Identification of human cytochromes *P*-450 analogous to forms induced by phenobarbital and 3-methylcholanthrene in the rat

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Antibodies to four rat liver forms of cytochrome *P*-450, two phenobarbital-inducible (PB₁ and PB₂) and two 3-methylcholanthrene-inducible (MC₁ and MC₂) proteins, have been used to make a structural and functional comparison of rat and human cytochromes *P*-450. Proteins from both species were identified on Western blots by their reaction with these antibodies. In the human liver preparations, structurally related proteins to PB₁ and to PB₂ were identified in all the samples tested with apparent *M_r* values of 51 800 and 54 800 for PB₁ and 53 600 and 57 200 for PB₂. Considerable variation in the content of the lower-*M_r* proteins was measured between samples and, as with the rat enzymes, samples which reacted well with anti-PB₁ also reacted with anti-PB₂, indicating that these proteins are regulated at least to some degree, co-ordinately. The apparent *M_r* values of the major human proteins identified with anti-MC₁ and anti-MC₂ were 54 400 and 57 000 respectively. Only six (of 31) human samples contained significant amounts of these proteins. The same six samples which reacted with anti-MC₁ also reacted with anti-MC₂, again indicating co-ordinate regulation of these two proteins. Antibody inhibition of microsomal 7-ethoxycoumarin and 7-ethoxyresorufin metabolism demonstrated a degree of conservation of substrate specificity related to specific *P*-450 isoenzymes between the species. However, the contributions of the different *P*-450 isoenzymes to the human microsomal activity were not always related to the rat enzyme with the highest activity towards these substrates.

INTRODUCTION

The recent studies demonstrating sex and strain differences as well as genetic polymorphisms in cytochrome *P*-450 isoenzymes (Johnson & Schwab, 1984; Kato & Kamataki, 1982; Nebert & Jensen, 1979; Vlasuk *et al.*, 1982) illustrate the ever-increasing complexity of this enzyme system. Genetic polymorphisms are not restricted to animal models but have also been demonstrated in man. In this case epidemiology studies indicate a possible relationship between these polymorphisms and individual susceptibility to cancer (Ayesh *et al.*, 1984). It is clearly very difficult to extrapolate data from animal sources on drug oxidation and drug toxicity to the metabolism of xenobiotics in man (Boobis & Davies, 1984). As a first step in such an extrapolation it is necessary to characterize the various human *P*-450 forms present and so compare their structural and functional similarities with forms found in other species.

Many different forms of cytochrome *P*-450 (*P*-450) have now been isolated from various animal species (Guengerich *et al.*, 1982; Johnson, 1979; Ryan *et al.*, 1982). The majority of these forms have been isolated from animals treated with *P*-450-inducing agents, e.g. PB, 3-MC or polychlorinated biphenyls, and it is now clear that many forms of *P*-450 are induced by a single agent. Following treatment with PB or 3-MC, specific cytochrome *P*-450 isoenzymes are induced with very marked

differences in their substrate specificities (Guengerich *et al.*, 1982; Johnson, 1979; Ryan *et al.*, 1982). The inducibility of these isoenzymes and the subsequent changes in enzyme activity vary markedly between species. For example 3-MC, which can significantly induce the metabolism of benzo[*a*]pyrene in rat or mouse liver microsomes preparations, has no such effect in rabbit liver microsomes (Atlas *et al.*, 1977). In spite of these differences in *P*-450 regulation, amino acid sequencing and the use of DNA probes have demonstrated that certain *P*-450 forms are structurally very similar between species (Fujita *et al.*, 1984; Kawajiri *et al.*, 1984; Simmons & Kasper, 1983; Tarr *et al.*, 1983).

In previous reports we have described the isolation, characterization and regulation of four forms of rat liver cytochrome *P*-450 (PB₁, PB₂, MC₁ and MC₂) (Wolf & Oesch, 1983; Wolf *et al.*, 1984). From *N*-terminal analysis *P*-450 PB₁ appears structurally related to, but not identical with, the PB₁ reported by Waxman & Walsh (1983). PB₂ is a novel protein also different from the PB-induced proteins reported by other laboratories but structurally related to form 'h' described by Ryan *et al.* (1984). MC₁ and MC₂ appear to be those proteins reported by Ryan *et al.* (1982) as forms 'd' and 'c' respectively. In the present study, antibodies raised against these four *P*-450 forms have been used to identify structurally analogous proteins in a series of human hepatic microsomal samples. At a functional level, we

Abbreviations used: *P*-450, cytochrome *P*-450; PB, phenobarbital; 3-MC, 3-methylcholanthrene; AR, Arochlor 1254.

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have investigated the ability of these antibodies to inhibit the metabolism of the *P*-450 substrates 7-ethoxyresorufin and 7-ethoxycoumarin.

MATERIALS AND METHODS

All chemicals were purchased from commercial sources and were of the highest grade available. Male CBA mice (25 g), Wistar rats (200 g) or New Zealand white rabbits (1.5 kg) were used. Rats were treated with either PB, AR or 3-MC as described previously (Wolf & Oesch, 1983). Human liver samples, from organ transplant donors, were frozen in liquid N₂ and stored at -80 °C within 2 h after removal from the donor, with the exception of sample 1 which was obtained after 5 h. Microsomal fractions were prepared by using the following procedure. Liver samples were thawed rapidly, scissor-minced and homogenized with a Silverson Laboratory Mixer Emulsifier in 3 vol. of 10 mM-phosphate buffer, pH 7.4, containing 1.15% KCl. All operations were carried out at 4 °C. Homogenates were centrifuged at 11000 *g*_{av.} for 20 min, the supernatants were centrifuged at 230000 *g*_{av.} for 1 h and microsomal pellets were washed twice with the KCl buffer and then stored at -80 °C in 0.25 M-sucrose buffered with 10 mM-phosphate, pH 7.4, at a protein concentration of 10–20 mg/ml. Using this procedure no cytochrome *P*-420 was detected in any of the samples used. Protein determinations were made according to the procedure described by Lowry *et al.* (1951).

Cytochromes *P*-450 PB₁, PB₂, MC₁ and MC₂ and cytochrome *P*-450 reductase were isolated from either PB- or 3-MC-treated Sprague-Dawley rats as described previously (Wolf & Oesch, 1983; Wolf *et al.*, 1984). All these proteins were of high purity and had specific *P*-450 contents of 14.7, 15.6, 16.5 and 16.4 nmol/mg of protein for PB₁, PB₂, MC₁ and MC₂ respectively. Antisera were prepared as described previously (Wolf & Oesch, 1983). IgG obtained by (NH₄)₂SO₄ precipitation yielded 10–15 mg of IgG/ml of antiserum. On Western blots antibodies to PB₁ and PB₂ did not react with the other proteins used. The antibody to MC₂ did react weakly with MC₁ due to known structural homology between these proteins. Cytochrome *P*-450 was determined by the method of Omura & Sato (1964). Cytochrome *P*-450 reductase activity was determined by measuring the NADPH-dependent rate of cytochrome *c* reduction at 30 °C (Yasukochi & Masters, 1976). 7-Ethoxyresorufin and 7-ethoxycoumarin de-ethylation were measured with the direct fluorimetric methods of Burke & Mayer (1975) and Ullrich & Weber (1972) respectively. The effects of *P*-450 antibodies on enzyme activities were investigated in human microsomal samples incubated for 5 min at room temperature with antiserum or its IgG fraction (1 ml of antiserum is equivalent to 10–15 mg of IgG) as described in the Figure legends. At these antibody concentrations, maximal inhibition was imposed on the metabolism of these substrates by rat liver microsomal samples. The amount of antiserum or IgG was always made up to a fixed concentration with preimmune samples. Following the preincubation period, aliquots were then taken for the fluorimetric assays. Results are expressed as the rate of metabolism in the presence of specific antibody relative to the rate measured in the presence of an equivalent amount of preimmune serum.

The detection of cytochrome *P*-450 isoenzymes

following electrophoretic transfer of the proteins from polyacrylamide gels to nitrocellulose sheets (Western blots) was as follows. All procedures were performed at room temperature. Samples (see Figure legends) were applied to 9% polyacrylamide gels and electrophoresis, in the presence of sodium dodecyl sulphate, was as described by Laemmli (1970). The transfer of proteins to nitrocellulose sheets (Anderman) was as described by Towbin *et al.* (1979) with modifications. The blot 'sandwich' contained sheets of 3 MM chromatography paper (Whatman) between polyacrylamide gel and scouring pad and between nitrocellulose sheet and scouring pad. The sandwich was placed in a Trans Blot cell (Bio-Rad) containing 50 mM-sodium phosphate, pH 6.5. A current of 0.25 A was applied for 16 h.

Portions of the nitrocellulose (*M*_r standards tracks) were stained for protein with Amido Black [0.05% in methanol/acetic acid/water (45:9:46, by vol.)] and destained with acetic acid/water (7:93, v/v).

Immunological detection of proteins on nitrocellulose was by the method of Badaracco *et al.* (1983) except that the peptides were not initially visualized by staining with Toluidine Blue, and other modifications were introduced. After electroblotting, the nitrocellulose sheets were immersed in blocking solution [0.01% thimerosal, 0.25% gelatin and 5% (v/v) heat-inactivated (55 °C for 1 h) horse serum in 0.15 M-NaCl/10 mM-Tris/HCl, pH 7.4] for 8 h, removed and incubated with antiserum appropriately diluted either 1:1000 or 1:10000 with blocking solution for 16 h. The nitrocellulose was washed with Tween in Tris-buffered saline (TTBS: 0.05% Tween-20 in 0.5 M-NaCl/20 mM-Tris/HCl, pH 7.5; three changes during 30 min), incubated with a 1:1000 dilution of horseradish peroxidase-conjugated goat anti-(rabbit IgG) (Miles), for 2 h, washed twice with TTBS (two changes during 20 min) and once with 0.17 M-NaCl/42 mM-Tris/HCl, pH 7.4, for 10 min. Immuno-reactive bands were visualized by incubating the sheets with a freshly prepared solution of 0.05% diaminobenzidine tetrahydrochloride and 0.01% H₂O₂ in 50 mM-Tris/HCl, pH 7.6, or a freshly prepared solution of 0.05% 4-chloro-1-naphthol, 0.01% H₂O₂ and 17% methanol in 42 mM-Tris/HCl, pH 7.4, containing 0.17 M-NaCl, for at least 10 min. The latter procedure was preferred as it did not involve the use of known carcinogenic materials and was more sensitive. The nitrocellulose sheets were rinsed with distilled water and dried.

RESULTS

In order to characterize the antibody preparations used, Western blots of various rat liver microsomal fractions from control, PB-, 3-MC- or AR-treated animals were carried out with the antisera to *P*-450 PB₁, PB₂, MC₁ and MC₂. These data are shown in Fig. 1. The major inducible protein band in each sample had the same *M*_r as the purified antigen but other bands were also noted. These bands were observed when either 4-chloro-1-naphthol or diaminobenzidine was used as peroxidase substrate. In the case of anti-PB₁, in addition to the major band at 53100 equivalent to this protein a second band at 48800 was also observed. [In previous publications the *M*_r of PB₁ was reported as 52000. In the present work, using a slightly different gel procedure, the *M*_r of this protein was consistently 53100.] This band was not

Fig. 1 Western blot of antibodies raised against PB_1 , PB_2 , MC_1 and MC_2 with liver microsomal samples from rats treated with various inducing agents

C, AR, PB and MC represent microsomes from control, AR-, PB- and 3-MC-treated rats respectively. The 'Pre' well comprised control microsomes where the nitrocellulose was reacted with rabbit pre-immune serum. Each lane contains 10 μ g of microsomal protein; other details are given in the Materials and methods section. Diaminobenzidine was used as peroxidase substrate.

induced by PB or any of the other inducing agents used. The major band at 53 100 was significantly induced by treatment of the animals with AR or PB. The synthesis of PB_1 was significantly suppressed by 3-MC treatment. The band at 48 800 was unaffected. PB_2 (M_r 53 600) was also induced by PB and AR and significantly reduced by 3-MC treatment. In confirmation of previous findings with the enzyme-linked immunosorbent assay (Wolf *et al.*, 1984), PB_1 is present in control samples at a higher concentration than PB_2 . The antibody to MC_1 recognized a protein in control microsomes with an M_r of 53 100. The synthesis of the protein was induced by PB and AR and suppressed by 3-MC treatment. The major protein induced by 3-MC, and also induced strongly by AR, had an M_r equivalent to that of purified MC_1 (54 400). This protein was not detectable in control preparations. In the experiment shown in Fig. 1 the antibody to MC_2 only reacted with a protein of M_r 57 700 equivalent to the purified antigen. However, in other blots, particularly when 4-chloro-1-naphthol was used, a second protein, of M_r 54 400 equivalent to MC_1 , was also identified. The intensity of both of these bands was much increased by 3-MC and AR treatment of the animals and suppressed by PB treatment.

The reactivity of the antibodies to the *P*-450s PB_1 , PB_2 , MC_1 and MC_2 with human liver microsomal samples is shown in Figs. 2 and 3, and Table 1. The human *P*-450s have been given a nomenclature analogous to the rat forms to indicate similarities and have been ordered on the basis of electrophoretic mobility. Using antibody

raised against rat PB_1 , major bands at M_r 51 800 and 54 800 were observed on Western blots of human microsomal samples from patients 1–7 (Fig. 3a). Considerable variation was observed between individuals. For example, in samples 2, 4 and 7 the band at M_r 51 800 was very faint. The intensity of the bands in the human samples was weaker than in the rat control, and this was due largely to the smaller amounts of human *P*-450 loaded on to the gel.

Two bands were identified for human samples 1–7 (M_r 53 600 and M_r 57 200) by antibody raised against rat PB_2 (Fig. 3b). As with anti- PB_1 , the intensity of the bands varied significantly between samples and that at M_r 53 600 was almost absent from samples 3, 4 and 7. In two of these samples, 4 and 7, and also in sample 5, the band with M_r 57 200 was much more intense than in the other samples. It is interesting to note that none of the differences between the human microsomal samples in their reactivity with anti- PB_1 or anti- PB_2 were apparent when the microsomal samples were examined on the basis of their Coomassie Blue staining patterns. In this regard all of the samples appeared remarkably similar (results not shown). It was also interesting that the extent of reactivity of anti- PB_1 in the various human samples with the protein of M_r 51 800 essentially followed the extent of reaction of anti- PB_2 with the protein of M_r 53 600, although some differences were apparent. No such similarity was observed between the high- M_r proteins (54 800 and 57 200).

Proteins which clearly reacted with the antibody to

Fig. 2. Western blots of antibodies to PB₁ (a) and PB₂ (b) with human liver microsomal samples

Each lane contains 15 µg of microsomal protein; other details are given in the Materials and methods section. 4-Chloro-1-naphthol was used as peroxidase substrate.

Fig. 3. Western blots of antibodies to MC₁ (a) and MC₂ (b) with human liver microsomal samples

Each lane contains 30 µg of microsomal protein; other details are given in the Materials and methods section. 4-Chloro-1-naphthol was used as peroxidase substrate.

Table 1. Major proteins in rat, mouse, rabbit and human microsomal samples identified by using Western blots

Antibody	Purified antigen	10 ⁻³ × Apparent <i>M_r</i> of reacting proteins			
		Rat	Rabbit	Mouse	Human
Anti-PB ₁	53.1 (PB ₁)	48.8	49.1	48.5	51.8 (human PB _{1a})
		53.1	52.7	52.9	54.8 (human PB _{1b})
Anti-PB ₂	53.6 (PB ₂)	53.6	54.5	54.4	53.6 (human PB _{2a})
					57.2 (human PB _{2b})
Anti-MC ₁	54.4 (MC ₁)	53.1	53.1	53.8	
		54.4	55.0	56.0	54.4 (human MC ₁)
Anti-MC ₂	57.7 (MC ₂)	54.4	55.0	56.1	
		57.7	59.1	57.0	57.0 (human MC ₂)

MC₁ or to MC₂ in the seven human samples used for the bulk of this study could not be identified. However, a further 24 samples were tested and six out of a total of 31 reacted strongly with anti-MC₁ (Fig. 3a), identifying a major cross-reacting protein of *M_r* 54 400. The antibody to MC₂ again only reacted with six out of the 31 samples

tested. In this case a major band of *M_r* 57 000 was detected (Fig. 3b). It was interesting to note that the majority of samples that reacted with anti-MC₁ also reacted with anti-MC₂, although this was not always the case. The difference in *M_r* of the proteins recognized by anti-MC₁ and anti-MC₂ was confirmed on Western blots

Table 2. Drug-metabolizing enzyme activities in human liver microsomal samples

Activities were measured as described in the Materials and methods section.

Sample	Cytochrome <i>P</i> -450 (nmol/mg)	<i>P</i> -450 reductase (units)	7-Ethoxycoumarin (nmol/min per mg)	7-Ethoxyresorufin (nmol/min per mg)
1	0.36	208	0.131	0.178
2	0.24	197	0.282	0.041
3	0.19	234	0.067	0.038
4	0.22	144	0.212	0.066
5	0.23	109	0.110	0.024
6	0.23	146	0.190	0.066

of a single gel containing human microsomes and MC₁ and MC₂ standards, the nitrocellulose being cut in half before staining with either antibody (results not shown). In addition to the reactivity of the *P*-450 antibodies with the human samples, we have also investigated the reactivity with rabbit and mouse liver microsomal proteins. These data are summarized in Table 1. All four antibodies reacted strongly with mouse and rabbit microsomal samples. The regulation of the mouse *P*-450 forms was extremely similar to the rat in all respects, the only difference being the apparent *M_r* values of the proteins identified (Table 1). Despite significant similarities, there were certain important differences in the rabbit from the data obtained in the rat. Both proteins identified with anti-PB₁, *M_r* values 49 100 and 52 700, were inducible with PB. Also the band at *M_r* 59 100 identified with anti-MC₂ was only weakly induced by treatment of the rabbits with 3-MC.

In addition to their use in the identification of structurally similar *P*-450 forms between species, the antibodies were used to investigate functional similarities by studying their effects on the metabolism of 7-ethoxyresorufin and 7-ethoxycoumarin in the human samples. The cytochrome *P*-450 contents, *P*-450 reductase activities and rates of 7-ethoxycoumarin and 7-ethoxyresorufin metabolism of the human samples are given in Table 2. In the majority of the human samples the rate of 7-ethoxyresorufin de-ethylation was much lower than the rate of 7-ethoxycoumarin metabolism. No relationship was found between the rates of metabolism of these two substrates or indeed with the cytochrome *P*-450 or *P*-450 reductase content.

The effects of the antibodies on the inhibition of 7-ethoxyresorufin metabolism and 7-ethoxycoumarin metabolism in the human liver samples are shown in Fig. 4. The values shown are expressed as percentages of the rates obtained in the presence of pre-immune serum. In some cases values slightly over 100% were obtained which may be due to serum effects; however, the reason is unclear. In liver microsomes from control, AR- and 3-MC-treated rats anti-MC₂ was by far the most effective inhibitor of 7-ethoxyresorufin metabolism; 50% inhibition was noted at approx. 0.5 µl of antiserum/incubation. Anti-MC₁ also inhibited this activity in these samples but at antibody concentrations an order of magnitude higher. In microsomes from PB-treated rats, it was interesting that anti-PB₁ and anti-MC₁ were the most effective inhibitors of 7-ethoxyresorufin de-ethylation, with anti-MC₂ having essentially no inhibitory effect here (results not shown). This is consistent with the

suppression of the synthesis of this protein (MC₂) by phenobarbital treatment. In the human microsomal samples anti-MC₁ was the most effective inhibitor of 7-ethoxyresorufin metabolism. The extent of inhibition with this antibody was not always directly related to the microsomal enzyme activity. However, most inhibition was observed in samples 1, 4 and 6, which did exhibit the highest rates of 7-ethoxyresorufin metabolism. Anti-MC₂ did partially inhibit this activity in samples 4, 5 and 6. However in sample 1, which had by far the highest activity, essentially no inhibition (13%) was observed. Anti-PB₁ and anti-PB₂ caused only slight inhibition in some of the samples. The inhibition of 7-ethoxycoumarin metabolism by anti-PB₁ was more marked, significant inhibition being measured in most of the samples. As with 7-ethoxyresorufin activity, anti-PB₂ was not an effective inhibitor. It was interesting that anti-MC₁ was no better an inhibitor of 7-ethoxycoumarin metabolism than was anti-MC₂, with inhibition by these antibodies only approaching 30% in samples 1 and 5. Again, no relationship between the extent of inhibition by the antibodies and the microsomal metabolism of 7-ethoxycoumarin was apparent.

DISCUSSION

The difficulties in interspecies comparisons of hepatic microsomal monooxygenases are manifold (Walker, 1980). Furthermore, there is currently a paucity of information concerning the structural and functional properties of human cytochromes *P*-450 and how these relate to those of other species. In the present study, four antibody preparations to PB- or 3-MC-inducible rat liver cytochromes PB₁, PB₂, MC₁ and MC₂ (Wolf & Oesch, 1983; Wolf *et al.*, 1984) have been used to compare the structural and catalytic properties of these isoenzymes in rat and human liver microsomes. Using the Western blot procedure we have identified forms of cytochrome *P*-450 in human liver which are structurally analogous to rat hepatic cytochromes *P*-450 and are likely candidates for regulation by either PB- or 3-MC-type inducers (Table 1). The human *P*-450s have been given a nomenclature analogous to the rat forms to indicate similarities. In addition, immunoblotting has been used to investigate further the regulation of rat PB₁, PB₂, MC₁ and MC₂ relative to other laboratory species (Table 1). The major proteins identified on Western blots with antibodies to MC₁ and MC₂ in rat, mouse and rabbit samples were those expected from the literature reports (see Nebert & Negishi, 1982). Comparison of PB₁ and PB₂ with other

Fig. 4. Effect of antibodies to PB₁ and PB₂ (a, b) and MC₁ and MC₂ (c, d) on 7-ethoxycoumarin (a, c) and 7-ethoxyresorufin (b, d) metabolic activity in human liver microsomal samples

Microsomes (0.2 mg of protein) in 100 μ l of 0.1 M-Tris/HCl, pH 7.4, were incubated for 5 min at 25 °C with 20 μ l of antiserum, or, in the case of anti-MC₁ and anti-MC₂, its IgG fraction (10 mg/ml). Then 0.9 ml of 0.1 M-Tris/HCl, pH 7.4, was added, followed by NADPH (final concentration 1 mM). Other details are described either in the Materials and methods section or by Burke & Mayer (1975).

species has not been reported in detail. It is clear that there are analogous proteins present in both rabbit and mouse liver.

Western blots using anti-MC₁ identified a second protein which was surprisingly induced by PB and not 3-MC. This finding is intriguing and suggests significant structural similarity between a PB-inducible and 3-MC-inducible protein. In support of this, Ashworth *et al.* (1984) have recently demonstrated that a cDNA probe for MC₁ will hybridize with a PB-inducible RNA species. Structural similarities between MC₁ and proteins such as PB₁ (form b) have been demonstrated (Kawajiri *et al.*, 1984) and it is feasible that epitopes on MC₁ are common to the phenobarbital-inducible protein. Also, Guengerich *et al.* (1982) have reported reactivity between anti-MC₁ (β NF/ISFG) and a cytochrome *P*-450 (PB-C) induced by PB.

Of the human microsomal samples studied, anti-PB₁ and anti-PB₂ reacted with nearly all the samples. However a considerable variation in the content of these proteins was observed between samples, suggesting a considerable variation in human hepatic *P*-450 isoenzyme distribution between individuals. No direct correlation between the drugs administered or the sex of these

patients was apparent. It was interesting that the low-*M_r* human protein reacting with anti-PB₁ appeared to be regulated in a manner similar to the low-*M_r* protein reacting with anti-PB₂ in that samples with high PB₁ content also had a high PB₂ content. This finding suggests that these two proteins are regulated, at least to some degree, co-ordinately in man in a manner similar to that already noted for other species (Wolf *et al.*, 1984). The co-ordinate regulation of *P*-450s was also striking for MC₁ and MC₂ where essentially only the samples which reacted with anti-MC₁ reacted with anti-MC₂. As with the PB-inducible proteins, there was considerable variation in the content of these proteins between individuals. None of the samples 1-7 used for the majority of this study were from smokers, which may account for our inability to detect MC₁ and MC₂ in these samples.

In a recent report Jaiswal *et al.* (1985) have reported the isolation of a human gene equivalent to mouse *P*₁ 450 or rat *P*-450 MC₂. They report that there only appears to be one gene in this gene family in humans. However, in the present study antibodies to MC₁ and MC₂ (which are both part of the same gene family in the rat) reacted with proteins of clearly different *M_r*. This suggests that this gene family contains more than one gene but may be

more diverged than in the mouse, or that one of the antibodies is recognizing a protein from a different gene family. The co-ordinate regulation argues against this latter possibility.

The identification of two bands by anti-PB₁ and anti-PB₂ on a Western blot of human microsomes is worthy of note, particularly in the case of PB₂ where one band had a much higher *M_r* (57200) than those associated with PB-inducible *P*-450s in animal species. To some degree there appeared to be an inverse relationship in the intensity of this band relative to that at *M_r* 53600. This raises the possibility that the high-*M_r* protein may be a zymogen and that there may be posttranslational modification of human hepatic *P*-450s. Such modification is well characterized for *P*-450s in the adrenal (Matocha & Waterman, 1984). Evidence for this possibility in rat liver samples is essentially negative but equivocal (Bresnick *et al.*, 1981; Dubois & Waterman, 1979; Kumar & Padmanaban, 1980). Initial studies using antibodies to further *P*-450 forms again show the presence of high-*M_r* proteins. In one case the second band observed was much higher than that associated with *P*-450 forms. The identity of the high-*M_r* proteins needs investigation.

The level of 7-ethoxyresorufin and 7-ethoxycoumarin metabolism detected in the human samples was generally much lower (5–10-fold) than that measured in the rat (results not shown). However, the different activities of the human microsome preparations towards these substrates clearly demonstrate that 7-ethoxyresorufin and 7-ethoxycoumarin are metabolized by different human *P*-450s, although some overlapping activity is of course possible. Although we were unable to detect MC₁ and MC₂ on Western blots of samples 1–7, a human protein structurally related to MC₁ appears to play a more significant role in the metabolism of 7-ethoxyresorufin than the other three *P*-450s investigated. This is interesting in view of the fact that the MC₂ isoenzyme is by far the most effective in the metabolism of this substrate in the rat. The metabolism of 7-ethoxyresorufin by the human samples is analogous to the situation in liver microsomes from PB-treated rats where anti-MC₁ is a more effective inhibitor of 7-ethoxyresorufin *O*-deethylation than is anti-MC₂. However, in liver microsomes from control and 3-MC-treated rats the reverse is true, with anti-MC₂ inhibiting 7-ethoxyresorufin metabolism much more effectively. It is interesting to note that proteins analogous to MC₁ or MC₂ do not always appear to be responsible for the metabolism of this substrate, as in certain samples, e.g. sample 3, neither anti-MC₁ nor anti-MC₂ were effective inhibitors. The proteins present in liver samples 1–7 may therefore be more characteristic of PB induction than 3-MC induction. This is consistent with the finding that anti-PB₁ did cause some inhibition of 7-ethoxycoumarin metabolism and that the PB-inducible *P*-450s were readily detectable on most of the Western blots of the human microsomes. Anti-MC₁, anti-MC₂ and anti-PB₂ were ineffective in inhibiting the metabolism of 7-ethoxycoumarin. The levels of inhibition imposed by the antibodies demonstrate some common substrate specificity between rat and human *P*-450s, in agreement with the results of Fujino *et al.* (1982), who have used monoclonal antibodies raised against rat MC₂ (MC-*P*-450) to inhibit benzo[*a*]pyrene metabolism in human lymphocytes.

In conclusion, the antibody inhibition experiments together with the Western blot data demonstrate (a) the

presence of human liver *P*-450s structurally analogous to those induced by PB or 3-MC in other species, (b) that these forms retain some similarities in substrate specificity between species and (c) that there is a considerable and potentially important variation in the levels of the *P*-450 isoenzymes between human samples. This information should prove important in our understanding of how individuals respond to drugs, toxins and chemical carcinogens.

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