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EFFECTS OF PHENOBARBITAL, 3-METHYLCHOLANTHRENE AND β-NAPHTHOFLAVONE PRETREATMENT ON MOUSE LIVER MICROSOMAL ENZYMES AND ON METABOLITE PATTERNS OF BENZO[*a*]PYRENE*†

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Abstract—C57B1/6 (B6), C3H, and DBA/2 (D2) strains of mice were pretreated with either a single dose of 3-methylcholanthrene (3-MC), β-naphthoflavone (β-NF) or α-naphthoflavone (α-NF), or with daily injections of phenobarbital (PB) for 4 days; control mice were injected with corn oil or saline only. Comparisons were made of the patterns of hepatic microsomal-protein bands on acrylamide gel electropherograms as well as the patterns of individual metabolites of benzo[*a*]pyrene (BP) extracted by ethyl acetate from incubation systems using these microsomes. Hepatic microsomes from control mice of all three strains produced similar protein bands and BP metabolite profiles, with B6 mice having slightly higher yields of the 9,10-diol and phenols of BP. α-NF had no apparent effect on the protein bands or the yields of BP metabolites. Sodium dodecylsulfate–polyacrylamide gel electrophoresis of hepatic microsomes revealed that PB pretreatment was followed by increases in the intensities of five protein bands with molecular weights between 43,000 and 68,000. The yields of BP metabolites were increased 1.5- to 3-fold for all three strains of mice after PB pretreatment. Pretreatment with 3-MC or β-NF had no apparent effect on the hepatic microsomal enzymes of D2 mice, either in terms of protein bands or metabolites of BP. For B6 and C3H mice, pretreatment with 3-MC or β-NF resulted in the appearance of a new protein band easily detectable in the range between molecular weights 53,000 and 58,000, as well as in increases of all BP metabolites. Pretreatment of B6 and C3H mice with 3-MC caused 10-fold increases in the microsomal yields of the 7,8-diol, and 5-fold increases in the yields of BP phenols. Pretreatment with β-NF caused a 7-fold increase of the 7,8-diol catalyzed by the hepatic microsomes of B6 mice and a 4-fold increase in the 7,8-diol in C3H mice; the yields of phenols were increased about 2.5-fold for both B6 and C3H mice. The results suggest that the induced microsomal proteins with molecular weights between 43,000 and 58,000 may contain induced forms of cytochromes(s) P-450 that activate different carbon atoms of the BP molecule and, therefore, lead to different metabolite profiles.

Benzo[*a*]pyrene is one of the carcinogenic polycyclic aromatic hydrocarbons (PAH).§ It is distributed widely in our environment [1]. PAH need to be metabolically activated to exert a carcinogenic, mutagenic or toxic effect [2]. Metabolites of PAH include epoxides, phenols, dihydrodiols, quinones and sulfate ester conjugates that are extracted by organic solvents, as well as glutathione and glucuronide conjugates in aqueous phase [3–7]. Active metabolic intermediates of BP, such as epoxides, are highly reactive in binding to cellular components such as DNA [8–11], whereas several other metabolites of BP, e.g. 3-OH-BP and the quinones of BP, have been reported to be toxic to cells in culture [12, 13]. Three dihydrodiols—the 4,5-diol, the 7,8-diol and the 9,10-diol—are generally detected metabolites of BP. Of particular interest is the 7,8-diol, which has been shown to be further metabolically activated to bind covalently to DNA [14]. Subsequent studies established that the 7,8-diol can be metabolized to 7,8-diol-9,10-epoxides of BP, which are both carcinogenic and mutagenic [15–19] and have been suggested as the ultimate carcinogenic form of BP [15]. The enzyme system metabolizing PAH has been referred to as aryl hydrocarbon hydropylase (AHH). Inducibility of AHH varies among species, strains and organs of animals [20], and the inducibility of AHH in inbred strains of mice

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§ Abbreviations: PAH, polycyclic aromatic hydrocarbons; BP or BaP benzo[*a*]pyrene; 3-MC, 3-methylcholanthrene; β-NF, β-naphthoflavone; α-NF, α-naphthoflavone; PB, phenobarbital; t.l.c., thin-layer chromatograph; 4,5-diol, *trans*-4,5-dihydroxy-4,5-dihydro-BP; 7,8-diol, *trans*-7,8-dihydroxy-7,8-dihydro-BP; 9,10-diol, *trans*-9,10-dihydroxy-9,10-dihydro-BP; 4,5-oxide, BP-4,5-oxide; 3-OH-BP, 3-hydroxy-BP; TCA, trichloroacetic acid; SDS–PAGE, sodium dodecylsulfate–polyacrylamide gel electrophoresis; citrate buffer (140 mM NaCl, 15 mM sodium citrate, pH 7.4); TKM–sucrose buffer (50 mM Tris, 25 mM KCl, 5 mM $MgCl_2$, 250 mM sucrose, pH 7.4); extraction buffer (15% glycerol, 2.5% SDS, 5% mercaptoethanol and 62.5 mM Tris, pH 6.8); B6, C57B1/6; and D2, DBA/2.

has been shown to be controlled genetically [21–25]. AHH has generally been measured by the yield of 3-OH-BP, which has not been shown to be carcinogenic. Therefore, comparison of yields of other metabolites of BP may reveal the presence of active metabolic intermediates that play a critical role in the initiation of cancer.

Substrate specificity in the metabolism of PAH and certain compounds has been shown to reside in microsomal cytochrome(s) P-450 [26]. Comparison of microsomal proteins separated by SDS–PAGE may establish the correlations between the induced enzymes and the metabolite profiles of BP.

MATERIALS AND METHODS

Materials. 3-MC, NADPH, α-NF, β-NF, and proteins used as molecular weight markers (bovine serum albumin, 68,000; bovine liver catalase, 58,000; bovine liver glutamate dehydrogenase, 53,000; and ovalbumin, 43,000) were obtained from the Sigma Chemical Co., St. Louis, MO; PB from the pharmacy of the Medical University of South Carolina, Charleston, SC; benzene and TCA from the Fisher Scientific Co., Pittsburg, PA; ethanol from the U.S. Industrial Chemical Co., New York; [^{3}H]BP (20–40 Ci/mmole) from Amersham/Searle, Arlington Heights, IL; corn oil (Mazola brand), Englewood Cliffs, NJ; specially purified SDS and ammonium persulfate from Accurate Chemical Co., Hicksville, NY; acrylamide, bis-*N,N*-methylene acrylamide,*N,N,N′,N′*-tetramethylenediamine and Coomassie brilliant blue R-250 from the Eastman Kodak Co., Rochester, NY; and t.l.c. plates (Brinkmann silica gel NH-R) from Brinkmann Instruments, Westbury, NY.

Pretreatment of mice. Four week-old male mice were purchased from the Jackson Laboratory, Bar Harbor, ME. The mice were maintained on Wayne Labblox diet and water *ad lib.* in the animal room at the Medical University of South Carolina 1 week before being treated. Groups of three mice of each strain were injected i.p., 24 hr before killing, with 3-MC, α-NF, or β-NF (4 mg/ml corn oil) in doses of 2 mg/100 g body weight; control mice were injected i.p. with corn oil only. Other groups of five mice of each strain were injected i.p. once daily for 4 days with PB (16 mg/ml saline) in doses of 8 mg/100 g body weight; control mice were injected with saline only.

Preparation of microsomes. Livers from each group of mice were rinsed with, and minced in, cold TKM–sucrose buffer (2 ml/g wet tissue). The homogenates were centrifuged at 10,000 *g* for 20 min, and the supernatant fractions were then centrifuged at 100,000 *g* for 60 min. The pellets from the 100,000 *g* centrifugation were resuspended in TKM–sucrose buffer (equivalent to 1 g wet tissue/ml). Protein concentrations were determined by the method of Lowry *et al.* [27], with bovine serum albumin as standard. Microsomal suspensions were then diluted to 1 mg/ml and 4 mg/ml in TKM–sucrose buffer.

Metabolism of BP. [^{3}H]BP (sp. act. 20–40 Ci/mmole) was purified by t.l.c. on Brinkmann silica gel NH-R t.l.c. plates, using benzene as the ascending solvent; the R_f of [^{3}H]BP was 0.73. The t.l.c. band containing [^{3}H]BP was extracted with benzene; the purified [^{3}H]BP was used within 1 week of purification. For detection of low-yield BP metabolites with a radioscanner, [^{3}H]BP was used without dilution with unlabeled BP. Freshly prepared microsomes were used in the incubation system for metabolism of BP. Each incubation mixture of 2 ml total volume in citrate buffer contained 0.5 mg microsomal proteins (in 0.5 ml TKM–sucrose buffer), 10 nmoles [^{3}H]BP (in 50 μl acetone), and 2.4 μmoles NADPH (in 50 μl citrate buffer). Incubation was initiated by the addition of NADPH solution and was carried out at 37° for 3 min.; duplicate incubation mixtures were used for each sample. The incubation mixture for background counts contained all the ingredients except microsomes and was incubated in the same manner as the other samples. At the end of incubation, each mixture was extracted immediately with 4 ml ethyl acetate; after centrifugation and removal of the organic extract, the aqueous phase was extracted again with 4 ml ethyl acetate. The two organic extracts were combined, and a 10-μl aliquot was taken to measure the amounts of [^{3}H]BP and its metabolites extracted by ethyl acetate. The rest of the organic extract was dried under nitrogen, and the dried residue was redissolved in 50 μl of a 1:1 mixture of acetone–ethyl acetate and spotted on two Brinkmann silica gel NH-R plates. Non-radioactive standards of BP derivatives were co-chromatographed with each sample. One of the plates was chromatographed with benzene only for the separation of 4,5-epoxides; the other plate was chromatographed with benzene–ethanol (92.5:7.5) for the separation of quinones, phenols, diols, and other polar metabolites of BP. All above procedures were carried out under yellow light. The resulting radiochromatograms were examined under u.v. light for fluorescent spots, and were scanned with a Varian–Berthold scanner. BP metabolites were quantified by counting, in scintillation solution, the cut segments of t.l.c. plates containing individual metabolites that had been detected by radioscanning and fluorescence under u.v. light. Details of the identification procedures for BP metabolites have been published previously [10, 14, 28].

SDS–PAGE of microsomes. One-tenth of a milliliter of diluted microsomes (protein, 4 mg/ml) was treated with 0.7 ml of extraction buffer, and the mixture was heated at 100° for 2 min. Molecular weight marker solution was prepared in the extraction buffer, and every milliliter of the solution contained 0.125 mg each of bovine serum albumin, bovine liver catalase, bovine liver glutamate dehydrogenase, and ovalbumin. Electrophoresis was performed with procedures described by Laemmli [29]. A slab gel apparatus (Bio-Rad model 220, Bio-Rad Co., Richmond, CA) was used. The gels were 0.75 mm thick. The separating gel (10 × 14 cm) contained 7.5% acrylamide, and the stacking gel contained 4.5% acrylamide with twenty sample wells. Ten microliters of the solubilized microsomes (0.5 mg/ml) or protein standards (0.125 mg/ml) was loaded into each sample well. Electrophoresis was carried out for two gels at

Table 1. Effect of pretreatment of mice on the patterns of BP metabolites produced in liver microsomal incubation systems*

Strain	Pretreatment	Metabolite							
		Polar†	9,10-Diol	7,8-Diol	4,5-Diol	Phenols‡	Quinones§	4,5-Oxide	Total
B6	Corn oil	19 ± 2	38 ± 6	18 ± 3	11 ± 4	181 ± 33	118 ± 20	12 ± 4	396 ± 18
	Saline	22 ± 2	34 ± 1	17 ± 4	11 ± 2	168 ± 23	117 ± 55	12 ± 4	381 ± 38
	α-NF	18 ± 3	31 ± 11	15 ± 2	10 ± 3	170 ± 39	107 ± 32	12 ± 4	362 ± 40
	PB	67 ± 9	87 ± 6	51 ± 27	29 ± 8	355 ± 46	313 ± 157	32 ± 12	936 ± 113
	β-NF	44 ± 10	66 ± 21	125 ± 40	35 ± 13	506 ± 81	356 ± 111	19 ± 11	1151 ± 120
	3-MC	62 ± 5	72 ± 24	197 ± 6	38 ± 9	1033 ± 51	513 ± 199	30 ± 19	1943 ± 249
C3H	Corn oil	16 ± 7	12 ± 5	11 ± 4	14 ± 8	126 ± 21	60 ± 27	11 ± 5	251 ± 73
	Saline	19 ± 4	16 ± 3	12 ± 1	13 ± 5	133 ± 18	75 ± 33	12 ± 5	280 ± 64
	α-NF	19 ± 4	16 ± 5	13 ± 3	14 ± 6	132 ± 30	75 ± 37	13 ± 5	282 ± 86
	PB	62 ± 17	45 ± 5	41 ± 16	29 ± 9	335 ± 168	249 ± 144	30 ± 15	791 ± 28
	β-NF	29 ± 13	35 ± 15	49 ± 12	22 ± 10	259 ± 61	193 ± 83	30 ± 13	617 ± 149
	3-MC	40 ± 15	41 ± 16	109 ± 42	32 ± 9	595 ± 251	243 ± 103	36 ± 13	1096 ± 377
D2	Corn oil	18 ± 2	13 ± 2	13 ± 3	17 ± 6	127 ± 32	82 ± 26	14 ± 8	284 ± 37
	Saline	20 ± 6	13 ± 2	12 ± 1	17 ± 6	129 ± 36	103 ± 37	14 ± 8	307 ± 72
	α-NF	15 ± 5	15 ± 5	12 ± 2	15 ± 8	112 ± 25	66 ± 7	13 ± 7	248 ± 38
	PB	71 ± 20	54 ± 27	46 ± 15	30 ± 9	317 ± 156	225 ± 41	39 ± 2	782 ± 168
	β-NF	17 ± 2	12 ± 4	12 ± 3	17 ± 5	130 ± 31	65 ± 11	13 ± 9	266 ± 31
	3-MC	17 ± 5	12 ± 1	12 ± 1	16 ± 7	119 ± 23	82 ± 27	14 ± 9	272 ± 38

* Mean yields ± S.D. of benzo[a]pyrene metabolites of three experiments. Ethyl acetate-extractable metabolites are expressed as pmoles·(min)$^{-1}$·(mg protein)$^{-1}$.

† Polar metabolites that did not migrate from origin of t.l.c.

‡ Mixtures of phenols (3-OH-, 6-OH-, and 9-OH-BP) used for co-chromatograph of samples.

§ Mixtures of 1,6-quinone, 3,6-quinone, and 4,5-quinone of BP.

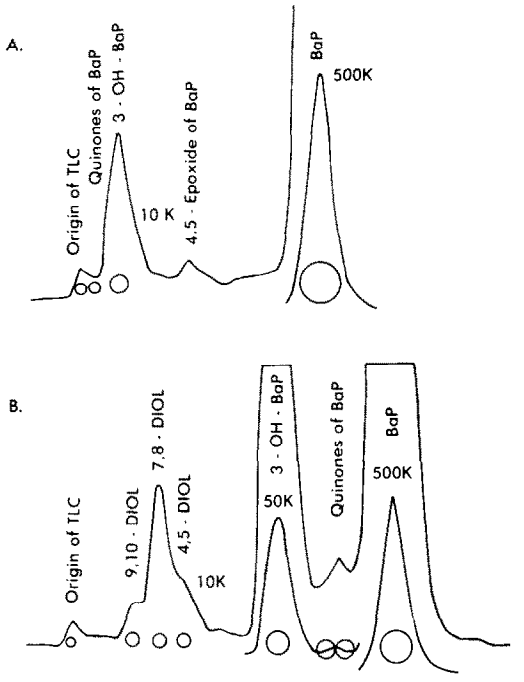

Fig. 1. Radioscans of t.l.c. plates. [^{3}H]BP and the metabolites generated by liver microsomes of B6 mice pretreated with 3-MC were separated with (A) benzene as ascending solvent, and (B) benzene–ethanol (92.5:7.5) as ascending solvent. The numbers 10K, 50K and 500K are the full-range scales of 10,000, 50,000 and 500,000 counts per sec used for scanning of peaks shown. The peak labeled 3-OH-BP also contained other phenols of BP. Quinones of BaP consisted of 1,6 quinone, 3,6-quinone, and 4,5-quinone.

20 mA/two gels and was terminated after about 6 hr when the tracking dye (bromophenol blue) was located about 1 cm above the bottom of the separating gels. The gels were fixed and stained with a solution containing 50% TCA and 0.1% Coomassie blue R-250 for 30 min, and then were destained with 7% acetic acid [29].

RESULTS

Figure 1 shows the radioscan tracing of a t.l.c. plate on which [^{3}H]BP and its metabolites that were produced by liver microsomes of B6 mice pretreated with 3-MC were separated. In Fig. 1A, the t.l.c. plate was chromatographed with benzene only to enable the separation of the 4,5-oxide from BP and its other metabolites. In Fig. 1B, the t.l.c. plate was chromatographed with solvent consisting of benzene–ethanol (92.5:7.5) as described above for the separation of quinones, phenols, diols, and other polar metabolites of BP; the 4,5-oxide could not be separated from BP after chromatography with the mixture of solvents. The circles in Fig. 1 indicate the fluorescent spots due to metabolites of [^{3}H]BP and to the unlabeled derivatives of BP used in the co-chromatography with each sample. The radioactive peak, indicated as 3-OH-BP, contained other phenols also (6-OH-BP and 9-OH-BP were also used as t.l.c. standards in this study).

Table 1 shows the mean yields of BP metabolites in three experiments. In each experiment, preparation of microsomes, metabolism of BP, and extraction of BP and its metabolites were carried out on the same day. The organic extracts were kept in a freezer overnight, and the t.l.c. of BP metabolites was carried out on the next day. Results in Table 1

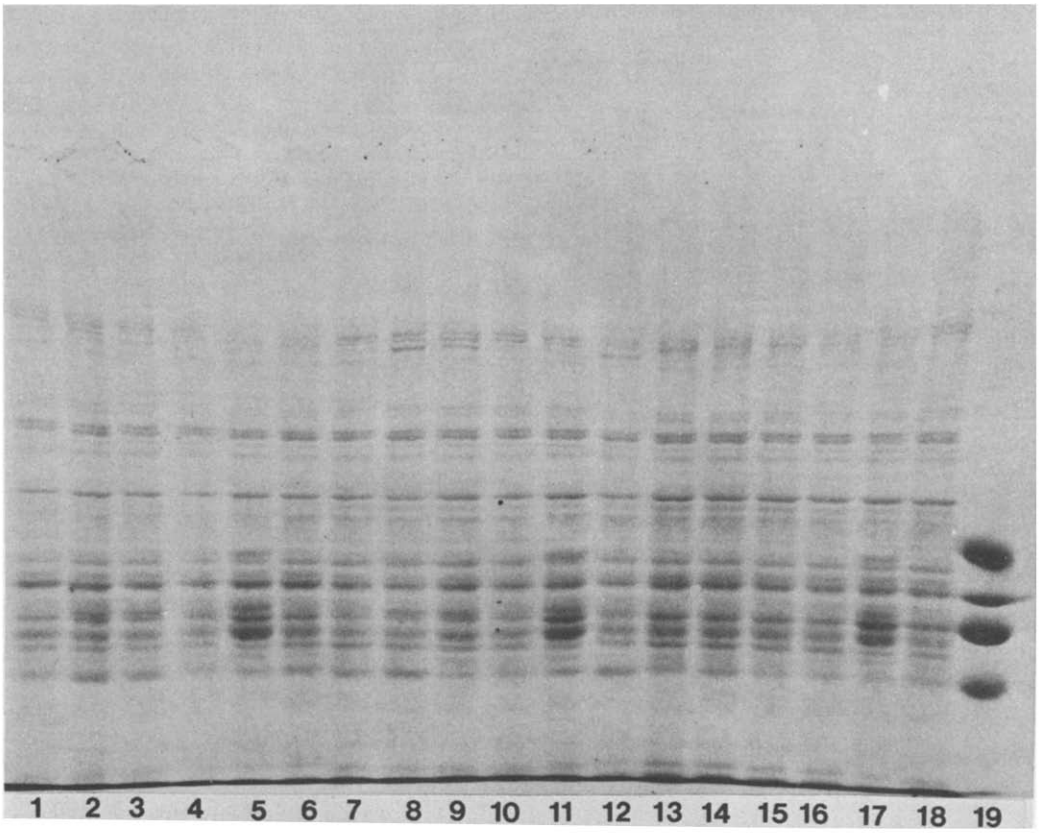

Fig. 2. Electrophoretogram of mouse liver microsomes. Positions 1–18 contained microsomes as follows: positions 1–6, B6 liver microsomes; positions 7–12, C3H liver microsomes; positions 13–18, D2 liver microsomes. Pretreatment of mice: corn oil (positions 1, 7, and 13); 3-MC (positions 2, 8, and 14); β-NF (positions 3, 9, and 15); α-NF (positions 4, 10, and 16); PB (positions 5, 11, and 17); and saline (positions 6, 12 and 18). Position 19: a mixture of 1.25 μg each of bovine serum albumin, catalase, glutamate dehydrogenase, and ovalbumin. Details of the electrophoresis are described in Materials and Methods.

demonstrate the following. First, there were notable differences in yields of BP metabolites produced by liver microsomes of different batches of mice of identical strain. The profiles of BP metabolites, however, were essentially similar for the particular strain of mice used in these experiments. Second, in terms of the total yield of BP metabolites extracted by ethyl acetate, the liver microsomes of control B6 mice metabolized about 30 per cent more BP than did control C3H and D2 mice. When comparing individual BP metabolities produced by liver microsomes of control mice, yields of polar metabolites of BP were about the same in all three strains of mice; the 9,10-diol yield by B6 mice was about 2.5-fold that of C3H and D2 mice; the 7,8-diol yield by B6 mice was about 40 per cent greater than the yields of C3H and D2 mice; D2 mice showed a slightly higher yield of the 4,5-diol than B6 and C3H mice; the yields of phenols and quinones in B6 mice were 30–70 per cent greater than those of D2 and C3H mice; the yields of the 4,5-oxide were about the same in the control mice of all three strains. Third, under the incubation conditions used in this study, the yields of phenols always were highest of the BP metabolites in each sample, followed by the yields of quinones, which might have been formed by air oxidation of phenols or by the action of microsomal enzymes. Fourth, pretreatment with α-NF had no apparent effect on the metabolism of BP by liver microsomal enzymes of all three strains of mice. Fifth, among BP-pretreated mice, BP metabolite profiles were quite similar in all three strains of mice. B6 mice, however, showed about a 20 per cent higher total-metabolite yield than C3H and D2 mice. In BP-pretreated B6 mice, the total-metabolite yield was about 2.5-fold that of control B6 mice; total metabolite yields of C3H and D2 mice pretreated with PB were about 3-fold those of corresponding control mice. In each strain of mice pretreated with PB, the yield of the 9,10-diol was the highest among the three diols, while yield of 4,5-diol was the lowest. In general, yields of BP metabilites produced by PB-pretreated mice were 2- to 3-fold those of the corresponding control mice. Sixth, at the dosage used in this study, neither 3-MC nor β-NF pretreatment showed any apparent effect on the BP metabolite profiles or on the yields produced by liver microsomes of D2 mice. Seventh, pretreatment with 3-MC caused large increases in yields of all BP metabolites produced by B6 and C3H mouse liver microsomes, whereas β-NF pretreatment caused smaller increases. The yield of total BP metabolites

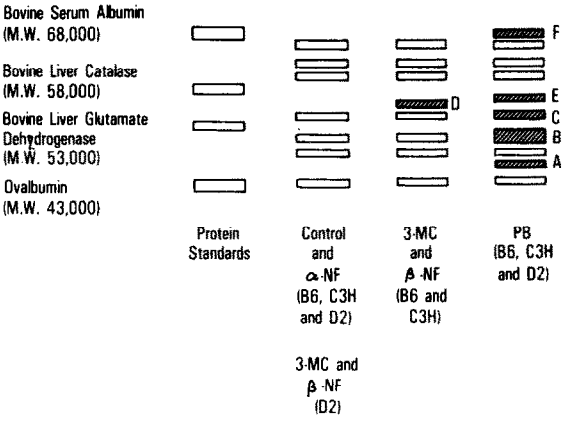

Fig. 3. Schematic diagram of protein bands between molecular weights 43,000 and 68,000, as shown in Fig. 2. The four bands in the first column from the left are those of protein standards (position 19 of Fig. 2). The seven protein bands in the second column are those of control mice (positions 1, 6, 7, 12, 13, and 18 of Fig. 2), of mice pretreated with α-NF (positions 4, 10, and 16 of Fig. 2), and of D2 mice pretreated with 3-MC or β-NF (positions 14 and 15 of Fig. 2). The eight bands in the third column are those of B6 and C3H mice pretreated with 3-MC or β-NF (positions 2, 3, 8, and 9 of Fig. 2). The ten protein bands in the last column are those of B6, C3H and D2 mice pretreated wth PB (positions 5, 11, and 17 of Fig. 2). Bands A through F are either new protein bands or bands having higher intensities compared to those of control mice.

produced by 3-MC-pretreated B6 mice was 5-fold that of control mice, and that catalyzed by β-NF-pretreated B6 mice was 3-fold that of the control B6 mice. The yield of total BP metabolites produced by 3-MC-pretreated C3H mice was 4-fold that of control C3H mice, and that produced by β-NF-pretreated C3H mice was 2.5-fold that of control C3H mice. The largest increase was in the yield of the 7,8-diol (about 11-fold for B6 mice pretreated with 3-MC, about 7-fold for B6 mice pretreated with β-NF, about 9-fold for C3H mice pretreated with 3-MC, and about 4-fold for C3H mice pretreated with β-NF). In B6 and C3H mice, pretreatment with 3-MC or β-NF was followed by 2- to 5-fold increases in other BP metabolites when compared with those of the corresponding control mice.

Figure 2 shows the protein band patterns of liver microsomes after electrophoresis on SDS–polyacrylamide gel. There were distinct differences in intensities of microsomal protein bands located at positions 2, 3, 5, 8, 9, 11 and 17 of the gel. Figure 3 is the schematic drawing for microsomal protein bands between molecular weight 43,000 and 68,000 as shown in Fig. 2. The intensities of bands A, B, C, and F of liver microsomes from PB-pretreated mice are heavier than those of the corresponding bands of liver microsomes of all control mice. Band E seems to be a new band. Pretreatment with 3-MC or β-NF caused the appearance of band D in liver microsomes of B6 and C3H mice but not in D2 mice, whereas pretreatment with PB had the same effect on liver microsomes of all three strains of mice. Band E shows slightly slower mobility on the gel compared to band D. Pretreatment with α-NF did not cause apparent change in microsomal protein band intensities compared to those of the control mice.

DISCUSSION

In this report, we have demonstrated that pretreating B6, C3H, and D2 mice with 3-MC, β-NF, α-NF, or PB had different effects on their liver microsomal protein band patterns separated by electrophoresis on SDS–polyacrylamide gel, as well as on patterns of BP metabolites generated by these liver microsomes. We used a low substrate concentration (5 nmoles/ml) in our incubation system for BP metabolism, and the incubation was carried out for only 3 min. Holder *et al.* [30] reported the dependence of BP metabolite profile on the substrate concentration. BP is a low-level contaminant in our environment; therefore, low substrate concentrations in the *in vitro* metabolism system may simulate the *in vivo* conditions better than the saturating substrate concentrations. In an earlier study [28], we reported the effects of BP pretreatment on the patterns of BP metabolites produced by liver microsomes of B6, C3H, D2, B6D2F$_1$, and C3HD2F$_1$ mice. Results in the earlier study and in this study indicated that BP and 3-MC had a similar effects on the pattern of BP metabolites produced by liver microsomes of B6, C3H, and D2 mice. Yields of BP metabolites produced by control mice in the previous study were twice as high as in this study. One of the contributing factors for the difference might be the difference in diet (laboratory diet by Foodstuffs Processing Co., Berkeley, CA, was used in the previous study, whereas Wayne Labblox was used in this study). Other laboratories have reported that 3-MC or β-NF induces AHH activity (measured by yield of 3-OH-BP) in liver microsomes of B6 and C3H mice but not in livers of D2 mice [21–25], whereas pretreatment with PB induces AHH activity to similar levels in livers of B6 and D2 mice [21]. Inducibility of AHH in livers of B6, C3H and D2 mice was reported to be correlated with the susceptibility to 3-MC- and BP-induced tumors in skin [31]. In this study, yields of all BP metabolites were increased in cases where liver microsomes of B6 and C3H mice pretreated with 3-MC or β-NF were used as enzyme sources. But there was a preferential increase in the yield of the 7,8-diol, as was reported by Holder *et al.* on B6 mice pretreated with 3-MC [30]. The 7,8-diol can be further activated metabolically to bind DNA *in vitro* [14]. It can be further metabolized to the 7,8-diol-9,10-epoxide [32], which has been proposed as the ultimate carcinogenic form of BP [15]. Therefore, the preferential increase in the yield of the 7,8-diol would indicate higher risk to the carcinogenic action of BP. The yield of the 4,5-oxide, which binds DNA without the need of further activation [10], was increased also. The 4,5-oxide, however, has been reported to be less carcinogenic than the 7,8-diol [33]. Our data on BP metabolite yields are also consistent with another report [34] that 3-MC is a more potent inducer of AHH than β-NF.

It was shown that pretreatment with β-NF caused *de novo* synthesis of microsomal proteins in B6 liver [35]. Our results with SDS–PAGE have also dem-

onstrated that pretreatment with 3-MC or β-NF led to the appearance of a new protein band (band D in Figs. 2 and 3) in liver microsomes of B6 and C3H mice, but not in D2 mice. The apparent molecular weight of protein at the position of band D was about the same as that of band 4 (55,000) reported by Haugen *et al.* [35]. Negishi and Nebert [36] reported isolating two inducible cytochrome(s) P-450 from liver microsomes of B6 mice pretreated with 3-MC, and that the molecular weight of these two forms was about 55,000, judged by SDS–PAGE. Therefore, band D in the present study may very likely contain the induced forms of cytochrome(s) P-450.

Although α-NF exhibited an inhibitory effect *in vitro* on AHH activity [37], pretreatment with α-NF did not show an apparent effect on either liver microsomal protein bands or on yields of BP metabolites produced by the corresponding liver microsomes of any of the three strains of mice.

PB pretreatment induced AHH activity to the same extent [21] and resulted in similar liver microsomal protein bands for both B6 and D2 mice [35]. Our results also showed that, in livers of all three strains of mice, PB pretreatment caused 2- to 3-fold increases in the yields of BP metabolites—the BP metabolite patterns were about the same in all three strains of mice. There was no apparent difference in the liver microsomal protein band patterns (as shown on gels after SDS–PAGE) for all three strains of mice. A new protein band was evident (band E of Figs. 2 and 3), which seems to have slightly slower electrophoretic mobility than band D of liver microsomes from B6 and C3H mice pretreated with 3-MC or β-NF. PB pretreatment also caused an increase in the intensities of several other protein bands: (bands A, B. C, and F of Figs. 2 and 3). Bands B and C seem to correspond, respectively, to bands 1 and 2 reported by Haugen *et al.* [35]. Band F had an electrophoretic mobility similar to that of the NADPH cytochrome P-450 reductase purified from liver microsomes of B6 mice pretreated with PB (unpublished results). Protein bands A, B, C, and E may contain different forms of cytochrome(s) P-450 or other enzymes (e.g. epoxide hydrase) induced by PB pretreatment. Huang *et al.* [38] reported the purification of two major forms of cytochrome P-450, A_2 and C_2 (mol. wt 50,000 and 56,000, respectively, on polyacrylamide gel), from $B6D2F_1$ mice pretreated with PB. Our band E may contain form C_2, reported by Huang *et al.* [38]; however, purified forms of liver microsomal cytochrome P-450 from the three strains of mice used in this study are needed for more definitive identification of the protein bands shown in Fig. 2. The purification of multiple forms of mouse liver microsomal cytochrome P-450 is in progress in our laboratory.

This report demonstrates a good correlation between mouse liver microsomal protein intensities (in the molecular weight range of 43,000–68,000) on polyacrylamide gels after electrophoresis and the patterns of BP metabolites produced by the corresponding liver microsomes: the appearance of band D in liver microsomes of B6 and C3H mice pretreated with 3-MC or β-NF corresponded to the preferential increase in the yield of the 7,8-diol among the increases of other metabolites; the similarity of BP metabolite yields and patterns among all control mice, mice pretreated with α-NF, and D2 mice pretreated with 3-MC or β-NF corresponded to the similarities between the liver microsomal protein bands of these microsomes; the mice of all three strains pretreated with PB showed similar BP metabolite yields and patterns corresponding to similar liver microsomal protein bands. However, purified forms of cytochrome(s) P-450 are needed for more definitive identifications of specific forms of cytochrome present in various tissues.

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