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IDENTIFICATION AND PARTIAL PURIFICATION OF HAMSTER MICROSOMAL CYTOCHROME P-450 ISOENZYMES

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Abstract—We have identified and partially purified three forms of cytochrome P-450 from hamster liver microsomes. Phenobarbital (PB) treatment induced three major polypeptides with relative mobilities (M_r) of 47,000, 50,000 and 51,500. The 47,000 polypeptide was assigned as epoxide hydrolase, since it was also enhanced by *trans*-stilbene oxide (TSO) treatment. Two polypeptides (M_r = 48,500 and 53,500) were induced by both 3-methylcholanthrene (3-MC) and β-naphthoflavone (BNF) treatments. Treatment with Aroclor 1254 induced three polypeptides (M_r = 48,500, 50,000 and 53,500), indicating the induction of both drug- and carcinogen-inducible cytochrome P-450s. Liver microsomal benzo[*a*]pyrene hydroxylase activity was not affected significantly by any of these inducers. In contrast, it was induced 2- to 3-fold in lung microsomes by 3-MC, BNF or Aroclor 1254 treatment. Benzphetamine *N*-demethylase and 7-ethoxycoumarin *O*-deethylase activities, expressed as nmoles of product formed per min per mg of liver microsomal protein, were increased 3- to 4-fold by either PB or Aroclor treatment. The activity of 7-ethoxycoumarin *O*-deethylase was the only one enhanced significantly by 3-methylcholanthrene or β-naphthoflavone treatment in liver microsomes. Pregnenolone-16-α-carbonitrile (PCN) and TSO did not alter any of these activities. The major polypeptides induced by PB (M_r = 50,000) and 3-MC (M_r = 48,500 and 53,500 respectively) were partially purified, to a specific content of 6–10 nmoles P-450/mg of protein and were active in catalyzing *N*-demethylation of benzphetamine, hydroxylation of benzo[*a*]pyrene, and *O*-deethylation of 7-ethoxycoumarin with different substrate specificity. None of these isoenzymes immuno-cross-reacted with antibodies prepared against rabbit cytochrome $P\text{-}450_{LM2}$ or $P\text{-}450_{LM4}$.

The cytochrome P-450-dependent mixed-function oxidase system catalyzes the oxidation of endogenous substrates, including steroids and fatty acids, and exogenous substrates, such as drugs and chemical carcinogens [1–3]. Cytochrome P-450 content and its associated drug metabolism activities are increased when animals are treated with drugs or polycyclic hydrocarbons [2]. Multiple forms of cytochrome P-450 have been identified in human, rabbit, rat and mouse liver microsomes [4]. At least eight forms of rat and rabbit liver cytochrome P-450 have been highly purified and their properties characterized [5, 6]. These forms of cytochrome P-450 have different but overlapping substrate specificities, and different spectral and immunochemical properties, electrophoretic mobilities, N- and C-terminal amino acid sequences, and polypeptide fingerprints [3–5].

The hamster has become increasingly popular in recent years as an animal model for the study of tumorigenesis, chemical carcinogenesis, and metabolic diseases. Hamster liver microsomes have relatively higher basal drug metabolism activities and are more efficient in activating chemical carcinogens [7–11]; in addition, the hamster is more resistant to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity than other animals [12]. It is not known at present how many forms of cytochrome P-450 are present in hamster liver microsomes. Recent reports indicate that benzo[*a*]pyrene hydroxylase activity in hamster liver microsomes is not stimulated by treatment with 3-MC [9–13]. There are also contradictory reports of the effect of 3-MC on the activation of benzo[*a*]pyrene to mutagenic intermediates by hamster liver microsomes [14, 15]. On the other hand, 3-MC treatment increases benzo[*a*]pyrene hydroxylase activity 3-fold in lung microsomes [10, 16]. This may explain why the hamster is prone to carcinogen-induced respiratory tract tumors [17]. To establish the hamster as a model for studying drug metabolism and carcinogenesis, it is necessary to first investigate the multiplicity of hamster cytochrome P-450. In this report, we have identified three major forms of hamster liver cytochrome P-450 by differential inductions with several inducers, partial purification of cytochrome P-450 and the reconstitution of cytochrome P-450-dependent monooxygenase activities.

MATERIALS AND METHODS

Materials. 3-Methylcholanthrene (3-MC) and phenobarbital (PB) were purchased from the Sigma Chemical Co. (St. Louis, MO); *trans*-stilbene oxide (TSO) was from ICN Pharmaceuticals (Irvine, CA); benzo[*a*]pyrene, β-naphthoflavone (BNF) and 7-ethoxy- and 7-hydroxycoumarin were from the Aldrich Chemical Co. (Milwaukee, WI); and *p*-nitro-

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phenetole was from Eastman (Rochester, NY). 3-Hydroxybenzo[*a*]pyrene was provided by Dr. Gelboin, National Cancer Institute. Aroclor 1254 was a gift from Monsanto (St. Louis, MD). Pregnenolone-16-α-carbonitrile (PCN) was a gift from Searle (Chicago, IL). Dilauroyl-glyceryl-3-phosphorylcholine (dilauroyl-GPC) was from the Serdary Research Laboratories (London, Ontario, Canada). *d*-Benzphetamine was provided by Dr. F. S. Sun of the Upjohn Co. (Kalamazoo, MI). All other reagents and chemicals used were of the highest grade and purity available.

Animals. Male Syrian golden hamsters weighing 80–100 g were purchased from the Charles River Breeding Laboratories (Wilmington, MA). The animals were treated as follows: 3-MC (25 mg/kg) [9], BNF (80 mg/kg) [18], and TSO (400 mg/kg) were injected daily for 3 days [13]; Aroclor 1254 (a mixture of polychlorinated biphenyls, 300 mg/kg) was given as a single injection [5]; PCN (50 mg/kg) was injected daily for 4 days [19]; PB (50 mg/kg) was one injection, followed by 0.1% PB in drinking water for 4 days. All inducers, except PB, were suspended in 0.2 ml corn oil and injected intraperitoneally. Control animals received the vehicle only. Each group consisted of eight hamsters. Animals were killed 20 hr after the last injection, except that the group receiving Aroclor 1254 treatment was killed 4 days after a single injection.

Liver microsomes were prepared according to routine procedures [20]. Lung microsomes were prepared according to Philpot *et al.* [21]. Microsomes were suspended in 50 mM Tris–acetate buffer, pH 7.4, containing 0.1 mM EDTA and 20% glycerol.

Enzyme activity assays and reconstitution of monooxygenase activities. Cytochrome P-450-dependent monooxygenase activities in microsomal preparations were measured in 50 mM Tris–Cl buffer, containing 5 mM $MgCl_2$, pH 7.5. Benzo[*a*]pyrene hydroxylase was assayed (0.5 nmole P-450, 80 μM benzo[*a*]pyrene) by the formation of 3-hydroxybenzo[*a*]pyrene according to Nebert and Gelboin [22]. Benzophetamine *N*-demethylase was assayed (5.0 nmoles P-450, 2 mM benzphetamine) for formaldehyde formation according to the method of Nash [23] as modified by Cochin and Axelrod [24]. 7-Ethoxycoumarin *O*-deethylase was assayed (0.1 nmole P-450, 0.4 mM 7-ethoxycoumarin) according to Greenlee and Poland [25] with minor modification [26]. *p*-Nitrophenetole *O*-deethylase (20 nmoles P-450, 2 mM *p*-nitrophenetole) was assayed by the formation of *p*-nitrophenol according to Shigematsu *et al.* [27].

Reconstitution of monooxygenases was done as described previously [26], using rabbit liver microsomal NADPH–cytochrome P-450 reductase. It has been reported that NADPH–cytochrome P-450 reductases from different animal species are interchangeable for catalysis [28], but each may be less efficient in transporting electrons to cytochrome P-450s of other than its own species.

Purification of hamster hepatic cytochrome P-450. 3-MC- or PB-treated hamster liver microsomes (3 g) were solubilized with sodium cholate (0.6 mg/mg of microsomal protein) and then diluted to 2 mg/ml with buffer containing 0.1 M K^+ phosphate, 1 mM EDTA, 0.6% sodium cholate and 20% glycerol, pH 7.25 (buffer A). The solubilized microsomes were centrifuged for 1 hr at 107,000 *g*. The supernatant fractions were loaded on an octylamino-Sepharose 4B column (5 × 60 cm) previously equilibrated with buffer A according to Imai and Sato [29]. After washing the column with buffer A containing 0.40% sodium cholate, cytochromes were eluted with buffer A containing 0.33% sodium cholate and 0.08% Emulgen 913, followed by the same buffer but with 0.2% Emulgen 913. For the purification of PB-induced cytochrome P-450, the cytochrome P-450 containing fractions eluted from the octylamino-Sepharose 4B column were combined, dialyzed against 10 mM K^+ phosphate buffer (pH 7.5) containing 0.1% Emulgen 913, 0.2% sodium cholate, 0.1 mM EDTA and 20% glycerol, and applied to a DEAE-Sepharose CL-6B column previously equilibrated with the same buffer at room temperature. After washing with equilibrium buffer, cytochrome P-450 was eluted with a 0 to 0.25 M KCl gradient in the equilibrium buffer. The cytochrome P-450 fractions eluted were then applied to a hydroxyapatite column and eluted stepwise with 0.035 M, 0.1 M and 0.15 M K^+ phosphate buffer containing 0.1 mM EDTA, 20% glycerol and 0.2% Emulgen 913, pH 7.4. The major cytochrome P-450 (relative mobility = 50,000, designated Form 2) was eluted with 0.1 M phosphate buffer.

For the purification of the 3-MC-inducible forms, cytochrome P-450 fractions that eluted from the octylamino-Sepharose 4B column were analyzed by sodium dodecylsulfate (SDS)–polyacrylamide gel electrophoresis. The fractions predominantly containing polypeptides of relative mobility = 48,500 (designated as Form 1) were pooled to give Fraction I. Fraction II contained mostly polypeptide of M_r = 53,500 (Form 4). Fraction I was applied to a hydroxyapatite column and Form 1 was eluted with 0.2 M potassium phosphate buffer. Fraction II was further purified by CM-Sepharose C-50 column, and Form 4 was eluted with 0.2 M potassium phosphate buffer. The final preparations of cytochrome P-450 were treated with Amberlite XAD-2, followed by absorption on calcium phosphate gel, and eluted with high ionic strength buffer to remove nonionic detergent as described [18].

Immunochemical studies. Antibodies against rabbit liver cytochrome $P\text{-}450_{LM2}$ or $P\text{-}450_{LM4}$ were raised in goats as described by Dean and Coon [30]. Ouchterlony double diffusion experiments were carried out in agarose gels containing 0.1% Emulgen 911 according to Thomas *et al.* [31].

Other methods. Rabbit liver cytochrome $P\text{-}450_{LM2}$, $P\text{-}450_{LM4}$, and NADPH–cytochrome P-450 reductase were prepared in this laboratory [26] according to the published procedures [18]. Cytochrome P-450 concentration was determined from the CO difference spectra of the reduced protein, using an extinction coefficient of 91 $mM^{-1}\,cm^{-1}$ [32] for the difference between A_{max} (in the 450 nm region) and $A_{490\,nm}$. Due to the contamination of hemoglobin, dithionite-difference spectroscopy was used to determine cytochrome P-450 in lung microsomes [33]. Protein concentrations were determined by the method of Lowry *et al.* [34] with bovine serum

Table 1. Cytochrome P-450 content and monooxygenase activities in hamster liver microsomes

Microsomal treatment	Specific content (nmoles/mg protein)	Benzphetamine *N*-demethylase*	7-Ethoxycoumarin *O*-deethylase*	Benzo[*a*]pyrene hydroxylase†	*p*-Nitrophenetole *O*-deethylase†
Control	1.85	3.42 (6.33)	4.60 (8.51)	286 (529)	181 (335)
3-MC	3.18	2.06 (6.56)	4.80 (15.3)	260 (827)	221 (703)
PB	2.66	9.18 (24.4)	7.16 (19.1)	281 (747)	264 (702)
Aroclor	3.87	5.70 (22.1)	4.36 (16.9)	259 (1002)	332 (1284)
BNF	2.90	2.20 (6.34)	7.03 (22.0)	303 (879)	191 (554)
PCN	1.71	3.28 (5.60)	3.48 (5.93)	263 (450)	209 (357)
TSO	2.40	3.82 (9.10)	5.08 (12.1)	288 (691)	254 (610)

* Activities are expressed as turnover number (nmoles product formed per min per nmole P-450) or specific activity (nmoles per min per microsomal protein), in parentheses. Each value is the average of at least four determinations.

† Activities are expressed as turnover number (pmoles product formed per min per nmole P-450) or specific activity (pmoles per min per mg microsomal protein), in parentheses. Each value is the average of at least four determinations.

albumin as the standard. SDS–polyacrylamide gel electrophoresis was the discontinuous pH buffer system of Laemmli [35]. The apparent molecular weights of purified cytochrome P-450 were determined by their relative mobilities (M_r) on the SDS–polyacrylamide gel, using proteins of known molecular weights (phosphorylase b, 94,000; bovine serum albumin (BSA), 68,000; catalase, 58,000; glutamine dehydrogenase, 53,000; ovalbumin, 43,000; and aldolase, 40,000).

RESULTS

Induction of cytochrome P-450 isoenzymes and monooxygenase activities in hamster liver microsomes. The basal level of cytochrome P-450 in control hamster liver microsomes was 1.85 nmoles cytochrome P-450/mg of microsomal protein, which is relatively higher than that of rat or mouse liver but similar to the content found in rabbit liver microsomes [18]. 3-MC, PB, Aroclor and BNF treatments increased cytochrome P-450 levels 1.5- to 2.0-fold (Table 1). TSO treatment also increased cytochrome P-450 content 40%; however, PCN had no effect on cytochrome P-450 content.

SDS–polyacrylamide slab gels were used to analyze polypeptide patterns of microsomal preparations (Fig. 1). A distinct cluster of polypeptides was detected in the molecular weight range (M_r) of 45,000 to 60,000. The treatment with Aroclor 1254 enhanced three major polypeptides (M_r = 48,500, 50,000 and 53,500). The 48,500 and 53,500 polypeptides were also induced by 3-MC or BNF, and the 50,000 polypeptide was also induced by PB treatment. These induced polypeptides were possibly cytochrome P-450 and were also present at lower levels in non-treated liver microsomes. In addition, PB treatment enhanced another polypeptide with an M_r of 47,000. This polypeptide was also enhanced, but to a lesser extent by the treatment with TSO. These experiments, therefore, suggest that this band might have been epoxide hydrolase [13, 36]. The third polypeptide which may also have been induced by PB had an M_r of 51,500.

Several cytochrome P-450-dependent monooxygenase activities were measured in these microsomal preparations. As shown in Table 1, PB treatment increased benzphetamine *N*-demethylase, 7-ethoxycoumarin and *p*-nitrophenetole *O*-deethylase activities 2- to 3-fold. 3-MC and BNF treatments significantly increased the specific activities (nmoles per min per mg of microsomal protein) of 7-ethoxycoumarin and *p*-nitrophenetole *O*-deethylase 2-fold; however, it did not change the turnover numbers (nmoles per min per nmole P-450). Benzo[*a*]pyrene hydroxylase activity was increased slightly by PB, 3-MC or BNF, when activity was expressed as specific activity. The turnover number of benzo[*a*]pyrene hydroxylase actually was decreased slightly by 3-MC treatment. Aroclor 1254 enhanced the specific activities of all four monooxygenases about 2- to 3-fold, but increased enzyme turnover numbers only slightly. Both PCN and TSO had no effect on any of these cytochrome P-450-dependent monooxygenase activities.

Induction of cytochrome P-450 and monooxygenase activities in hamster lung microsomes. The specific content of cytochrome P-450 in lung microsomes was only one-tenth of that found in liver microsomes. As shown in Table 2, only 3-MC treatment significantly increased cytochrome P-450 content. The specific activities of 7-ethoxycoumarin *O*-deethylase and benzo[*a*]pyrene hydroxylase in lung microsomes were much lower than in liver microsomes, even though the turnover rates were only about 2- and 5-fold lower respectively. 3-MC, Aroclor and BNF all significantly increased benzo[*a*]pyrene hydroxylase activity in the lung. PCN and TSO treatments had no effect on these two monooxygenase activities.

We also analyzed SDS–polyacrylamide gel pat-

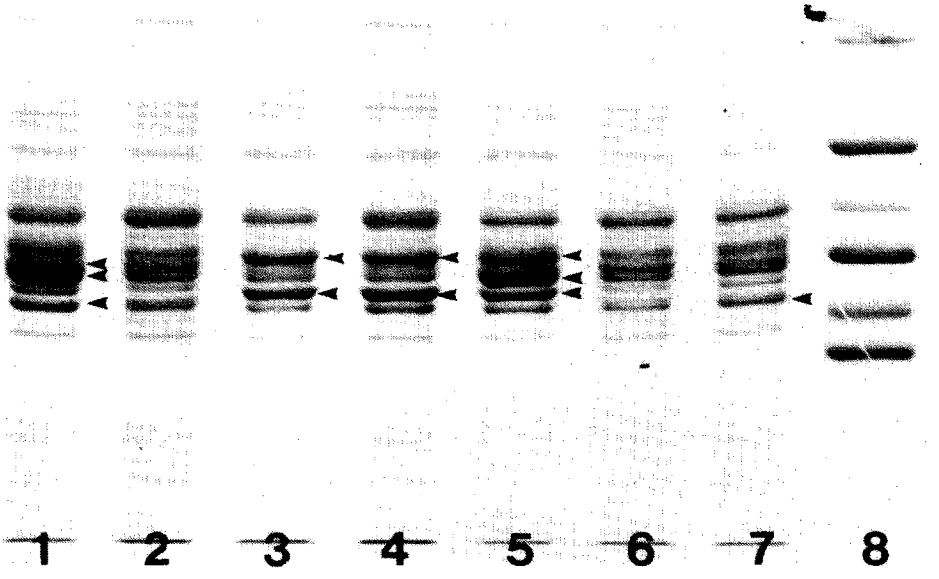

Fig. 1. SDS–polyacrylamide gel electrophoresis of hamster liver microsomes. A 1.5 mm slab gel was used as described in Materials and Methods. Samples analyzed were: wells 1–7 contained liver microsomes (25 μg) from PB, control, 3-MC, BNF, Aroclor 1254, PCN, and TSO treated hamsters respectively. Well 8 contained molecular weight standards: phosphorylase b (94,000), BSA (68,000), catalase (58,000), glutamine dehydrogenase (53,000), ovalbumin (43,000), and aldolase (40,000), 3.0 μg each.

Table 2. Cytochrome P-450 content and monooxygenase activities in hamster lung microsomes

Microsomal treatment	Specific content (nmoles P-450/mg protein)	7-Ethoxycoumarin *O*-deethylase*	Benzo[*a*]pyrene hydroxylase†
Control	0.160	2.056 (0.331)	53 (8)
3-MC	0.336	3.693 (0.640)	166 (56)
PB	0.157	2.163 (0.343)	34 (5)
Aroclor	0.170	2.340 (0.412)	117 (20)
BNF	0.206	2.344 (0.480)	126 (26)
PCN	0.157	2.114 (0.416)	51 (8)
TSO	0.080	2.750 (0.229)	ND‡ (ND)

* Activities are expressed as turnover numbers (nmoles product formed per min per nmole P-450) or specific activity (nmoles per min per mg microsomal protein), in parentheses. Each value is the average of at least four determinations.

† Activities are expressed as turnover number (pmoles product formed per min per nmole P-450) or specific activity (pmoles per min per mg microsomal protein), in parentheses. Each value is the average of at least four determinations.

‡ Not determined.

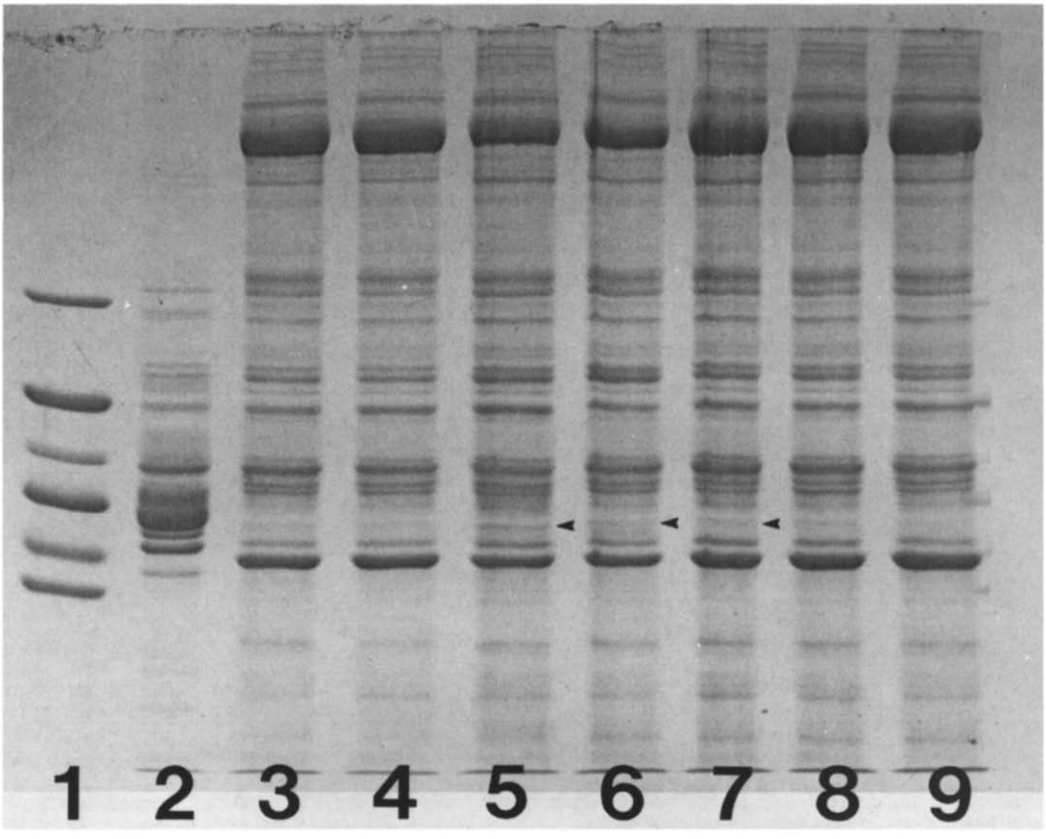

Fig. 2. SDS–polyacrylamide gel electrophoresis of hamster lung microsomes. A 1.5 mm slab gel was used as described in Materials and Methods. Samples analyzed were: well 1, molecular weight standards, same as used in Fig. 1; well 2, liver microsomes from a PB treated hamster, 25 μg; wells 3–9 contained lung microsomes (50 μg each) from control, PB, 3-MC, BNF, Aroclor 1254, PCN and TSO treated hamsters respectively.

terns of these lung microsomal preparations (Fig. 2). The overall polypeptide patterns were similar to those of liver microsomes. However, the polypeptides in the relative mobility region between 45,000 and 60,000 were less intense. Only one band with an M_r of 47,000 was enhanced in lung microsomes of hamsters treated with polycyclic hydrocarbons. The identity of this band is not known at present.

Purification and reconstitution of cytochrome P-450-dependent monooxygenase activities. The major form of cytochrome P-450 induced by PB (Form 2) was purified to 6 nmoles/mg of protein. The M_r of this cytochrome P-450 was 50,000, which is similar to that of cytochrome P-450$_{LM2}$, the major form of rabbit liver cytochrome P-450 induced by PB [18]. The 51,500 polypeptide (Form 3) was not purified. Two forms of cytochrome P-450 induced by 3-MC treatment (Forms 1 and 4) were both partially purified to 10 nmoles/mg of protein. Form 4 had an M_r similar to that of BNF-induced rabbit liver cytochrome P-450$_{LM4}$ and had a similar absorption maximum of 448.5 nm in the CO complex of the reduced cytochrome. The SDS–polyacrylamide gels of these partially purified forms are shown in Fig. 3. It appears that these three isoenzymes correspond to the three major polypeptides induced by 3-MC, PB, or Aroclor in hamster liver microsomes. Some high and low molecular weight contaminating polypeptides were also present in these preparations.

We reconstituted cytochrome P-450-dependent monooxygenase activities using partially purified hamster liver microsomal cytochrome P-450, rabbit liver NADPH–cytochrome P-450 reductase, and dilauroyl-GPC. As shown in Table 3, Form 1 was the most active in catalyzing the hydroxylation of benzo[*a*]pyrene and *O*-deethylation of 7-ethoxycoumarin, and it was also active in catalyzing *N*-demethylation of benzphetamine. Form 2 was the most active in catalyzing *N*-demethylation of benzphetamine. In contrast, Form 4 had no activity toward benzphetamine, very low activity toward benzo[*a*]pyrene, but was as active as Form 1 in catalyzing *O*-deethylation of 7-ethoxycoumarin.

Immunochemical studies. The test of immunocross-reactivity between rabbit and hamster liver microsomal cytochrome P-450 isoenzymes is shown in Fig. 4. Goat antibody against rabbit P-450$_{LM2}$ or P-450$_{LM4}$ did not cross-react with any of these hamster P-450 isoenzymes. No precipitation line was formed between these antibodies and liver microsomes of control, PB-, or 3-MC-treated hamsters.

DISCUSSION

We have identified three inducible forms of cytochrome P-450 in hamster liver microsomes by the use of different inducers, partial purification of individual cytochrome P-450s, and by the reconsti-

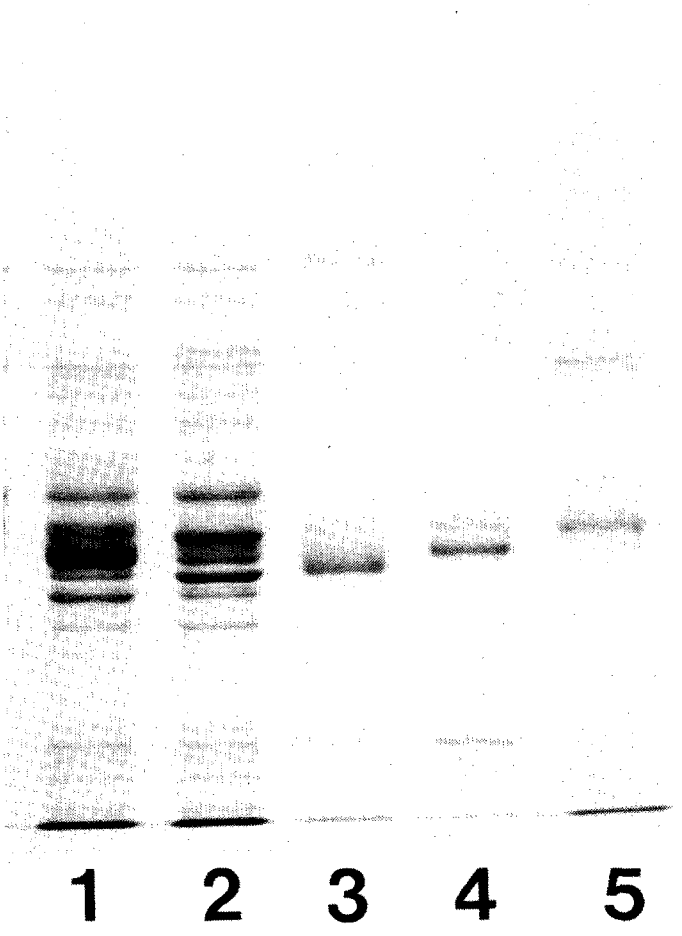

Fig. 3. SDS–polyacrylamide gel electrophoresis of partially purified hamster liver cytochrome P-450s. A 1.5 mm slab gel was used as described in Materials and Methods. Microsomes (30 μg) or purified cytochrome P-450 (3 μg) was applied to each well. Well 1, PB microsomes; well 2, 3-MC microsomes; well 3, Form 1; well 4, Form 2; and well 5, Form 4.

tution of cytochrome P-450-dependent monooxygenase activities. The basal level of cytochrome P-450 in hamster liver microsomes was relatively higher than in rats and mice but was comparable with rabbit liver microsomes [18, 37, 38]. The specific contents of cytochrome P-450 in liver microsomes reported here are much higher than that reported by Thorgeirsson *et al.* [9]; however, they are comparable with those reported by Burke and Prough [10].

It is interesting to note that 3-MC and BNF induced an identical polypeptide pattern in the liver microsomes; however, BNF treatment increased 7-ethoxycoumarin *O*-deethylase activities more than did 3-MC. Aroclor 1254 induced both drug- and carcinogen-inducible isoenzymes in hamster liver microsomes, similar to that observed in rats [5, 39]. In contrast, Aroclor 1254 only induced P-450$_{LM4}$, a carcinogen-inducible form, in rabbit liver micro-

Table 3. Substrate specificity of hamster cytochrome P-450 isoenzymes

	Reconstituted activity		
Isoenzyme	Benzphetamine *N*-demethylase*	7-Ethoxycoumarin *O*-deethylase*	Benzo[*a*]pyrene† hydroxylase
Form 1	7.80	2.93	238
Form 2	11.30	1.74	142
Form 4	0	2.78	26

* Activities are expressed as nmoles product formed per min per nmole P-450. Each value is the average of four determinations.

† Activities are expressed as pmoles product formed per min per nmole P-450. Each value is the average of four determinations.

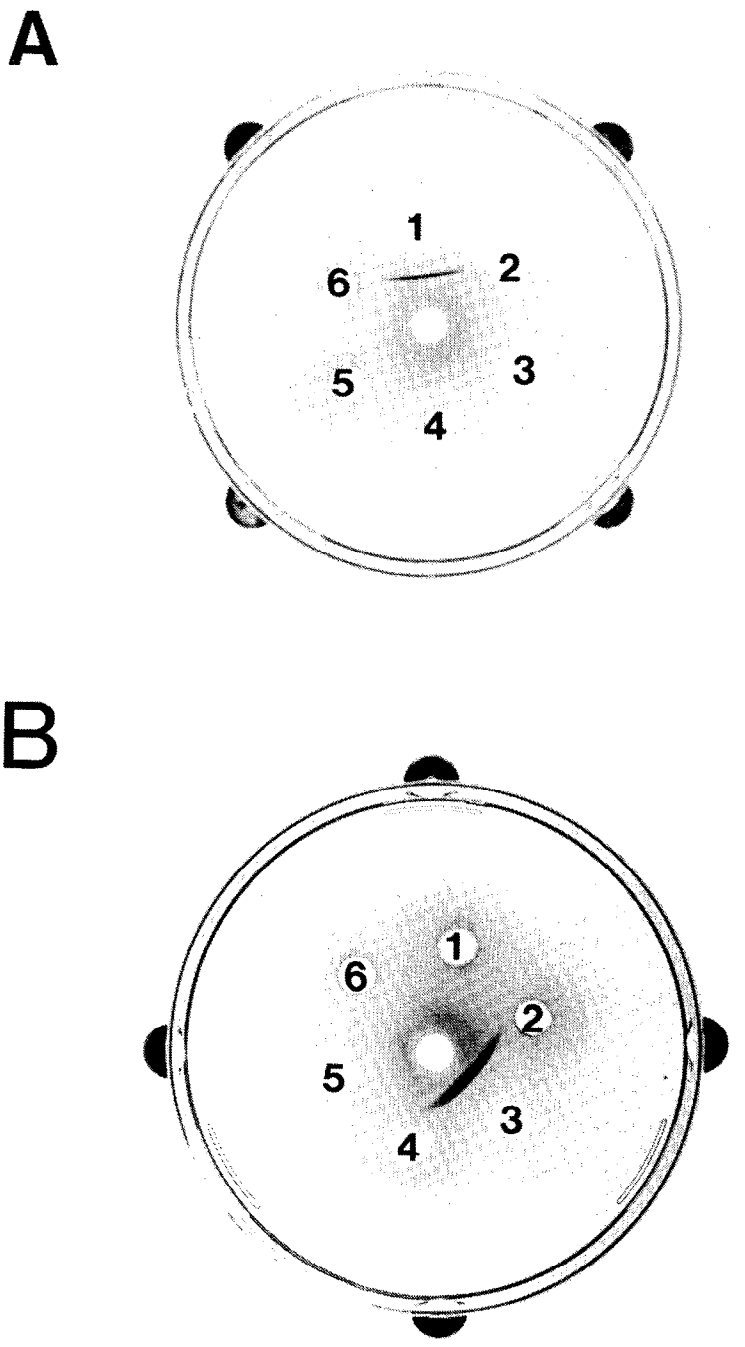

Fig. 4. Ouchterlony double diffusion study of immuno-cross-reactivity of rabbit and hamster liver cytochrome P-450. Microsomes and purified cytochrome P-450 were solubilized with Emulgen 911 as described in Materials and Methods. Antigen or antibodies (15 μl) was applied in each well. (A) Center well, anti P-450$_{LM4}$ IgG, 80 mg/ml; well 1, P-450$_{LM4}$, 1.1 mg/ml; well 2, hamster P-450 Form 1, 1.7 mg/ml; well 3, hamster P-450 Form 2, 1.8 mg/ml; well 4, hamster P-450 Form 4, 1.5 mg/ml; well 5, hamster PB microsomes, 21.5 mg/ml; and well 6, hamster 3-MC microsomes, 15.6 mg/ml. (B) Center well, anti-P-450$_{LM2}$ IgG, 88 mg/ml; well 1, hamster PB microsomes, 21.5 mg/ml; well 2, hamster 3-MC microsomes, 15.6 mg/ml; well 3, P-450$_{LM2}$, 1.2 mg/ml; well 4, hamster P-450 Form 1, 1.7 mg/ml; well 5, hamster P-450 Form 2, 1.8 mg/ml; and well 6, hamster P-450 Form 4, 1.5 mg/ml.

somes [40]. TSO treatment induced epoxide hydrolase only slightly, which is similar to the report of Oesch and Schmassmann [13] that epoxide hydrolase activity increases only 50% in hamster liver microsomes after treatment with TSO.

Benzo[*a*]pyrene hydroxylase activity was not altered by any of these inducers. It should be mentioned, however, that benzo[*a*]pyrene hydroxylase activity was measured as the formation of 3-hydroxybenzo[*a*]pyrene, the major hydroxylation product [22, 41]. It is possible that 3-MC-induced isoenzymes may be more active in catalyzing the conversion of benzo[*a*]pyrene 7,8-dihydrodiol to diolepoxide, the ultimate carcinogen [42]. We have reported previously that rabbit P-450$_{LM4}$ is seven times more active than P-450$_{LM2}$ toward benzo[*a*]pyrene 7,8-dihydrodiol, although P-450$_{LM2}$ is ten times more active in catalyzing benzo[*a*]pyrene hydroxylation than P-450$_{LM4}$ [41]. The induction of aryl hydrocarbon hydroxylase activity in hamster liver is much more pronounced in the fetus and neonate than in the adult animals [43]. The response of the hamster to polycyclic hydrocarbon inductions is similar to that of the rabbit; however, Thorgeirsson *et al.* [9] reported that hamster microsomes responded to α-naphthoflavone inhibition differently than rabbit microsomes and more closely resemble those of rats and mice.

7-Ethoxycoumarin *O*-deethylation is catalyzed by both drug- and carcinogen-inducible cytochrome P-450 isoenzymes [25]. In hamster liver microsomes, the induction of this activity is paralleled by the change in the drug-inducible benzphetamine *N*-demethylase activity. The specific activity of 7-ethoxycoumarin *O*-deethylase was increased 2-fold by PB, 3-MC, BNF or Aroclor treatment. In contrast, this enzyme activity in rabbit liver microsomes increases 30% or decreases 50% by PB and 3-MC treatments respectively [44]. In the case of hamster liver microsomes, the drug-induced forms of cytochrome P-450 were largely responsible for 7-ethoxycoumarin *O*-deethylase activity.

Our results on the induction of lung microsomal cytochrome P-450 are similar to that reported by Burke and Prough [10]. In contrast to liver microsomes, benzo[*a*]pyrene hydroxylase activity was stimulated 3-fold in the lung microsomes by polycyclic hydrocarbons. We also identified a polypeptide, possibly a cytochrome P-450, in lung microsomes from 3-MC-, BNF- and Aroclor 1254-treated hamsters. This polypeptide may be equivalent to Form 1 from the liver microsomes, which is the most active isoenzyme in catalyzing liver microsomal benzo[*a*]pyrene hydroxylation.

Form 2, the major form of hamster liver cytochrome P-450, induced by PB treatment, was most active toward benzphetamine in the reconstituted enzyme system. However, the turnover number of this hamster enzyme was much lower than rabbit P-450$_{LM2}$ or rat P-450B [5, 26]. This isoenzyme was also active in catalyzing benzo[*a*]pyrene hydroxylation. Form 1 was active toward all three substrates studied and was the most active isoenzyme in catalyzing benzo[*a*]pyrene hydroxylase activity. Form 4 was as active as Form 1 in catalyzing 7-ethoxycoumarin *O*-deethylation, but it was completely inactive toward benzphetamine and was much less active in catalyzing benzo[*a*]pyrene hydroxylation. This may explain the very low stimulation of benzo[*a*]pyrene hydroxylase activity by polycyclic hydrocarbons, since both Form 1 and Form 4 were induced by both these inducers. These three isoenzymes thus have overlapping, but rather different substrate specificities.

Several other laboratories have reported that 3-MC treatment increased biphenyl hydroxylase and *N*-hydroxylation of 2-acetylaminofluorene in hamster liver microsomes [10, 11]. Therefore, it will be interesting to identify the cytochrome P-450 isoenzymes responsible for catalyzing these two enzyme activities. Isolation and reconstitution of cytochrome P-450-dependent monooxygenase activities are essential steps in establishing Syrian hamsters as an animal model for the study of human disease.

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