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The Formation of 3-Methylcholanthrene-Initiated Lung Tumors Correlates with Induction of Cytochrome P450IA1 by the Carcinogen in Fetal but Not Adult Mice[1]

MARK STEVEN MILLER,*,[2] ANN B. JONES,* SANG S. PARK,† AND LUCY M. ANDERSON*

*Perinatal Carcinogenesis Section, *Laboratory of Comparative Carcinogenesis, and †Laboratory of Molecular Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701-1013*

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The Formation of 3-Methylcholanthrene-Initiated Lung Tumors Correlates with Induction of Cytochrome P450IA1 by the Carcinogen in Fetal but Not Adult Mice. MILLER, M. S., JONES, A. B., PARK, S. S., AND ANDERSON, L. M. (1990). *Toxicol. Appl. Pharmacol.* **104,** 235–245. The administration of 3-methylcholanthrene (MC) to pregnant mice results in the formation of lung tumors in the offspring. Previous work has shown that fetuses demonstrating inducibility of aryl hydrocarbon metabolism develop two to five times more lung tumors than induction-nonresponsive littermates. In this study, the effects of fetal versus adult MC exposure were compared with regard to both induction of aryl hydrocarbon hydroxylase activity (AHH) in lung and dependence of lung tumorigenesis on the Ah genotype. In inducible (C57BL/6 × DBA/2)F1 fetal lung supernatants, a single ip injection of 100 mg/kg of MC to the mothers resulted in a maximal 50-fold induction of AHH activity by 8 hr, which persisted for 48 hr. The enzyme data agreed well with RNA blot analysis, as MC caused maximal induction of P450IA1 RNA by 4 hr. For comparison, adult (F1 × DBA/2) mice were given three weekly injections of 100 mg/kg MC and tumor incidences were determined after 16 weeks. No differences were observed between responsive and nonresponsive mice of either sex in the number of mice bearing lung tumors, nor did the tumor multiplicity differ between responsive and nonresponsive males. However, noninducible female mice had a significantly higher tumor multiplicity than their inducible counterparts ($p < 0.025$). Single ip injections of MC to adult F1 mice revealed that lung AHH activity was increased only 4- to 7-fold in the adult animal compared to the large fetal induction ratio. The difference in the magnitude of induction was due to the higher constitutive levels of AHH activity seen in adult tissue (4- to 14-fold greater than maximal basal fetal levels), as fetal and adult supernatants showed similar levels of induced activity following MC treatment. These results suggest that the correlation between susceptibility to MC-initiated lung tumors and induction of cytochrome P450IA1 is a unique property of the fetus and may be due, in part, to the low basal levels of fetal activating enzymes and their high induction ratio during the fetal period.

The *in vivo* exposure of experimental animals and humans to certain classes of chemical carcinogens results in the induction of specific isoforms of the cytochrome P450 enzyme system (Conney, 1967; Miller, 1978; Gonzalez, 1988). One of the most widely investigated carcinogen/P450 interactions is the induction by polycyclic aromatic hydrocarbons (PAH)[3] of cytochromes P450IA1

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[2] To whom reprint requests should be addressed.

[3] Abbreviations used: AHH, aryl hydrocarbon hydroxylase; BP, benzo[*a*]pyrene; DEPC, diethyl pyrocarbonate; 3-OH-BP, 3-hydroxybenzo[*a*]pyrene; MAb, monoclonal antibody; MC, 3-methylcholanthrene; PAH, polycyclic aromatic hydrocarbon; 1× SSC, 0.15 M NaCl/0.015 M Na citrate.

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and P450IA2[4] (Nebert *et al.*, 1981; Pelkonen and Nebert, 1982; Conney, 1982; Nebert and Jones, 1989). Studies on the induction of cytochrome P450IA1 by PAH have been aided by the development of mouse strains which differ genetically in the inducibility of the P450IA1 gene locus (Gielen *et al.*, 1972; Thomas *et al.*, 1972). Induction by PAH is apparently mediated by the product of the regulatory Ah gene locus, which codes for a receptor protein that binds PAH and then enhances the transcription rate of the P450IA1 gene (reviewed in Nebert *et al.*, 1981; Whitlock, 1987; Nebert and Jones, 1989). C57Bl/6 mice are genotypically inducible, or responsive (Ah^b Ah^b) to PAH-mediated induction, while the DBA/2 strain is genotypically noninducible, or nonresponsive (Ah^d Ah^d), due to the presence of a low-affinity binding form of the Ah receptor (Okey *et al.*, 1989). A cross between these two strains results in offspring that are phenotypically inducible and contain the hybrid genotype (Ah^b Ah^d), thus demonstrating that the Ah receptor gene segregates as an autosomal dominant trait (Gielen *et al.*, 1972; Thomas *et al.*, 1972). A further backcross between the inducible F1 hybrid offspring and the noninducible DBA strain will yield a second generation litter in which half the offspring are responsive and half are nonresponsive to P450IA1 inducing agents. Previous studies from this and other laboratories, using these mouse genetic models, have shown that transplacental exposure to PAH results in fetal toxicity, teratogenicity, and tumorigenicity in the offspring, the incidences of which are strongly influenced by the sensitivity of both maternal and fetal tissues to induction of AHH activity (Shum *et al.*, 1979; York and Manson, 1984; York *et al.*, 1984; Legraverend *et al.*, 1984; Anderson *et al.*, 1985, 1989; George and Manson, 1986). Administration of 3-methylcholanthrene (MC) as a single ip injection on the 17th day of gestation to backcrossed pregnant females resulted in a higher incidence of lung tumors in the responsive progeny of both sexes compared to their nonresponsive littermates (Anderson *et al.*, 1985, 1989). In addition, liver tumor incidence was somewhat higher in responsive than in nonresponsive male offspring following transplacental exposure to MC. PAHs do not cause liver tumors in adult mice. For both lung and liver tumors, offspring from noninducible mothers generally had higher tumor incidences than did the offspring from inducible mothers.

The transplacental experiments cited above suggest that drug-metabolizing enzymes may play an important role in modulating the tumorigenic effects of chemical carcinogenic agents in fetuses. Cytochrome P450-dependent monooxygenase activities have been demonstrated in a variety of animal species during the fetal period (reviewed in Pelkonen, 1985; Anderson *et al.*, 1989), and fetal liver AHH activity has been observed in several rodent species (Nebert and Gelboin, 1969). These results have been confirmed at the molecular level by the use of specific cDNA probes to the mouse P450IA1 and P450IA2 genes to determine expression of these genes in fetal mouse liver (Ikeda *et al.*, 1983; Kimura *et al.*, 1987; Miller *et al.*, 1989b). Thus, it appears that mouse fetal liver has the capacity to metabolize PAH and can respond to these agents by inducing the isozyme(s) of P450 responsible for PAH metabolism. Studies using liver preparations have suggested that fetal tissues show much lower levels of drug metabolic activity than adults, but have relatively high induction ratios. Little is known about the effects of PAHs in fetal lung tissue, a major target organ for MC-induced tumor initiation in both fetal and adult mice. Recent studies from this laboratory have shown that induction of AHH activity in responsive fetal lung tissue correlates with expression of P450IA1 RNA (Miller *et al.*, 1989b). In this report, the relative lung tumor incidences in backcrossed mice treated as adults with MC were compared with earlier studies (Anderson *et al.*, 1985, 1989) in which tumors were produced as a

[4] Cytochromes P450IA1 and P450IA2 are the accepted nomenclature for mouse P_1450 and P_3450, respectively (Nebert *et al.*, 1987).

result of transplacental MC exposure. The results show that the incidence of lung tumors in adult treated animals did not correlate positively with the relative responsivity of the animals to cytochrome P450 inducers, as seen after fetal exposure. Biochemical and molecular analysis suggest that the differing sensitivities of fetal and adult mice to MC-mediated tumorigenesis may be due to the marked differences in P450IA1 enzyme levels and induction ratios in fetal versus adult tissues.

MATERIALS AND METHODS

Chemicals. Olive oil, MC, NADPH, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, bovine serum albumin (fraction V), Ficoll, and polyvinylpyrrolidone were purchased from Sigma Chemical Co. (St. Louis, MO), guanidine thiocyanate was obtained from Bethesda Research Laboratories, (Gaithersburg, MD), formamide was purchased from Fluka Chemical Corp. (Hauppauge, NY), glyoxal was obtained from Fisher Scientific Co. (Springfield, NJ), dextran sulfate was purchased from 5 Prime → 3 Prime, Inc. (Paoli, PA), and [α-^{32}P]dCTP (~3000 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL).

Animals and treatment protocols. C57BL/6NCr female and DBA/2NCr male mice were obtained from the Animal Production Area of the Frederick Cancer Research Facility. The mice were housed in a pathogen-free environment in plastic cages with hardwood shavings as bedding and were allowed free access to food (NIH-31 Open Formula Autoclavable Diet) and tap water. A 12-hr fluorescent light/dark cycle was maintained. For studies involving transplacental exposure, B6 females were mated with D2 males and the pregnant mice were treated ip on the 17th day of gestation (Day 1 was considered the day when the vaginal plug was detected) with either olive oil alone (0.5 ml/35 g) or 100 mg/kg of MC dissolved in olive oil, as described in the previous tumorigenicity study (Anderson *et al.*, 1985). Mothers were euthanized at various times after injection and the fetuses removed and processed as described below. For determination of adult AHH activity, 6- to 9-month-old (C57BL/6 × DBA/2)F1 females were given a single ip injection of either olive oil alone or MC, as described above, and euthanized 8 to 48 hr after injection. Microsomal or 800*g* supernatant fractions were prepared as indicated below. For tumor studies, 6-week-old male and female backcrossed mice (F1 × DBA/2) were phenotyped with regard to induction responsiveness by the zoxazolamine sleeping-time test (Robinson and Nebert, 1974) by pretreatment with 150 mg/kg of β-naphthoflavone 48 hr prior to a 200 mg/kg dose of zoxazolamine. We have found that this treatment protocol unequivocally distinguishes inducible from noninducible animals. At 8 weeks they were given three weekly ip injections of 100 mg/kg of MC. The mice were euthanized 16 weeks after the last treatment and tumors were counted in 1-mm hard-sectioned slices of Bouin's fixed lungs with the use of a dissecting microscope as described previously (Anderson *et al.*, 1985, 1989). There were 16–18 mice of each sex and phenotype per group.

Preparation of microsomal and 800g supernatant fractions. Supernatants were prepared by a modification (Miller *et al.*, 1989b) of a previously described method (Jannetti and Anderson, 1981). Liver and lung tissues were removed and rinsed briefly in 0.1 M potassium phosphate buffer (pH 7.25). Fetal livers and lungs from the same litter were pooled. The tissues were homogenized in 0.1 M potassium phosphate buffer (pH 7.25) by 10 passes with a Teflon pestle. The homogenates were subjected to three cycles of freezing in a dry ice/ethanol bath and thawing in a room temperature water bath. Supernatant fractions were then isolated by centrifugation for 10 min at 800*g* at 4°C in a Sorvall RT6000 centrifuge. Microsomes were prepared as by Nebert (1978). Tissue fractions were stored at −80°C for 2–6 months without loss of activity.

Western blot analysis. The 800*g* supernatant fractions were prepared as described above and the proteins separated by SDS–PAGE (Laemmli, 1970) on an 8.5% gel. The proteins were then transferred from the gel to a nitrocellulose membrane filter by a modification of a previously described method (Towbin *et al.*, 1979), using the Trans-Blot Cell from Bio-Rad Laboratories (Richmond, CA). Transfer was accomplished by overnight electrophoresis at approximately 35 V in a buffer system containing 25 mM Tris/192 mM glycine/20% methanol (pH 8.3). MC-inducible protein was detected by overnight incubation with MAb 1-7-1, raised in the mouse against rat cytochrome P450IA1 (Park *et al.*, 1982), following incubation in a blocking solution consisting of 3% gelatin in Tris-buffered saline (20 mM Tris/500 mM NaCl, pH 7.5). Proteins which bound MAb 1-7-1 were visualized by incubating the membrane with goat anti-mouse IgG alkaline phosphatase conjugate (Bio-Rad) for 2 hr and then reacting with *p*-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt according to the manufacturer's instructions.

Determination of AHH activity. AHH activity was measured by a fluorescence assay using quinine sulfate as the standard (Nebert and Gelboin, 1968; Nebert, 1978). Protein content was determined by the method described by Lowry *et al.* (1951).

Isolation and characterization of total cellular RNA. RNA was purified as described by MacDonald *et al.* (1987). Tissues from the same litter were pooled. Fetal liver and lung tissues were removed and placed immediately in 8 ml of a cold 4 M guanidine thiocyanate/0.1 M Tris–HCl (pH 7.5)/1% 2-mercaptoethanol solution. The tissues were homogenized by a 15-sec burst with a Polytron homogenizer and then passed through an 18-gauge syringe needle six times to shear DNA and decrease the

viscosity of the solution. Sodium sarkosyl (*N*-lauroylsarcosine, sodium salt) was added to a final concentration of 0.5% and the homogenates were layered over a 6.1 M CsCl/25 mM NaAc (pH 5.2)/10 mM EDTA solution. RNA was collected by centrifugation for approximately 20 hr at 20°C in a Beckman SW41 rotor at 110,000*g*. The RNA pellets were redissolved in 0.5 ml of autoclaved, glass-distilled, DEPC-treated water and precipitated by centrifugation from a 70% ethanol/0.3 M NaAc (pH 5.2) solution. The pellets were rinsed once with 70% ethanol and once with absolute ethanol and air-dried. RNA was then dissolved in 10 mM sodium phosphate buffer (pH 7.0), denatured by treatment with glyoxal, and blotted onto a Biodyne A nylon membrane filter (Pall Ultrafine Filtration Corp., Glen Cove, NY) after fractionation by electrophoresis in a 1% agarose gel with constant recirculation of the 10 mM sodium phosphate (pH 7.0) buffer (Thomas, 1983). A duplicate set of lanes was stained with acridine orange to determine the relative levels of rRNA in each lane.

The blots were baked, prehybridized, and hybridized as described (Thomas, 1983). The mouse pmP_1450-3′ plasmid (Kimura *et al.*, 1984) was kindly provided by Dr. Daniel Nebert and contained a 3′-specific cDNA insert of the P450IA1 gene cloned into pBR322. The insert was isolated by digesting the plasmid with *Bgl*II/*Ssp*I to yield a 745-bp fragment that hybridized specifically to the IA1 RNA (Gonzalez *et al.*, 1985). The fragment was labeled by the random primer labeling technique (Feinberg and Vogelstein, 1983, 1984). Following hybridization, the blots were washed four times with 2× SSC/0.1% SDS at room temperature for 5–10 min followed by three washes with 0.1× SSC/0.1% SDS at 50°C for 15 min. The blots were wrapped in Saran-Wrap and autoradiographed in the presence of an intensifying screen (Cronex Lightning Plus, DuPont, Wilmington, DE) with preflashed Kodak X-Omat XAR-5 film at −80°C.

Statistical methods. For the tumorigenicity studies, differences between the means were determined by a paired *t* test. For the enzymology, differences between the means for a given treatment time were determined by Duncan's multiple comparison test (Steel and Torrie, 1980). Trends for time-dependent effects were first tested by Jonckheere's test for ordered alternatives and regression analysis (Hollander and Wolfe, 1973) followed by individual *t* testing when trends were significant, with correction for unequal variances (Snedecor and Cochran, 1967). Differences between samples were considered statistically significant at the $p = 0.05$ level.

RESULTS

Although treatment of backcrossed adult mice with MC caused the appearance of lung tumors, there were no differences in the number of tumor-bearing animals between responsive and nonresponsive mice of either sex (Table 1). While a similar lack of effect was observed for tumor multiplicity between male responsive and nonresponsive mice, noninducible female mice had a significantly higher lung tumor multiplicity than inducible females ($p < 0.025$). These results contrast with those obtained in transplacentally exposed mice, where a greater incidence and multiplicity of lung tumors was seen in responsive versus nonresponsive littermates (Anderson *et al.*, 1985, 1989).

TABLE 1

INCIDENCE OF 3-METHYLCHOLANTHRENE-INITIATED LUNG TUMORS IN [(C57BL/6 × DBA/2)F1 × DBA/2]BACKCROSSED ADULT MICE

	Percentage of mice with tumors	Average number of tumors per mouse in tumor-bearing mice
Males		
Responsive	13/16 (81%)	3.6 ± 2.8
Nonresponsive	13/18 (72%)	2.5 ± 2.6
Females		
Responsive	10/16 (62%)	1.3 ± 0.5[a]
Nonresponsive	9/19 (47%)	3.1 ± 2.3[a]

[a] Statistically significant difference, $p < 0.025$, using the two-tailed Student *t* test.

In order to delineate the biochemical basis for the observed biological differences between adult and fetal animals, F1 mice were treated with a single injection of MC and AHH activity was measured in the lung and liver at various times after injection. Because of the unusual properties of fetal tissues, 800*g* supernatants were prepared for determination of enzyme activity since fetal tissues have been shown to be somewhat resistant to tissue disruption by standard homogenization procedures (Ackermann *et al.*, 1972) and a portion of the microsomes in fetal tissues sediments at centrifugation speeds as low as 10,000*g* (Ackermann *et al.*, 1972; Cresteil *et al.*, 1979). Adult tissues were prepared in a similar manner and compared to microsomal preparations to be sure that the adult 800*g* supernatant tissue fraction accurately reflected oxidative microsomal metabolism.

TABLE 2

3-METHYLCHOLANTHRENE-MEDIATED INDUCTION OF LUNG ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN RESPONSIVE B6D2F1 MICE

	AHH activity[a]		
Treatment	8 hr	24 hr	48 hr
Fetus (800*g*)[b]			
Oil	<0.1	<0.1	0.24 ± 0.13
MC[c]	4.96 ± 3.15	2.58 ± 0.90	6.00 ± 2.70
Adult (800*g*)			
Oil	2.65 ± 1.73	1.35 ± 0.32	0.95 ± 0.17
MC[c]	6.45 ± 2.08	4.32 ± 0.86	6.52 ± 0.91

[a] AHH activity is expressed as pmol of 3-OH-BP formed/min/mg protein and the values represent the mean ± SD of at least three determinations.

[b] Fetal enzymatic data are from a previous publication (Miller *et al.*, 1989b).

[c] MC administered as a single 100 mg/kg ip injection.

In fetal and adult lung tissues, AHH activity was maximally induced by 8 hr postinjection and remained elevated for at least 48 hr, whereas adult liver tissue reached maximal levels by 48 hr (Tables 2 and 3). The differences between the induced fetal values at the various time points were not statistically significant, whereas the difference between induced adult liver values at 24 vs 48 hr were significant at the $p < 0.01$ level. Fetal lung AHH activity was increased 25- to 50-fold after treatment with MC (Table 2). In agreement with the enzyme data, MC caused a rapid, early induction of fetal lung P450IA1 RNA by 4 hr (Fig. 1A). In addition, in accordance with the low levels of constitutive AHH activity detected in fetal lung tissue, hybridization with the P450IA1-specific probe gave no detectable signal in total RNA prepared from oil-treated mice, in agreement with previous data which demonstrated the lack of a detectable signal 8 to 48 hr after administration of the olive oil vehicle (Miller *et al.*, 1989b). Thus, the results obtained by RNA blot analysis agreed well with functional enzyme data. Although there were some differences in the amount of RNA loaded in the gel lanes, these differences could not account for the large induction of RNA at 4 hr relative to the other time points (Fig. 1B).

Basal levels of fetal lung AHH activity were barely detectable on Gestation Days 17 (8 hr postinjection) and 18 (24 hr postinjection) and were still rather low on the 19th day (48 hr postinjection). Basal adult levels in 800*g* lung supernatants were at least 4-fold higher than the corresponding fetal levels. Following treatment with MC, fetal lung supernatants demonstrated a rather high induction ratio for AHH activity (25- to 50-fold) compared to adult lung supernatants (2- to 7-fold). However, the absolute values for induced AHH activity in the lung of adult and fetal mice were very similar, demonstrating that the high induction ratios seen in fetal lung tissue were due to the correspondingly low basal levels observed in the fetus. As expected, adult lung microsomal preparations had much higher enzymatic turnover values for AHH activity than the supernatant fractions, with MC treatment causing a 4-fold induction of microsomal AHH activity from 4.9 ± 0.9 to 19.2 ± 2.9 pmol/min/mg protein. However, the relative induction ratios for microsomal and 800*g* supernatants were quite

TABLE 3

3-METHYLCHOLANTHRENE-MEDIATED INDUCTION OF LIVER ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN RESPONSIVE B6D2F1 MICE

	AHH activity[a]		
Treatment	8 hr	24 hr	48 hr
Fetus (800*g*)[b]			
Oil	0.5 ± 0.1	0.7 ± 0.1	2.8 ± 0.4
MC[c]	20.5 ± 8.1	14.1 ± 4.3	15.4 ± 1.7
Adult (800*g*)			
Oil	111 ± 27	49 ± 17	106 ± 07
MC[c]	158 ± 29	164 ± 26	463 ± 90

[a] AHH activity is expressed as pmol of 3-OH-BP formed/min/mg protein and the values represent the mean ± SD of at least three determinations.

[b] Fetal enzymatic data are from a previous publication (Miller *et al.*, 1989b).

[c] MC administered as a single 100 mg/kg ip injection.

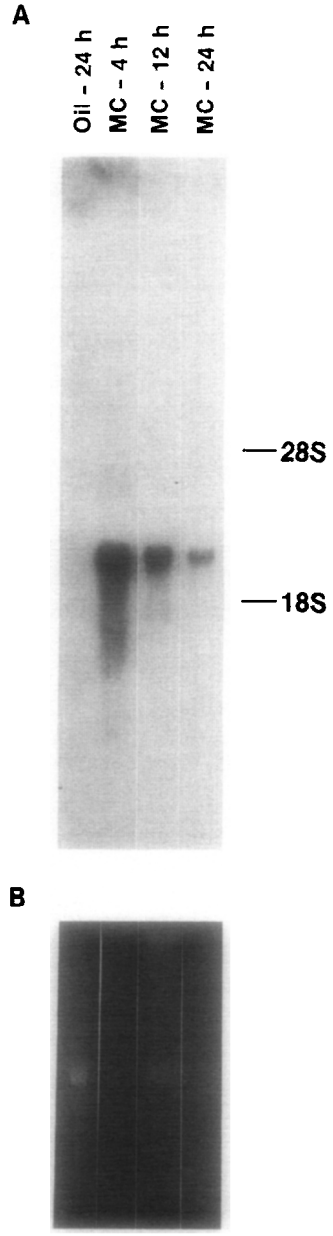

FIG. 1. Induction of P450IA1 total RNA in fetal lung tissue. Pregnant mothers were treated ip on Day 17 of gestation with either olive oil alone or 100 mg/kg of MC and euthanized at various times after injection. (A) Total RNA was isolated from fetal lung tissue and blotted onto nylon membrane filters, and the filters were hybridized with a 745-bp *Bgl*II/*Ssp*I fragment from the pmP$_1$450-3′ plasmid. The figure is a composite of individual lanes from the same autoradiograph. Exposure time = 24 hr. (B) A duplicate set of lanes was stained with acridine orange to visualize the rRNA bands.

similar (4- and 7-fold, respectively, 48 hr after injection of MC), demonstrating that the adult supernatant preparations accurately predicted the relative levels of microsomal monooxygenase activities. Similar results were obtained with adult liver preparations (see below). Western blot analysis of lung supernatant preparations from adult and fetal mice confirmed these results (Fig. 2). Constitutive levels of cytochrome P450IA1 were barely detectable in either adult or fetal lung preparations. Treatment with MC markedly increased the amount of protein cross-reactive to MAb 1-7-1 in both the adult and the fetus to similar levels. We have identified this 56.5-kDa band as cytochrome P450IA1 by comparison to adult MC-treated liver microsomes (data not shown). The faint lower molecular weight band seen in induced fetal samples is probably cytochrome P450IA2. A previous study by our laboratory has demonstrated the presence of low levels of P450IA2 RNA in fetal lungs following transplacental injection of MC (Miller *et al.*, 1989b). The faint higher molecular weight bands were not seen consistently in different blots and were probably due to nonspecific binding.

Since the liver is a major site of carcinogen metabolic activation/detoxication pathways and also differs in fetal versus adult mice with regard to susceptibility to PAH tumorigenesis, liver AHH activity was also examined (Table 3). As expected, both the basal and induced enzyme activities in the liver were much greater than the corresponding values in the lung, due to the relatively high concentration of metabolic enzymes found in the liver. As seen in lung tissue, liver AHH activity was generally induced to a greater extent relative to the basal level in fetal than in adult tissue. In the fetus, induction of AHH activity was again maximal 8 hr following administration of MC. From 8 to 24 hr after injection of MC, fetal liver AHH activity was induced 20- to 41-fold over constitutive levels compared to the 3-fold induction seen in adult liver supernatants. At 48 hr after injection of MC, the relative induction of fetal liver AHH activity was only about 5.5-fold. This decline

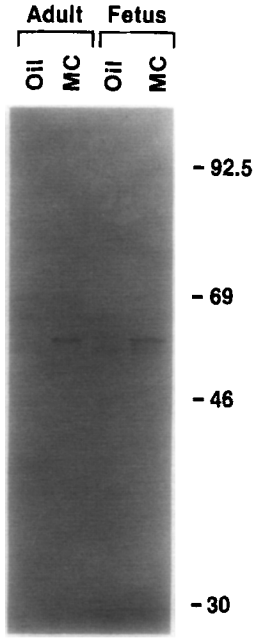

FIG. 2. Induction of P450s in the lung that are immunologically cross-reactive to MAb 1-7-1. Pregnant mothers were treated ip on Day 17 of gestation with either olive oil alone or 100 mg/kg of MC and euthanized 48 hr after injection. An 800*g* supernatant fraction was prepared from adult or fetal lung preparations. Ten micrograms of supernatant protein was fractionated by electrophoresis on an 8.5% polyacrylamide gel, transferred to nitrocellulose, and probed with MAb 1-7-1 as described under Materials and Methods. "Rainbow" prestained molecular weight markers (Amersham Corp., Arlington Heights, IL) were used to determine the relative molecular weight of the stained band.

in the induction ratio in the fetus was due to the marked increase in basal AHH activity observed between the 18th and 19th days of gestation, as the absolute turnover numbers for the induced samples were practically identical over this time period. In the adult, MC caused approximately a 4-fold induction of AHH activity 48 hr after injection in both supernatant and microsomal liver preparations. Adult liver activities in the supernatants were at least 12-fold higher than the corresponding values in fetal liver supernatants at all time points examined. At 48 hr after injection, adult microsomal activities were about 4-fold greater than the corresponding adult supernatant activities, with MC treatment inducing microsomal AHH activity from 421 ± 27 to 1501 ± 186 pmol/min/mg protein.

DISCUSSION

Several studies have shown that, following transplacental exposure to PAHs, the incidence of lung tumors in the exposed offspring is strongly influenced by the maternal and fetal phenotype for induction of cytochrome P450 by the Ah locus regulatory gene product (Shum *et al.*, 1979; York and Manson, 1984; York *et al.*, 1984; Legraverend *et al.*, 1984; Anderson *et al.*, 1985; George and Manson, 1986; Miller *et al.*, 1989b). Attempts to show a correlation of inducibility of AHH enzyme activity with relative lung tumor incidence following administration of MC has previously not been reported in adult animals. Earlier studies have demonstrated that the sc injection of MC into different genetic strains of mice resulted in the formation of sc sarcomas, the incidences of which correlated positively with the inducible phenotype of the strains studied (Kouri *et al.*, 1973a,b; Atlas *et al.*, 1976). In addition, a limited study by Kouri *et al.* (1980) has provided evidence suggesting that the intratracheal administration of MC causes a higher incidence of lung tumors in responsive mice than in nonresponsive strains. However, oral administration of BP (Nebert and Jensen, 1979) or the topical application of MC (Duran-Reynals *et al.*, 1978) resulted in a greater incidence of leukemia in the nonresponsive mice. These studies have suggested that the direct application of some PAH carcinogens to a particular target tissue may result in an increased tumor incidence in inducible strains of mice, presumably because of the increased activation of the proximate carcinogen to reactive electrophiles by the responsive target tissue. At more distal sites, however, the higher tumor incidence is more likely to occur in the nonresponsive strain, since the lower hepatic metabolic activity in noninducible animals allows more parent compound to reach these distal

sites. While this has been demonstrated for leukemia induction, few other studies exist that show differences in tumor incidence at other target tissues. This suggests that in induction responsive animals, the protective aspect of induced P450-mediated oxidative metabolism limits the parent PAH from mediating toxic damage, a hypothesis confirmed by the protective effect of the noncarcinogenic inducer, β-naphthoflavone, when given prior to MC in responsive mouse strains (Anderson and Seetharam, 1985). The protective effect was not observed in nonresponsive mice. Our present experiments found no differences in lung tumor incidence between responsive and nonresponsive mice following ip MC treatment. Only the nonresponsive females showed a significantly greater lung tumor multiplicity than their responsive female counterparts (Table 1), again a demonstration of the protective effects of metabolic enzymes for distal target organs.

The marked difference seen in the correlation of lung tumor incidence in fetal versus adult tissue to the inducibility phenotype of the individual may be partially explained by their very different biochemical properties. With the exception of induced lung tissue, the fetus generally has much lower levels of AHH activity than the adult. In the adult, the liver supernatants displayed enzyme activities that were 12- to 70-fold higher than those of comparably treated fetal liver supernatants. Constitutive adult lung values were 4- to 14-fold greater than constitutive fetal enzymatic activities. When treated with MC, however, adult and fetal enzymatic turnover numbers in lung were practically identical, as confirmed by Western blotting. This suggests that, in the fetal lung, gene regulatory mechanisms are already in place that allow full induction of cytochrome P450IA1 to adult levels. This is in contrast to the liver, where both constitutive and inducible enzyme activities were severalfold greater in adult tissues compared to the fetus. The 4- to 14-fold increases in constitutive levels of AHH activity may thus be due to the increased expression of other isozymic forms of cytochrome P450. Thus, at 24 and 48 hr postinjection, MC causes a 25-fold increase in the levels of AHH activity in the fetal lung. In the adult lung, the higher constitutive level of AHH activity is maximally increased only 7-fold by MC treatment while the absolute induced activity remains constant relative to fetal values. Clearly, the decreased relative inducibility of adult lung tissue is due to the increased expression of another enzyme that metabolizes BP but is resistant to induction by MC. Similar differences in the levels of AHH activity between liver and lung tissues and between adult and fetal animals have been demonstrated in previous studies (Nebert and Gelboin, 1969; Bürki, *et al.*, 1973a,b; Shum *et al.*, 1979; Jannetti and Anderson, 1981; Pelkonen, 1985; George and Manson, 1986; Anderson *et al.*, 1989; Miller *et al.*, 1989a,b).

Fetal and adult tissues display other interesting differences. Fetal and adult lung tissues attain maximal levels of enzyme activity by 8 hr following MC injection, whereas adult liver tissue reached its highest value 48 hr after exposure to MC. This was corroborated by the fact that maximal levels of P450IA1 RNA in the fetal lung were achieved by 4 hr (Fig. 1); similar results were obtained with the fetal liver (Miller *et al.*, 1989b). This is in contrast to the slow but steady rise in P450IA1 RNA accumulation seen in the adult liver which reached maximal levels 12–15 hr after administration of MC to mice or rats (Gonzalez *et al.*, 1984; Kawajiri *et al.*, 1984; Chen and Negishi, 1982; Bresnick *et al.*, 1981). Thus, following MC treatment fetal tissues demonstrate much higher induction ratios than adult tissues and, at least in the liver, attain their maximal enzymatic activities at earlier time points than adult hepatic tissue. It thus appears that, during the fetal period, responsive mice can boost their levels of AHH enzyme activity over that seen in nonresponsive mice to a much greater extent than is possible in the adult animal. This may account, in part, for the correlation in lung tumor incidence and P450 inducibility observed during the fetal period. The metabolic capacity of the liver may also play a role in

modulating the susceptibility of fetal and adult mice to MC-induced lung tumors. In the fetus, liver AHH activity was approximately 2.5 times that seen in the fetal lung, whereas the ratio of liver to lung AHH activity in the adult was 71.0. Adult livers thus have a much greater capacity to detoxify exogenous chemicals and may be able to eliminate or inactivate a large percentage of the total dose of MC before it reaches the adult lung. This could limit the amount of parent compound available both for activation to toxic intermediates and for induction of adult lung P450s. In contrast, the fetus will allow a much greater portion of the transplacental dose of MC to pass through the liver unchanged, leaving more available for activation to reactive electrophiles that can bind to and damage cellular macromolecules. A recent study by our laboratory has shown that the metabolic capacity of maternal livers can strongly influence the degree of tumor induction following transplacental exposure to MC (Miller *et al.*, 1989a). Thus, differences in metabolic capacity at both the target organ and the more distal sites, as well as differences in the pharmacokinetic distribution of MC between the various fetal and maternal compartments, may strongly influence the susceptibility of mice to chemically induced tumors during growth and development of the animal. The differences between adult and fetal animals to tumor initiation by MC is not well understood at this time. This could be due to other subtle differences in the biochemical composition of adult and fetal tissues, such as the relative levels of other constitutive forms of cytochrome P450, phase II enzymes, or DNA repair enzymes. A continued comparison of the carcinogenic, biochemical, and molecular consequences of chemical carcinogen exposure in adult and fetal tissues may provide further insight into the complex array of gene systems that impinge on the neoplastic process.

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