Biochemical Pharmacology, Vol. 36, No. 21, pp. 3613–3619, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

CHARACTERIZATION OF THE CYTOCHROME P-450 MONOOXYGENASE SYSTEM OF HAMSTER LIVER MICROSOMES
EFFECTS OF PRIOR TREATMENT WITH ETHANOL AND OTHER XENOBIOTICS*

C. MURRAY ARDIES,† JEROME M. LASKER‡ and CHARLES S. LIEBER

Alcohol Research and Treatment Center, Bronx Veterans Administration Medical Center and Mt. Sinai School of Medicine, New York, NY 10468, U.S.A.

(*Received* 6 *October* 1986; *accepted* 27 *March* 1987)

Abstract—The cytochrome P-450 monooxygenase system of hamster liver microsomes and its response to prior treatment with ethanol and other xenobiotics have been examined. Male Syrian golden hamsters were administered ethanol (ETOH), phenobarbital (PB), 5,6-benzoflavone (BF) or isoniazid (INH). Each treatment resulted in a moderate increase (20–60%) in the specific content of liver microsomal cytochrome P-450 along with a unique hemeprotein ferrous carbonyl Soret maximum. Sodium dodecyl sulfate–polyacrylamide gel electrophoretic analysis of liver microsomes revealed distinctive changes in protein banding patterns in the cytochrome P-450 (45–60 kDa) region with each treatment. NADPH : cytochrome *c* reductase activity was increased by both PB and INH, whereas cytochrome b_5 content was increased by INH only. Microsomal oxidation of ETOH and aniline *p*-hydroxylation (expressed per nmol cytochrome P-450) were enhanced dramatically by ETOH and INH, whereas PB and BF had no effect on these enzymatic activities. Both ETOH and INH also increased zoxazolamine 6-hydroxylation but, in contrast to other rodent species, this drug-metabolizing activity was decreased in hamster liver microsomes after treatment with either PB or BF. Microsomal benzphetamine *N*-demethylation was decreased by ETOH, INH and BF administration and was only modestly enhanced after treatment with PB. ETOH and INH had no effect on the *O*-deethylation of 7-ethoxycoumarin, and enzymatic activity increased by BF but decreased by PB. These results demonstrate that the cytochrome P-450-dependent monooxygenase system of hamster liver microsomes responds to treatment with ETOH and other xenobiotics in a manner that is quantitatively and, in certain respects, qualitatively different from that reported for the rat, rabbit, and mouse.

The microsomal cytochrome(s) P-450-dependent monooxygenases of mammalian liver are important enzymes controlling the metabolism of a vast number of xenobiotics and endogenous compounds [1–3]. Previous studies from this laboratory have demonstrated that rat liver microsomes, when fortified with NADPH, oxidize ETOH§ to acetaldehyde via a cytochrome P-450-dependent process [4, 5] that is inducible in rats [4, 5], deermice [6], and rabbits [7] by chronic treatment with ETOH, concomitant with the appearance of a unique form of cytochrome P-450 [8]. When purified and reconstituted with NADPH : cytochrome P-450 reductase and phospholipid, this cytochrome P-450 isozyme catalyzes alcohol oxidation at rates significantly higher than other rabbit or rat liver cytochrome P-450 isozymes [9–11]. Antibodies produced against this hemeprotein inhibit almost 75% of the total ETOH oxidation catalyzed by liver microsomes from ETOH-treated rabbits, but only 30% of that catalyzed by microsomes from untreated rabbits [12]. Such results indicate the existence of a form of liver microsomal cytochrome P-450 that not only specifically oxidizes ETOH but is also inducible by this compound.

One drawback of the animal models utilized in the above-cited studies concerns the relatively modest induction of liver microsomal ETOH oxidation observed following ETOH treatment. ETOH oxidation catalyzed by liver microsomes (per mg protein) from ETOH-treated rats or from deermice which lack liver alcohol dehydrogenase activity have been shown to increase 2-fold and 3-fold, respectively, compared to untreated animals [6, 13]. However, since ETOH treatment also increases the total microsomal cytochrome P-450 content, only a 6–39% [14, 15] increase in microsomal ETOH oxidation is observed if these reported activities are expressed per nmol cytochrome P-450. A 3-fold increase in rates of ETOH oxidation by rabbit liver microsomes has been reported following treatment with ETOH [7], but the turnover number for this substrate by rabbit liver microsomes is very low when compared to the rat or deermouse. At present, an animal model which exhibits both high rates of microsomal cytochrome P-450-dependent ETOH oxidation as well as pronounced induction of this enzymatic activity following chronic alcohol exposure has yet to be developed.

Recently, the hamster has become increasingly

*This work was supported in part by DHHS Grants AA 05934, AA 03508 and AA 06371 and by the Veterans Administration. A preliminary account of this research has been published in abstract form [J. M. Lasker, C. M. Ardies and C. S. Lieber, *Fedn Proc.* **44**, 1449 (1985)].

†Postdoctoral fellow supported by DHHS Grant AA07275.

‡Address correspondence to: Dr. Jerome M. Lasker, Alcohol Research and Treatment Center, Veterans Administration Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468.

§Abbreviations: ETOH, ethanol; INH, isoniazid; PB, phenobarbital; BF, 5,6-benzoflavone; EtICN, ethylisocyanide; and SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

popular as an animal model in studies of toxicity and carcinogenesis mediated via the bioactivation of xenobiotics by cytochrome P-450. Studies utilizing hamsters have focused primarily on species comparisons performed with untreated animals [16–19], while reports concerning drug-induced alterations of the hamster liver monooxygenase system are few in number [20–22]. Whereas one study did utilize ETOH [22], the effects of ETOH treatment on cytochrome P-450-dependent ETOH oxidation were not examined. To date, no single investigation has attempted to provide an in-depth characterization of the cytochrome P-450-dependent monooxygenase system of hamster liver microsomes as well as its response to treatment with different xenobiotics.

In preliminary studies, we found that chronic ETOH administration to hamsters produced an increase in liver cytochrome P-450, enhanced the turnover rates of ETOH oxidation and aniline *p*-hydroxylation by liver microsomes, and increased the content of a single protein band in the cytochrome P-450 region ($M_r = 54{,}000$), as determined by SDS–PAGE [23]. Based on these results, the hamster may be an excellent model species with which to study the effects of ETOH treatment on hepatic microsomal monooxygenases. The purpose of the present study was 2-fold: (a) to fully characterize the cytochrome P-450-dependent monooxygenase system of hamster liver microsomes, and (b) to describe the response of this drug-metabolizing enzyme system to prior treatment with ETOH, PB, BF, and INH.

MATERIALS AND METHODS

Chemicals. Aniline, zoxazolamine, isoniazid, 5,6-benzoflavone, 7-ethoxycoumarin and NADPH were obtained from the Sigma Chemical Co. (St. Louis, MO). 7-Hydroxycoumarin was from the Aldrich Chemical Co. (Milwaukee, WI), and *p*-aminophenol was from Eastman (Rochester, NY). Sodium phenobarbital was purchased from the J. T. Baker Chemical Co. (Phillipsburg, NJ). Desferrioxamine mesylate (Desferyl) was provided by CIBA (Summit, NJ), and benzphetamine was provided by Upjohn (Kalamazoo, MI). [4,6-^{3}H]Zoxazolamine was donated by Mr. Wayne Levin, Department of Experimental Carcinogenesis and Metabolism, Hoffmann-La Roche Inc. (Nutley, NJ). Chelex 100 metal-chelating resin (100–200 mesh) and chemicals employed for SDS–PAGE were obtained from Bio-Rad Laboratories (Richmond, CA). All other reagents used were of the highest grade commercially available.

Animals and treatments. Male Syrian golden hamsters [Lak: LVG (SYR), 50–60 g body weight] were purchased from the Charles River Breeding Laboratories (Wilmington, MA). ETOH was administered as a 10% solution in drinking water for 10 days. In preliminary studies, hamsters were found to consume 18–20 g ETOH·kg^{-1}·day^{-1} when given ethanol in this manner (J. M. Lasker, unpublished observations). INH was administered as a 0.1% solution in drinking water (following neutralization with HCl) for 10 days. PB was given intraperitoneally in isotonic saline at a dose of 80 mg·kg^{-1}·day^{-1} for 4 days, and BF was given intraperitoneally in corn oil at a dose of 50 mg·kg^{-1}·day^{-1} for 3 days. Animals receiving ETOH and INH were allowed access to the solutions until the time they were killed, whereas animals treated with PB and BF were killed 24 and 48 hr, respectively, after the last injection. In preliminary experiments, neither saline nor corn oil administration alone was found to influence any of the variables described in this study. Therefore, for sake of simplicity, animals designated as controls were those allowed access to only Purina rodent chow and water *ad lib.*

The animals within each treatment group were divided into pairs and killed by decapitation. The livers from each pair were combined and microsomes were prepared according to the method of Lu and Levin [24], with the exception that 5 mM potassium phosphate was added to the KCl wash (pH 7.4) while EDTA was omitted. The final microsomal pellets were resuspended in 10 mM potassium phosphate buffer (pH 7.4) containing 0.25 M sucrose at a final cytochrome P-450 concentration of 10 nmol/ml and stored at −80° until used.

Enzyme assays. All enzyme assays were performed in 100 mM potassium phosphate buffer (pH 7.4) that had been treated with Chelex 100 resin to remove as much extraneous iron as possible. Incubation mixtures contained microsomal protein equivalent to 0.5 nmol cytochrome P-450 in a final volume of 1.0 ml. The following substrate concentrations were used: aniline, 10 mM; benzphetamine, 1 mM; ethanol, 50 mM; 7-ethoxycoumarin, 1 mM; and zoxazolamine, 0.5 mM. Reactions were initiated by the addition of 1 μmol NADPH and were terminated after 10 min at 37° with 1 ml of cold 10% trichloroacetic acid; zoxazolamine hydroxylase assays were terminated with 0.2 ml of cold 4 N acetic acid. Aniline hydroxylation was determined by colorimetric measurement of *p*-aminophenol formation [25], 7-ethoxycoumarin *O*-deethylation was measured using a fluorimetric assay for 7-hydroxycoumarin formation [26], and 6-hydroxylation of zoxazolamine was determined in a radiometric assay according to Tomaszewski *et al.* [27]. Benzphetamine *N*-demethylation was determined using a colorimetric assay for formaldehyde as described by Nash [28] and modified by Cochin and Axelrod [29]. ETOH oxidation was determined by measurement of acetaldehyde formation using a head-space gas chromatographic method [8]; incubation volumes were 2 ml and contained 1 mM azide plus 0.5 mM desferrioxamine. These assays performed in glass vials sealed with rubber septa and were terminated after 10 min at 37° with 0.4 ml of 35% perchloric acid injected through the vial septum by means of a repeating dispenser fitted with a gas-tight syringe (Hamilton Co., Reno, NV). All enzymatic measurements were performed under conditions of saturating substrate concentration and were linear with respect to both time and cytochrome P-450 concentration. Turnover numbers were calculated for each enzymatic activity and the results are expressed as nmol product formed·min^{-1}·(nmol cytochrome P-450)$^{-1}$.

Other assay methods. Spectral determinations were performed using either an Aminco DW-2a or a Perkin–Elmer model 320 dual-beam recording spectrophotometer. Cytochrome P-450 content was determined from the CO-difference spectrum of the

reduced hemeprotein using the extinction coefficient of 91 $mM^{-1}cm^{-1}$ for the difference between the maximum at or near 450 nm and at 490 nm [30]. Cytochrome b_5 content was determined from the NADH-reduced minus oxidized difference spectrum using an extinction coefficient of 185 $mM^{-1}\,cm^{-1}$ between 424 and 409 nm [30]. EtICN difference spectra were generated in 100 mM potassium phosphate buffer (pH 7.4) according to the method of Sladek and Mannering [31]; the concentrations of cytochrome P-450 and EtICN used were 1 μM and 2 mM, respectively. NADPH:cytochrome *c* reductase activity was measured by the method of Phillips and Langdon [32] where one unit of reductase activity is defined as that amount catalyzing the reduction of 1 nmol of cytochrome *c*/min at 22°. An extinction coefficient of 21 $mM^{-1}\,cm^{-1}$ at 550 nm was used. Protein concentration was determined by the biuret reaction [33] using crystalline bovine serum albumin as the standard.

An aliquot of each of the microsomal preparations was treated with SDS plus 2-mercaptoethanol, heated, and electrophoresed on slab gels in the presence of SDS as described by Laemmli [34]. The separating gel was 12 cm long, 0.75 mm thick, and contained 7.5% acrylamide. Molecular weights were estimated by comparison to protein standards of known molecular weight.

Statistics. As described above, liver microsomes were prepared from pairs of animals within each treatment group, producing an N of at least 4 for each treatment group. Individual determinations with each sample of microsomes were performed in quadruplicate. Data from the various assays were first analyzed by a one-way analysis of variance. When significant differences were detected ($P < 0.05$), a Neuman–Kuels post-hoc test [35] was utilized to locate the source of the differences.

RESULTS

Treatment of hamsters with ETOH, INH, or BF resulted in a 22% increase in the aggregate content of cytochrome P-450 in liver microsomes (Table 1), whereas a 60% increase was observed in PB-treated animals. Differences between treatment groups were also observed in the spectral Soret maximum of the reduced cytochrome P-450 carbon monoxide complex (Table 1). This Soret maximum exhibited a red shift from 450.5 to 451.5 nm in liver microsomes following ETOH and INH treatment, whereas a hypsochromic blue shift to 449.5 nm was noted in microsomes from animals treated with BF. Treatment with PB did not alter the cytochrome P-450 ferrous carbonyl Soret maximum.

Addition of the cytochrome P-450 ligand EtICN to liver microsomes from untreated hamsters resulted in spectral Soret peaks at 455 and 428 nm. The 455:428 nm peak-height ratio of the EtICN binding spectra was increased significantly following ETOH, INH, and BF treatment; this effect was greatest following INH administration (Table 1).

SDS–PAGE analysis of hamster liver microsomes revealed unique changes in protein banding patterns in the 45,000–60,000 molecular weight region with each treatment (Fig. 1). Both ETOH and INH administration appeared to increase the microsomal content of only a single protein with $M_r = 54{,}000$. In contrast, increased levels of at least four microsomal polypeptides ($M_r = 45{,}000$, 51,000, 52,000 and 55,000) were observed following PB treatment. BF administration caused increases in the microsomal content of two proteins with $M_r = 49{,}000$ and 53,000.

As shown in Table 2, NADPH:cytochrome *c* reductase activity was enhanced following both PB and INH treatment. This treatment effect was modest, however, and represents only a 23–25% increase in enzymatic activity. The specific content of another microsomal hemeprotein, cytochrome b_5, was affected only by INH administration (Table 2).

Analysis of cytochrome P-450-dependent monooxygenase activities (Table 3) revealed alterations in the capacity for substrate oxidation which were unique for the different treatments used. ETOH oxidation to acetaldehyde by hamster liver microsomes was enhanced dramatically following chronic administration of either ETOH or INH. Microsomal ETOH oxidation increased 115% with ETOH treatment and 123% with INH treatment when compared to control values (Table 3). It should be emphasized that the ethanol oxidation rates presented in Table 3 are those rates catalyzed directly by cytochrome P-450. The contribution of catalase and/or hydroxyl radicals ($OH^{\cdot}$) to the rates reported here would be expected to be negligible, since ETOH oxidation

Table 1. Effects of xenobiotic treatment on hamster liver microsomal cytochrome P-450

Treatment	Cytochrome P-450 (nmol/mg protein)	CO-reduced max (nm)	Ethyl isocyanide binding spectra* (OD_1/OD_2)
C (N = 4)	1.05 ± 0.03†	450.5	5.23 ± 0.6†
ETOH (N = 5)	1.30 ± 0.11‡	451.5	11.1 ± 2.5‡
INH (N = 5)	1.27 ± 0.04‡	451.5	25.0 ± 6.3‡
PB (N = 4)	1.63 ± 0.07‡	450.5	7.1 ± 1.0
BF (N = 4)	1.28 ± 0.06‡	449.5	9.1 ± 1.7‡

*OD_1 = optical density at or near 455 nm minus that at 490 nm; OD_2 = optical density at 430 nm minus that at 490 nm. Measurements were made in 100 mM potassium phosphate buffer, pH 7.4, at 20°.

†Values represent the mean ± SEM.

‡$P < 0.05$ versus control values (C).

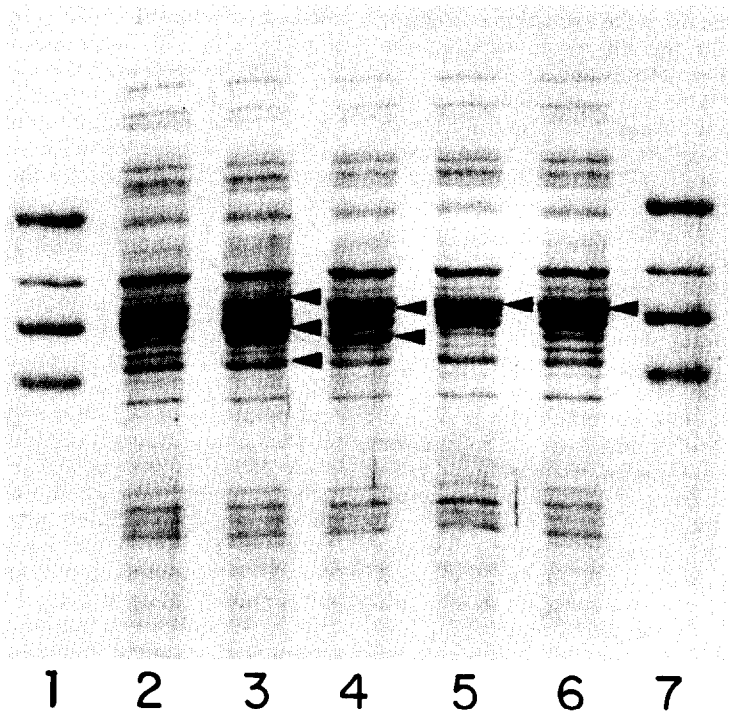

Fig. 1. SDS–PAGE of hamster liver microsomes following treatment with various xenobiotics. The samples were analyzed at protein levels of 7.5 μg each on slab gels (0.75 mm thick) containing 7.5% acrylamide using the discontinuous buffer system. Migration proceeds from top to bottom. Lanes 2–6 contain liver microsomes from untreated, PB-treated, BF-treated, INH-treated, and ETOH-treated hamsters respectively. Lanes 1 and 7 contain protein standards (0.5 μg each) with molecular weights of 68,000, 58,000, 53,000, and 43,000 (top to bottom). Arrows denote positions of induced polypeptides.

assays were performed in the presence of azide, a catalase inhibitor [36], and desferrioxamine, an inhibitor of microsomal hydroxyl radical formation [37].

In addition to ETOH oxidation, microsomal aniline *p*-hydroxylation was also dramatically increased by both ETOH and INH (Table 3). This treatment effect represented a 242% increase in catalytic activity after ETOH and 325% increase after INH. Both ETOH and INH also caused a slight enhancement of microsomal zoxazolamine 6-hydroxylase activity. In contrast, all of the above microsomal drug-metabolizing activities were decreased following PB treatment (Table 3). BF administration also resulted in decreases in microsomal zoxazolamine 6-hydroxylation and aniline *p*-hydroxylation but did not effect ETOH oxidation. Benzphetamine *N*-demethylase activity of hamster liver microsomes was increased following PB treatment but decreased after treatment with ETOH, INH, and BF. Finally, 7-ethyoxycoumarin *O*-deethylation was enhanced modestly (20%) by BF, whereas PB depressed this microsomal drug-metabolizing activity.

Table 2. Effects of xenobiotic treatment on hamster liver microsomal NADPH: cytochrome reductase and cytochrome b_5

Treatment	NADPH: cytochrome *c* (P-450) reductase (units/mg protein)*	Cytochrome b_5 (nmol/mg protein)
C (N = 4)	225.7 ± 8.0†	0.70 ± 0.1†
ETOH (N = 5)	215.6 ± 6.7	0.72 ± 0.1
INH (N = 5)	281.5 ± 12.0‡	0.94 ± 0.1‡
PB (N = 4)	278.0 ± 12.7‡	0.68 ± 0.1
BF (N = 4)	211.9 ± 12.0	0.65 ± 0.1

*One unit of enzyme activity is defined as that amount catalyzing the reduction of 1 nmol of cytochrome *c* per min in 0.3 M potassium phosphate buffer, pH 7.7, at 22°.

†Values represent the mean ± SEM.

‡$P < 0.05$ versus control values (C).

DISCUSSION

The cytochrome P-450-dependent monooxygenase system of hamster liver microsomes has been characterized, in part, both prior to and after treatment with ETOH and other commonly-employed "inducers" of these drug-metabolizing enzymes. Our results indicate not only that important differences exist between cytochrome P-450-dependent monooxygenases of naive hamsters when compared to these enzymes in other rodents but also suggest that hamster liver monooxygenases respond to xenobiotic treatment in a manner that is both qualitatively and quantitatively different from other species. Such information is important when interpreting results

Table 3. Cytochrome P-450-dependent monooxygenase activities in hamster liver microsomes after treatment with various xenobiotics*

Treatment	Ethanol oxidation	Aniline *p*-hydroxylase	Benzphetamine *N*-demethylase	7-Ethoxycoumarin *O*-deethylase	Zoxazolamine 6-hydroxylase
	[nmol product formed·min^{-1}·(nmol cytochrome P-450)$^{-1}$]				
C (N = 4)	5.3 ± 1.3	1.2 ± 0.1	7.7 ± 0.2	5.6 ± 0.2	2.8 ± 0.1
ETOH (N = 5)	11.4 ± 0.1†	4.1 ± 0.1†	6.1 ± 0.3†	5.9 ± 0.3	3.4 ± 0.2†
INH (N = 5)	12.1 ± 1.4†	5.1 ± 0.1†	6.1 ± 0.2†	5.9 ± 0.3	3.4 ± 0.1†
PB (N = 4)	3.8 ± 0.03†	1.0 ± 0.1†	9.1 ± 0.5†	4.2 ± 0.2†	2.1 ± 0.2†
BF (N = 4)	5.6 ± 1.8	1.0 ± 0.1†	4.0 ± 0.4†	6.7 ± 0.2†	1.8 ± 0.1†

*All values represent the mean ± SEM.
†$P < 0.05$ versus control values (C).

obtained in studies of xenobiotic-mediated toxicity and/or carcinogenesis using hamsters. Species differences in xenobiotic disposition may aid in explaining, in part, why hamsters are more susceptible than rats to cancers caused by dimethylnitrosamine and *N*-acetylaminofluorene administration, i.e. this increased susceptibility may result from greater bioactivation of these procarcinogens by hamster versus rat liver oxidative enzymes [38]. Our results also indicate that the hamster is a suitable animal model to study the effects of ETOH on cytochrome P-450-dependent monooxygenases. An appropriate animal model to examine such effects is essential since ETOH is often consumed (and chronically abused) by humans, and has been implicated as a risk factor associated with human liver [39, 40] and alimentary tract [41, 42] cancers.

In naive hamsters, we found the aggregate content of liver cytochrome P-450 roughly equivalent to that found in rats [43] and mice [44] but somewhat lower than that reported in rabbits [45]. The specific content values reported here are also similar to those described in other studies using untreated hamsters [16, 18]. Upon xenobiotic treatment, modest increases in the total cytochrome P-450 content in hamster liver microsomes were observed; these increases followed the same qualitative pattern (i.e. PB > BF >ETOH) found in other rodent species. Whereas much larger increases in liver cytochrome P-450 content have been reported in rats, rabbits, and mice [43, 45, 46] after PB and/or BF treatment, the small increase in aggregate hamster liver cytochrome P-450 content after ETOH treatment described here parallels similar small increases reported previously in this species [47] and other rodents [13, 14]. In contrast to this study, INH administration to rats did not effect liver cytochrome P-450 content [48, 49].

Alterations in the spectral characteristics of microsomal P-450 (detected with ligands such as CO and EtICN) are brought about primarily by a redistribution of the P-450 microsomal subpopulation which, in rats and rabbits, consists of at least ten forms of cytochrome P-450, each with unique spectral and/or catalytic properties [49, 50]. In rats [8, 48, 49], a red shift in the cytochrome P-450 ferrous carbonyl Soret maximum is also observed after ETOH and INH treatments. ETOH- and INH-inducible cytochrome P-450 isozymes purified from rabbit [9] or rat liver microsomes [11, 49] possess CO-reduced Soret maximum at 452 nm. Induction of a homologous cytochrome P-450 isozyme in hamster liver following ETOH or INH treatment could explain the spectral shift towards this wavelength. Fujii *et al.* [47] did not observe any cytochrome P-450 spectral perturbations in liver microsomes from ETOH-treated hamsters, although the animals used had consumed an amount of ETOH in drinking water similar to that consumed by the animals employed in this investigtion. At present, we cannot readily explain this apparent discrepancy in results. Consistent with reports in other rodent species [46, 48, 51], PB treatment did not alter the cytochrome P-450 CO-reduced Soret maximum in hamster liver microsomes. The hypsochromic spectral shift from 450.5 to 449.5 nm noted following BF administration to hamsters also occurs in rats and mice [52]. In the presence of another cytochrome P-450 ligand, EtICN, typical Soret maxima were observed in hamster liver microsomes. Although no peak shifts were observed, xenobiotic treatment did alter significantly the 428/455 absorbance ratios; in all cases, the height of the 428 nm peak increased relative to the peak at 455 nm.

Alterations in spectral properties described above, when combined with the results of SDS–PAGE and catalytic activity measurements (see below), confirmed that each treatment produced unique changes in the subpopulation of cytochromes P-450 in hamster liver microsomes. The content of only a single major protein with $M_r = 54{,}000$ increased in liver microsomes after either ETOH or INH treatment (Fig. 1). Based on its molecular weight and xenobiotic inducibility, this protein apparently is an isozymic form of hamster liver cytochrome P-450, and may be similar to the 51,000 dalton hemeprotein observed to increase in rat liver microsomes following ETOH [8, 43] or INH [49] administration. The increases observed in the content of four hamster liver proteins with $M_r = 45{,}000$, 51,000, 52,000 and 55,000 after PB treatment and two proteins with $M_r = 49{,}000$ and 53,000 following BF administration have been described in part previously [20, 21].

Treatment of hamsters with xenobiotics produced significant alterations in the activities of specific cytochrome P-450-dependent monooxygenases in liver microsomes. Prior treatment with ETOH or INH caused greater than a 2-fold enhancement of microsomal ETOH oxidation rates, and 3.4- and 4.3-fold increases, respectively, in rates of aniline *p*-hydroxylation. Increases in these microsomal drug-

metabolizing activities following ETOH treatment have been described in rats [53] and rabbits [7]. However, rates of ETOH oxidation by rabbit liver microsomes are 10-fold less than hamster liver microsomes, while a much smaller increase in turnover number (rates expressed per nmol cytochrome P-450) for this activity is observed in rats [54]. The large increase in liver microsomal aniline *p*-hydroxylation seen in rats following INH treatment [48] is similar to that found in the present study. Both Fujii *et al.* [47] and McCoy [55] have reported the induction of microsomal ETOH oxidation and aniline *p*-hydroxylation in ETOH-treated hamsters but to a lesser extent than that observed here. Such variation may reflect differences between laboratories in the assay procedures used or, more likely, in the ETOH treatment protocols. We have reported previously that the extent of induction of hamster liver microsomal ETOH oxidation and aniline *p*-hydroxylation by ETOH treatment is much greater if the animals are fed ETOH until killed rather than fasted overnight [56].

Microsomal benzphetamine *N*-demethylase activity was decreased following ETOH and INH treatment, whereas no change was noted in 7-ethoxycoumarin *O*-deethylation. Decreased benzphetamine oxidation has been reported previously in ETOH-treated hamsters, and contrasts with the enhancement of this activity observed in rabbits [7] and rats [57] treated with ETOH. In the latter species, increases in microsomal benzphetamine metabolism are most likely due to the general increase in cytochrome P-450 content following ETOH treatment [13, 14]. In contrast to the often-described induction of rat liver microsomal 7-ethoxycoumarin *O*-deethylase activity by ETOH, neither we nor Fujii *et al.* [47] found an increase in this activity in ETOH-treated hamsters. Moreover, zoxazolamine 6-hydroxylation by hamster liver microsomes was enhanced after treatment with either ETOH or INH, whereas in rats this reaction is stimulated by polycyclic aromatic hydrocarbon treatment [27], an effect not observed here.

Compared to ETOH and INH treatment, both PB and BF administration produced a more marked substrate specificity in the hamster monooxygenase system. For example, treatment with PB increased only one monooxygenase activity, benzphetamine *N*-demethylase, while all other activities decreased. In other rodents, PB has been shown to increase microsomal 7-ethoxycoumarin *O*-deethylase and ethanol oxidation in addition to benzphetamine *N*-demethylase [7, 26]. Similarly, treatment of hamsters with BF enhanced only 7-ethoxycoumarin *O*-deethylation, whereas rats treated with this polycyclic aromatic hydrocarbon (or with 3-methylcholanthrene) display increased rates of microsomal 7-ethoxycoumarin *O*-deethylation and zoxazolamine 6-hydroxylation [27]. Chiang and Steggles [21] have reported that benzo[a]pyrene hydroxylase is not induced in hamsters by BF, 3-methylcholanthrene, or Arochlor 1254 treatment; this enzymatic activity is highly inducible in rats and mice administered these compounds [46, 52].

In summary, our results demonstrate that the hamster is unlike those other rodents commonly used to study hepatic microsomal monooxygenases. The cytochrome P-450-dependent monooxygenase system of naive hamsters displays inherently higher catalytic activity towards many substrates when compared to rats, rabbits or mice. Certain drug-metabolizing activities are inducible to a larger extent in hamsters after xenobiotic administration whereas other activities, previously shown to be inducible in different rodents, are either unaffected or repressed in the hamster. In particular, treatment with ETOH dramatically enhances microsomal ETOH oxidation without concomitant increases in benzphetamine *N*-demethylase or 7-ethoxycoumarin *O*-deethylase activities. These characteristics, coupled with the hamster's avid voluntary consumption of alcohol solutions [58], make this species an attractive model to study alterations in cytochrome P-450-dependent ETOH oxidation after treatment with ETOH. We recognize, however, that the differences among rodents themselves in the response of the liver monooxygenase system to xenobiotic administration, as outlined in this paper, make extrapolation of results obtained in these species to humans a somewhat tenuous proposition.

Acknowledgements—We would like to thank Barbara P. Bloswick for her expert technical assistance and Rene Cabell for preparation of this manuscript.

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