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Genetic Expression of Aryl Hydrocarbon Hydroxylase Induction

III. Changes in the Binding of *n*-Octylamine to Cytochrome P-450

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SUMMARY

Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons is expressed as a simple autosomal dominant trait; in any mouse homozygous or heterozygous for the allele *Ah*, the monooxygenase activity is generally induced by aromatic hydrocarbons in many tissues regularly containing the inducible enzyme system. Induction of hydroxylase activity by 3-methylcholanthrene administration to C57BL/6N mice is associated with an approximately equal conversion of hepatic type *b* to type *a* P-450, as measured by *n*-octylamine binding to the cytochrome. This conversion of mouse hepatic P-450 from one form to the other may also occur in rat and hamster liver microsomes and in kidney microsomes of the mouse, rat, or hamster. However, there is not a precise correlation in liver or kidney microsomes from control, 3-methylcholanthrene-treated, or phenobarbital-treated mice, rats, or hamsters between the sum of type *a* plus type *b* P-450 and the total P-450 concentration, as measured by difference spectra of the reduced P-450–CO complex.

In the *AhAh* or *Ahah* mouse but not in the *ahah* mouse, the hydroxylase is induced by a variety of aromatic compounds: polycyclic hydrocarbons such as 3-methylcholanthrene or benz[*a*]anthracene, flavones, and 2-phenylbenzothiazoles. Phenobarbital treatment of *AhAh*, *Ahah*, or *ahah* mice similarly induces the hepatic aryl hydrocarbon hydroxylase system about 100% and causes identical increases of about 65% in both type *a* and *b* P-450. Chlorpromazine administration to *AhAh*, *Ahah*, or *ahah* mice induces the oxygenase activity to levels similar to those stimulated by phenobarbital, but increases hepatic type *b* P-450 preferentially more than the type *a* form.

INTRODUCTION

Many mixed-function oxygenases (3) are membrane-bound, multicomponent enzyme systems which require NADPH, NADH, and molecular oxygen for the oxidative metabolism of drugs, insecticides, polycyclic hydrocarbons, and numerous lipophilic endogenous substrates (4). Any rise or fall in these enzyme activities may therefore affect the intensity and duration of drug action as well as the metabolic rate at which these various compounds are metabolized.

Studies from this laboratory have been directed at understanding the subcellular processes which regulate the induction[1] and

The first two papers of this series are refs. 1 and 2. These studies were presented in part before the Symposium on Pharmacogenetics, Federation of the American Society for Experimental Biology, Chicago, April 1971; at the Meeting of the American Society for Pharmacology and Experimental Therapeutics, Burlington, Vt., August 1971; and at the 13th Annual Meeting of the Pharmacological Society of the Deutsche Demokratische Republik, Weimar, East Germany, November 1971.

[1] The process of induction denotes a relative

decay of mixed-function oxygenase activities (5, 6). For this purpose we have studied in detail aryl hydrocarbon hydroxylase activity,[2] one of the few monooxygenases that can be induced by either polycyclic hydrocarbons or phenobarbital (2, 4, 6, 8–12). With the use of cell culture, we have delineated a chronological sequence of events occurring during the process of aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons (5, 6). The rapid entry of polycyclic hydrocarbons into the cell is independent of temperature (13), and induction-specific RNA is presumably synthesized during the first 30 min (14). Aryl hydrocarbon hydroxylase induction (15) and the concomitant appearance of a new, spectrally distinct, CO-binding cytochrome (16, 17) depend upon translation involving this RNA. Aryl hydrocarbon hydroxylase induction by either polycyclic hydrocarbons or PB[3] is initially sensitive to inhibition by actinomycin D, and with either type of inducer there is also an effect at the post-translational level, in which the regular rate of decay of the induced enzyme activity is retarded (12).

As reported in previous papers of this series (1, 2), the aryl hydrocarbon hydroxylase system is inducible in certain strains of inbred mice and noninducible in others. Compared with fetal cell cultures from the C57BL/6N mouse, for example, in which the enzyme is highly inducible, similar cultures from DBA/2N mice show (a) a relative lack of inducible activity in response to polycyclic hydrocarbons, (b) a diminished formation of the new, spectrally distinct, CO-binding cytochrome, and (c) indirect evidence for a decreased expression of induction-specific RNA (1). On the other hand, we found no differences between these two mouse strains in culture (1) with respect to the rate of uptake of polycyclic hydrocarbons by these cells, in the gross binding of polycyclic hydrocarbons to subcellular fractions, or in the rate of degradation of the aryl hydrocarbon hydroxylase activities. Most recently we have found (2, 18) that the presence of aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons is expressed in many tissues of the mouse as a simple autosomal dominant trait. This interpretation of data was based on the various genetic crosses between the C57BL/6N mouse, in which the enzyme is inducible by MC, and the DBA/2N, NZW/BLN, and NZB/BLN strains, in which activity is not induced by MC (2).

Monooxygenases have as an active site for their oxidative functions (4) cytochrome P-450, so named (19) because the reduced form of this pigment on combination with CO usually has a Soret maximum at about 450 nm. The microsomal CO-binding hemoprotein comprises a mixture of at least two distinguishable forms (20–28), which probably fundamentally reflect two different spin states of the octahedral iron. Further characterization of the terminal oxidase active sites used for polycyclic hydrocarbon hydroxylation may help us to understand the mechanisms by which aryl hydrocarbon hydroxylase induction is regulated. We have shown that the spectral blue shift in the Soret maximum of the reduced hemoprotein-CO complex from 450 nm to about 448 nm *in vivo* (2, 6) and to about 446 nm in cell culture (1, 5, 16, 17) is clearly associated with induction of this enzyme by polycyclic hydrocarbons. Yet an unlikely possibility exists that this spectral shift is related to the physical or covalent binding of aromatic hydrocarbon molecules at or near the P-450 active site so as to perturb the iron-CO bond. In this report this possibility is ruled out with certainty by the use of mice genetically non-

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increase in the rate of synthesis *de novo*, in the rate of activation of enzyme activity from pre-existing moieties, or both, compared with the rate of breakdown. Since this enzyme is a multicomponent, membrane-bound system, there are technical difficulties at present in attempting to distinguish between enzyme synthesis *de novo* and activation. Thus the *rate of enzyme induction* is used here only to express the rate at which the induced monooxygenase activity accumulates.

[2] This enzyme is also called *benzpyrene hydroxylase* and *aryl hydroxylase*. The nomenclature aryl hydrocarbon hydroxylase is preferred, since the enzyme from cells grown in culture (7) or from mammalian liver microsomes (8) converts a variety of aromatic hydrocarbons to phenolic derivatives and is not specific for benzo[*a*]pyrene.

[3] The abbreviations used are: PB, phenobarbital; MC, 3-methylcholanthrene.

responsive to polycyclic hydrocarbons. Hence, whereas aromatic hydrocarbon binding to P-450 and metabolism by the constitutive aryl hydrocarbon hydroxylase occur in microsomes from these mice, the induction process is completely lacking. With the use of such a negative control, we examine here differences in type *a* and type *b* cytochrome P-450 with the use of *n*-octylamine as a probe. In the accompanying paper (29), interactions of the constitutive and induced enzyme systems with various lipophilic compounds provide yet another means for gaining information about the active sites involved in aromatic hydrocarbon metabolism.

MATERIALS AND METHODS

The polycyclic hydrocarbons benzo[*a*]pyrene and benz[*a*]anthracene, obtained from Sigma, and MC, from J. T. Baker Chemical Company, were purified by recrystallization twice from benzene. PB was purchased from Merck and Company, Inc.; 2-chloro-10-(3-dimethylaminopropyl)phenothiazine HCl, from Smith Kline & French Laboratories; *n*-octylamine, α-naphthoflavone, and β-naphthoflavone, from Aldrich Chemical Company; and 2,5-diphenyloxazole, from New England Nuclear. Dr. J. L. Leong generously provided us with 2-(4′-bromophenyl)benzothiazole, 2-(4′-chlorophenyl)benzothiazole, 2-(4′-formylphenyl)-benzothiazole, and rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rutinoside). NADPH and NADH were obtained from Sigma. Instrument-grade CO gas was purchased from Matheson Company, Inc., and a holmium oxide standard cuvette was obtained from Arthur Thomas Company. National Institutes of Health Animal Supply provided us with all the animals used in these studies: various inbred strains of mice, Sprague-Dawley rats, and golden Syrian hamsters.

Treatment of animals. The mice, rats, and hamsters were kept in standard hardwood bedding (beech, birch, and maple sawdust) in plastic cages and fed normal laboratory chow (Ralston Purina Company) ad libitum until death. We attempted to control as completely as possible the animal room environment: i.e., an automatic day-night (16-hr–8 hr) cycle and avoidance of exposure to pharmacologically active compounds such as cigarette smoke and insecticides. At the time of experiments, mice of either sex (2) were used between 4 and 8 weeks of age, and male rats and hamsters were used at 3–4 months of age. For studies of enzyme induction by aromatic hydrocarbons, the animals were given an intraperitoneal injection of the compound (80 mg/kg of body weight unless otherwise stated) in corn oil 24 hr before death; controls received corn oil only. PB-treated animals received intraperitoneally 80 mg of PB in 0.90% NaCl per kilogram of body weight on each of 3 successive days before death. All tissues were obtained from the animals at the same hour of the day. Immediately upon exsanguination, minced tissues from individual animals were separately washed free of blood in ice-cold 0.15 M KCl–0.25 M potassium phosphate buffer, pH 7.25. Tissue homogenates of bowel and kidney at protein concentrations between 3 and 10 mg/ml were prepared for the enzyme assay. Liver homogenates were centrifuged at 15,000 × *g* for 15 min, and the supernatant fraction from this was recentrifuged at 78,000 × *g* for 90 min. The surface of the microsomal pellet was washed several times. The microsomes were then suspended in 30% glycerol–0.25 M potassium phosphate buffer, pH 7.25, and immediately used for determinations of aryl hydrocarbon hydroxylase specific activity, cytochrome P-450 content, and *n*-octylamine binding from each individual animal. Kidney microsomes from an individual rat or hamster or from 8–12 mice were prepared and studied similarly.

Enzyme assay. The determination of enzyme activity and tissue or microsomal protein concentration in duplicate was done exactly as previously described (2, 5, 6). One unit of aryl hydrocarbon hydroxylase activity is defined (17) as that amount of enzyme catalyzing the formation per minute at 37° of hydroxylated product causing fluorescence equivalent to that of 1 pmole of 3-hydroxybenzo[*a*]pyrene. The limit of sensitivity for the assayed specific hydroxylase activity is about 0.10 unit/mg of protein, and duplicate determinations normally vary less than 10% (5, 6).

Spectrophotometry. The method of Omura and Sato (19) was used for the determination of cytochrome P-450 concentrations in 1-cm

cuvettes at room temperature in a Shimadzu model MPS-50L multipurpose recording spectrophotometer. Wavelength measurements were standardized periodically by the use of a holmium oxide crystal. The concentrations of the CO-binding hemoproteins were determined from the CO difference spectra: extinction coefficients used were 91 mM^{-1} cm^{-1} for the difference in absorption between the Soret maximum and 490 nm for the pigments absorbing maximally in the 450 nm region, and 110 mM^{-1} cm^{-1} for the change in absorbance between 420 and 490 nm for P-420 (19). For these calculations, it was kept in mind (30) that the absorption of P-450 below the baseline at 420 nm is about 45% of the Soret maximum around 450 nm and that the absorbance of P-420 above the baseline at 450 nm is about 10% of the P-420 spectral maximum around 420 nm.

The method of Jefcoate and co-workers (26) was used for determination of the two types of P-450 as a function of binding to *n*-octylamine.[4] The difference in absorption between 392 and 500 nm reflects the species of CO-binding hemoprotein having iron in the high-spin state and an extinction coefficient of 65 mM^{-1} cm^{-1} (i.e., type *a*), and the change in optical density between 410 and 500 nm represents the low-spin iron species of P-450, which has an extinction coefficient of 25 mM^{-1} cm^{-1} (i.e., type *b*) (26). Ten microliters of *n*-octylamine in methanol were added to 2.5 ml of microsomes to make a final concentration of 1.0 mM *n*-octylamine; 10 μl of methanol alone were added to the microsomal suspension in the reference cuvette.

RESULTS

Difference spectra caused by n-octylamine binding to P-450 in the mouse. Figure 1 shows the *n*-octylamine difference spectra for hepatic microsomes from MC-treated and control C57BL/6N (B6) and DBA/2N (D2) mice. With the spectrum from control B6 microsomes, we found a value of 0.76 for the ratio of type *b* to type *a* P-450. After MC treatment of B6 mice, this ratio decreased to 0.42. In spectra from the livers of D2 mice, however, the ratio remained about the same, increasing slightly from 0.87 in control microsomes to 0.97 after MC treatment. This ratio was also decreased after MC treatment of C3H/HeN, $C3H_f$/HeN, BALB/cAnN, A/HeN, and AL/N inbred strains and of random-bred Swiss general-purpose mice, in all of which aryl hydrocarbon hydroxylase is inducible by MC. This ratio was not decreased after MC treatment of NZW/BLN, NZB/BLN, or AKR/N inbred mice, in all of which the hepatic hydroxylase activity is not inducible by MC.

Mendelian expression of aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons in various tissues and of a change in type b to type a ratio of P-450. Figure 2 illustrates the genetic variance of aryl hydrocarbon hydroxylase induction by MC among various tissues of inbred and hybrid B6 or D2 mice and of changes in the type of hepatic cytochrome P-450. In the liver of each inbred B6 mouse (Fig. 2A), MC administration induced the enzyme activity about 5-fold, whereas it was not at all inducible in the D2 inbred mice (18). In B6D2 F_1 offspring and in offspring from the B6 × B6D2 F_1 cross, the hepatic aryl hydrocarbon hydroxylase system was always inducible. MC treatment of D2 × B6D2 F_1 offspring, however, produced a distinctly bimodal distribution, with approximately 50% induced to levels as high as those found in the inbred B6 mouse and the remaining half totally nonresponsive to MC. Among the B6D2 F_2 generation, administration of MC again gave a bimodal distribution: about three-fourths of the animals were fully inducible, and the remaining one-fourth were not significantly different from control values. Aryl hydrocarbon hydroxylase induction by MC in kidney (Fig. 2B) and bowel (Fig. 2C) similarly segregates as a simple Mendelian dominant gene.[5] Because

[4] That these two forms of P-450 may represent high-spin (type *a*) and low-spin (type *b*) iron of P-450 is considered at length under DISCUSSION.

[5] Genetic expression of aryl hydrocarbon hydroxylase induction in lung (2) and skin (2, 31) by aromatic hydrocarbons differs from that in kidney and bowel in that the basal specific activities are higher, and there is a slight induction (as much as 2-fold) of the enzyme by polycyclic hydrocarbons

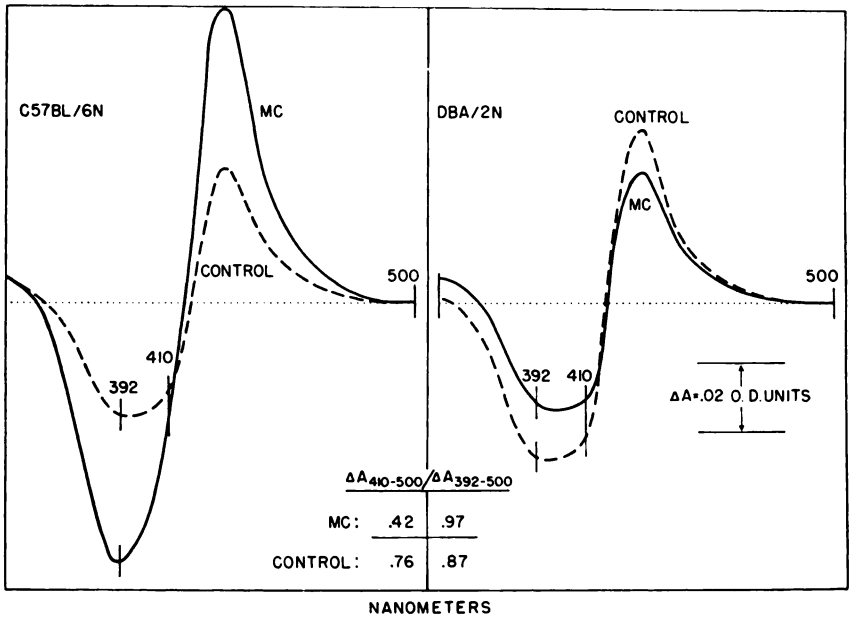

FIG. 1. *n-Octylamine difference spectra for liver microsomes from MC-treated and control C57BL/6N and DBA/2N mice*

Protein concentrations were 3.5, 2.9, 3.3, and 3.5 mg/ml, and the cytochrome P-450 content, as calculated by the method of Omura and Sato (19), was 4.7, 2.4, 2.2, and 3.3 nM for the microsomal fractions from the MC-treated C57BL/6N, control C57BL/6N, MC-treated DBA/2N, and control DBA/2N animals, respectively.

of the relatively low basal hydroxylase activity in these tissues (Fig. 2B and C), the increases in enzyme activity were 5- to more than 50-fold. We have further found that the presence or absence of hepatic aryl hydrocarbon hydroxylase induction in an individual mouse is a good indicator for determining its relative presence or absence in nonhepatic tissues of that mouse (2, 18) and that this genetic expression can be detected *in utero* or in cell cultures derived from D2 × B6D2 F_1 fetuses (18). These studies also have been extended to crosses between other inbred mouse strains (2) and have led us to propose that the alleles *Ah* and *ah* at one genetic locus regulate induction of the enzymes by aromatic hydrocarbons. Hence, in any mouse homozygous or heterozygous for *Ah*, aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons generally occurs as an all-or-none response in tissues regularly containing the inducible enzyme (2). Of further interest is the finding (2) that induction of the hepatic hydroxylase system by PB is about the same in both *AhAh* and *ahah* mice.

Figure 2D shows that a decrease in the type *b* to type *a* ratio of P-450 is directly associated with the genetic response of aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons. During these studies we similarly found that the 2-nm blue spectral shift in the Soret maximum of the reduced hemoprotein–CO complex was present in liver or kidney microsomes of each mouse in which enzyme activity was in-

in certain inbred and hybrid mice in which induction does not occur in liver, kidney, and bowel. Still, induction in lung and skin by aromatic hydrocarbons in *AhAh* and *Ahah* mice is 2–10-fold higher than in *ahah* mice. This phenomenon may represent an adaptation in response to the environment, since the lung and skin are constantly exposed to air and dust particles which may contain polycyclic hydrocarbons, insecticides, pesticides, and aromatic substances in woods and waxes. A careful, long-term genetic study of the expression of basal and inducible aryl hydrocarbon hydroxylase activity in lung and skin, as a function of environmental changes, is necessary.

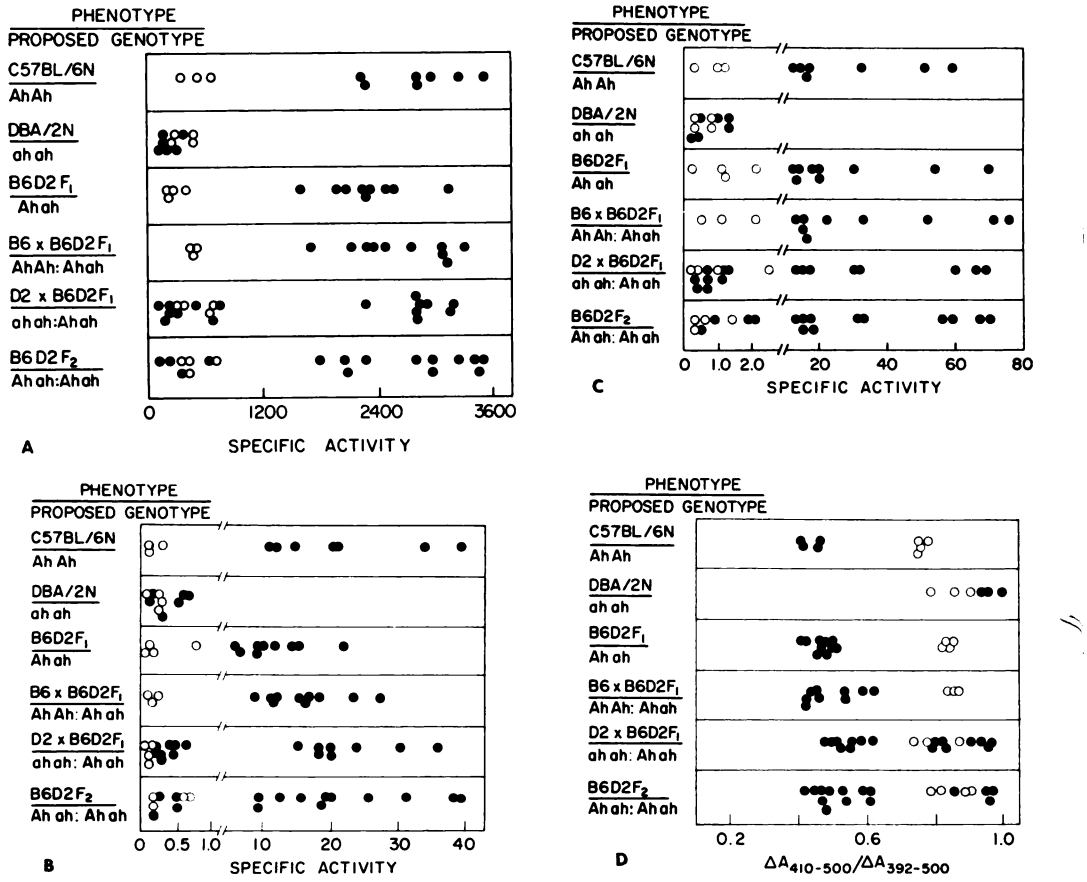

FIG. 2. *Genetic variance of aryl hydrocarbon hydroxylase activity in hepatic microsomes (A), kidney homogenates (B), or bowel homogenate (C), and genetic variance of ratio of type b to type a P-450 in liver microsomes (D)*

●, a determination from an individual MC-treated mouse; O, the corresponding control value obtained from a mouse that received corn oil only. In this figure and the tables, as in the text, specific activity indicates units per milligram of protein.

duced and was absent from microsomal fractions from each MC-nonresponsive mouse. To date, in other appropriate crosses between inbred or hybrid C57BL/6N, DBA/2N, NZW/BLN, and NZB/BLN mice, hepatic aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons, the decrease in the type *b* to type *a* ratio, and the blue spectral shift in the Soret maximum of the reduced cytochrome–CO complex are always directly related. This relationship is also true in the inbred C3H/HeN, BALB/cAnN, A/HeN, AL/N, and CBA/N strains and in random-bred NIH Swiss general-purpose mice, in which the enzyme is MC-inducible, and in the inbred AKR/N strain, in which it is not.

Changes in n-octylamine binding to P-450 in mice after MC or PB treatment. The values for hepatic aryl hydrocarbon hydroxylase activity and P-450 concentrations are summarized in Table 1. The mice are separated into four distinct groups: control; genetically nonresponsive to MC; MC-responsive; and PB-treated. Several interesting points should be noted. (a) Compared with the baseline specific enzyme activity of 520 for both the control and MC-nonresponsive groups, the maximally inducible levels were 5 times higher (i.e., 2590) after MC treatment of genetically responsive animals and 2-fold greater (i.e., 1000) after PB administration. (*b*) Concomitant with enzyme induction by MC, there was

TABLE 1

Relationship between content of types a, b, and total CO-binding cytochrome and aryl hydrocarbon hydroxylase activity in mice

Treatment of the mice and the preparation of liver and kidney microsomes are described under MATERIALS AND METHODS. The 28 mice from which kidney microsomes were examined represent determinations from three pooled samples of 8–12 mice in each experiment. The type *a* and *b* iron-containing cytochromes were calculated according to the method of Jefcoate *et al.* (26), and total P-450 was calculated by the method of Omura and Sato (19). The values of aryl hydrocarbon hydroxylase activity and cytochrome concentrations are expressed as means ± standard deviations. These data represent gruops of inbred C57BL/6N and DBA/2N mice.

Strain	Treatment	*N*	Aryl hydrocarbon hydroxylase specific activity	CO-binding cytochrome			Total P-450/(type *a* + *b*)
				Type *a*	Type *b*	Total	
			units/mg microsomal protein	*pmoles/mg microsomal protein*			
Liver							
B6 or D2	None	41	520 ± 200	200 ± 56	500 ± 140	720 ± 230	1.0
D2	MC	38	520 ± 210	210 ± 61	500 ± 120	630 ± 160	0.89
B6	MC	36	2590 ± 560	300 ± 84	390 ± 150	980 ± 230	1.4
B6 or D2	PB	31	1000 ± 330	330 ± 76	830 ± 210	1140 ± 360	1.0
B6	MC + PB	12	3080 ± 770	420 ± 97	730 ± 200	1450 ± 390	1.3
Kidney							
B6 or D2	None	28	0.9 ± 0.3	80 ± 22	240 ± 48	200 ± 54	0.62
D2	MC	28	0.8 ± 0.3	80 ± 19	240 ± 40	220 ± 52	0.68
B6	MC	28	23 ± 14	90 ± 36	250 ± 110	240 ± 110	0.70
B6 or D2	PB	28	1.2 ± 0.4	60 ± 29	160 ± 49	200 ± 71	0.91

approximately a 100-pmole gain in type *a* P-450 and a 100-pmole loss in type *b* P-450. By using the Fisher *F*-test for analysis of variance, we found that the sum of type *a* + type *b* P-450 content in the control, MC-treated nonresponsive, and MC-treated responsive groups was not significantly different at a level of confidence of 0.0001. With aryl hydrocarbon hydroxylase induction by PB, a 65% increase in both type *a* and type *b* forms of the CO-binding hemoprotein occurred. (c) Estimating the total P-450 content of mouse liver microsomes by the method of Omura and Sato (19), and the type *a* and *b* P-450 species by the method of Jefcoate and co-workers (26), we found a discrepancy in the [total P-450/(*a* + *b*)] ratio for MC-treated genetically responsive mice. Whereas the expected value of about 1.0 was found to be the same ($p > 0.05$) for control, MC-nonresponsive, and PB-induced animals, the average ratio of 1.4 for mice having the MC-induced activity was slightly ($p > 0.02 < 0.05$) different. We found no difference ($p > 0.05$) in the extinction coefficient of 91 mM^{-1} cm^{-1} for P-450 as estimated by Omura and Sato (19) in either control or MC-treated B6 or D2 mice.[6] Thus aryl hydrocarbon hydroxylase induction by MC may cause a decrease in the extinction coefficient of either or both the type *a* and type *b* CO-binding pigments. Since the conversion of type *b* to type *a* P-450 and the [total P-450/(*a* + *b*] ratio of 1.4 was not found in MC-treated D2 mice, these MC-evoked changes in B6 mice most likely are not caused either by displacement of other endogenous substrates from the P-450 active site by MC or by metabolites of MC covalently bound closely enough to the active site to influence the ligand field of the P-450 iron. (d) Mice treated with both MC and PB showed an additive response with respect to specific aryl hydrocarbon hydroxylase activity and type *a*, type *b*, and total P-450 content.

Similar studies were carried out with kidney microsomes from these same groups

[6] Unpublished observations.

of mice (Table 1). The magnitude of enzyme induction by MC was more than 20-fold, whereas activity after PB treatment was not significantly ($p > 0.05$) greater than that of the control or D2 mice. This general lack of induction by PB in non-hepatic tissues has been previously noted (8). Among all four groups, the concentrations of type *a*, type *b*, and total P-450 were not significantly different ($p > 0.05$) from one another. A trend statistically significant at the 0.05 level of confidence in the kidney microsomes of PB-treated mice was a decrease in both type *a* and type *b* P-450, whereas the determination of total P-450 by the method of Omura and Sato (19) showed no change. This decrease is also reflected in the [total P-450/(*a* + *b*)] ratio of 0.91 after PB administration, compared with values between 0.62 and 0.70 for the other groups of kidney microsomes. One possible explanation is that PB itself binds, or PB treatment causes other endogenous substrates to bind more firmly, to the P-450 active site so as to decrease the amount of *n*-octylamine binding during our determination *in vitro* for types *a* and *b* P-450. Because the [total P-450/(*a* + *b*)] ratio is less than 1.0 in all four groups, it would appear that either or both techniques for estimating total P-450 (19) and high- and low-spin P-450 content (26) are unsatisfactory for kidney microsomes. Also, there was no 40% increase in the [total P-450/(*a* + *b*)] ratio for MC-treated genetically responsive mice, as we had found with liver microsomes, indicating that if any change occurs in the extinction coefficient for renal microcomal type *a* or *b* P-450, it is too small to detect.

Changes in n-octylamine binding to P-450 in rats and hamsters after MC or PB treatment. Table 2 shows the results of similar studies with rat and hamster liver and kidney. In rat liver aryl hydrocarbon hydroxylase activity was induced more than 8-fold by MC and less than 2-fold by PB. Contrary to our finding in the mouse, how-

TABLE 2

Relationship between content of type a, type b, and total CO-binding cytochrome and aryl hydrocarbon hydroxylase activity in rats and hamsters

Treatment of the animals and the preparation of liver or kidney microsomes are described under MATERIALS AND METHODS. The calculations and expressed values are the same as those in Table 1.

Treatment	*N*	Aryl hydrocarbon hydroxylase specific activity	CO-binding cytochrome			Total P-450/(type *a* + *b*)
			Type *a*	Type *b*	Total	
		units/mg microsomal protein	*pmoles/mg microsomal protein*			
Rat liver						
None	10	170 ± 61	160 ± 46	370 ± 140	820 ± 150	1.5
MC	11	1390 ± 380	330 ± 82	550 ± 200	1110 ± 240	1.3
PB	11	310 ± 140	290 ± 81	740 ± 270	1730 ± 400	1.7
Rat kidney						
None	6	2.5 ± 1.7	28 ± 5.2	81 ± 15	85 ± 23	0.78
MC	6	200 ± 34	48 ± 4.9	130 ± 23	140 ± 28	0.79
PB	6	3.8 ± 2.0	35 ± 4.8	110 ± 14	100 ± 26	0.69
Hamster liver						
None	3	520 ± 140	280 ± 120	870 ± 300	1490 ± 510	1.3
MC	3	640 ± 110	610 ± 160	1500 ± 260	2400 ± 510	1.1
PB	2	1100 ± 280	670 ± 240	1200 ± 180	2300 ± 370	1.2
Hamster kidney						
None	3	52 ± 10	86 ± 11	190 ± 62	330 ± 26	1.2
MC	3	130 ± 43	85 ± 30	190 ± 59	260 ± 69	1.0
PB	2	54 ± 14	60 ± 28	140 ± 26	240 ± 28	1.2

ever, there was an increase in both type *a* and *b* hepatic P-450 following MC treatment: about a 100% increase in type *a* hemoprotein (from 160 to 330 pmoles/mg of microsomal protein) and about a 50% rise in type *b* pigment (from 370 to 550 pmoles/mg of microsomal protein). PB administration to the rat produced a less than 2-fold induction of enzyme activity and nearly equal (approximately 80% and 100%) increases in type *a* and type *b* P-450, respectively, findings similar to those in mouse liver. With rat kidney microsomes, MC caused approximately 80-fold induction of hydroxylase activity, i.e., a 20-pmole increase in type *a* and about a 50-pmole rise in type *b* P-450, whereas after PB administration the specific enzyme activity was not significantly ($p > 0.05$) greater, and the increases in type *a* and *b* P-450 were smaller. In hamster liver, MC produced about 120% and 70% increases in type *a* and type *b* P-450, respectively, whereas PB effected approximately 140% and 40% increases in these pigment concentrations. In hamster kidney, hydroxylase induction by MC was less than 3-fold, owing to the high constitutive level of the enzyme; the lack of change in type *a* and *b* P-450 after MC administration and the significant ($p > 0.01 < 0.05$) decrease after PB treatment were identical with observations with mouse kidney. The relationship between aryl hydrocarbon hydroxylase induction and increase in type *a* P-450 found in mouse liver was also seen in rat or hamster liver; the main difference in the mouse was the approximately equal conversion of type *b* to type *a* P-450, compared with at least small increases in type *b* P-450 seen in rat or hamster hepatic microsomes. This relationship was not observed in the kidney, where enzyme induction occurred without significant increases in type *a* or *b* P-450 in the mouse and hamster and where induction by MC treatment of the rat occurred with similar rises in the amount of both type *a* and *b* CO-binding pigment.

The association between aryl hydrocarbon hydroxylase induction and a significantly ($p > 0.02 < 0.05$) increased [total P-450/($a + b$)] ratio was found in mouse liver but not in rat or hamster liver or in kidney microsomes from mouse, rat, hamster, or rabbit.[7] This ratio significantly exceeded unity (e.g., between 1.3 and 1.7) in rat liver and was less than 1.0 in mouse and rat kidney; we therefore suggest that the extinction coefficients of type *a* and *b* P-450, which were based on *n*-octylamine binding (26) relative to rabbit hepatic P-450 concentration estimated by the method of Omura and Sato, may vary among species or even tissues of the same animal. On the other hand, relatively good agreement between these two methods (19, 26) was seen with liver microsomes from the mouse or hamster and with hamster kidney microsomes.

Genetic response of inbred and hybrid mice to other aromatic hydrocarbons. How specific is the *Ah* allele in responding to other foreign compounds? Table 3 shows that a variety of aromatic hydrocarbons induce aryl hydrocarbon hydroxylase activity in the liver or kidney of *AhAh* or *Ahah* mice and not in *ahah* mice. β-Naphthoflavone was as good an inducer as MC, whereas benz[*a*]anthrazene and the bromo and chloro derivatives of 2-phenylbenzothiazole were intermediate as inducers. Two other flavones, α-naphthoflavone and rutin, caused smaller rises ($p > 0.05$) in genetically responsive mice. The 4′-formyl derivative of 2-phenylbenzothiazole was not an inducer. These results with the flavones and 2-phenylbenzothiazoles were expected, since α-naphthoflavone (32), β-naphthoflavone and rutin (33), and the bromo and chloro substituents of 2-phenylbenzothiazole (34) are inducers of aryl hydrocarbon hydroxylase activity to varying degrees. The reason for expecting this response in *AhAh* or *Ahah* but not in *ahah* mice is that each of these classes of compounds, as is the case for the polycyclic hydrocarbons, stimulates the hydroxylase activity in nonhepatic tissues such as lung (33, 34), bowel (33, 35), and skin (32, 33), as well as in liver (33–35). Also, in a structure-activity study (34), other derivatives of 2-phenylbenzothiazole, such as 4′-formyl, are not inducers of the enzyme system in

[7] Unpublished observation based on single determinations of kidney microsomes from each of two rabbits.

TABLE 3

Genetic variance of aryl hydrocarbon hydroxylase induction in mouse liver and kidney by various aromatic hydrocarbons

Treatment of the mice and preparation of liver microsomes are described under MATERIALS AND METHODS. The hydroxylase specific activity for liver is based on 1 mg of microsomal protein, and that for kidney is based on 1 mg of whole tissue protein; values are expressed as means ± standard deviation, with a minimum of four individual mice assayed per group. These compounds similarly induce aryl hydrocarbon hydroxylase activity in C57BL/6N, C3H/HeN, C3H$_f$/HeN, BALB/cAnN, A/HeN, AL/N, and CBA/N inbred strains and in random-bred NIH Swiss general-purpose mice; similarly, they do not stimulate the liver or kidney hydroxylase in DBA/2N, NZW/BLN, NZB/BLN, or AKR/N inbred strains.

Proposed genotype of *ah* locus	Treatment	Aryl hydrocarbon hydroxylase specific activity		$\Delta A_{410-500}$: $\Delta A_{392-500}$ for liver microsomes
		Liver	Kidney	
		units/mg protein		
AhAh or *Ahah*	None	570 ± 180	0.4 ± 0.3	0.92[a]
ahah		530 ± 180	0.3 ± 0.3	0.89
AhAh or *Ahah*	MC	2510 ± 470	17 ± 13	0.44
ahah		540 ± 170	0.3 ± 0.4	0.95
AhAh or *Ahah*	Benz[*a*]anthracene	1540 ± 180	11 ± 8.1	0.49
ahah		580 ± 200	0.5 ± 0.3	0.91
AhAh or *Ahah*	α-Naphthoflavone	750 ± 140	0.6 ± 0.3	0.86
ahah		520 ± 160	0.3 ± 0.2	0.94
AhAh or *Ahah*	β-Naphthoflavone	2480 ± 530	52 ± 22	0.43
ahah		550 ± 110	0.3 ± 0.3	0.91
AhAh or *Ahah*	Rutin	610 ± 150	0.7 ± 0.4	0.90
ahah		510 ± 100	0.2 ± 0.2	0.89
AhAh or *Ahah*	2-(4′-Bromophenyl)benzothiazole	1410 ± 330	15 ± 12	0.53
ahah		530 ± 140	0.5 ± 0.3	0.96
AhAh or *Ahah*	2-(4′-Chlorophenyl)benzothiazole	940 ± 190	3.9 ± 2.7	0.68
ahah		560 ± 160	0.3 ± 0.1	0.91
AhAh or *Ahah*	2-(4′-Formylphenyl)benzothiazole	500 ± 98	0.7 ± 0.3	0.82
ahah		530 ± 120	0.2 ± 0.3	0.94
AhAh or *Ahah*	Chlorpromazine[b]	1080 ± 220	0.5 ± 0.2	1.10
ahah		1100 ± 190	0.4 ± 0.2	1.08
AhAh or *Ahah*	Sodium phenobarbital	1000 ± 230	0.7 ± 0.3	0.88
ahah		1030 ± 200	0.4 ± 0.3	0.88

[a] When further experiments were carried out in the autumn instead of in the spring, the $\Delta A_{410-500}$:$\Delta A_{392-500}$ ratio ranged between 1.0 and 1.3 for control mice and between 0.50 and 0.80 for mice in which aryl hydrocarbon hydroxylase was induced. The reason for this slight change in the ratio is not known but may be the result of seasonal variations or dietary or environmental differences in the animals before they arrived in our laboratory animal quarters. However, the conversion of type *b* to type *a* P-450 remained approximately the same.

[b] Chlorpromazine was given intraperitoneally at 45 mg/kg of body weight on 2 successive days, and the tissues were assayed 24 hr after the second dose.

any tissue. 2-Chloro-10-(3-dimethylaminopropyl)phenothiazine (chlorpromazine) induced the hepatic hydroxylase system in both *AhAh* and *ahah* mice to levels similar to those induced by PB. Thus, by some regulatory mechanism not yet understood, the allele *Ah* responds to specific foreign aromatic chemicals which include certain flavones, 2-phenylbenzothiazoles, and carcinogenic and noncarcinogenic polycyclic hydrocarbons, but which do not include PB or phenothiazines.

Also in Table 3, a general decrease in the $\Delta A_{410-500}$:$\Delta A_{392-500}$ ratio for hepatic P-450 and an increase in kidney aryl hydrocarbon hydroxylase induction are clearly proportional to the magnitude of hepatic enzyme induction by polycyclic hydrocarbons, fla-

vones, and the bromo and chloro derivatives of 2-phenylbenzothiazole in genetically responsive mice. On the other hand, this ratio remained unchanged after PB treatment and increased because of a larger rise in the type *b* P-450 species after chlorpromazine administration.[8] Also, neither of these two compounds induced hydroxylase activity in the kidney. This latter finding is not in agreement with the data of Wattenberg and Leong (35), who found that chlorpromazine and other phenothiazines induced the hydroxylase in nonhepatic tissues as well as in rat liver. Therefore, compounds such as PB and phenothiazones, which do not stimulate the kidney aryl hydrocarbon hydroxylase system, induce the hepatic enzyme in both *AhAh* and *ahah* mice. On the contrary, certain polycyclic hydrocarbons, flavones, and 2-phenylbenzothiazoles induce both the hepatic and nonhepatic hydroxylase systems in mice homozygous or heterozygous for the *Ah* allele. Moreover, during treatment of the genetically responsive animal by this latter class of aromatic chemicals, type *b* is converted to type *a* hepatic P-450 concomitantly with enzyme induction.

DISCUSSION

We have shown in this report an association between aryl hydrocarbon hydroxylase induction by various aromatic hydrocarbons and an approximately equal conversion of type *b* to type *a* P-450 in mouse liver. The important negative control was the *ahah* mouse, in which neither enzyme induction nor this change in the type of P-450 occurred, although binding of aromatic hydrocarbons such as MC to the P-450 active site and metabolism of the aromatic hydrocarbons undoubtedly take place. Thus, during MC treatment of an animal, the amount of MC bound to microsomes does not measurably influence the type *b*:*a* ratio of P-450 in mouse liver or kidney. This conclusion is important, in light of the facts that dietary differences and tetracycline ingestion are known (26) to change the type *b*:*a* ratio of P-450 in rabbit liver. This conclusion does not agree with the suggestion by Schenkman (36) that the type I spectral alterations in the hepatic P-450 from benzo[*a*]pyrene-treated rats are caused by bound inducer or metabolite molecules.

Williams (37) was the first to suggest that two distinct spin states of P-450 might exist. Jefcoate and co-workers have demonstrated (23) that changes in shape of the difference spectra obtained with primary aliphatic amines afford a sensitive method for estimating the proportions of type *a* and *b* microsomal P-450 species. The increase of a high-spin hemoprotein from rabbits treated with MC (38–40) was equated (24) with the type *a* P-450. However, this $g = 6.6$ signal did not decrease when high-spin P-450 had been converted to a low-spin form by *n*-octylamine binding *in vitro*, and the signal was lost while the Soret maximum of the reduced cytochrome–CO complex persisted at 448 nm in microsomes aged 1 day at −15° (24). These findings might be explainable on the basis of changes in the thermal equilibrium between high- and low-spin iron in hemoprotein (41, 42). For example, the thermal equilibrium between the high- and low-spin electronic configurations of the heme iron of methemoglobin (43) stands in contrast with the much larger energies required for spin conversions in most iron complexes (44) and suggests that the apoprotein energetically poises the spin state. Perhaps one cannot directly compare results obtained with EPR spectroscopy at liquid helium temperatures and spectrophotometric data obtained at room temperature.

Peisach and Blumberg have claimed (45) that no spin Hamiltonian for high-spin iron (46) can give rise to a self-consistent set of $g = 6.6$ and 3.0 values. P-450 in the high-spin form from intact microsomes has instead been associated with a $g = 7.9$ signal (45, 47, 48). With the use of EPR spectroscopy below 10°K, we have recently found (49) two high-spin and one low-spin species of P-450.

[8] Chlorpromazine administration for 2 days in these mice produced about 4- and 3-fold rises in type *b* and type *a* P-450 content, respectively. This effect *in vivo* differed from that found *in vitro*, where addition of chlorpromazine to a microsomal suspension just before *n*-octylamine caused a 20% decrease in apparent type *b* P-450 without a substantial change in the type *a* species (unpublished data).

Whereas all three forms of P-450 increase after PB treatment, only the lower-field, high-spin form (g = 8.0) is associated with the MC-treated *AhAh* or *Ahah* genetically responsive mouse (49). Moreover, detergents such as Lubrol, sodium deoxycholate, Triton X-100, and sodium dodecyl sulfate, organic solvents, and freezing and thawing irreversibly destroy the g = 8.0 signal, while the g = 6.6 signal is increased (49). Our findings are in agreement with recent Mossbauer spectroscopic evidence (50) indicating an increase in one of the two distinct high-spin species of P-450 after treatment of rats with MC. Therefore, we suggest that the type *a* P-450 as determined by *n*-octylamine difference spectra perhaps reflects both forms of high-spin P-450. Furthermore, all these data are consistent with the report (36) that the change in shape of type II spectra, formed between P-450 and basic amines such as *n*-octylamine, reflects both the high- and low-spin binding sites of the cytochrome.

With Fig. 3 we attempt to explain our results with mouse liver, based on the extinction coefficients of type *a* and *b* P-450 estimated by Jefcoate and co-workers (23). Genetic information is depicted by the horizontal lines. In the control mouse, which has an average specific aryl hydrocarbon hydroxylase activity of about 500, the *Ah* or *ah* allele at the *ah* locus is repressed so that little or no induction-specific RNA and protein are synthesized. The letters M, N, O, P, ··· designate other genetic loci responsible for synthesizing RNA and protein necessary for the various moieties of the microsomal electron transport pathway (5, 51). After secondary and tertiary structural changes occur, these proteins (e.g., flavoprotein and lipoprotein), along with phospholipids, fatty acids, and cholesterol, are arranged into the completed membrane, in which we found the ratio of two type *a* for every 5 type *b* P-450 molecules. We have determined that the type *b* and not type *a* P-450 is responsible for benzo[*a*]pyrene metabolism by the constitutive aryl hydrocarbon hydroxylase system (29). We cannot distinguish between two possibilities. A small portion of the type *b* P-450 may be responsible for control enzyme activity, as represented in Fig. 3 by 5% of the total type *b*

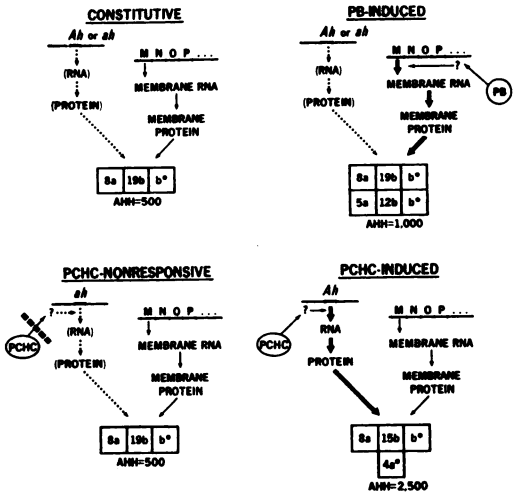

FIG. 3. *Hypothetical scheme showing relationship between genetic differences in aryl hydrocarbon hydroxylase (AHH) induction by polycyclic hydrocarbons (PCHC), induction by drugs such as phenobarbital, and changes in form of mouse hepatic cytochrome P-450*

Each "unit" of *a* represents 25 pmoles of type *a* CO-binding pigment, and each "unit" of *b* stands for 25 pmoles of type *b* P-450 per milligram of hepatic microsomal protein. *a** represents 1 "unit" of newly synthesized CO-binding cytochrome, which presumably metabolizes aromatic hydrocarbons very specifically and which is inhibited *in vitro* by *a** inhibitors (29), such as α- and β-naphthoflavone, 2,5-diphenyloxazole, and Lindane. *b** depicts 1 "unit" of type *b* P-450, which perhaps metabolizes aromatic hydrocarbons less specifically and which can be inhibited *in vitro* by *b** inhibitors such as several phenylimidazoles, 2-diethylaminoethyl-2,2-diphenyl valerate, metyrapone, 17β-estradiol, and numerous other compounds (29). Thus, for example, the mean values for the control mouse depict 200 pmoles of type *a* cytochrome and 500 pmoles of type *b* iron-containing pigment, 25 pmoles of the latter being responsible for the constitutive hepatic hydroxylase specific activity of 500.

being rather specific for aromatic hydrocarbon metabolism, depicted as *b**. Alternatively, we could postulate that all of the low-spin P-450 participates to some extent, but less specifically, in polycyclic hydrocarbon metabolism.

In the polycyclic hydrocarbon-nonresponsive mouse, the specific aryl hydrocarbon hydroxylase activity and amounts of high- and low-spin forms of P-450 are the

same as in the control mouse. Therefore, in spite of uptake, binding, and metabolism (1, 29) of aromatic hydrocarbons, the *ah* allele for some unknown reason cannot respond to this class of foreign compounds in the way that the *Ah* allele can. An alternative possibility is that the induction-specific RNA and protein are synthesized normally, but that the membrane is genetically defective and cannot incorporate the protein or respond in a manner conducive to a change from low- to high-spin P-450.

In the genetically responsive mouse, aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons occurs through some regulatory process involving the *Ah* allele, while the loci M, N, O, P, ··· regulating microsomal membrane biosynthesis continue to operate at the same rate as in the control or MC-treated *ahah* mouse. Induction-specific RNA and protein are now synthesized in response to the derepressed *Ah* allele, and by some mechanism this protein stoichiometrically converts 4 "units" (i.e., 20% of the total low-spin P-450) of type *b* pigment to type *a*, concomitant with a 5-fold induction of enzyme activity. This newly synthesized protein, in causing the conversion of type *b* to *a* P-450, may be only loosely associated with the microsomal membrane or may be incorporated covalently into the membrane as the "substrate-specific protein" apoenzyme portion of P-450 (5). We have designated these four newly converted "units" of high-spin P-450 as *a**, or hemoprotein specifically involved in aromatic hydrocarbon metabolism. Whether or not the type *a* P-450 in control mice that was not previously metabolizing benzo[*a*]pyrene is now somehow activated into metabolizing the inducer is not known. However, some compounds binding preferentially to high-spin P-450 do not affect the MC-induced aryl hydrocarbon hydroxylase activity *in vitro* (29), indicating that types *a* and *a** are two types of high-spin P-450. Our recent EPR data (49) further corroborate this hypothesis. Also, we know (29) that type *a* P-450 is largely responsible for benzo[*a*]pyrene hydroxylation by the induced enzyme system in both mouse liver and kidney microsomes. Moreover, the spectral blue shift coupled with hydroxylase induction *in vivo* (2) or in cell culture (1, 16, 17) is related to this conversion of type *b* to high-spin P-450 of the *a** type. With respect to measurements of P-450 concentration by the method of Omura and Sato (19), however, we found that MC treatment of the genetically responsive mouse increases total P-450 content by about 35%, although there is no change in the total amount of type *a* plus type *b* P-450 (Table 1). Therefore, hydroxylase induction by MC may cause preferential synthesis of *a** P-450 (i.e., the high-spin form having the $g = 8.0$ EPR signal) and little or no synthesis of the low-spin and other forms of high-spin P-450 (49). Hence the apparent conversion of type *b* to type *a** P-450, as determined by *n*-octylamine binding, may be fortuitous. For example, the extinction coefficient for the cytochrome–*n*-octylamine complex of type *a** may be similar to that of type *b* (25 mM^{-1} cm^{-1}) rather than that of type *a* (65 mM^{-1} cm^{-1}).

One point should be noted with respect to work from other laboratories. Administration of MC is reported to increase in the nucleus of hepatic cells the RNA polymerase activity, the RNA:DNA ratio, the incorporation of orotic acid into RNA (50, 51), and the initiation of new RNA chains (52). Because aryl hydrocarbon hydroxylase induction by MC involves a single autosomal dominant gene, we suggest that these observed changes (52–54) are not specifically associated with the induction process and would in all likelihood be found in the MC-treated *ahah* mouse. Alternatively, this simple genetic expression in the mouse might not exist in other species such as the rat, with which these studies (52–54) were performed.

In the PB-treated mouse, specific aryl hydrocarbon hydroxylase activity is doubled along with a 65% increase in both type *a* and *b* P-450. We postulate in Fig. 3 that PB, acting through some mechanism,[9]

[9] Whether this stimulation reflects increased synthesis or decreased degradation, or both, requires further study. However, we have determined (12) that aryl hydrocarbon hydroxylase induction by PB is initially sensitive to actinomycin D, that PB can also stabilize the induced enzyme activity at the post-translational level,

stimulates RNA and protein synthesis regulated by loci M, N, O, P, ···. Hence, type *a* P-450 is increased from 8 to 13 "units," and type *b* P-450 from 20 to 33 "units," while the type *a*:*b* ratio of 2:5 is maintained. We have suggested (29) that the PB-induced hydroxylase activity is inhibited by numerous compounds which interact with type *b* P-450, just as the constitutive enzyme system is; therefore an increase from 1 to 2 *b** "units" in Fig. 3 represents the 2-fold induction of activity by PB. However, the rise in enzyme activity (100%) is significantly ($p < 0.01$) greater than the increase in type *b* P-450 content (65%). This finding suggests that the proliferative changes of the membrane by PB are unequal and that control microsomes cannot be equated with microsomes from PB-treated mice.[10] PB administration to rats is known (55) to increase the rate of synthesis of some, and to decrease the rate of synthesis of other, proteins of the hepatic endoplasmic reticulum, a finding which supports our suggestion. A marked different inhibitory effect on benzo[*a*]pyrene hydroxylation by hexachlorobenzene between the control and PB-induced aryl hydrocarbon hydroxylase systems (29) is further evidence for detectable differences between constitutive and PB-induced microsomal membranes.

A stoichiometric conversion of type *b* to type *a* P-450 in mouse kidney was not observed. From Table 1 and Fig. 3, we can estimate that an increase of about 2000 in specific enzyme activity is associated in the liver with 100 pmoles of type *b* converted to type *a* P-450. Thus 1 pmoles of *a** P-450 represents an induction of about 20 units of hydroxylase activity per milligram of microsomal protein. Since MC treatment caused a rise of 20 in the specific enzyme activity from mouse kidney, a 1-pmole change in type *b* to type *a* hemoprotein could not be detected under our experimental conditions. Actually MC produced an increase of about 200 and 80 in the specific aryl hydrocarbon hydroxylase activity of rat and hamster kidney, respectively; threoretical 10- and 4-pmole rises, respectively, in type *a* P-450 are in agreement with the experimental data. A similar association between enzyme induction by MC and increases in type *a* P-450 is not inconsistent with our observations on rat and hamster liver, but the fact that MC also enhanced rather than decreased the type *b* P-450 content in these species makes the stoichiometric relationship less clear.

NOTE ADDED IN PROOF: At the recommendation of Dr. Margaret Green, Chairman of the Committee on Standardized Genetic Nomenclature for Mice, the locus for aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons will be *Ah* (for *a*romatic *h*ydrocarbon responsiveness). The allele Ah^b will designate the dominant gene, *i.e.* expression of the hydroxylase induction —to the same extent as that found in the dominant parent—in liver, kidney, or bowel of F_1 progeny from the cross between C57BL/6 and DBA/2, NZB/BLN, or NZW/BLN (2); the allele Ah^d will represent the recessive gene. The C57BL/6 strain (or B6) is arbitrarily designated the prototype strain for the Ah^b allele, and the DBA/2 strain (or D2) the prototype strain for the Ah^d allele. Therefore, in this manuscript *Ah* denotes Ah^b and *ah* denotes Ah^d. The previously named *In* locus on chromosome #1, named for the inflammatory response to topical application of polycyclic hydrocarbons (B. A. Taylor (1971) *Life Sci. 10* (I), 1127), in all likelihood coincides with the *Ah* locus (P. E. Thomas, B. A. Taylor, and J. J. Hutton, unpublished observations). From crosses between certain other strains, we now have evidence for codominance, and

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and that induction by PB may require rRNA synthesis more than the induction process by polycyclic hydrocarbons.

[10] Several compounds interacting with type *a* P-450 inhibited aryl hydrocarbon hydroxylase activity from PB-treated mice to a significantly greater extent than the control enzyme activity, and almost as much as the MC-induced activity (29). One possible explanation of these data is that gene activation or amplification by PB may include derepression of the *ah* locus in both the *AhAh* and the *ahah* mouse, resulting in a rise in *a** as well as *b** P-450 content (29). We have EPR data (49) that support this hypothesis.

complete repression, of hydroxylase induction (D. W. Nebert, I. S. Owens, and N. Considine, unpublished observations), and in time these findings may be shown to reflect additional alleles at the same locus or different loci.

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