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Hepatic Glutathione Transferase Activity Induced by Polycyclic Aromatic Compounds

Lack of Correlation with the Murine *Ah* Locus

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SUMMARY

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Hepatic cytosolic glutathione transferase activity with 1-chloro-2,4-dinitrobenzene as substrate was induced by 3-methylcholanthrene or β-naphthoflavone in C57BL/6N inbred mice and in (C57BL/6N)(DBA/2N)F_1 but not in DBA/2N inbred mice. High doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induced the transferase activity in both C57BL/6N and DBA/2N mice. The glutathione transferase activity with benzo[*a*]pyrene 4,5-oxide as substrate was induced by 3-methylcholanthrene in C57BL/6N but not DBA/2N mice. The transferase activity with styrene 7,8-oxide as substrate was different from either of the above activities in that about twofold induction by 3-methylcholanthrene occurred in both C57BL/6N and DBA/2N mice. Among progeny from the (C57BL/6N)(DBA/2N)F_1 × DBA/2N backcross, however, no association was found between the transferase induction process by 3-methylcholanthrene and the presence of the *Ah* receptor [i.e., inducible aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase activity]. It is therefore concluded that induction of glutathione transferase activity by polycyclic aromatic compounds is mediated by a gene(s) distinct from the *Ah* regulatory genes. These data emphasize the importance of examining progeny from the appropriate backcross before making conclusions about the genetic linkage of any two expressed traits.

INTRODUCTION

The cytosolic glutathione transferases (EC 2.5.1.18) are enzymes of ubiquitous distribution in mammalian tissues and catalyze the nucleophilic attack by glutathione on a large number of electrophilic substrates differing widely in their chemical structures (reviewed in Ref. 1). From rat liver at least five forms have been obtained in a homogeneous state, as judged by mobility of a single electrophoretic band. Although each of the transferases can be recognized by its pattern of activity with different substrates, these enzymes are best described as possessing an overlapping substrate specificity among compounds as varied as 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, 1,2-epoxy-3-(*p*-nitrophenoxy)-propane, benzo[*a*]pyrene 4,5-oxide, styrene 7,8-oxide, ethacrynic acid, and menaphthyl sulfate.

The murine *Ah* locus controls the induction (by polycyclic aromatic compounds such as 3-methylcholanthrene, benzo[*a*]pyrene, β-naphthoflavone, or TCDD[1]) of numerous drug-metabolizing enzyme activities in the liver and in virtually all nonhepatic tissues examined (reviewed in Ref. 2). A cytosolic receptor highly specific for polycyclic aromatic inducers appears to be essential for the induction process. The livers of genetically "responsive" C57BL/6N mice (Ah^b/Ah^b) and (C57BL/6N)(DBA/2N)F_1 heterozygotes (Ah^b/Ah^d) have at least 50 times and 30 times, respectively, more functional receptor molecules than the livers of DBA/2N (Ah^d/Ah^d) and other genetically "nonresponsive" inbred strains (3). The cytosolic receptor is thus viewed as the major *Ah* regulatory gene product.

[1] Abbreviations used: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; B6, the C57BL/6N inbred mouse strain; D2, the DBA/2N inbred mouse strain.

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Several structural genes of the *Ah* complex are apparently turned on during the sequence of events following exposure of the animal to polycyclic aromatic inducers. Not only two or more forms of polycyclic aromatic-inducible microsomal cytochrome *P*-450 (with their associated monooxygenase activities), but also microsomal UDP glucuronosyltransferase (EC 2.4.1.17) and two cytosolic enzymes [reduced NAD(P):menadione oxidoreductase (EC 1.6.99.2) and ornithine decarboxylase (EC 4.1.1.17) activities] are induced, probably by means of the same receptor for polycyclic aromatic compounds (reviewed in Ref. 2).

In addition to the two inducible forms of *P*-450 that monooxygenate benzo[*a*]pyrene, these other inducible enzymes are viewed as metabolically coordinated in the breakdown of polycyclic hydrocarbons such as benzo[*a*]pyrene: The glucuronosyltransferase conjugates benzo[*a*]pyrene phenols and diols with glucuronic acid (4), and the oxidoreductase reduces benzo[*a*]pyrene quinones (5). Ornithine decarboxylase is known to increase during various subcellular processes requiring a certain amount of gene activation (6).

The question therefore arises as to how many other metabolically coordinated enzymes are 3-methylcholanthrene inducible and associated with the murine *Ah* locus. Microsomal epoxide hydrase (EC 4.2.1.63) is not inducible in mouse liver by 3-methylcholanthrene and was shown (7) not to be controlled by the *Ah* regulatory gene. Glutathione transferase also may be regarded as a metabolically coordinated enzyme because benzo[*a*]pyrene arene oxides are conjugated with glutathione by one or more of these enzymes (1). Mouse or rat liver, lung, kidney, or intestine glutathione transferase activities are known to be high (8, 9) and at least slightly inducible by 3-methylcholanthrene (10–13) or TCDD (14); other reports have concluded that the transferase is not statistically significantly inducible by 3-methylcholanthrene (15), TCDD (16), or β-naphthoflavone (12). The quantity of inducer given, the pharmacokinetics of inducer uptake, the kinetics of enzyme induction, and the choice of substrate for assaying the transferases, however, may explain the reported differences in these data. In this report we examine mouse liver for a possible correlation between polycyclic aromatic-inducible glutathione transferase activity and the Ah^b allele and show that there is no association.

MATERIALS AND METHODS

Materials and animals. All chemicals were purchased from sources cited previously (3, 17, 18). B6 and D2 inbred mice were obtained from the Veterinary Resources Branch, National Institutes of Health (Bethesda, Md.). Breeding to obtain responsive F_1 hybrids from the B6 × D2 cross and to obtain responsive heterozygotes and nonresponsive homozygotes from the B6D2F_1 × D2 backcross was carried out in the Developmental Pharmacology Branch mouse colony, National Institute of Child Health and Human Development, National Institutes of Health (Bethesda, Md.). Sexually immature weanlings of either sex (4 to 6 week olds) were always used. The rigid environmental conditions of the animal room and the diet of the mice have been recently described in detail (3).

Treatment of mice. 3-Methylcholanthrene or β-naphthoflavone in corn oil was administered as a single intraperitoneal dose (200 mg/kg). Controls received the vehicle corn oil alone (25 ml/kg). TCDD in *p*-dioxane was given intraperitoneally as a single dose (100 μg/kg). Phenobarbital in normal saline (0.85% NaCl) was given as intraperitoneal doses of 30 mg/kg on the first day, followed by 30 mg/kg twice about 12 h apart on the second, third, fourth, and fifth days. Zoxazolamine in corn oil was administered as a single intraperitoneal dose (250 mg/kg) to progeny of the B6D2F_1 × D2 backcross that had been treated 36 h previously with β-naphthoflavone; 2 weeks after the progeny had been phenotyped for the *Ah* regulatory gene by this paralysis test (19), the mice were then treated with various inducers and assessed for inducible enzyme activities.

Preparation of subcellular fractions. During kinetics of induction studies, two or three mouse livers per time point were combined and minced. The minced tissue was washed as free of blood as possible with 0.25 M potassium phosphate buffer, pH 7.4; 5 vol of buffer was used per vol of minced tissue. In other experiments, individual livers were minced and homogenized. The microsomal (20) and supernatant (17) fractions were prepared as described before. Samples frozen at −80°C retained enzymatic activities for at least 6 months. For the determinations with styrene 7,8-oxide and benzo[*a*]pyrene 4,5-oxide as substrates, these transferases were assayed in cytosolic samples that had been packaged with dry ice and shipped to the Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Enzyme assays. The hepatic cytosolic glutathione transferases were assayed with the substrates 1-chloro-2,4-dinitrobenzene (1.0 mM; 5.0 mM glutathione) (17), 1,2-dichloro-4-nitrobenzene (1.0 mM; 1.0 mM glutathione) (17), 1,2-epoxy-3-(*p*-nitrophenoxy)propane (5.0 mM; 5.0 mM glutathione) (21), uniformly labeled [^{14}C]styrene 7,8-oxide (1.0 mM; 5.0 mM glutathione) (9), and generally labeled [^{3}H]benzo[*a*]pyrene 4,5-oxide (0.01 mM; 5.0 mM glutathione) (22), as previously described. Kinetics with each substrate were studied to ensure linear product formation as a function of both incubation time and protein concentration. *One unit* of activity is defined as that amount of enzyme catalyzing at 37°C the formation of 1.0 nmol of glutathione-conjugated product in 1 min; *specific activity* denotes units per milligram of cytosolic protein.

Hepatic microsomal aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (EC 1.14.14.2) was assayed as described (20). *One unit* of activity is defined as that amount of enzyme catalyzing in 1 min at 37°C the formation of hydroxylated products causing fluorescence equivalent to that of 1.0 pmol of the 3-hydroxybenzo[*a*]pyrene recrystallized standard. *Specific activity* denotes units per milligram of microsomal protein.

RESULTS

Preliminary experiments. No sex differences in basal or inducible glutathione transferase activities were ob-

TABLE 1

Immunoprecipitable glutathione transferase activities from mouse liver cytosol in the presence of antibodies raised against highly purified transferases from rat liver

Each "+" indicates that at least 50% inhibition was obtained by the use of IgG preparations isolated from sera obtained from appropriately immunized animals; control samples with preimmune serum were concomitantly run. Each "–" indicates that more than 90% of the enzyme activity remained in the supernatant fraction after exposure to the antibody. Experimental procedures were identical to those already described (17, 25).

Substrate	Substrate concentration	Glutathione concentration	Antibodies to homogeneous forms of rat transferase		
			Anti-A	Anti-B	Anti-E
	mM	*mM*			
1-Chloro-2,4-dinitrobenzene	1.0	1.0	–	–	–
1,2-Dichloro-4-nitrobenzene	1.0	5.0	+	–	–
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	5.0	5.0	–	–	+
Bromosulfophthalein	0.03	5.0	–	–	–
trans-4-Phenyl-3-butene-2-one	0.05	0.25	+	–	–
Ethacrynic acid	0.2	0.25	–	–	–
4-Nitropyridine-*N*-oxide	0.2	5.0	+	–	–

served among sexually immature B6 or D2 mice. Basal and 3-methylcholanthrene-inducible glutathione transferase activities with 1-chloro-2,4-dinitrobenzene were highest between 3 and 6 weeks of age, and 60 to 85% of these highest values were present at 2 or 12 weeks of age. These developmental data are quite similar to those reported in rat liver (23). We therefore used interchangeably males and females that were sexually immature (between 4 and 6 weeks of age).

With each substrate, the optimal pH for measurement of glutathione transferase activity was established. The optimal pH values at which transferase substrates were assayed were similar to those used with the rat liver enzymes: 6.5 for 1-chloro-2,4-dinitrobenzene; 7.5 for 1,2-dichloro-4-nitrobenzene; 6.5 for 1,2-epoxy-3-(*p*-nitrophenoxy)propane; and 7.6 for both styrene 7,8-oxide and benzo[*a*]pyrene 4,5-oxide.

The antibody prepared against rat liver transferase A (17) cross-reacted with and immunoprecipitated the activity for three of seven substrates examined in mouse (Table 1). The antibody prepared against rat transferase E (24) immunoprecipitated only transferase activity with 1,2-epoxy-3-(*p*-nitrophenoxy)propane. The antibody prepared against rat transferase B (25) did not immunoprecipitate transferase activity with any of the substrates examined. Clearly the enzyme specificities of the mouse do not correspond to those of the rat.

Effects of microsomal enzyme inducers on several glutathione transferase activities. 3-Methylcholanthrene, β-naphthoflavone, and TCDD are known (3) to interact with the *Ah* receptor. The best induction of transferase activity by these agents (Fig. 1) occurred with 1-chloro-2,4-dinitrobenzene as substrate; less response was seen with 1,2-dichloro-4-nitrobenzene and little, if any, effect was found with 1,2-epoxy-3-(*p*-nitrophenoxy)propane. In D2 mice the lack of transferase induction

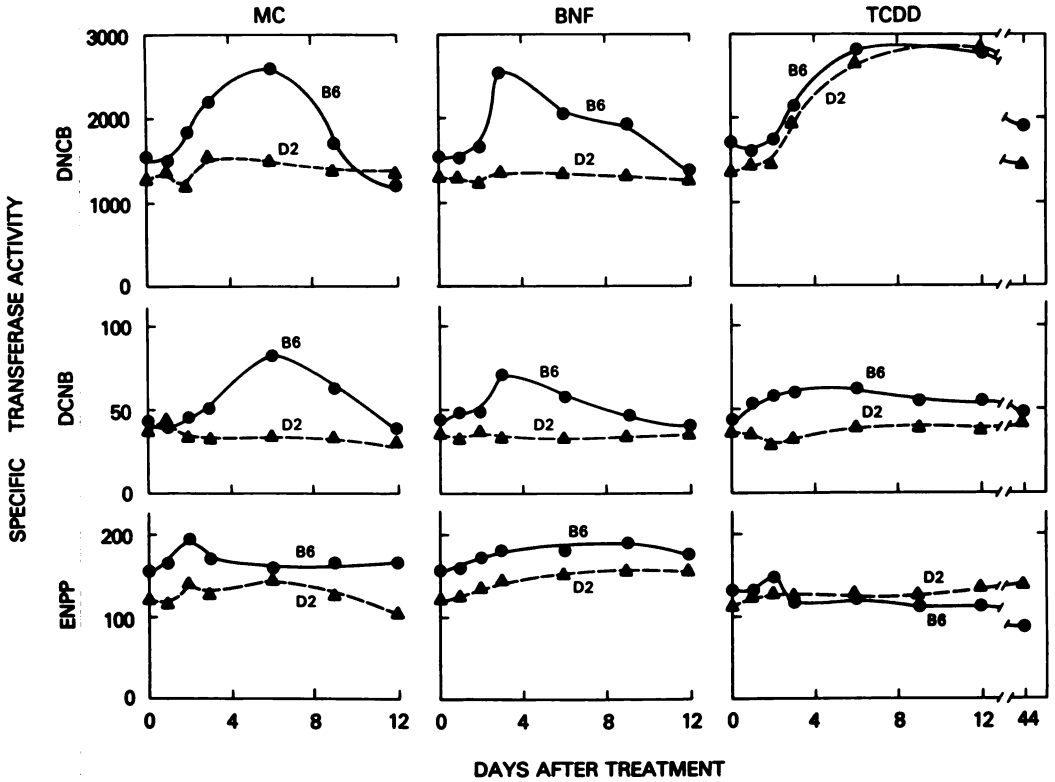

FIG. 1. *Effect of microsomal inducers 3-methylcholanthrene (MC), β-naphthoflavone (BNF), or TCDD on hepatic glutathione transferase activities in B6 and D2 mice as a function of days after treatment*

Transferase substrates assayed for include 1-chloro-2,4-dinitrobenzene (DNCB), 1,2-dichloro-4-nitrobenzene (DCNB), and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENPP). A second experiment yielded similar kinetics of induction, or lack thereof.

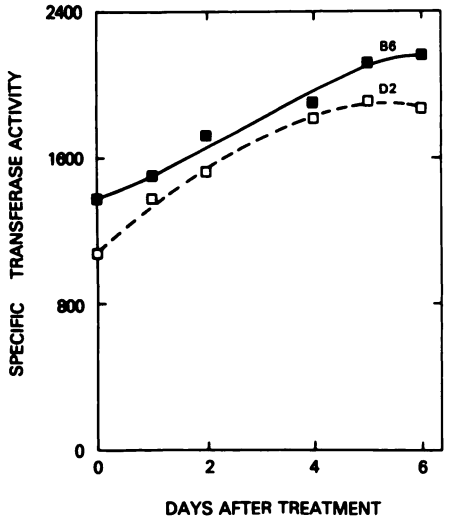

FIG. 2. *Effect of phenobarbital on hepatic glutathione transferase activity in B6 and D2 mice as a function of days after treatment*

The substrate used in this experiment was 1-chloro-2,4-dinitrobenzene; a second experiment yielded similar results. Similar fold induction and kinetics of induction by phenobarbital were also seen with 1,2-dichloro-4-nitrobenzene as substrate (data not illustrated).

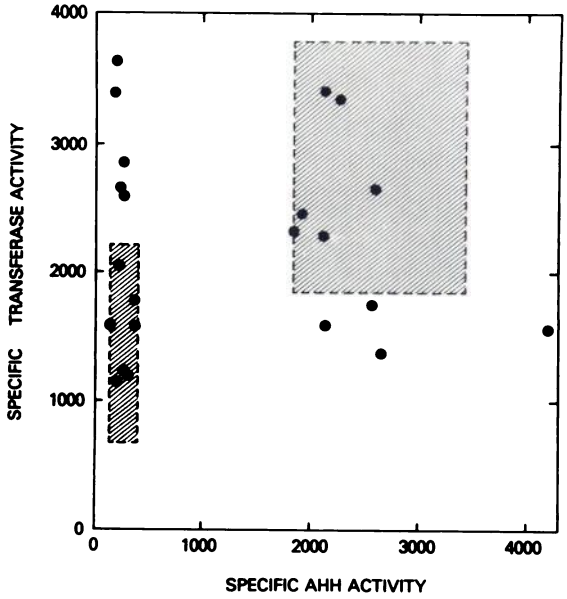

FIG. 3. *Distribution of hepatic glutathione transferase and aryl hydrocarbon hydroxylase (AHH) activities in individual 3-methylcholanthrene-treated offspring from the B6D2F$_1$ × D2 backcross*

The substrate used in this experiment was 1-chloro-2,4-dinitrobenzene. Each point represents an individual mouse treated 6 days previously with a single dose of 3-methylcholanthrene. The shaded regions denote the means ± SD for the transferase and aryl hydrocarbon hydroxylase activities in eight B6 mice (upper right) and eight D2 mice (lower left)—each individually assayed for the two enzyme activities 6 days after a single dose of 3-methylcholanthrene.

by 3-methylcholanthrene or β-naphthoflavone and the same degree of induction by high doses of TCDD as that found in B6 mice are similar to data with aryl hydrocarbon hydroxylase induction or with UDP glucuronosyltransferase, reduced NAD(P):menadione oxidoreductase, and ornithine decarboxylase induction. The lack of a strain difference when B6 and D2 mice were treated with phenobarbital (Fig. 2) is also observed for these other inducible enzymes controlled by the *Ah* regulatory gene (reviewed in Ref. 2).

Glutathione transferase induction among progeny of the appropriate genetic crosses. With 1-chloro-2,4-dinitrobenzene as substrate, 3-methylcholanthrene-inducible transferase activity was also found in the B6D2F$_1$ (Table 2). From all these data with B6 and D2 mice and with the F$_1$ heterozygotes, therefore, one is tempted to conclude that this induction process is closely associated with the *Ah*^{b} allele.

The experiment illustrated in Fig. 3 demonstrates the importance of examining progeny from the B6D2F$_1$ × D2 backcross before making such a conclusion. The 10 offspring having 3-methylcholanthrene-inducible aryl hydrocarbon hydroxylase activities of 1900 or more are known (3) to have sufficient quantities of the *Ah* receptor. i.e., the *Ah* regulatory gene product. The 12 3-methylcholanthrene-treated offspring having aryl hydrocarbon hydroxylase activities of 400 or less are known (3) to have undetectable *Ah* receptor. Five of twelve *Ah*-nonresponsive backcross individuals had transferase activities of greater than 2500, and 4 of 10 *Ah*-responsive individuals

TABLE 2

Effect of 3-methylcholanthrene on glutathione transferase and aryl hydrocarbon hydroxylase activities in B6 and D2 mice and in progeny from the appropriate genetic crosses

The mice were killed 6 days after a single dose of 3-methylcholanthrene; controls received corn oil. The values expressed are means ± SD. Further details are described in the text.

Mouse	*In vivo* treatment	*N*	Glutathione transferase specific activities			Specific aryl hydrocarbon hydroxylase activity
			1-Chloro-2,4-dinitrobenzene	Styrene 7,8-oxide	Benzo[*a*]pyrene 4,5-oxide	
B6	3-Methylcholanthrene	8	2820 ± 480	288 ± 28	12.6 ± 1.4	2630 ± 400
	Control	6	1560 ± 245	138 ± 33	6.3 ± 0.4	330 ± 59
D2	3-Methylcholanthrene	8	1440 ± 380	159 ± 27	6.4 ± 1.0	270 ± 69
	Control	6	1290 ± 270	66 ± 13	4.7 ± 0.6	310 ± 64
B6D2F$_1$	3-Methylcholanthrene	8	2410 ± 310			
	Control	6	1490 ± 170			
Ah^b/*Ah*^{da}	3-Methylcholanthrene	10	2270 ± 720	126 ± 16	18.1 ± 4.0	2430 ± 670
Ah^d/*Ah*^{da}	3-Methylcholanthrene	12	2120 ± 860	115 ± 16	16.2 ± 3.1	240 ± 66

[a] Progeny from the B6D2F$_1$ × D2 backcross that had been phenotyped 2 weeks earlier by the zoxazolamine paralysis test (19).

had transferase activities of less than 1800; these mice are regarded as "recombinants" (26).

Similar data were found for glutathione transferase induction by 3-methylcholanthrene with benzo[*a*]pyrene 4,5-oxide as substrate (Table 2). Induction occurred in B6 and little or no induction was seen in D2 mice. Among the Ah^b/Ah^d and Ah^d/Ah^d individuals from the $B6D2F_1$ × D2 backcross, no association of glutathione transferase induction by 3-methylcholanthrene with the presence of the *Ah* receptor was observed.

Data with styrene 7,8-oxide were different: Induction by 3-methylcholanthrene occurred in both B6 and D2 mice and no association between this induction process and the presence of the *Ah* receptor was seen among Ah^b/Ah^d and Ah^d/Ah^d offspring from the backcross. We therefore must consider that the transferase activity with styrene 7,8-oxide as substrate may be different from the transferase with either benzo[*a*]pyrene 4,5-oxide or 1-chloro-2,4-dinitrobenzene.

DISCUSSION

The data in this report show that the glutathione transferase induction process by 3-methylcholanthrene is not associated with the *Ah* receptor and, therefore, the Ah^b allele. Thus, the gene(s) controlling glutathione transferase induction by 3-methylcholanthrene cannot be linked with the *Ah* regulatory gene, i.e., the two genes must be more than 40 cMorgans apart on the same chromosome or on different chromosomes (26).

Because the effect of 3-methylcholanthrene, β-naphthoflavone, or TCDD on the *aggregate* glutathione transferase activity is only about twofold, it remains possible that highly purified mouse cytosolic enzymes would demonstrate a larger inducing effect on a specific transferase protein. It is also feasible that an additional cytosolic glutathione transferase for a substrate that was not examined would be inducible by 3-methylcholanthrene and controlled by the *Ah* regulatory gene. The possibility of an association between the *Ah* receptor and other 3-methylcholanthrene-inducible microsomal enzyme activities should also be explored, e.g., membrane-bound glutathione transferases and sulfotransferases.

The mechanism by which 3-methylcholanthrene induces cytosolic glutathione transferase activity in mice lacking detectable amounts of *Ah* receptor is not understood. However, β-naphthoflavone has been shown to induce aminopyrine *N*-demethylase activity about twofold in D2 mice (27). β-Naphthoflavone also induces aniline *p*-hydroxylase, *d*-benzphetamine *N*-demethylase, chlorcyclizine *N*-demethylase, ethylmorphine *N*-demethylase, pentobarbital 3′-hydroxylase, and testosterone 7α-hydroxylase activities between 40% and twofold in the liver of several *Ah*-nonresponsive strains (28). In view of these previous results and with the additional data presented here, we can conclude only that polycyclic aromatic compounds are capable of some degree of induction of *P*-450-mediated monooxygenase activities and other drug-metabolizing enzymes by a process independent of the *Ah* receptor. Whether some other receptor or a "nonreceptor"[2] mechanism is responsible for this subcellular response to polycyclic aromatic inducers remains to be determined.

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REFERENCES

1. Jakoby, W. B. The glutathione S-transferases: A group of multifunctional detoxification proteins, in *Advances in Enzymology and Related Areas of Molecular Biology* (A. Meister, ed.). John Wiley & Sons, New York, Vol. 46, 383–414 (1978).
2. Nebert, D. W., and N. M. Jensen. The *Ah* locus: Genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450-mediated monooxygenases, in *CRC Critical Reviews in Biochemistry* Vol. 6 (G. D. Fasman, ed.). CRC Press, Cleveland, Ohio, 401–437 (1979).
3. Okey, A. B., G. T. Bondy, M. E. Mason, G. F. Kahl, H. J. Eisen, T. M. Guenthner and D. W. Nebert. Regulatory gene product of the *Ah* locus. Characterization of the cytosolic inducer-receptor complex and evidence for its nuclear translocation. *J. Biol. Chem.* **254:** 11636–11648 (1979).
4. Nemoto, N., and H. V. Gelboin. Enzymatic conjugation of benzo(a)pyrene oxides, phenols and dihydrodiols with UDP-glucuronic acid. *Biochem. Pharmacol.* **25:** 1221–1226 (1976).
5. Lind, C., H. Vadi and L. Ernster. Metabolism of benzo(a)pyrene-3,6-quinone and 3-hydroxybenzo(a)pyrene in liver microsomes from 3-methylcholanthrene-treated rats. *Arch. Biochem. Biophys.* **190:** 97–108 (1978).
6. Tabor, C. W., and H. Tabor. 1,4-Diaminobutane (putrescine), spermidine, and spermine. *Annu. Rev. Biochem.* **45:** 285–306 (1976).
7. Oesch, F., N. Morris, J. W. Daly, J. E. Gielen and D. W. Nebert. Genetic expression of the induction of epoxide hydrase and aryl hydrocarbon hydroxylase activities in the mouse by phenobarbital or 3-methylcholanthrene. *Mol. Pharmacol.* **9:** 692–696 (1973).
8. Litterst, C. L., E. G. Mimnaugh, R. L. Reagan and T. E. Gram. Comparison of in vitro drug metabolism by lung, liver, and kidney of several common laboratory species. *Drug Metab. Disp.* **3:** 259–265 (1975).
9. James, M. O., J. R. Fouts and J. R. Bend. Hepatic and extrahepatic *in vitro* metabolism of an epoxide (8-^{14}C-styrene oxide) in the rabbit. *Biochem. Pharmacol.* **25:** 187–193 (1976).
10. Darby, F. J., and R. K. Grundy. Glutathione S-aryltransferase: The effect of treating male and female rats with phenobarbitone on the apparent kinetic parameters for the conjugation of 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene with glutathione. *Biochem. J.* **28:** 175–177 (1972).
11. Kaplowitz, N., J. Kuhlenkamp and G. Clifton. Drug induction of hepatic glutathione S-transferases in male and female rats. *Biochem. J.* **146:** 351–356 (1975).
12. Mukhtar, H., and E. Bresnick. Effects of phenobarbital and 3-methylcholanthrene administration on glutathione-S-epoxide transferase activity in rat liver. *Biochem. Pharmacol.* **25:** 1081–1084 (1976).
13. Hales, B. F., and A. H. Neims. Induction of rat hepatic glutathione S-transferase B by phenobarbital and 3-methylcholanthrene. *Biochem. Pharmacol.* **26:** 555–556 (1977).
14. Ryan, A. J., M. O. James, Z. Ben-Zvi, F. C. Law and J. R. Bend. Hepatic and extrahepatic metabolism of ^{14}C-styrene oxide. *Environ. Health Perspect.* **17:** 135–144 (1976).
15. Mukhtar, H., and E. Bresnick. Mouse liver and lung glutathione S-epoxide transferase: Effects of phenobarbital and 3-methylcholanthrene administration. *Chem Biol. Interact.* **15:** 59–67 (1976).
16. Kouri, R. E., T. H. Rude, R. Joglekar, P. M. Dansette, D. M. Jerina, S. A. Atlas, I. S. Owens and D. W. Nebert. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: Cocarcinogen which enhances 3-methylcholanthrene-initiated subcutaneous tumors in mice genetically "nonresponsive" at *Ah* locus. *Cancer Res.* **38:** 2777–2783 (1978).

[2] Unknown nonreceptor mechanisms leading to increases in enzyme activity might include (i) direct binding of chemicals to cytochromes *P*-450 or other membrane moieties; (ii) perturbation of various membrane components; (iii) interaction of nonmetabolized parent drug, or its metabolites, with nucleic acids or proteins in the nucleus; (iv) activation of preexisting inactive enzyme protein; and (v) inhibition of degradation. In cultured hepatocytes from fetal rat liver (29), for example, aryl hydrocarbon hydroxylase activity can be stabilized, and even enhanced, in the presence of the inducer benz[*a*]anthracene or phenobarbital and in the almost complete absence of RNA and/or protein synthesis.

17. Habig, W. H., M. J. Pabst and W. B. Jakoby. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249:** 7130–7139 (1974).
18. Mukhtar, H., R. M. Philpot and J. R. Bend. Epoxide-metabolizing enzyme activities and cytochrome P-450 content of rat ovaries during pregnancy. *Biochem. Biophys. Res. Commun.* **81:** 89–98 (1978).
19. Robinson, J. R., and D. W. Nebert. Genetic expression of aryl hydrocarbon hydroxylase induction. Presence or absence of association with zoxazolamine, diphenylhydantoin, and hexobarbital metabolism. *Mol. Pharmacol.* **10:** 484–493 (1974).
20. Nebert, D. W. Genetic differences affecting microsomal electron transport: The *Ah* locus. *Methods Enzymol.* LII: 226–240 (1978).
21. Fjellstedt, T. A., R. H. Allen, B. K. Duncan and W. B. Jakoby. Enzymatic conjugation of alkyl epoxides with glutathione. *J. Biol. Chem.* **248:** 3702–3707 (1973).
22. Aitio, A. and J. R. Bend. Inhibition of rat liver glutathione *S*-transferase activity by aprotic solvents. *FEBS Lett.* **101:** 187–190 (1979).
23. Mukhtar, H., and E. Bresnick. Glutathione-S-epoxide transferase activity during development and the effect of partial hepatectomy. *Cancer Res.* **36:** 937–940 (1976).
24. Jakoby, W. B., J. N. Ketley and W. H. Habig. Rat glutathione S-transferases: Binding and physical properties, in *Glutathione: Metabolism and Function* (I. M. Arias and W. B. Jakoby, eds.). Raven Press, New York, 213–223 (1976).
25. Habig, W. H., M. J. Pabst, G. Fleischner, Z. Gatmaitan, I. M. Arias and W. B. Jakoby. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. *Proc. Natl. Acad. Sci. USA* **71:** 3879–3882 (1974).
26. Nebert, D. W. Genetic differences in the induction of monooxygenase activities by polycyclic aromatic compounds. *Pharmacol. Ther.* **6:** 395–417 (1979).
27. Nebert, D. W., N. Considine and I. S. Owens. Genetic expression of aryl hydrocarbon hydroxylase induction. VI. Control of other aromatic hydrocarbon-inducible monooxygenase activities at or near the same genetic locus. *Arch. Biochem. Biophys.* **157:** 148–159 (1973).
28. Atlas, S. A., B. A. Taylor, B. A. Diwan and D. W. Nebert. Inducible monooxygenase activities and 3-methylcholanthrene-initiated tumorigenesis in mouse recombinant inbred sublines. *Genetics* **83:** 537–550 (1976).
29. Nebert, D. W., and J. E. Gielen. Aryl hydrocarbon hydroxylase induction in mammalian liver cell culture. II. Effects of actinomycin D and cycloheximide on induction processes by phenobarbital or polycyclic hydrocarbons. *J. Biol. Chem.* **246:** 5199–5206 (1971).

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