidation.* Liver mitochondria were prepared as described by McGarry *et al.* [9]. An aliquot was used to determine mitochondrial protein by the method of Lowry *et al.* [10]. The *in vitro* β-oxidation of [U-^{14}C]palmitic acid by mouse liver mitochondria was assessed as described by Fréneaux *et al.* [2]. Mitochondria (2 mg protein/2 mL) were incubated with ATP (0.2 mM), DL-carnitine (50 μM), coenzyme A (15 μM), the tetracycline derivative (1 mM), and [U-^{14}C]palmitic acid (40 μM; 0.05 μCi/2 mL). The latter was added after 5 min of preincubation at 30°. The reaction was then carried out for 10 min at 30°. Acid-soluble β-oxidation products were measured as previously described [2].

In vivo *oxidation of fatty acids.* The tetracycline derivatives (0.25 mmol/kg i.p.) were dissolved in water (0.5 mL/mouse) followed by the addition of NaOH to