Biochemical Pharmacology, Vol. 40, No. 6, pp. 1255–1261, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + .00

MONOCLONAL ANTIBODY-DIRECTED CHARACTERIZATION OF BENZENE, ETHOXYRESORUFIN AND PENTOXYRESORUFIN METABOLISM IN RAT LIVER MICROSOMES

TAMIE NAKAJIMA,*† EIVOR ELOVAARA,‡ SANG S. PARK,§ HARRY V. GELBOIN,§ EINO HIETANEN‖ and HARRI VAINIO‖

*Department of Hygiene, Shinshu University School of Medicine, Matsumoto, 390, Japan; ‡Department of Industrial Hygiene and Toxicology, Institute of Occupational Health, Topeliuksenkatu 41 a A, SF-00250 Helsinki, Finland; §Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD 20892, U.S.A.; and ‖International Agency for Research on Cancer, 150 Cours Albert Thomas, F-69372 Lyon, France

(*Received* 1 *February* 1990; *accepted* 23 *April* 1990)

Abstract—The contribution of cytochrome P450IA, P450IIB, P450IICII and P450IIEI to the oxidative metabolism of benzene, 7-ethoxyresorufin and 7-pentoxyresorufin was investigated using monoclonal antibodies (MAb) in liver microsomes from fed, one-day fasted, phenobarbital (PB)-, 3-methylcholanthrene (MC)- and ethanol-treated rats. Overall catalytic activity varied with different pretreatments and thereby the contribution of different P450s. MAb 1-91-3 against P450IIE1 did not influence alkoxyresorufin dealkylation but inhibited benzene aromatic hydroxylase (BAH) in relation to its increasing inducibility as follows: MC, PB (≤48%) < fed (≤59%) < fasted (≤70%) < ethanol (≤91%). MAbs 2-66-3, 4-7-1 and 4-29-5, all against P450IIB, had no effect on 7-ethoxyresorufin *O*-deethylase (EROD) but inhibited the activities of high-K_m BAH (≥58%) and 7-pentoxyresorufin *O*-depentylase (PROD) (≥96%) in PB-treated microsomes. MAb 1-7-1 against P450IA inhibited EROD (79%), PROD (50%) and high-K_m BAH (42%) activities in MC-microsomes. MAb 1-68-11 against P450IIC11 inhibited EROD, PROD and high-K_m BAH activities. Thus, P450IIE1 contributed to benzene metabolism as a low-K_m BAH but not to alkoxyresorufin metabolism. P450IIB was responsible besides for the major part of 7-pentoxyresorufin metabolism also, selectively, for benzene hydroxylation at high benzene concentrations. P450IA contributed primarily to 7-ethoxyresorufin metabolism and only slightly to PROD and high-K_m BAH activities. P450IIC11 contributed slightly to high-K_m BAH and to alkoxyresorufin metabolism.

Cytochrome P450 is a key enzyme in the biotransformation of xenobiotics. The multiplicity of forms makes it difficult to define the responsibility of individual isozymes in the metabolism of individual xenobiotics. Recently, monoclonal antibodies (MAbs¶) against different forms of cytochrome P450 have been developed [1–4] and shown to make possible accurate and quantitative identification and classification of the P450 families and forms [5, 6]. Previously, we used three MAbs (clones 1-7-1, 2-66-3 and 1-91-3 against cytochromes P450IA, P450IIB and P450IIE1 [7], respectively) to establish that P450IIE1 and P450IIB contribute to benzene metabolism at both high and low affinity sites for benzene in rat liver microsomes, respectively: P450IIE1 was induced by fasting, pyrazole and ethanol treatments, but decreased by treatment with phenobarbital (PB) [8]. Different affinities of P450IIE1 and P450IIB towards benzene have also been reported by Johansson and Ingelman-Sundberg [9], and Koop *et al.* [10].

The O-dealkylation of 7-ethoxyresorufin [11–14] and 7-pentoxyresorufin [13–15] are known to be catalysed by different forms of cytochromes P450; P450IA is selectively inducible by 3-methylcholanthrene (MC) and P450IIB by PB. However, there is little information on whether other P450 isozymes, e.g. those expressed constitutively (P450IIE1 or P450IIC11 [16]), also contribute to their metabolism. These two isozymes differ in inducibilities, and substrate specificities [17].

In the present study, we examined the contributions of P450IA (MC-inducible), P450IIB (PB-inducible), P450IIE1 (constitutive and ethanol-inducible), and P450IIC11 (constitutive form) to the metabolism of benzene, 7-ethoxyresorufin and 7-pentoxyresorufin in rat liver microsomes using MAb-directed inhibition for reaction phenotyping, i.e. measuring the contribution of the specific cytochrome P450s.

MATERIALS AND METHODS

Animals. Male Wistar rats (6 weeks of age) were obtained from Nippon SLC Inc. (Shizuoka, Japan), housed in an air-conditioned room (20 ± 2°) with a 12-hr light–dark cycle and maintained on pellet feed (Nippon Clea, CE-2, Tokyo, Japan) and water *ad libitum* until they reached the age of 8 weeks. They were then separated into five groups: control, fasted,

† To whom correspondence should be addressed.

¶ Abbreviations: MAb, monoclonal antibody; BAH, benzene aromatic hydroxylase; EROD, 7-ethoxyresorufin *O*-deethylase; PROD, 7-pentoxyresorufin *O*-depentylase; PB, phenobarbital; MC, 3-methylcholanthrene.

Table 1. Protein and cytochrome P450 contents in liver microsomes of variously treated rats

Treatment	Body weight (g)	Liver weight (g)	Microsomal protein (mg/g liver)	Cytochrome P450 (nmol/mg protein)
Fed	271 ± 9	12.1 ± 0.6	22.5 ± 3.5	0.61 ± 0.08
Fasted	253 ± 6	8.3 ± 0.3*	23.1 ± 2.6	0.72 ± 0.05
PB	257 ± 12	14.7 ± 0.9*	31.8 ± 3.6*	1.24 ± 0.10*
MC	252 ± 8	14.2 ± 1.1*	23.4 ± 3.1	1.17 ± 0.05*
Ethanol	220 ± 12*	8.9 ± 0.5*	34.3 ± 1.3*	0.96 ± 0.06*

Each value represents the mean ± SD of five rats.
* Significantly different ($P \leq 0.05$) from the fed group.

Table 2. Effects of different treatments on oxidation of benzene, 7-ethoxyresorufin and 7-pentoxyresorufin in rats

Treatment	BAH		EROD	PROD
	0.23 mM*	6.26 mM*		
Fed	0.33 ± 0.08	0.38 ± 0.06	0.092 ± 0.022	0.047 ± 0.010
Fasted	0.87 ± 0.17‡	0.79 ± 0.14‡	0.138 ± 0.021‡	0.059 ± 0.006
PB	0.42 ± 0.11	1.34 ± 0.19‡†	0.340 ± 0.068‡	3.530 ± 0.650‡
MC	0.28 ± 0.08	0.66 ± 0.06‡†	20.400 ± 6.030‡	0.313 ± 0.042‡
Ethanol	2.26 ± 0.19‡	1.97 ± 0.32‡	0.212 ± 0.031‡	0.063 ± 0.014

Each value represents the mean ± SD of five rats; BAH, benzene aromatic hydroxylase; EROD, 7-ethoxyresorufin *O*-deethylase; PROD, 7-pentoxyresorufin *O*-dependentylase.
All activities are expressed as nmol/mg microsomal protein/min. BAH activity at 6.26 mM was not corrected for the concentration at the lower concentration.
* Benzene concentration used in enzyme assays.
† Significant by different between the low-K_m (0.23 mM) and high-K_m (6.26 mM) BAH.
‡ Significant by different ($P \leq 0.05$) from the fed rat group.

PB, MC and ethanol groups. The first four groups received a nutritionally adequate liquid diet (basal diet) prepared according to the method of DeCarli and Lieber [18] with a slight modification [19]. The fasted group was deprived of food the day before decapitation; rats in the PB and MC groups were treated with PB (80 mg/kg per day for 4 days) or MC (20 mg/kg per day for 4 days) intraperitoneally. Rats in the ethanol group received an ethanol-containing diet prepared by adding 2.5 g of ethanol per 100 mL of basal diet in which the sucrose content had been reduced to 5.0 g/100 mL and the oil content increased to 5.0 g/100 mL, so that the ethanol diet was isocaloric with the basal diet. These liquid diets were given once a day, 80 mL/rat at 16:00 hr for 3 weeks as the only source of food and water.

Isolation of liver microsomes. Livers were homogenized in three vol. of 1.15% (w/v) KCl. Microsomes were isolated by differential centrifugation at 105,000 *g* for 60 min, and pellets were washed once in the salt solution and subsequently suspended in distilled water. After gassing with N_2, microsomes were stored at −85° at a concentration of 20 mg protein/mL.

Enzyme and protein assays. Benzene metabolism was determined by measuring the formation of phenol and hydroquinone using 200 μg of microsomal protein/0.6 mL of reaction mixture containing a final concentration of 0.83 mM NADP, 16.7 mM glucose-6-phosphate, 41.7 mM magnesium chloride, 2 I.U. glucose-6-phosphate dehydrogenase, 50 mM potassium-phosphate buffer (pH 7.4) and 0.23 or 6.26 mM benzene. After extraction of phenol and hydroquinone by ether, the concentrations were measured by a Shimazu high-performance liquid chromatograph equipped with a UV detector (SPD-6A) and a data processor (Chromatopac C-R3A) according to the method of Nakajima *et al.* [8]. 7-Ethoxyresorufin *O*-deethylase (EROD) and 7-pentoxyresorufin *O*-dependentylase (PROD) activities were measured kinetically at 1 μM final concentration by the methods of Prough *et al.* [20] and Burke *et al.* [13], respectively. The protein content was measured by the method of Lowry *et al.* [21] and the concentration of cytochrome P450 by the spectrophotometric method of Omura and Sato [22].

Monoclonal antibody inhibition. MAbs were produced by the hybridoma technique at the U.S. National Cancer Institute Laboratory of Molecular Carcinogenesis (Bethesda, MD, U.S.A.) using a modification of the method of Koehler and Milstein [23], tested and characterized as described previously [1–4]. Six MAbs that have been shown to be specific towards different cytochromes P450s were used as follows: clone 1-7-1 [1] to MC-inducible P450c, which crossed P450d, clones 2-66-3, 4-7-1 and 4-29-5 [2] to PB-inducible P450b, which crossed P450e, clone 1-91-3 [3] to ethanol-inducible P450IIE1 (P450j), and clone 1-68-11 [4] to one of the constitutive cytochrome P450 isozymes (P450IIC11, P450h) in rats.

Table 3. Inhibition of benzene aromatic hydroxylase (BAH) activity by six monoclonal antibodies (MAbs) in rat liver microsomes

Treatment	Hy-Hel	MAb (Nomenclature of P450)† 1-7-1 (P450IA)	2-66-3 (P450IIB)	4-7-1 (P450IIB)	4-29-5 (P450IIB)	1-68-11 (P450IIC11)	1-91-3 (P450IIE1)
0.23 mM*							
Fed	0.39 (100)	0.42 (108)	0.39 (100)	0.39 (100)	0.34 (87)	0.47 (121)	0.16 (41)
Fasted	0.61 (100)	0.61 (100)	0.78 (128)	0.59 (87)	0.71 (116)	0.54 (89)	0.18 (30)
PB	0.27 (100)	0.29 (107)	0.27 (100)	0.28 (104)	0.23 (85)	0.36 (133)	0.14 (52)
MC	0.25 (100)	0.25 (100)	0.26 (104)	0.28 (112)	0.24 (96)	0.22 (88)	0.14 (56)
Ethanol	2.23 (100)	2.47 (111)	2.58 (116)	2.69 (121)	3.09 (139)	2.21 (99)	0.20 (9)
6.26 mM*							
Fed	0.37 (100)	0.38 (103)	0.36 (97)	0.33 (89)	0.39 (105)	0.22 (59)	0.22 (59)
Fasted	0.72 (100)	0.75 (104)	0.94 (131)	0.62 (86)	0.78 (108)	0.52 (72)	0.30 (42)
PB	1.80 (100)	2.13 (118)	0.75 (42)	0.52 (29)	0.54 (30)	1.62 (90)	2.02 (112)
MC	0.76 (100)	0.44 (58)	0.74 (97)	0.86 (113)	0.90 (118)	0.63 (83)	0.56 (74)
Ethanol	1.90 (100)	2.32 (122)	2.22 (117)	1.82 (103)	1.83 (103)	1.75 (92)	0.38 (20)

* Benzene concentration used in enzyme assays.

Figures represent remaining BAH activity (nmol/mg protein/min) and, in parentheses, the % remaining activity expressed as (activity with MAb/activity with Hy-Hel) × 100. BAH activity at 6.26 mM was not corrected for the contribution at the lower concentration.

The coefficient of variation (%) in the immunoinhibition was ≤15.2%.

† See Ref. 7.

Table 4. Inhibition of 7-ethoxyresorufin *O*-deethylase (EROD) activity by six monoclonal antibodies (MAbs) in rat liver microsomes

Treatment	Hy-Hel	MAb (Nomenclature of P450)* 1-7-1 (P450IA)	2-66-3 (P450IIB)	4-7-1 (P450IIB)	4-29-5 (P450IIB)	1-68-11 (P450IIC11)	1-91-3 (P450IIE1)
Fed	0.087 (100)	0.085 (98)	0.095 (109)	0.109 (125)	0.087 (100)	0.023 (26)	0.087 (100)
Fasted	0.118 (100)	0.102 (86)	0.118 (100)	0.134 (114)	0.128 (108)	0.044 (37)	0.118 (100)
PB	0.350 (100)	0.496 (142)	0.331 (95)	0.350 (100)	0.340 (97)	0.056 (16)	0.343 (98)
MC	22.600 (100)	4.740 (21)	23.300 (103)	24.400 (108)	23.700 (105)	19.400 (86)	23.300 (103)
Ethanol	0.236 (100)	0.227 (96)	0.257 (109)	0.227 (96)	0.257 (109)	0.092 (39)	0.281 (119)

Figures represent remaining EROD activity (nmol/mg protein/min) and, in parentheses, the % remaining activity expressed as (activity with MAb/activity with Hy-Hel) × 100.

The coefficient of variation in the immunoinhibition was ≤14.0%.

* See Ref. 7.

As a reference protein, a control MAb (Hy-Hel) against chicken lysozyme was used to determine any non-specific reaction. MAbs and reference protein were added to pooled microsomes and buffer at room temperature 30 min before starting the enzyme assay by adding NADPH-generating system and substrate (benzene, 7-ethoxyresorufin or 7-pentoxyresorufin), as described [8]. In preliminary experiments, maximal inhibition of each MAb was obtained below a MAb protein/microsomal protein ratio of 0.5 for assays of EROD and PROD, and 1.0 for assays of benzene metabolism; these relative concentrations were used in the assays.

Statistics. Means were tested by Student's *t*-test. The 0.05 level of probability was the criterion of significance.

RESULTS

Microsomal protein and cytochrome P450

One-day fasting did not influence the microsomal protein or total cytochrome P450 content, whereas PB and ethanol increased both. MC treatment increased the cytochrome P450 content but not the microsomal protein content (Table 1).

Benzene, 7-ethoxyresorufin and 7-pentoxyresorufin metabolism.

The effects of the different treatments on the metabolism of benzene, 7-ethoxyresorufin and 7-pentoxyresorufin are shown in Table 2. Two benzene concentrations (0.23 mM and 6.26 mM) were used to examine the differences between catalytic activities of low-K_m and high-K_m benzene aromatic

hydroxylase (BAH) [8]. One-day fasting increased both forms of BAH activity 2- to 3-fold. Chronic ethanol consumption enhanced benzene oxidation 7-fold, which was greater than with any other treatment. PB and MC treatments increased oxidation only at the high benzene concentration: the increase was smaller with MC than with PB.

All treatments enhanced EROD activity. One-day fasting, ethanol and PB treatments elevated the activity by 1.5-, 2.3- and 3.7-fold when compared with fed control rats, respectively. MC induced this activity 222-fold. On the other hand, neither one-day fasting nor ethanol treatment influenced PROD activity, which was enhanced with MC 7-fold and with PB 75-fold.

Inhibition of BAH activity by MAbs

The high-K_m BAH activity was selectively inhibited by MAb 1-7-1 in microsomes from MC-treated rats, and by clones 2-66-3, 4-7-1 and 4-29-5 in microsomes from PB-treated rats. The percentages of inhibition of high-K_m BAH by the latter three MAbs were greater than that by 1-7-1.

MAb 1-68-11 inhibited only the high-K_m BAH activity in fed, one-day fasted and MC-treated microsomes. The inhibition was greater in the control microsomes than in any other preparation.

MAb 1-91-3 inhibited both BAH activities in all microsomes, except for high-K_m BAH measured in microsomes from PB-treated rats. The inhibition, both relative (%) and actual (nmol/mg/min), was greater in the ethanol-pretreated group, followed by the one-day fasted group. The percentage inhibition was lower in microsomes from MC- and PB-treated rats than in those from control rats. Following maximal inhibition of low-K_m BAH by MAb 1-91-3, the remaining activities of MAb-insensitive BAH were compatible to each others in all microsome preparations (Table 3).

Inhibition of EROD activity by MAbs

MAbs 1-91-3, 2-66-3, 4-7-1 and 4-29-5 did not inhibit EROD activity in any of the rat microsomes (Table 4), although one-day fasting, ethanol and PB treatments slightly increased the activity (Table 2). MAb 1-7-1 inhibited EROD activity only in microsomes from MC-treated rats. Clone 1-68-11 inhibited EROD activity in all microsomes except in MC-induced microsomes.

Inhibition of PROD activity by MAbs

MAb 1-91-3 did not inhibit the PROD activity in any of the microsome preparations, whereas clone 1-7-1 and clones 2-66-3, 4-7-1 and 4-29-5 inhibited this activity in MC- and PB-induced microsomes, respectively (Table 5). However, the degree of inhibition differed widely: the 1-7-1 MAb inhibited by 50%, and the 2-66-3, 4-7-1 and 4-29-5 MAbs by 96–99%.

MAb 1-68-11 completely inhibited PROD activity in fed, fasted and ethanol-treated microsomes, whereas the inhibition in MC-treated microsomes was relatively very small and in PB-microsomes non-existent.

DISCUSSION

The contribution of epitope-specific cytochrome P450 isozymes to the total P450-dependent metabolism of benzene, 7-ethoxyresorufin and 7-pentoxyresorufin in rat liver is summarized in Table 6. The effects of different MAbs on BAH (low-K_m and high-K_m forms), EROD and PROD can be clearly differentiated. On the basis of the relative inhibitory power and differences in total catalytic activity (i.e. the magnitude of induction), it is possible to compare the actual inhibition levels due to MAb-sensitive P450s in different microsomal preparations. It is clear that the pattern of isozymes induced and/or repressed varied with treatment, and more than one isozyme contributed to BAH, EROD and PROD activities.

Benzene metabolism (measured as the formation of phenol and hydroquinone) was separated into low-affinity and high-affinity forms in terms of cytochromes P450. At least four isoenzymes were shown to be responsible for benzene metabolism; cytochrome P450IA (high-K_m), P450IIB (high-K_m), P450IIC11 (high-K_m) and P450IIE1 (low-K_m). MAb against P450IIE1 inhibited BAH activity in the following order (when expressed as percentage of inhibition); ethanol > fasted > fed > PB = MC. The actual inhibition (expressed as nmol/mg/min) followed the same order of MAb-sensitive activities of low-K_m BAH/high-K_m BAH: ethanol, 2.03/1.42; fasted, 0.43/0.42; fed, 0.23/0.15; PB, 0.13/0; MC, 0.11/0.20. The remaining, MAb-insensitive activity levels of low-K_m BAH were similar for various microsomal preparations (Table 3, last column). The data suggest that (i) P450IIE1, one of the constitutive cytochrome P450s, contributes to benzene metabolism, and (ii) P450IIE1 is slightly inhibited at high concentrations of benzene. These results are consistent with those of previous reports by us [8, 24], and by others [9, 10, 25].

MAbs against P450IA, P450IIB and P450IIC11 inhibited high-K_m BAH activity selectively in microsomes from MC-treated, PB-treated, and fed, fasted and MC-treated rats, respectively. These results suggest that P450IIC11, which is expressed constitutively [16, 17, 26], belongs to the same category as MC- and PB-inducible P450IA and P450IIB with regard to their affinities toward benzene. P450IIC11 seems to display high xenobiotic metabolizing activity, comparable to that of P450IIB (26).

The MAb-sensitive high-K_m BAH activities deriving from P450IIC11 were as follows (nmol/mg/min): fed, 0.15; fasted, 0.20; PB, 0.18; MC, 0.13; ethanol, 0.15, indicating that (i) P450IIC11 contributes to benzene metabolism as a high-K_m BAH and, unlike P450IIE1, is not induced by one-day fasting or ethanol treatment, and (ii) the catalytic turnover is smaller than that of P450IIE1 for the low-K_m BAH.

In spite of the finding that cytochrome P450IIC11 contributed only to the high-K_m BAH activity, there was no difference between the microsomal BAH activities measured at low and high benzene concentrations in fed, fasted and ethanol-treated rats. A plausible explanation is offered by the fact that

Table 5. Inhibition of 7-pentoxyresorufin *O*-depentylase (PROD) activity by six monoclonal antibodies (MAbs) in rat liver microsomes

		MAb (Nomenclature of P450)†					
Treatment	Hy-Hel	1-7-1 (P450IA)	2-66-3 (P450IIB)	4-7-1 (P450IIB)	4-29-5 (P450IIB)	1-68-11 (P450IIC11)	1-91-3 (P450IIE1)
Fed	0.051 (100)	0.051 (100)	0.053 (104)	0.050 (98)	0.045 (88)	ND (0)	0.045 (88)
Fasted	0.043 (100)	0.037 (86)	0.043 (100)	0.047 (109)	0.050 (116)	ND (0)	0.050 (116)
PB	3.760 (100)	4.630 (123)	0.150 (4)	1.670 (44) 0.046 (1.2)*	1.500 (40) 0.038 (1.0)*	3.720 (99)	4.300 (114)
MC	0.335 (100)	0.168 (50)	0.335 (100)	0.302 (90)	0.335 (100)	0.288 (86)	0.335 (100)
Ethanol	0.071 (100)	0.071 (100)	0.069 (97)	0.062 (87)	0.063 (89)	ND (0)	0.075 (106)

Figures represent remaining activity (nmol/mg protein/min) and, in parentheses, the % remaining activity expressed as (activity with MAb/activity with Hy-Hel) × 100.

* At MAb-protein/microsomal protein ratio 1 (200 μg/200 μg).

ND, not detectable.

The coefficient of variation in the immunoinhibition was ⩽ 14.3%.

† See Ref. 7.

Table 6. Summary of cytochromes P450 reaction phenotyping of benzene, 7-ethoxyresorufin and 7-pentoxyresorufin metabolism by monoclonal antibody (MAb) inhibition

		MAbs‡ (Nomenclature of P450)§					
Treatment	Magnitude of induction (-fold)*	1-7-1 (P450IA)	2-66-3 (P450IIB)	4-7-1 (P450IIB)	4-29-5 (P450IIB)	1-68-11 (P450IIC11)	1-91-3 (P450IIE1)
Inhibition of low-K_m/high-K_m BAH							
Fed	1.0/1.0	0/0	0/0	0/0	0/0	0/+	++/+
Fasted	2.6/2.1	0/0	0/0	0/0	0/0	0/+	++/++
PB	1.3/3.5	0/0	0/++	0/++	0/++	0/0	+/0
MC	0.9/1.7	0/+	0/0	0/0	0/0	0/+	+/+
Ethanol	6.9/5.2	0/0	0/0	0/0	0/0	0/0	+++/+++
Inhibition of EROD							
Fed	1.0	0	0	0	0	++	0
Fasted	1.5	0	0	0	0	++	0
PB	3.7	0	0	0	0	+++	0
MC	222.0	+++	0	0	0	0	0
Ethanol	2.3	0	0	0	0	++	0
Inhibition of PROD							
Fed	1.0	0	0	0	0	+++	0
Fasted	1.3	0	0	0	0	+++	0
PB	75.0	0	+++	++(+++)†	++(+++)†	0	0
MC	6.7	++	0	0	0	0	0
Ethanol	1.3	0	0	0	0	+++	0

BAH, benzene aromatic hydroxylase; EROD, 7-ethoxyresorufin *O*-deethylase; PROD, 7-pentoxyresorufin *O*-depentylase.

* Compared to fed controls calculated from Table 2.

† 200 μg MAb/200 μg microsomal protein.

‡ Relative inhibitory effect of MAb: 0, ⩽ 15%; +, 16–49%; ++, 50–74%; +++, ⩾ 75%.

§ See Ref. 7.

the activity of P450IIE1, the major catalyst of low-K_m BAH, was slightly inhibited at a high benzene concentration (Table 2).

No difference in the inhibition of high-K_m BAH activity was found among the three MAbs against P450IIB1. MAbs 2-66-3 and 4-7-1 are known to be cross-reactive with P450IIB2 and/or P450IIA1, but MAb 4-29-5 cross-reacts only with P450IIB2 (26). We deduce that these three MAbs inhibit BAH activity in PB-induced microsomes by interaction with cytochromes P450IIB1 and P450IIB2, but not with P450IIA1.

Our MAb studies are in agreement with the concept that 7-ethoxyresorufin and 7-pentoxyresorufin metabolism is catalysed by MC-inducible and PB-inducible cytochromes P450, respectively. MAb

against P450IIC11 strongly inhibited the metabolism of both substrates in normally fed control rats, indicating that the metabolism of 7-ethoxyresorufin and 7-pentoxyresorufin in control microsomes is catalysed mainly by P450IIC11. The fact that MAb against P450IIC11 strongly inhibited EROD activity in PB-induced microsomes is inconsistent with the report that PB reduces this cytochrome [27]. MAb 1-68-11 cross-reacts with P450IIC6 [26], suggesting that the effects of this MAb on EROD activity are derived not only from P450IIC11 but also from P450IIC6, which exhibits affinity to 7-ethoxyresorufin [12] and is induced 2- to 3-fold by phenobarbital [27].

P450IIE1 did not contribute to 7-ethoxyresorufin or 7-pentoxyresorufin metabolism, although one-day fasting and ethanol-treatment enhanced the metabolism 1.5- to 2.3-fold. Isozymes other than P450IIE1 or P450IIB, which catalyse the metabolism of ethoxyresorufin but are not identical immunochemically, may be induced by one-day fasting, ethanol and PB-treatment. This hypothesis is supported, in part, by the fact that pyrazole, which is a potent inducer of P450IIE1 in rats [8, 28, 29], did not influence ethoxyresorufin metabolism (data not shown). Recently, Khani *et al.* [30] reported that the rabbit alcohol-inducible P450IIE subfamily is coded by the genes for cytochromes P450IIE1 and P450IIE2. However, as the latter isozyme has not been found in rat or man [30, 31], it is unlikely that the ethoxyresorufin-metabolizing P450 induced by ethanol in the present study is identical with P450IIE2.

Ethanol has been reported to induce P450IIB [32]; however, we found neither induction of PROD by ethanol treatment nor inhibition by MAbs against P450IIB. This suggests that chronic ethanol treatment does not induce P450IIB. In other experiments (not reported here), a dose of ethanol twice as high as that used here enhanced PROD activity 2-fold. P450IIB may be induced by ethanol provided that the dose is large.

REFERENCES

1. Park SS, Fujino T, West D, Guengerich FP and Gelboin HV, Monoclonal antibodies that inhibit enzyme activity of 3-methylcholanthrene-induced cytochrome P-450. *Cancer Res* **42**: 1798–1808, 1982.
2. Park SS, Fujino T, Miller H, Guengerich FP and Gelboin HV, Monoclonal antibodies to phenobarbital-induced rat liver cytochrome P-450. *Biochem Pharmacol* **33**: 2071–2081, 1984.
3. Ko IY, Park SS, Song BJ, Patten C, Tan Y, Hah YC, Yang CS and Gelboin HV, Monoclonal antibodies to ethanol-induced rat liver cytochrome P-450 that metabolizes aniline and nitrosamines. *Cancer Res* **47**: 3101–3109, 1987.
4. Park SS, Waxman DJ, Lapenson DP, Schenkman JB and Gelboin HV, Monoclonal antibodies to rat liver cytochrome P-450 2C/RLM5 that regiospecifically inhibit steroid metabolism. *Biochem Pharmacol* **38**: 3067–3074, 1989.
5. Fujino T, Park SS, West D and Gelboin HV, Phenotyping of cytochromes P-450 in human tissues with monoclonal antibodies. *Proc Natl Acad Sci USA* **79**: 3682–3686, 1982.
6. Gelboin HV and Friedman FK, Monoclonal antibodies for studies on xenobiotic and endobiotic metabolism. *Biochem Pharmacol* **34**: 2225–2234, 1985.
7. 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: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* **8**: 1–13, 1989.
8. Nakajima T, Elovaara E, Park SS, Gelboin HV, Hietanen E and Vainio H, Immunochemical characterization of cytochrome P-450 isozymes responsible for benzene oxidation in the rat liver. *Carcinogenesis* **10**: 1713–1717, 1989.
9. Johansson I and Ingelman-Sundberg M, Benzene metabolism by ethanol-, acetone-, and benzene-inducible cytochrome P-450 (IIE1) in rat and rabbit liver microsomes. *Cancer Res* **48**: 5387–5390, 1988.
10. Koop DR, Laethem CL and Schnier GG, Identification of ethanol-inducible P450 3a(P450II E1) as a benzene and phenol hydroxylase. *Toxicol Appl Pharmacol* **98**: 278–288, 1989.
11. Burke MD, Prough RA and Mayer RT, Characteristics of a microsomal cytochrome P-448-mediated reaction. Ethoxyresorufin *O*-de-ethylation. *Drug Metab Dispos* **5**: 1–8, 1977.
12. Åström A and DePierre JW, Metabolism of 2-acetylaminofluorene by eight different forms of cytochrome P-450 isolated from rat liver. *Carcinogenesis* **6**: 113–120, 1985.
13. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T and Mayer RT, Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem Pharmacol* **34**: 3337–3345, 1985.
14. Dutton DR and Parkinson A, Reduction of 7-alkoxyresorufins by NADPH-cytochrome P450 reductase and its differential effects on their O-dealkylation by rat liver microsomal cytochrome P450. *Arch Biochem Biophys* **268**: 617–629, 1989.
15. Lubet RA, Mayer RT, Cameron JW, Nims RW, Burke MD, Wolff T and Guengerich FP, Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch Biochem Biophys* **238**: 43–48, 1985.
16. Waxman DJ, Rat hepatic cytochrome P-450 isoenzyme 2c. *J Biol Chem* **259**: 15481–15490, 1984.
17. Ryan DE, Iida S, Wood AW, Thomas PE, Lieber CS and Levin W, Characterization of three highly purified cytochrome P-450 from hepatic microsomes of adult male rats. *J Biol Chem* **259**: 1239–1250, 1984.
18. DeCarli LM and Lieber CS, Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate liquid diet. *J Nutr* **91**: 331–336, 1967.
19. Nakajima T, Okino T, Okuyama S, Kaneko T, Yonekura I and Sato A, Ethanol-induced enhancement of trichloroethylene metabolism and hepatotoxicity: different from the effect of phenobarbital. *Toxicol Appl Pharmacol* **94**: 227–237, 1988.
20. Prough RA, Burke MD and Mayer RT, Direct fluorometric methods for measuring mixed-function oxidase activity. In: *Methods in Enzymology (L11 Part C)* (Eds. Fleischer S and Packer L), pp. 372–377. Academic Press, New York, 1978.
21. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
22. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. 1. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
23. Koehler G and Milstein C, Continuous cultures of fused

cells secreting antibody of predefined specificity. *Nature* **256**: 495–497, 1975.

24. Nakajima T, Okino T and Sato A, Kinetic studies on benzene metabolism in rat liver—possible presence of three forms of benzene metabolizing enzymes in the liver. *Biochem Pharmacol* **36**: 2799–2804, 1987.
25. Thomas PE, Bandiera S, Maines SL, Ryan DE and Levin W, Regulation of cytochrome P-450j, a high-affinity *N*-nitrosodimethylamine-demethylase, in rat hepatic microsomes. *Biochemistry* **26**: 2280–2289, 1987.
26. Waxman DJ, Lapenson DP, Park SS, Attisano C and Gelboin HV, Monoclonal antibodies inhibitory to rat hepatic cytochromes P-450: P-450 form specificities and use as probes for cytochrome P-450-dependent steroid hydroxylations. *Mol Pharmacol* **32**: 615–624, 1987.
27. Waxman DJ, Dannan GD and Guengerich FP, Regulation of rat hepatic cytochrome P-450: age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isozymes. *Biochemistry* **24**: 4409–4417, 1985.
28. Koop DR, Crump BL, Nordblom GD and Coon MJ, Immunochemical evidence for induction of the alcohol-oxidizing cytochrome P-450 of rabbit liver microsomes by diverse agents: ethanol, imidazole, trichloroethylene, acetone, pyrazole and isoniazid. *Proc Natl Acad Sci USA* **82**: 4065–4069, 1985.
29. Tu YY, Sonneberg J, Lewis KF and Yang CS, Pyrazole induced cytochrome P-450 in rat liver microsomes: an isozyme with high affinity for demethylnitrosamine. *Biochem Biophys Res Commun* **103**: 905–912, 1981.
30. Khani SC, Porter TD, Fujita VS and Coon MJ, Organization and differential expression of two highly similar genes in the rabbit alcohol-inducible cytochrome P-450 subfamily. *J Biol Chem* **263**: 7170–7175, 1988.
31. Song BJ, Gelboin HV, Park SS, Yang CS and Gonzalez FJ, Complementary DNA and protein sequences of ethanol-inducible rat and human cytochrome P-450s. *J Biol Chem* **261**: 16689–16697, 1986.
32. Johansson I, Ekström G, Scholte B, Puzycki D, Jörnvall H and Ingelman-Sundberg M, Ethanol-, fasting-, and acetone-inducible cytochrome P-450 in rat liver: regulation and characteristics of enzymes belonging to the IIB and IIE gene subfamilies. *Biochemistry* **27**: 1925–1934, 1988.