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THE INDUCIBILITY AND CATALYTIC ACTIVITY OF CYTOCHROMES P450c (P450IA1) AND P450d (P450IA2) IN RAT TISSUES

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Abstract—The metabolism of phenacetin is primarily by cytochrome P450-dependent O-deethylation to paracetamol (POD activity). In untreated rats, microsomal POD activity is detectable in both the liver and lung, but not in the small intestine or the kidney. POD activity is highly induced in both hepatic and extrahepatic tissues of the rat following treatment with polycyclic aromatic hydrocarbons such as 3-methylcholanthrene (MC). Only cytochrome P450c (P450IA1) is inducible in rat extrahepatic tissues by MC or isosafrole, whereas in the liver both cytochromes P450c and P450d (P450IA2) are inducible by these compounds. Specific antibodies to cytochromes P450c and P450d were used to study the expression and function of these two related isoenzymes in rat liver and extrahepatic tissues before and after induction with MC. Whereas cytochrome P450d is responsible for all of the high affinity POD activity in hepatic microsomal fractions of both untreated and MC treated rats, this activity is mediated only by P450c in microsomal fractions from extrahepatic tissues following MC treatment. POD activity of microsomal fractions from lung of untreated rats was not mediated by either cytochrome P450c or P450d.

Although the liver is the main site of drug oxidation, many extrahepatic tissues, particularly the kidney, small intestine and lung, contain cytochromes P450 [1, 2], the regulation and expression of which frequently differ from those of the liver [3, 4]. Amongst such isoenzymes are the polycyclic aromatic hydrocarbon (PAH¶)-inducible forms, products of the P450IA genes [5]. These comprise two isoenzymes which share 70% homology of their primary structure. In the rat, these have been termed cytochromes P450c (P450IA1) and P450d (P450IA2) [5, 6]. Not only are these isoenzymes inducible by environmental pollutants, both in laboratory animals and in man [7–9], but they also catalyse the transformation of a number of otherwise innocuous compounds into highly reactive intermediates which can cause toxicity or carcinogenicity.

It has been shown, by immunological methods [2, 3, 8–10], that cytochromes P450c and P450d are differentially expressed in the liver of untreated rats. Cytochrome P450d comprises about 5% of total, spectrophotometrically determined, cytochrome P450 content, whereas cytochrome P450c accounts for only 0.1–1% of total cytochrome P450 content. Such studies have also established that although these two isoenzymes show coincident induction in the liver [9, 10] this is not so in extrahepatic tissues [3, 11], where it appears only cytochrome P450c is inducible. Immunohistochemical studies with a monoclonal antibody (MAb) specific to cytochrome P450c in the rat have confirmed the induction of this isoenzyme in a number of extrahepatic tissues in the rat [12].

The metabolism and activation of a number of xenobiotic chemicals, including PAHs, have been demonstrated in many extrahepatic tissues. Although constitutive activity is often low, induction following treatment with an inducing agent is often more pronounced than in the liver. This, coupled with the relatively low activity of detoxication pathways, has been implicated as a critical factor in determining the susceptibility of such tissues to carcinogenicity.

Although many isoenzymes of cytochrome P450 may be capable of catalysing a particular oxidation reaction at high substrate concentrations, at concentrations likely to be encountered *in vivo* some isoenzymes show a high degree of substrate and regio-selectivity, so that a single isoenzyme can catalyse most of a given metabolic reaction [13]. PAH-inducible forms of cytochrome P450 are amongst those that show such substrate specificity. For example, in the rat, cytochrome P450c is some 50 times more active than cytochrome P450d in the metabolism of benzo[*a*]pyrene [6, 14] and, conversely, cytochrome P450d is much more active that cytochrome P450c at converting promutagenic pyrolysis products and other aromatic amines to mutagenic and carcinogenic intermediates [15–17].

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¶ Abbreviations used: BSA, bovine serum albumin; FPLC, fast protein liquid chromatography; HRP, horseradish peroxidase; IGSS, immunogold silver stain; ISF, isosafrole; MAb, monoclonal antibody; MC, 3-methylcholanthrene; P450, cytochrome P450; PAGE, polyacrylamide gel electrophoresis; PAH, polycyclic aromatic hydrocarbon; PB, phenobarbitone; POD, phenacetin *O*-deethylase; PBS, phosphate buffered saline.

In the present study, the expression of cytochromes P450c and P450d in the rat has been quantified, using specific monoclonal antibodies, in liver and extrahepatic tissues, before and following treatment of the animals with PAH. The O-deethylation of phenacetin has been used as a specific index of the activity of PAH-inducible forms of cytochrome P450 [18, 19]. However, the isoenzyme catalysing this reaction in liver is different from that catalysing O-deethylation in extrahepatic tissues. The contribution of cytochromes P450c and P450d to this reaction in hepatic and extra-hepatic tissues has now been determined using specific inhibitory antibodies.

MATERIALS AND METHODS

Chemicals. Phenacetin and paracetamol were purchased from BDH Chemicals Ltd (Poole, U.K.). Deuterated [$^2H_3$]paracetamol was synthesized as previously described [20]. NADPH, *n*-undecane, phenobarbitone (PB), 3-methylcholanthrene (MC) and prestained molecular weight markers were from Sigma Chemical Co. (Poole, U.K.). Isosafrole (ISF, *cis* and *trans*) was purchased from Eastman-Kodak (Liverpool, U.K.). 3,5-Bistrifluoromethylbenzoyl chloride was supplied by Fluorochem Ltd (Glossop, U.K.). All gel filtration and ion-exchange resins were from Pharmacia (Milton Keynes, U.K.). All reagents for sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting were obtained from either BioRad Laboratories (Watford, U.K.) or National Diagnostics (Aylesbury, U.K.). Nitrocellulose membrane (Hybond-C) was obtained from Amersham International Plc (Amersham, U.K.). Anti-mouse Ig conjugated to horseradish peroxidase (HRP) was obtained from ICN Biomedicals (High Wycombe, U.K.) as were gold-labelled anti-mouse IgG, blotting grade, and all reagents used for immunogold detection (IGSS). All other chemicals were obtained from either Sigma or BDH and were of AnalaR grade or best equivalent.

Treatment of animals and preparation of microsomal fractions. Male Wistar rats (Olac, Bicester, U.K.), weighing between 180–250 g, were maintained as previously described [19]. Groups of six animals were treated with inducing compounds as follows: MC, 80 mg/kg in corn oil by intraperitoneal injection 48 hr before they were killed; ISF, 160 mg/kg in corn oil by intraperitoneal injection daily for 4 days. Control animals received no treatment. Animals were killed by stunning and exsanguination. Livers, lungs, kidneys and small intestine (proximal 30 cm) (gut) were removed and microsomal fractions prepared by differential ultracentrifugation and processed as previously described [19, 21]. Heparin, 3 units/mL of homogenate, was added to the intestinal samples to aid clean sedimentation of the microsomal pellet.

Preparation of antibodies to cytochromes P450. The production and characterization of all of the antibodies used in the present study have been described previously [8, 14, 19, 22, 23]. Monoclonal antibodies were prepared in quantity and purified as detailed elsewhere [19, 24]. Monoclonal antibodies C8 and C9, and the monospecific polyclonal anti-P450d(-c) were prepared and purified as previously described [8, 14].

Immunoquantification. Western blotting was performed essentially as described previously [23]. Proteins and prestained molecular weight standards were subjected to SDS–PAGE (10% polyacrylamide) [25], and then transferred to nitrocellulose membrane. Probe monoclonal antibodies were used as before [19]. The detecting antibody, anti-mouse Ig, conjugated to either horseradish peroxidase or gold, was used at dilutions of 1:2000 and 1:100, respectively, as recommended by the manufacturers, in phosphate buffered saline (PBS: 10 mM sodium–potassium phosphate buffer, pH 7.4 containing 137 mM sodium chloride and 2.6 mM potassium chloride) containing 0.1% (w/v) BSA. The location of peroxidase activity was detected with 4-chloro-1-naphthol and hydrogen peroxide [26] and of gold particles by precipitation of silver using IGSS reagents [27].

Immunoquantification of rat cytochrome P450d was by computerized integration of the peaks obtained by densitometric scanning of Western blots probed with MAb 12/2/3/2. Immunoquantification of rat cytochrome P450c was accomplished using MAb 12/2/3/2 or 3/4/2, after either Western or dot blotting, in the latter case using a Bio-Dot microfiltration apparatus (BioRad Laboratories, Watford, U.K.). Stained bands were quantified by reflectance densitometry using a model GS 300 Transmittance/Reflectance Scanning Densitometer (Biotech Instruments Ltd, Luton, U.K.) and analysed using a Waters model 840 Data and Chromatography control station (Millipore (U.K.) Ltd, Harrow, U.K.). Standard curves were constructed using rat cytochrome P450c (specific content 14 nmol/mg), purified as previously described [23]. On Western blotting, the limit of detection with MAb 3/4/2 was 30 fmol P450c. There was a linear relationship between the amount of P450c to 1 pmol and intensity of staining on the blot. Probit transformation extended the linear relationship to 4 pmol P450c. With this antibody the limit of detection of P450c for microsomal fractions was 0.5 pmol/mg protein. For MAb 12/2/3/2, the limit of detection of the purified isoenzyme was 0.6 pmol of P450c, with a linear relationship between 1 and 14 pmol. With this antibody, the limit of detection with microsomal fractions was 10 pmol P450/mg protein which was improved to 2 pmol/mg by the use of gold-labelled detecting antibody, together with IGSS reagent.

Phenacetin O-deethylase assay. Microsomal POD activity was determined by the method of Murray and Boobis [20] exactly as described previously [19], at a final phenacetin concentration of 4 or 20 μM and a final protein concentration of 0.2–2.0 mg/mL. Immunoinhibition of metabolic activity was assessed as before [19].

Other assays. Protein concentration was determined by the method of Lowry *et al.* [28], using crystalline BSA (fraction V) as standard. Total hepatic cytochrome P450 content was measured by the method of Omura and Sato [29], assuming an extinction coefficient of 91/mM/cm, between 450–480 nm. Total microsomal cytochrome P450 content of extrahepatic tissues was determined by the method of Johannsen and DePierre [30], in which the difference between oxidized CO-bound and reduced

Table 1. Microsomal phenacetin *O*-deethylase activity in rat tissues following treatment with 3-methylcholanthrene

Tissue	[S] μM	Treatment	
		Untreated	MC
		(POD activity pmol/min/mg)	
Liver	4	23.6 ± 14.6	545 ± 164*
Liver	20	59.7 ± 6.40	1600 ± 154*
Lung	20	4.10 ± 0.840	9.26 ± 1.20*
Kidney	20	<0.2	10.9 ± 2.48*
Gut	20	<0.2	15.4 ± 4.57*

MC-treated animals received a single dose of 80 mg/kg of the hydrocarbon by intraperitoneal injection, as described in Materials and Methods. Phenacetin *O*-deethylase activity was determined at substrate concentrations of 4 and 20 μM in liver and at 20 μM in all extrahepatic tissues. Values are means ± SE for three different livers or, for extrahepatic tissues, three different pools each containing organs from three different animals.

* $P < 0.05$ compared with corresponding untreated group by Student's *t*-test using either one or two sample analysis as appropriate.

CO-bound cytochrome P450 was determined, assuming an extinction coefficient of 105/mM/cm between 450–475 nm. Additional details may be found in Ref. 19.

Analysis of data. Results have been expressed as means ± SE, where appropriate. Statistical analysis was performed by Student's *t*-test, for unpaired samples using a commercial software package (StatGraphics 2.1, Statistical Graphics Corporation, Maryland U.S.A.) running on an Olivetti M24 Personal Computer. The null hypothesis was rejected at $P < 0.05$. Correlations were assessed using the Spearman rank correlation coefficient (r_s).

RESULTS

Hepatic and extrahepatic phenacetin O-deethylase activity

POD activity was determined in both hepatic and extrahepatic tissues of untreated rats and rats treated with MC. With liver samples, substrate concentrations of 4 and 20 μM were used, the lower concentration being close to the K_m of the high affinity component of POD activity [19, 31]. However, in extrahepatic tissues, activity could be detected with concentrations only at or above 20 μM (Table 1). This concentration was, therefore, used in all studies of POD activity in extrahepatic tissues. In untreated rats POD activity, with 20 μM substrate, was detectable in microsomal fractions only from liver and lung, with activity in the liver some 14-fold higher than in the lung. POD activity of kidney and proximal small intestine from untreated animals was below the limit of detection of the assay (0.2 pmol/min/mg microsomal protein). Treatment of rats with MC resulted in a significant increase in POD activity of all of the tissues studied (Table 1). In MC-treated rats, specific POD activity in all of the extrahepatic tissues was comparable, but was some 100–170-fold lower than that of the liver (liver >> gut > kidney > lung). However, fold induction of POD activity, following treatment of rats withMC, was greater for kidney and gut than for liver (>60-fold c.f. 27-fold in the liver). In the lung, POD activity was increased by only 2.2-fold following MC treatment of the rats. Thus, the rank order of fold-induction of POD activity following MC-treatment was gut, kidney > liver > lung.

Immunoquantification

Hepatic microsomal fractions from untreated, MC and ISF-treated rats were subjected to Western blotting with MAb 12/2/3/2, which recognizes only cytochromes P450c and P450d (Fig. 1). It was not possible to detect either isoenzyme in the liver of untreated rats, using HRP-conjugated second antibody. However, when a gold-labelled detecting antibody was used together with IGSS, cytochrome P450d could be detected in the liver of untreated animals (data not shown). With MAb 3/4/2, specific to P450c and which increased the sensitivity of the assay by 20-fold, this isoenzyme was still not detectable on Western blots of hepatic microsomes from untreated rats [19, 23].

The microsomal content of cytochromes P450c and P450d was quantified by scanning densitometry of stained Western blots (Table 2). Whereas the hepatic cytochrome P450c content of untreated rats was less than 0.5 pmol/mg microsomal protein, the content of P450d was 9.0 ± 1.0 pmol/mg.

After treatment of rats with either MC or ISF there was a considerable increase in the intensity of both the cytochrome P450c band, at M_r 57,000 and the cytochrome P450d band, at M_r 54,000 in hepatic microsomes (Fig. 1). The results of immunoquantification of the two isoenzymes in liver are summarized in Table 2, in which the data shown are for the same samples as those used to produce the Western blot shown in Fig. 1. In rats treated with MC, P450d content was increased by 15-fold. The content of P450c in these animals was three-fold greater than that of P450d. Following treatment of rats with ISF, induction of P450d was some two-fold greater than that of P450c.

Cytochrome P450c, determined by Western blotting with MAbs 3/4/2 (Fig. 2) and 12/2/3/2 (Fig. 3), was not detectable in extrahepatic tissues (lung, kidney and gut) of untreated rats. Treatment of the animals with either MC or ISF increased the microsomal cytochrome P450c content of all of the extrahepatic tissues studies (Fig. 2). As in the liver, MC was a more potent inducer of this isoenzyme than ISF. The constitutive levels of cytochrome P450d, determined by Western blotting with MAb 12/2/3/2 (Fig. 3), in the lung, kidney and gut were below the limits of detection of the technique. Western blotting of these samples with MAb 12/2/3/2 confirmed the induction of P450c by MC and ISF, but there was no evidence for any increase in the content of cytochrome P450d in any of the extrahepatic tissues studied, following treatment with MC or ISF.

Treatment of rats with MC caused an approximately two-fold increase in total cytochrome P450 content of microsomal fractions from the extrahepatic tissues (Table 2). The cytochrome P450d content of all three extrahepatic tissues, both before and after treatment of the animals with MC or ISF, was less than 2 pmol P450/mg, the detection limit of the IGSS assay (Table 1). Amongst all of the

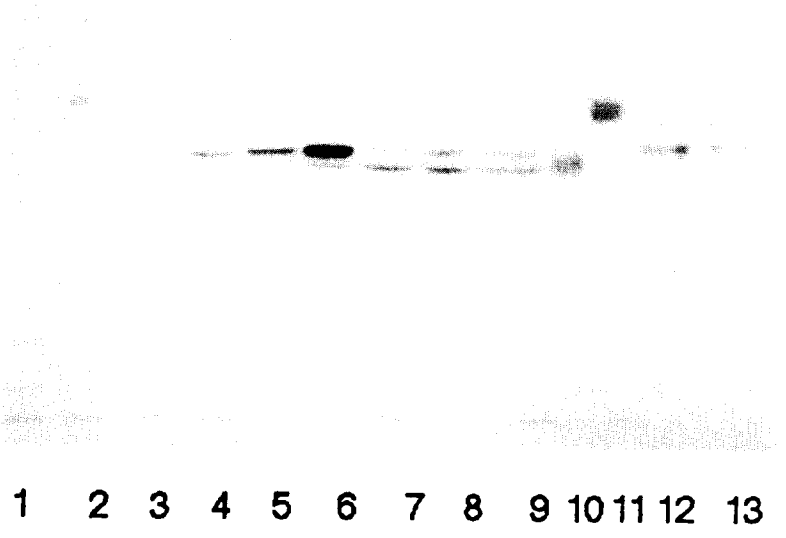

Fig. 1. Western blot with MAb 12/2/3/2 (which recognizes only cytochromes P450c and P450d) of hepatic microsomal fractions from rats. Microsomal proteins (20 μg) from untreated (lanes 1–3), MC-treated (lanes 4–6) and ISF-treated (lanes 7–9) rats, together with prestained molecular weight markers at M_r 48,500 (lane 10) and M_r 58,000 (lane 11) and purified cytochrome P450c, 4 pmol (lane 12) and 2 pmol (lane 13), were separated by SDS polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane and subjected to Western blotting.

Table 2. Quantification of cytochromes P450c and P450d in rat tissues following induction

Tissue	Treatment	Cytochrome P450 (total)	P450c	P450d
		(pmol P450/mg microsomal protein)		
Liver	Untreated	722 ± 5	<0.5	9.0 ± 0.6
	MC	989 ± 4*	356 ± 60*	124 ± 22*
	ISF	1030 ± 4*	106 ± 32*	183 ± 42*
Lung	Untreated	38.0 ± 4.0	<0.5	<2
	MC	98.7 ± 28.4	18.8 ± 1.2*	<2
	ISF	ND	2.0 ± 0.6*	<2
Kidney	Untreated	119 ± 3	<0.5	<2
	MC	217 ± 2*	34.3 ± 2.9*	<2
	ISF	268 ± 2*	8.6 ± 0.4*	<2
	Untreated	45.7 ± 8.3	<0.5	<2
Gut	MC	116 ± 18.0*	107 ± 14.5*	<2
	ISF	ND	9 ± 0.6*	<2

Rats were treated with MC and ISF as described in Materials and Methods. Total cytochrome P450 content was determined by difference spectroscopy by established methods [29, 30]. Immunoquantification of cytochromes P450c and P450d was accomplished by Western or dot blotting with MAbs 3/4/2 or 12/2/3/2 by the methods described in the text. Values are means ± SE for three different pools of tissue, each from three different animals, except for livers which were from individual rats.

* $P < 0.05$ compared with corresponding untreated group by Student's t-test using either one or two sample analysis as appropriate.

ND, not determined.

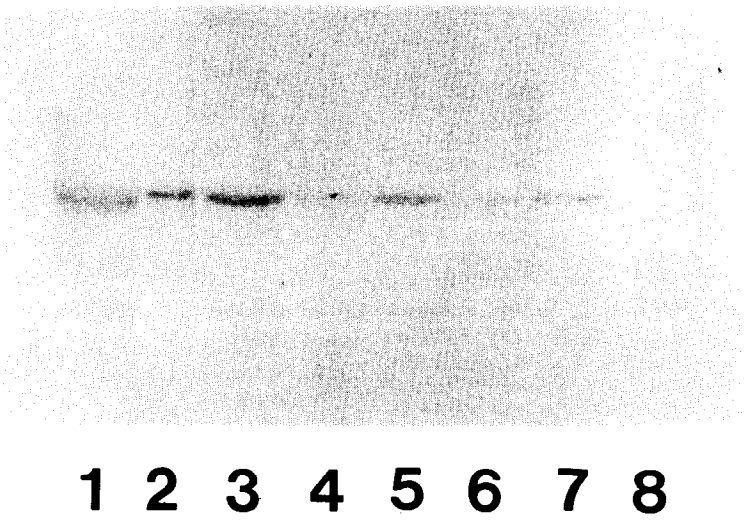

Fig. 2. Western blot with MAb 3/4/2 (specific to cytochrome P450c) of hepatic and extrahepatic tissues of rats. Microsomal proteins (5 μg) from tissues of rats pretreated with MC (lanes 1, 3, 5 and 7) and ISF (lanes 2, 4, 6 and 8) were subjected to Western blotting. Tissues were from liver (lanes 1 and 2), gut (lanes 3 and 4), kidney (lanes 5 and 6) and lung (lanes 7 and 8).

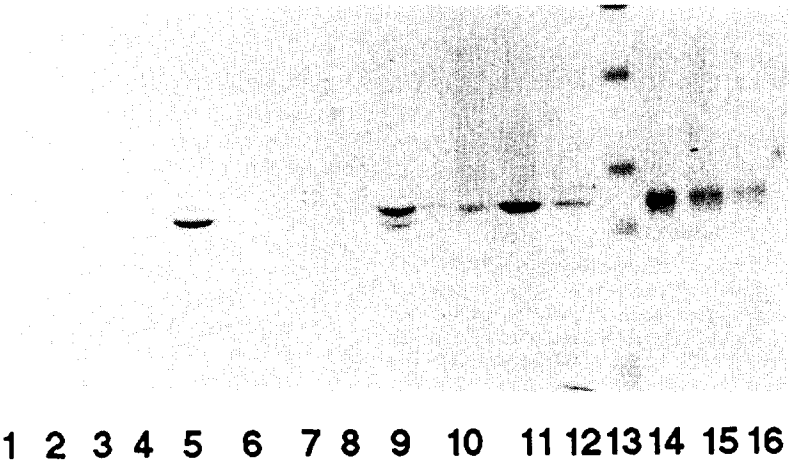

Fig. 3. Western blot with MAb 12/2/3/2 of extrahepatic tissues of rats. Microsomal proteins (20 μg) from tissues of untreated (lanes 1–4), ISF-treated (lanes 5–8) and MC-treated (lanes 9–12) animals, together with mixed prestained molecular weight markers (lane 13) and purified cytochrome P450c, 10 pmol, (lane 14), 8 pmol, (lane 15) and 4 pmol, (lane 16), were subjected to Western blotting. Tissues were from liver (lanes 1, 5 and 9), kidney (lanes 2, 6 and 10), gut (lanes 3, 7 and 11) and lung (lanes 4, 8 and 12)

tissues, cytochrome P450c was most highly induced in the liver. However, whereas this isoenzyme accounted for 40% of total cytochrome P450 in the liver after MC treatment, in the gut of MC treated rats, P450c accounted for more than 90% of total, spectrophotometrically determined, content of cytochrome P450.

Following the treatment of rats with MC there was a highly significant correlation between POD activity and immunologically determined content of cytochrome P450c ($r_s = 0.927$, $P < 0.005$, $N = 9$) amongst all of the extrahepatic tissues. A comparison between POD activity and cytochrome P450d content was not possible, as the content of this isoenzyme in extrahepatic tissues was below the detection limit of the assay in all cases.

Immunoinhibition of hepatic and extrahepatic microsomal POD activity

The contribution of cytochromes P450c and P450d to the O-deethylation of phenacetin in microsomal fractions from liver and extrahepatic tissues was determined by immunoinhibition, using highly specific antibodies. POD activity of hepatic microsomal fractions from both untreated and MC-treated rats was inhibited by 75–80% by both anti-P450d-

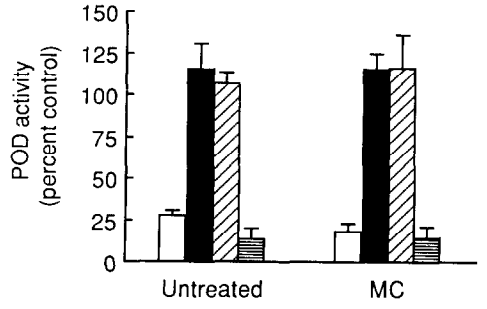

Fig. 4. Effect of antibodies to cytochromes P450c and P450d on hepatic POD activity. Activity was determined with hepatic microsomal fractions of untreated and MC-treated rats using 4 μM phenacetin and antibodies 12/2/3/2 (□); C8 (■); C9 (▨) and anti-P450d(-c) (▤). Results are expressed as per cent of control activity in the absence of antibody and are means + SE of the averaged determinations with three different pools of tissues. The concentrations of the antibodies used was as described in Materials and Methods.

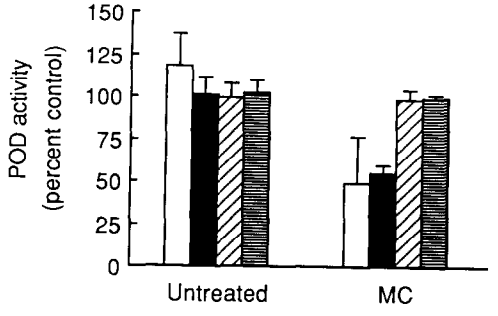

Fig. 5. Effect of antibodies to cytochromes P450c and P450d on lung POD activity. Activity was determined with microsomal fractions from lungs of untreated and MC-treated rats using 20 μM phenacetin and antibodies 12/2/3/2 (□); C8 (■); C9 (▨) and anti-P450d(-c) (▤). The results are means ± SE of the average of duplicate determinations with three different pools, each containing tissues from three animals.

(-c), specific to cytochrome P450d, and MAb 12/2/3/2, which reacts only with cytochromes P450c and P450d (Fig. 4). A specific, inhibitory MAb to P450c, C8, was without effect, activity being comparable to that obtained with MAb C9, which is also specific to P450c, but which is not inhibitory to the purified isoenzyme [14].

With microsomal fractions from lung of untreated rats none of the antibodies affected POD activity (Fig. 5). In lung from MC-treated rat, MAbs C8 and 12/2/3/2, both of which react with cytochrome P450c, inhibited 50% of POD activity. Unlike in liver, POD activity in the lung was unaffected by the antibody against cytochrome P450d (anti-P450d(-c)).

Immunoinhibition studies could not be performed on microsomal fractions from kidney or gut of untreated rats, as activity was below the limits of detection (Table 1). In MC-treated rats, microsomal POD activity of both kidney and gut was inhibited

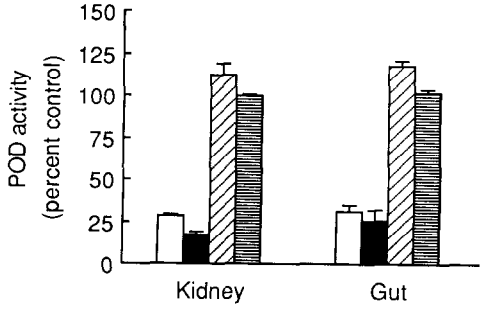

Fig. 6. Effect of antibodies to cytochromes P450c and P450d on kidney and gut POD activity. Activity was determined with microsomal fractions of kidney and gut from MC-treated rats using 20 μM phenacetin and antibodies 12/2/3/2 (□); C8 (■); C9 (▨) and anti-P450d(-c) (▤). The results are means ± SE of the average of duplicate determinations with three different pools each containing tissues from three animals.

by more than 75% by both C8 and 12/2/3/2. Again, as in the lung, the antibody specific to cytochrome P450d was without effect.

DISCUSSION

In the liver of untreated rats, the content of cytochrome P450d exceeds that of P450c, by at least 18-fold, confirming previous reports [3, 8–10]. Although the level of cytochrome P450c is below the limit of detection of the present assay, it has previously been shown by immunocytochemistry [12] and by immunoblotting [14] that this isoenzyme is expressed in the liver of uninduced animals. These data on hepatic P450 expression in untreated animals are in agreement with those on mRNA levels, where the level of the mRNA coding for cytochrome P450d exceeds that coding for cytochrome P450c [32].

In extrahepatic tissues the content of both isoenzymes is below the detection limit of the assay. However, immunocytochemical studies have shown that cytochrome P450c is expressed in such tissues [12]. It is not known whether or not cytochrome P450d is expressed, but in preliminary studies the presence of cytochrome P450d has been detected in rat kidney, albeit at low levels, by immunocytochemistry (unpublished data).

Following treatment of rats with either MC or ISF, both isoenzymes of cytochrome P450 are induced in liver. As reported by others [2, 8–10], treatment with MC results in greater induction of cytochrome P450c than of P450d, whereas treatment with ISF results in higher levels of P450d than of P450c. Over the course of several experiments it was noted that there is large variation in the extent of induction of both cytochromes P450c and P450d amongst individual MC-treated rats. The induction of cytochrome P450c ranged from 8-fold to equal that of cytochrome P450d. There was a greater variation in the absolute amount of cytochrome P450d than of cytochrome P450c, with no correlation between them amongst different MC-treated rats ($r_s = 0.420$, $P > 0.1$, $N = 10$). This supports the recent suggestion that

although the induction of these two isoenzymes in liver is tightly linked, in that inducers of one isoenzyme always induce the other, they are not coordinately regulated [10]. Due to the extremely low expression of cytochrome P450c in untreated animals, the fold-increase of this isoenzyme from its constitutive level is greater than that of cytochrome P450d for both MC and ISF treatment, despite the greater amount of P450d induced by ISF.

Cytochrome P450c is inducible by both MC and ISF in all three of the extrahepatic tissues studied (kidney, lung and small intestine), whereas cytochrome P450d does not appear to be inducible in any of these tissues by either compound, at least within the limits of detection of the assay. The lack of induction of cytochrome P450d in the kidney was confirmed by preliminary immunocytochemical studies. The relative extent of induction of cytochrome P450c in extrahepatic tissues, following treatment of the animals with MC, is in good agreement with previous data obtained using immunocytochemistry [12]. Of the tissues studied here, lung is least inducible for cytochrome P450c. This is consistent with the observation that this isoenzyme is present in only very few of the cell types in lung [12]. These data are also in agreement with the reported accumulation of mature mRNA coding for cytochrome P450c, but not that coding for cytochrome P450d, in kidney, heart and lung of PAH-treated rats. However, both mRNAs were detected in the liver of the same animals [33].

The amount of immunoreactive cytochrome P450 in a tissue may not necessarily equate with the contribution of that isoenzyme to a given metabolic reaction. This is probably best estimated by the use of specific inhibitory antibodies. The catalytic activity of cytochromes P450c and P450d has been investigated in this way. In a previous study [18, 19] we have shown that both isoenzymes, when reconstituted, catalyse POD activity.

In the present study it was found that in hepatic microsomal fractions from control rats only cytochrome P450d catalyses high affinity POD activity. Residual activity can be accounted for entirely by the contribution of the low affinity component of POD activity. Cytochrome P450d represents less than 5% of total, spectrophotometrically determined, content of cytochrome P450. The fact that such low constitutive levels of cytochrome P450d are still responsible for all of high affinity POD activity is a reflection of the specificity of the isoenzyme for this reaction. Lung is the only extrahepatic tissue in untreated rats in which POD activity can be detected. This activity, unlike that of liver, is not mediated by either of the PAH-inducible forms of cytochrome P450. High constitutive levels of the major PB-inducible form of cytochrome P450, P450b/e, in the lung [4] may contribute towards POD activity in this tissue, since it was previously found that treatment of rats with PB induces low affinity POD activity in the liver [19, 34].

Whereas in liver from rats treated with MC there is a very significant correlation between microsomal high affinity POD activity and immunologically determined content of cytochrome P450d ($r_s = 0.888$, $P < 0.01$, $N = 10$ rats), but not of cytochrome P450c ($r_s = 0.333$, $P > 0.1$, $N = 10$), in all extrahepatic tissues taken together POD activity correlates with cytochrome P450c content. It is perhaps salutary to note that POD activity amongst all of the tissues (liver and extrahepatic) correlates with the content of both cytochrome P450c and P450d (P450c: $r_s = 0.896$, $P < 0.0001$; P450d: $r_s = 0.921$, $P < 0.0001$). Thus, correlation between a monooxygenase activity and the content of an isoenzyme of cytochrome P450, or indeed between two monooxygenase activities, cannot be used as the sole evidence for the involvement of a single isoenzyme of cytochrome P450. Similar results have been obtained with 7-ethoxyresorufin [10].

In animals treated with ISF theres is no correlation between hepatic POD activity and content of cytochrome P450d. However, this is because ISF inactivates cytochrome P450d by forming a metabolically inactive complex [6, 19].

After treatment of rats with MC, cytochrome P450d is still the only isoenzyme in liver that catalyses high affinity POD activity, despite the considerable induction of cytochrome P450c that occurs [19]. Indeed, in this tissue the content of cytochrome P450c exceeds that of cytochrome P450d. In both the kidney and small intestine, POD activity is catalysed by the induced levels of P450c that occur in these tissues, in the absence of any apparent increase in the content of P450d. In the lung, MC treatment of rats causes a two-fold increase in POD activity, which increase is entirely due to the induction of P450c. Thus, substrate specificity in one tissue, even after induction, cannot always be extrapolated to that in another tissue.

The hydrocarbon-inducible isoenzymes of cytochrome P450, P450c and P450d in the rat, have been implicated in the conversion of a number of drugs and other chemicals to toxic or carcinogenic intermediates. The tissue-specific toxicity of such compounds may be due, in part, to their selective activation at their site of toxicity. Thus, the differential expression or induction of one of these isoenzymes in a given tissue, such as demonstrated in the present study, could help explain the susceptibility of that tissue to such chemicals.

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