Genetic Regulation of Aryl Hydrocarbon Hydroxylase Induction

II. SIMPLE MENDELIAN EXPRESSION IN MOUSE TISSUES IN VIVO*

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JACQUES E. GIELEN, FRANCINE M. GOUJON, AND DANIEL W. NEBERT

From the Section on Developmental Pharmacology, Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014

SUMMARY

In the inbred C57BL/6N mouse, aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons increases rapidly in the neonatal period; this response to these pharmacological, exogenous compounds does not appear in the DDA/2N, NZW/BLN, or NZB/BLN strains. The development of the constitutive hydroxylase activity in the neonatal period is the same in all four strains. Small differences in pH optima, substrate affinities, relative thermalability, or benz[a]pyrene metabolism, which might have affected the enzyme assay, do not account for the large genetic difference in the response to polycyclic hydrocarbons.

The hydroxylase induction by aromatic hydrocarbons is inherited as a simple autosomal dominant trait, designated the ah locus. In the individual mouse that is genetically responsive to polycyclic hydrocarbons, the hydroxylase activity is induced as an all-or-none phenomenon in all tissues which regularly contain the polycyclic hydrocarbon-inducible enzyme. The magnitude of induction of the hydroxylase is 4- to 5-fold in liver, and ranges from 5- to more than 50-fold in kidney, bowel, lung, and skin. The formation of a spectrally distinct carbon monoxide-binding cytochrome from liver microsomes is associated with the polycyclic hydrocarbon-inducible enzyme activity and is not seen in the liver of mice which are genetically nonresponsive to aromatic hydrocarbons. Phenobarbital induces the hepatic oxygenase to similar levels (i.e. about 2-fold) in all four strains.

We have been studying (1-14) the mechanisms regulating micrococal enzyme induction from two fundamental points of interest. First, the stimulation of enzyme activity in response to a foreign, pharmacological stimulus such as a polycyclic hydrocarbon or a drug serves as an interesting experimental system for studying genetic expression in eukaryotic cells and for comparing this experimental model with other systems in which endogenous compounds cause enzyme induction. Second, these membrane-bound mixed function (15) oxygenases are important for the oxidative metabolism of most drugs, polycyclic hydrocarbons, and insecticides, as well as lipophilic endogenous substrates (16) such as steroids, bilirubin, indoles, thyrroxine, sympathomimetic amines, hemin (17), and fatty acids (18). Thus, the day-to-day fluctuations in the levels of these oxidases influence the intensity and duration of drug action and the rate of metabolism of chemical carcinogens (10), insecticides (19), toxic chemicals (20), and numerous normal body substrates (16).

Aryl hydrocarbon hydroxylase activity* is one of the few mixed-function oxygenases that can be induced2 by either polycyclic hydrocarbons or PB3 (2, 5, 12-14, 16). This NADPH-linked enzyme has as an active site for its oxidative function the CO-binding cytochrome P450 (21), so named because the reduced form of this pigment upon combination with CO has a Soret maximum at about 450 nm. This hydroxylase activity in liver microsomes from rats treated with MC can be reconstituted from three components: flavoprotein, lipid, and a cytochrome-containing fraction; substrate specificity is presumably determined by the type of cytochrome comprising the active oxidative site (22).

There is growing evidence (1, 2, 7, 22-28) that cytochrome

1 This enzyme is also called benzopyrene hydroxylase and aryl hydroxylase. The nomenclature aryl hydrocarbon hydroxylase is preferred, since the enzyme from cells grown in culture (3) or from mammalian liver microsomes (5) converts a variety of polycyclic hydrocarbons to phenolic derivatives and is not specific for benz[a]pyrene. The substrate specificity of either the constitutive or the induced hydroxylase from the various mammalian tissues in culture and in vivo requires more study. For example, in mouse liver in vitro 17a-estradiol and testosterone competitively inhibit polycyclic hydrocarbon hydroxylatation (10).

2 The process of induction denotes a relative increase in the rate of de novo synthesis or in the rate of activation of enzyme activity from pre-existing moieties, or in the rate of both, compared to the rate of breakdown. Since this enzyme may be a multicomponent membrane-bound system, there are technical difficulties in attempting to distinguish between enzyme de novo synthesis and activation. Thus, the rate of enzyme induction is being used here only to express the rate at which induced hydroxylase activity is accumulating.

3 The abbreviations used are: PB, sodium phenobarbital; and MC, 3-methylcholanthrene.
hydroxylase induction in mammalian cell culture by polycyclic hydrocarbons. Furthermore, a direct correlation exists between the formation of high spin iron-containing cytochrome \( P_{450} \) and aryl hydrocarbon hydroxylase induction in mouse liver by polycyclic hydrocarbons (2). It is conceivable that an incidental increase in this type of cytochrome \( P_{450} \) occurs during the PB-stimulated nonspecific proliferation of the hepatic smooth endoplasmic reticulum and that this incidental increase explains the induction of the hydroxylase activity by PB. Although the polycyclic hydrocarbon-inducible hydroxylase activity exists in most extrahepatic tissues as well (5, 10, 29-31), it is not known whether or not a multicomponent membrane-bound enzyme system similar to that in liver is responsible for polycyclic hydrocarbon hydroxylase in any of these other tissues.

A chronological sequence of events occurs during the process of hydroxylase induction in mammalian cell culture by polycyclic hydrocarbons (2, 6-9, 11). The rapid entry of polycyclic hydrocarbons into the cell is independent of temperature (8), and synthesis of an induction-specific RNA takes place during the first 20 min (9). The oxygenase induction (6, 9) and the appearance of a new spectrally distinct CO-binding cytochrome (7) are concomitantly dependent upon translation involving this RNA species. The parent polycyclic hydrocarbon molecule is not metabolized by the control enzyme and subsequently by the induced hydroxylase as well; this process results in metabolites covalently bound to cellular material (8, 9) and water-soluble derivatives excreted into the growth medium (8). More recently (14) we found that the primary action of PB, as well as that of polycyclic hydrocarbons, on aryl hydrocarbon hydroxylase induction may be transcriptional and that with either inducer there is also a secondary effect at the posttranslational level in which the regular rate of decay of the induced enzyme is retarded.

In the previous paper (1) of this series, we found differences between C57BL/6N and DBA/2N fetal cells in culture with respect to this chronological sequence of events. Thus, compared with fetal cultures from the C57BL/6N mouse, similar cell cultures from the DBA/2N mouse show (a) a relative lack of inducible hydroxylase 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. On the other hand, we found (I) no differences between these two cell types in 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 induced hydroxylase activities. By examining various tissues from the inbred C57BL/6N, DBA/2N, NZW/BLN, and NZB/BLN mouse strains and from offspring of the appropriate genetic crosses between these inbred strains, we show in this report that aryl hydrocarbon hydroxylase induction and formation of the spectrally distinct CO-binding hepatic cytochrome in response to polycyclic hydrocarbons follow simple Mendelian genetics.

Materials

The polycyclic hydrocarbons benzo[a]pyrene, obtained from Sigma (St. Louis, Missouri), and MC, purchased from J. T. Baker Chemical Company (Phillipsburg, New Jersey), were purified by reprecipitation twice from benzene. The PB was obtained from Merck and Company, Inc., Rahway, New Jersey. Nuclear Chicago Solubilizer (a solubilizing quaternary ammonium base) and generally labeled [\(^{3}H\)]benzo[a]pyrene (500 mCi per millimole) were purchased from Nuclear Chicago, Arlington Heights, Illinois. NADPH and NADDI were obtained from Sigma. Bovine serum albumin was bought from Armour Pharmaceutical Company, Chicago, Illinois. Instrumental grade carbon monoxide gas was purchased from the Matheson Company, Inc., East Rutherford, New Jersey. National Institutes of Health Animal Supply provided us with all the strains of mice mentioned in this report.

Methods

Treatment of Animals—The mice were kept for breeding in standard hardwood bedding (composed of a beech, birch, and maple sawdust) in plastic cages and fed normal laboratory chow (Ralston Purina Company, St. Louis, Missouri) ad libitum until time of sacrifice. We attempted to control as completely as possible the animal room environment: i.e. an automatic day-night (16 hours to 8 hours) cycle and avoidance of exposure to pharmacologically active compounds such as cigarette smoke and insecticides. Except where indicated, the mice ranged between 3 and 10 weeks of age at the time the hydroxylase specific activities and cytochrome \( P_{450} \) content were determined. Among inbred mice of the same strain, we found no statistically significant difference (\( p > .05 \)) in the control, MC-inducible or PB-inducible enzyme or hepatic hemoprotein levels between male and female littermates or between 3-week-old and 10-week-old animals of the same genetic constitution. MC-treated mice were injected once intraperitoneally with 80 mg of MC in corn oil per kg of body weight 24 hours before death; control mice received corn oil only. PB-treated mice received intraperitoneally 50 mg of PB in normal (0.90%) saline per kg of body weight on each of 3 successive days before sacrifice. All experiments were begun at approximately the same hour of the day.

Immediately upon exsanguination of the animal, the minced tissues from each individual mouse 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 the bowel, lung, kidney, and skin at concentrations between 3 and 10 mg of protein per ml were prepared for the enzyme assay. Liver homogenates were centrifuged at 15,000 \( \times g \) for 15 min, and the supernatant fraction from this was recentrifuged at 78,000 \( \times g \) 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 consecutively for the determinations of hydroxylase activity and cytochrome \( P_{450} \) content from each individual mouse.

Enzyme Assay—The determination of hydroxylase activity and tissue protein concentration in duplicate was similar to that (2, 3, 8) previously described. For determining the hepatic enzyme activity, the 1.00 ml reaction mixture included 50 \( \mu \)moles of potassium phosphate buffer, pH 7.2, 0.36 \( \mu \)mole of NADPH, 0.39 \( \mu \)mole of NADH, 600 \( \mu \)g of bovine serum albumin, 3 \( \mu \)moles...
of MgCl₂, 0.10 ml of microsomes (containing 80 to 300 μg of microsomal protein), and 80 nmol of the substrate benzo[a]pyrene added in 40 μl of methanol just prior to the 10-min incubation. We found (2) that when low amounts of protein are present in the assay mixture, additional protein in the form of albumin aids the solubility of the benzo[a]pyrene and therefore the reproducibility of the assay. For assaying the nonhepatic enzyme activity, the 1.00-ml reaction mixture contained 50 nmol of potassium phosphate buffer, pH 7.5, 0.36 nmol of NADPH, 0.59 nmol of NADH, 0.10 ml of tissue homogenate (containing 0.30 to 1.0 mg of protein), and 80 nmol of benzo[a]pyrene added in 40 μl of methanol just prior to the 30-min incubation. Following incubation at 37°C, addition of cold acetone, and extraction with hexane (3), the alkali-extractable metabolites were examined with an Amino-Bowman model 4-S202 SPF recording spectrophotofluorometer (American Instrument Company, Baxter Laboratories, Silver Spring, Maryland); fluorescence corresponding to 3-hydroxybenzo[a]pyrene has an activation peak at 396 nm and an emission maximum at 522 nm (2). The fluorescence of a blank sample, to which benzo[a]pyrene had been added after the incubation and addition of acetone, was subtracted from the fluorescence of each experimental sample. One unit of aryl hydrocarbon hydroxylase activity has been defined (7) as that amount of enzyme catalyzing the formation per min at 37°C of hydroxylated product causing fluorescence equivalent to that of 1 pmol of 3-hydroxybenzo[a]pyrene. Protein concentrations were determined by a slight modification of the method as described by Lowry et al. (32), with crystalline bovine serum albumin as the standard. The limit of sensitivity for the assayed specific hydroxylase activity is about 0.10 unit per mg of protein, and duplicate determinations normally vary less than 10% (2).

In separate experiments the optimal pH conditions for the hydroxylase activity from different tissue sources were determined. In other separate experiments the substrate [3H]benzo[a]pyrene was used and the total amount of alkali-extractable radioactivity was measured; 0.10- or 0.20-ml aliquots of the 1 N NaOH were dissolved in 1 ml of Nuclear Chicago Solubilizer, and the solution was mixed with 10 ml of a toluene scintillation mixture and counted in a Packard scintillation counter.

Spectrophotometry—Difference spectra of turbid microsomal fractions were measured as previously described (7, 21) in 1-cm cuvettes at room temperature in a Shimadzu model MPS-50L multipurpose recording spectrophotometers (American Instrument Company, Baxter Laboratories, Silver Spring, Maryland). Wave length measurements were standardized by the use of a hemolium oxide crystal.

The method of Oniura and Sato (21) for determining the concentrations of CO-binding microsomal cytochromes was used. The experimental sample was saturated with CO by bubbling the gas gently for 1.3 to 30 min, and a base-line for the oxidized fraction was measured as previously described (7, 21) in 1-cm cuvettes at room temperature in a Shimadzu model MPS-50L fluorometer (American Instrument Company, Baxter Laboratories, Silver Spring, Maryland). Fluorescence corresponding to fluorescence of cytochrome P₄₅₀ was measured in the proximity of the hemoprotein active site is indeed different from that of the other cytochrome species. The membrane structure or configuration which the newborn and especially the premature have not yet acquired the necessary microsomal enzymes for metabolizing chloramphenicol or bilirubin. The constitutive hydroxylase in utero appeared significantly (p < .05) earlier in the C57BL/6N mouse liver, compared with that in DBA/2N liver; however, the postnatal rise in the control hepatic enzyme activity from either strain was not significantly different. Twenty-four hours after a single intraperitoneal dose of MC to the pregnant C57BL/6N mouse, the hydroxylase activity was induced transeptally to detectable levels as early as 9 days before parturition. Induction of this enzyme response to the polycyclic hydrocarbon also increased markedly during the first week post partum and slowly declined after the weaning period. In the DBA/2N mouse this age-dependent response of aryl hydrocarbon hydroxylase induction by MC did not appear. If one compares the control and MC-inducible hydroxylase activities at each age (first two columns in Table I), there is a 5- to more than 50-fold difference at the various ages, indicating a poor correlation between the constitutive and inducible enzyme levels. We have also found in fetal rat hepatocyte cultures that the maximally inducible hydroxylase activity is not dependent upon the constitutive enzyme level.

This marked increase in hydroxylase induction by MC as a function of age also occurred in C57BL/6N nonhepatic tissues, whereas the lack of response to MC was found in DBA/2N nonhepatic tissues. Furthermore, we found a similar complete absence of response to polycyclic hydrocarbons in the NZW/BLN and NZB/BLN strains, and MC-inducible hydroxylase activity in the National Institutes of Health General-Purpose and the C3H/Hen mouse strains. On the contrary, the transplacental and post partum stimulation of this oxygenase activity in the liver by PB was similar in each of the six strains mentioned. The transplacental induction of hydroxylase activity by polycyclic hydrocarbons occurs in fetal tissues of the hamster or rat, respectively.

**RESULTS**

**Presence or Absence of Age-dependent Response of Hydroxylase Induction by 3-Methylcholanthrene—**Table I compares the hepatic hydroxylase activity of C57BL/6N and DBA/2N mice in response to MC as a function of age. The constitutive level of this enzyme in either mouse strain was detectable in utero, increased markedly during the first post partum week, and was maximal in the 3-week-old weaning. The phenomenon of this "physiologic" induction of hepatic oxygenase activity occurring immediately post partum is presumably related to such clinical entities as the Gray syndrome (34) and neonatal hyperbilirubinemia, in which the newborn and especially the premature have not yet acquired the necessary microsomal enzymes for metabolizing chloramphenicol or bilirubin. The constitutive hydroxylase in utero appeared significantly (p < .05) earlier in the C57BL/6N mouse liver, compared with that in DBA/2N liver; however, the postnatal rise in the control hepatic enzyme activity from either strain was not significantly different. Twenty-four hours after a single intraperitoneal dose of MC to the pregnant C57BL/6N mouse, the hydroxylase activity was induced transeptally to detectable levels as early as 9 days before parturition. Induction of this enzyme response to the polycyclic hydrocarbon also increased markedly during the first week post partum and slowly declined after the weaning period. In the DBA/2N mouse this age-dependent response of aryl hydrocarbon hydroxylase induction by MC did not appear. If one compares the control and MC-inducible hydroxylase activities at each age (first two columns in Table I), there is a 5- to more than 50-fold difference at the various ages, indicating a poor correlation between the constitutive and inducible enzyme levels. We have also found in fetal rat hepatocyte cultures that the maximally inducible hydroxylase activity is not dependent upon the constitutive enzyme level.

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**Whether the constitutive and inducible hydroxylase activities represent different proteins or a different membrane configuration is presently not clear. However, the ferric iron of cytochrome P₄₅₀ involved in the two enzyme active sites probably has different spin states (2), and the two enzymes interact differently with various lipophilic compounds (D. W. Nebert, F. M. Goujon, and J. E. Gielen, submitted for publication). These two types of evidence suggest to us that the membrane structure or configuration in the proximity of the hemoprotein active site is indeed different between the "control" and the "aromatic hydrocarbon-induced" hydroxylase activities.**

J. E. Gielen and D. W. Nebert, unpublished data.
Hepatic aryl hydrocarbon hydroxylase induction in response to MC as a function of age in the C57BL/6N or DBA/2N mouse

Hydroxylase activity was measured from the homogenate of hepatic tissue. In this table and in subsequent tables the values of enzyme activity or cytochrome content are given as the mean ± standard deviation, with the number of individual samples assayed shown in parentheses.

| Age (days) | Control | MC | Control | MC |
|-----------|---------|----|---------|----|
| - 9 b     | <0.10(6)| 0.70±0.15(8) | <0.10(5) | <0.10(5) |
| - 5       | 0.50±0.04(4) | 7.7±1.0(7) | <0.10(6) | <0.10(5) |
| - 3       | 0.72±0.11(7) | 5.0±9.0(7) | 0.35±0.06(6) | 0.30±0.04(5) |
| 9         | 68±11(10) | 19±0.40(5) |
| 13        | 13±2.2(8) | 110±12(10) | 18±4.0(11) | 13±2.9(9) |
| 21        | 130±28(12) | 270±200(15) | 110±29(12) | 92±11(12) |
| 70        | 86±18(6) | 610±140(7) | 80±19(6) | 77±13(7) |

$^a$Units per mg of whole liver homogenate protein.

$^b$Minus numbers represent the estimated days prior to birth at which the hydroxylase activities in the individual livers of 5 or more fetuses were determined. The mother had been treated with MC or corn oil alone 24 hours before assaying the fetal hepatic enzyme. "Zero" denotes individual mice born less than 24 hours after their mother had received the MC.

and the marked rise in both constitutive and inducible oxygenase activities immediately post partum has been previously noted (5, 11, 35).

Effect of pH on Hydroxylase Activities from 3-Methylcholanthrene-treated and Control C57BL/6N or DBA/2N Mice—The enzyme active sites may differ in such a way that MC-inducible and MC-noninducible hydroxylase activities are different with respect to pH optima, substrate affinity, relative thermostability, or product formation. These possible considerations, which might affect the enzyme assay, are ruled out in the following two figures in which we compare the enzymes from C57BL/6N and DBA/2N mice. Similar results were obtained with MC-inducible and MC-noninducible hydroxylase activities from the other mouse strains.

Fig. 1 illustrates the effects of pH on the hydroxylase activities from hepatic and renal microsomes of MC-treated and control C57BL/6N or DBA/2N mice. A pH range of 6.0 to 7.2 was optimal for the MC-induced and control liver oxygenase from C57BL/6N mice and for the enzyme from DBA/2N liver microsomes after MC treatment. A pH optimum of about 6.4 was found for the constitutive hydroxylase from DBA/2N liver. The reason for this unusually low pH optimum is unknown but suggests some structural differences between the constitutive enzyme system from DBA/2N liver and the control hepatic hydroxylase from C57BL/6N or the liver oxygenase from MC-treated C57BL/6N or DBA/2N mice. Of further interest, we found this same low pH optimum of 6.4 for the constitutive enzyme from both NZW/BLN and NZB/BLN strains. However, the small differences in pH optimum for the hydroxylase activities of constitutive and MC-treated C57BL/6N and DBA/2N mice do not account for the 5-fold to more than 50-fold stimulation of enzyme activity by MC that develops in the neonatal C57BL/6N mouse (Table I).

The stability of hepatic cytochrome P450 is greatest between pH 7.2 and 7.5 (36). However, the CO-binding pigments cannot be compared at different pH values, since the stability of the MC-induced, PB-induced, and control forms of the cytochrome is pH-dependent (28). Thus, we chose to assay the hepatic hydroxylase activity and CO-binding hemoproteins at pH 7.2 for the remainder of the results presented in this report.

A pH optimum of about 7.5 was observed for the MC-induced enzyme activity from C57BL/6N kidney microsomes. Because of the low enzyme activity in C57BL/6N kidney control microsomes and in renal microsomes from the MC-treated or control DBA/2N mouse, the pH profile on these samples could not be accurately determined. This higher pH optimum of the enzyme
from an extrahepatic tissue is consistent with that found (2, 3) in secondary cell cultures from rodent embryos, since the cells in culture are predominantly nonhepatic tissue (i.e., fibroblasts). Therefore, the extrahepatic hydroxylase activity was examined at pH 7.5 for all data shown in the remainder of this paper.

**Apparent** $K_m$ **Values and Heat Inactivations for Hydroxylase Activities from 3-Methylcholanthrene-treated and Control C57BL/6N or DBA/2N Mice**—The apparent Michaelis constants for the hepatic oxygenases from MC-treated and control C57BL/6N or DBA/2N mice ranged from 10 to 50 μM and were not significantly different from one another in several experiments. Since the hydroxylase activity apparently represents a functional membrane-bound multicomponent electron chain (22), it must be emphasized (3, 13) that the apparent $K_m$ values reflect the rate-limiting step in the electron transport pathway. The apparent $K_m$ value from the MC-induced enzyme activity from C57BL/6N kidney microsomes ranged from 0.5 to 0.8 μM in several experiments. This high affinity of the hydroxylase for benzo[a]pyrene in an extrahepatic tissue is in agreement with that (3) estimated for the benzo[a]anthracene-induced aryl hydrocarbon hydroxylase activity in secondary fetal hamster cell cultures which are predominantly nonhepatic cells. The constitutive enzyme from C57BL/6N kidney microsomes and the enzyme in renal microsomes from either MC-treated or control DBA/2N mice were too low for accurately determining an apparent $K_m$ value.

We found that the constitutive hepatic enzymes from C57BL/6N, DBA/2N, NZW/BLN, and NZB/BLN strains, the liver oxygenase activities in all four strains after MC treatment, and the MC-induced kidney hydroxylase activity in the C57BL/6N mouse were not different with respect to heat inactivation. A 5-min preliminary treatment period of the enzyme in phosphate buffer without cofactors or substrate at 50° before the assay destroyed about one-half, and 5 min at 60° inactivated virtually all, of the hydroxylase activity in each case. This finding indicates to us that, if a multicomponent enzyme system is responsible for polycyclic hydrocarbon hydroxylation in each case, the most thermolabile moiety is the same in each of the basal and MC-inducible hydroxylases.

**Equating 3-Hydroxybenzo[a]pyrene Formation with Hydroxylase Activity in 3-Methylcholanthrene-treated and Control C57BL/6N or DBA/2N Mice**—Various oxygen-containing metabolites of benzo[a]pyrene are known (37, 38) to be formed by the oxygenase in vivo and in cell culture. Thus, it is possible that our equating 3-hydroxybenzo[a]pyrene formation with aryl hydrocarbon hydroxylase activity from each mouse strain may be erroneous. For example, the major metabolite might be 3-hydroxybenzo[a]pyrene in the C57BL/6N mouse and 9,10-dihydro-9,10-dihydroxybenzo[a]pyrene in the DBA/2N mouse. If these two products possess sufficiently different fluorescent activation and
### Table II

**Genetic variance of hepatic aryl hydrocarbon hydroxylase induction by MC or PB in inbred and hybrid C57BL/6N, DBA/2N, NZW/BLN, and NZB/BLN mouse strains**

| Genetic cross | Phenotype | Theoretical genotype | Hydroxylase specific activity$^a$ |
|---------------|-----------|-----------------------|----------------------------------|
|               |           | Control | MC | PB |
| **Hydroxylase specific activity$^a$** | | | | |
|  |  | | 2700 ± 380 (10) | 1160 ± 140 (6) |
| CC | AhAh x AhAh | 630 ± 120 (7) | | |
| NN | ahah x ahah | 590 ± 150 (8) | 550 ± 180 (10) | 1120 ± 110 (6) |
| MW | ahah x ahah | 770 ± 270 (12) | 760 ± 190 (13) | 1270 ± 290 (8) |
| BB | ahah x ahah | 590 ± 180 (6) | 560 ± 180 (7) | 1270 ± 300 (7) |
| DW | ahah x ahah | 780 ± 280 (6) | 660 ± 120 (9) | | |
| Ws | ahah x ahah | 580 ± 140 (6) | 590 ± 210 (9) | 1090 ± 250 (6) |
| WB | ahah x ahah | 770 ± 240 (7) | 710 ± 210 (11) | |
| CD | AhAh x ahah | 590 ± 220 (6) | 7500 ± 380 (8) | 1170 ± 290 (8) |
| CM | AhAh x ahah | 760 ± 210 (6) | 2970 ± 500 (8) | |
| CB | AhAh x ahah | 620 ± 180 (5) | 3020 ± 430 (13) | 1200 ± 310 (5) |
| C/CD | AhAh x AhAh | 670 ± 250 (7) | 2770 ± 570 (10) | 1300 ± 190 (6) |
| CD/D | Ahah x ahah | 620 ± 180 (7) | 580 ± 100 (20) | 2660 ± 490 (23) | 1120 ± 140 (8) |
| CM/W | Ahah x ahah | 760 ± 230 (6) | 720 ± 240 (8) | 2390 ± 400 (7) | |
| CW/D | Ahah x ahah | 700 ± 200 (4) | 590 ± 150 (5) | 2840 ± 100 (7) | |
| CD/CN | Ahah x Ahah | 670 ± 170 (6) | 590 ± 320 (6) | 2980 ± 590 (42) | 1220 ± 240 (7) |
| CW/CN | Ahah x Ahah | 770 ± 240 (6) | 730 ± 480 (4) | 3090 ± 560 (12) | |
| CD/ON | Ahah x Ahah | 660 ± 200 (5) | 660 ± 180 (8) | 2760 ± 390 (9) | |

*Units per mg of hepatic microsomal protein, expressed as the mean ± standard deviation with the number of individual mice assayed shown in parentheses.

$^a$ In this table and in subsequent tables the C57BL/6N, DBA/2N, NZW/BLN, and NZB/BLN strains are referred to as C, D, W, and B, respectively. Thus, CC represents an inbred C57BL/6N mouse, C/CD indicates the homozygous inbred C57BL/6N mouse crossed with F1 hybrid from a previous cross between a C57BL/6N and a DBA/2N mouse, CW/CW stands for a cross between F1 hybrids of C57BL/6N and NZW/BLN parents, yielding the F2 generation, etc.
emission spectral maxima, conceivably it could appear that the hydroxylase activity is inducible in the C57BL/6N mouse and not in the DBA/2N mouse strain.

Fig. 2 demonstrates that more than 95% of the alkali-extractable radioactivity can be accounted for by the 3-hydroxybenzo[a]pyrene determined spectrophotofluorometrically and that this comparison is true for the hepatic and renal microsomal hydroxy-

Hepatic hydroxylase induction by polycyclic hydrocarbons as simple autosomal dominant trait—Table II shows the genetic expression of the constitutive hydroxylase and of the enzyme in induction by MC or PB in offspring from various crosses between inbred and hybrid mice. The oxygenase activity was induced by MC more than 4-fold in the C57BL/6N mouse, whereas the levels of the enzyme system were not significantly different from the constitutive levels in MC-treated DBA/2N, NZW/BLN, or NZB/BLN inbred mice or in F1 offspring from the three possible genetic crosses between the DBA/2N, NZW/BLN, and NZB/BLN strains. In the F1 hybrids from crosses between C57BL/6N and any one of the DBA/2N, NZW/BLN, or NZB/BLN strains, and in offspring from the cross between inbred C57BL/6N and the F1 hybrid of C57BL/6N and DBA/2N parents (i.e., CD/C), the hydroxylase activity in response to MC rose about 4-fold. In offspring from the backcross between inbred DBA/2N or NZW/BLN mice and F1 hybrids from previous crosses between C57BL/6N and DBA/2N or C57BL/6N plus NZW/BLN (i.e. CD/D, CW/W, or CW/D), distribution of the oxygenase activity after MC treatment was distinctly bimodal: in approximately one-half of the population the enzyme was induced about 4-fold; in the other half the specific activities were not significantly different from control values. In MC-treated offspring from F1 hybrids of C57BL/6N and either DBA/2N or NZW/BLN parents (i.e., F2 generation), the hepatic microsomal hydroxylase activities were again distributed in a bimodal manner: in about three-fourths of the population the enzyme was induced about 4-fold by MC, whereas the enzyme levels in the remaining one-fourth of the MC-treated mice were not different from the constitutive levels. We found no correlation between the extent of MC-inducible hydroxylase activity and the coat color or sex in any of the genetic crosses examined. Also, we observed that other polycyclic hydrocarbons similarly induce the enzyme in the same pattern among these various inbred and hybrid mice.

Therefore, the expression of aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons is inherited as a simple autosomal dominant trait, for which we postulate the genes $Ah$ and $Ah$.
It can be seen in Table II that if we decide that strain C57BL/6N is homozygous for \( Ah \) and the other three strains are homozygous for \( ah \), the microsomal oxygenase activity is inducible by MC in any mouse homozygous or heterozygous for \( Ah \). A \( p < .001 \) was found by chi square analysis in which MC-inducible hepatic hydroxylase activity was observed with the expected frequencies of 50 and 75% in the \( AhAh \times ahah \) and \( AhAh \times Ahah \) crosses, respectively: there were 37 mice in which the oxygenase was inducible by MC among a total of 70 offspring from the \( AhAh \times ahah \) backcrosses; among 81 \( F_2 \) offspring from the various genetic crosses, MC induced the hydroxylase system in 63 of these mice. Moreover, by MC administration to pregnant mice containing fetuses of an \( AhAh \times ahah \) genetic backcross (39), we can detect in utero fetuses having the MC-inducible hydroxylase and those nonresponsive to MC. Also, if such fetuses are individually placed in cell culture (39), the bimodal distribution in response to polycyclic hydrocarbons in the growth medium is found.

Among the MC-treated animals there are clearly two groups of specific activities: (a) those ranging from 550 to 760 in which the means are not significantly different from the means of the constitutive enzyme activities, and (b) those ranging from 2390 to 3090 in which the hydroxylase activity is induced between 4- and 5-fold. Actually, the mean value of 3090 is significantly different \( (p < .01) \) from the means of 2500 and 2390. However, we feel that the variations in these maximally inducible levels probably reflect small differences in the assay (e.g. pH, chemical reagents, efficiency of the spectrophotofluorometer) or in the mice (e.g. effective dose of MC absorbed, age, hormonal, nutritional, or environmental factors) for the 6-month period during which these determinations were made. Two general observations which were not significantly \( (p > .05) \) different should also be noted. First, among the inbred NZW/BLN and crosses involving NZW/BLN with the other three strains, we observed a greater variation and generally higher mean values for the constitutive hydroxylase specific activities. Second, in the MC-treated mice in which the enzyme was noninducible the mean specific activities of the oxygenase were generally less than those of the constitutive enzyme.

For all genetic groups examined in Table II, PB induced the

### Table IV

Genetic variance of kidney aryl hydrocarbon hydroxylase induction by MC or PB in inbred and hybrid C57BL/6N, DBA/2N, NZW/BLN, and NZB/BLN mouse strains

| Genetic cross | Theoretical genotype | Hydroxylase specific activitya | Control | MC | PB |
|---------------|----------------------|--------------------------------|---------|----|----|
| UC            | AhAh x AhAh          | 0.35 ± 0.12 (14)               | 12 ± 11 (15) | 0.06 ± 0.05 (4) |
| DD            | ahah x ahah          | 0.20 ± 0.20 (5)                | 0.24 ± 0.25 (7) | 0.28 ± 0.22 (4) |
| WW            | ahah x ahah          | 0.19 ± 0.07 (5)                | 0.36 ± 0.14 (6) | 0.47 ± 0.24 (4) |
| BB            | ahah x ahah          | 0.09 ± 0.06 (5)                | 0.23 ± 0.15 (10) | 0.23 ± 0.19 (7) |
| DW            | ahah x ahah          | 0.19 ± 0.12 (6)                | 0.25 ± 0.06 (9) |
| DB            | ahah x ahah          | 0.32 ± 0.26 (6)                | 0.35 ± 0.21 (9) | 0.16 ± 0.10 (4) |
| BW            | ahah x ahah          | 0.10 ± 0.07 (7)                | 0.23 ± 0.09 (11) |
| CD            | AhAh x ahah          | 0.37 ± 0.16 (5)                | 14 ± 9.2 (15) | 1.3 ± 1.0 (9) |
| CW            | AhAh x ahah          | 0.18 ± 0.05 (6)                | 12 ± 10 (15) | 0.16 ± 0.09 (4) |
| CB            | AhAh x ahah          | 0.26 ± 0.18 (5)                | 15 ± 7.3 (10) | 0.15 ± 0.11 (5) |
| C/CW          | AhAh x AhAh          | 0.35 ± 0.08 (7)                | 15 ± 9.3 (10) | 0.19 ± 0.04 (4) |
| CD/D          | Ahah x ahah          | 0.27 ± 0.14 (6)                | 0.28 ± 0.11 (6) | 19 ± 20 (9) | 1.1 ± 0.97 (4) |
| CW/NW         | Ahah x ahah          | 0.50 ± 0.33 (6)                | 0.36 ± 0.43 (8) | 19 ± 16 (7) |
| CW/CW         | Ahah x Ahah          | 0.72 ± 0.44 (6)                | 0.66 ± 0.43 (4) | 17 ± 6 (12) |

aUnits per mg of kidney homogenate protein.
hepatic hydroxylase activity to the same extent. The mean values ranged from 1090 to 1320 and were not significantly (p > .05) different from each other, but were significantly (p < .01) different from those of the constitutive oxygenase levels. The magnitude of induction by PB was about 2-fold or slightly less. Hence, induction of the enzyme by polycyclic hydrocarbons is expressed in an autosomal dominant character; however, among the strains of mice examined, this simple Mendelian expression was not found for the hydroxylase induction by PB. This result is consistent with other studies (12-14, 26-28, 40-42) indicating that microsomal enzyme induction by PB and by polycyclic hydrocarbons involves processes which are distinctly different at least at one step in the sequence of events. For example, the combination of PB and a polycyclic hydrocarbon in vivo (41) or in cell culture (12, 13) stimulates certain hepatic oxygenases to levels which are the sum of that induced by either inducer alone.

**Genetic Expression of Formation of Spectrally Distinct Hepatic CO-binding Cytochrome after 3-Methylcholanthrene Treatment—**

Table III shows the levels of hepatic CO-binding pigments in some of the same groups of control, MC-treated, and PB-treated mice shown in the previous table. The mean values of constitutive cytochrome P₄₅₀ ranged between 700 and 950 pmoles per mg of microsomal protein and were not significantly (p > .05) different from each other. The CO-binding hemoprotein in the inbred NZW/BLN and the C57DL/6N-NZW/BLN F₁ hybrid appeared more variable and again the mean values were generally higher than those for the other inbred and hybrid strains. Distribution of the hemoprotein concentration in the MC-treated mice was also distinctly bimodal and followed classical Mendelian genetics. In those groups having no hydroxylase induction in response to MC, the CO-binding cytochrome content was not significantly (p > .05) different from constitutive levels; in mice possessing the MC-inducible oxygenase, the CO-binding pigment levels ranged between 1130 and 1710 pmoles per mg of microsomal protein. The means of the groups in this latter population are not significantly different from each other but are significantly (p < .01) greater than those of cytochrome P₄₅₀ in the control mice. Furthermore, a correlation between the oxygenase induction (Table I) and the rise in cytochrome P₄₅₀ content (Table III) among MC-treated mice can be clearly seen. This observation has been investigated further, and we found a stoichiometric relationship between the induction by MC and the increase in high-spin iron-containing CO-binding pigment in mouse liver microsomes (2).

Polycyclic hydrocarbon administration in vivo causes about a 2-nm blue spectral shift in the Soret maximum of the reduced hemoprotein-CO complex from hepatic microsomes (24-26). Similarly we found that this hyperspectral shift was present in each MC-treated mouse in which the enzyme was inducible and absent in each MC-treated mouse in which the hydroxylase did not respond to MC. From additional studies, we conclude that an increase in high spin iron-containing cytochrome P₄₅₀ is directly related to this blue shift in the absorption peak of the reduced hemoprotein-CO complex (2).

**Stimulation of cytochrome P₄₅₀ content by PB—**

Stimulation of cytochrome P₄₅₀ content by PB was similar in all groups of inbred and hybrid mice examined and ranged between 1430 and 1950 pmoles per mg of microsomal protein. The means of the groups within this range are not significantly (p > .05) different from each other, but are significantly (p < .01) different from those of the constitutive oxygenase levels. The magnitude of induction by PB was about 2-fold or slightly less. Hence, induction of the enzyme by polycyclic hydrocarbons is expressed in an autosomal dominant character; however, among the strains of mice examined, this simple Mendelian expression was not found for the hydroxylase induction by PB. This result is consistent with other studies (12-14, 26-28, 40-42) indicating that microsomal enzyme induction by PB and by polycyclic hydrocarbons involves processes which are distinctly different at least at one step in the sequence of events. For example, the combination of PB and a polycyclic hydrocarbon in vivo (41) or in cell culture (12, 13) stimulates certain hepatic oxygenases to levels which are the sum of that induced by either inducer alone.

**Genetic Expression of Hydroxylase Induction by 3-Methylcholanthrene in Kidney—**

Table IV shows the enzyme levels from the kidney of the various control, MC-treated, and PB-treated inbred and hybrid mice. The mean hydroxylase specific activities from control and PB-treated mice ranged from 0.09 to 0.72 and were not significantly (p > .05) different from each other. It is known that PB is not an effective inducer of aryl hydrocarbon hydroxylase activity in nonhepatic tissues in vivo (5) or in nonhepatic cells in culture (3). In the MC-treated mice the oxidase activity was again distributed as before: in one population the means were not different from constitutive levels, whereas in the other population the means of the specific activities ranged from 12 to 19 and were significantly (p < .01) different from the control values. In mice possessing the MC-inducible hydroxylase, the specific activities in the kidney were highly variable, ranging from 3 to more than 40. Fig. 3 illustrates that if one plots the hydroxylase-specific activities of the liver versus that of the kidney for MC-treated mice from Ahah × ahah genetic crosses, a bimodal distribution is again appreciated. Furthermore, there is a high correlation in the magnitude of enzyme induction between the liver and kidney. We found similarly high correlations in the magnitude of hydroxylase induction in bowel, lung, and skin.

FIG. 3. Relationship between aryl hydrocarbon hydroxylase activity in liver and kidney of 38 representative mice from the Ahah × ahah backcross. Each closed circle depicts the hepatic microsomal and kidney enzyme activity from an individual MC-treated mouse. The correlation coefficient ρ is 0.83 (p < 0.001).

1 D. W. Nebert, F. M. Goujon, and J. E. Gielen, manuscript in preparation.
Genetic variance of bowel aryl hydrocarbon hydroxylase induction by MC or PB in inbred and hybrid C57BL/6N, DBA/2N, NZW/BLN, and NZB/BLN mouse strains

| Genetic cross | Phenotype | Theoretical genotype | Hydroxylase specific activitya | Control | MC | PB |
|--------------|-----------|----------------------|-------------------------------|---------|----|----|
|              |           |                      |                               |         |    |    |
| CC           | AhAh x AhAh | 0.96 ± 0.65 (6) | 31 ± 28 (17)                  | 0.98 ± 0.31 (4) |
| DD           | ahah x ahah  | 0.24 ± 0.27 (5) | 0.47 ± 0.37 (5)               | 0.48 ± 0.20 (4) |
| WW           | ahah x ahah  | 2.0 ± 1.3 (6) | 2.0 ± 1.2 (6)                  | 1.7 ± 0.45 (4) |
| BB           | ahah x ahah  | 0.47 ± 0.11 (4) | 1.7 ± 0.51 (9)                | 0.45 ± 0.12 (5) |
| DW           | ahah x ahah  | 3.0 ± 0.82 (4) | 3.4 ± 2.6 (9)                 |          |    |    |
| BB           | ahah x ahah  | 0.31 ± 0.22 (4) | 0.45 ± 0.35 (9)               | 0.20 ± 0.30 (4) |
| BB           | ahah x ahah  | 1.6 ± 1.1 (4) | 1.4 ± 0.52 (11)               |          |    |    |
| CW           | AhAh x ahah  | 1.2 ± 0.70 (6) | 47 ± 29 (15)                  | 1.5 ± 1.3 (4) |
| CW           | AhAh x ahah  | 1.1 ± 0.91 (6) | 66 ± 48 (8)                   | 0.77 ± 0.16 (4) |
| GB           | AhAh x ahah  | 0.93 ± 0.68 (5) | 81 ± 42 (10)                  | 0.37 ± 0.19 (3) |
| CD/D         | Ahah x ahah  | 0.71 ± 0.46 (4) | 35 ± 20 (10)                  | 0.71 ± 0.38 (4) |
| CD/D         | Ahah x ahah  | 0.70 ± 0.38 (7) | 0.57 ± 0.22 (6)               | 0.60 ± 0.15 (3) |
| CD/D         | Ahah x ahah  | 1.8 ± 1.3 (6) | 0.96 ± 0.60 (8)               | 66 ± 38 (7) |
| CW/CW        | Ahah x Ahah  | 1.6 ± 0.88 (6) | 1.0 ± 0.62 (4)                | 70 ± 32 (12) |

aUnits per mg of bowel homogenate protein.

Thus, if the hepatic hydroxylase is highly inducible by MC, so is the enzyme in the kidney, bowel, lung, and skin; if the hepatic oxygenase does not respond to MC, then hydroxylase activity in these other tissues is also noninducible by MC. Most likely, the extent of enzyme induction is partially dependent upon how much MC is taken up by each tissue or is related to such factors as age, sex, nutrition, and stress of each individual animal.

Genetic Expression of Hydroxylase Induction in Bowel, Lung, and Skin by 3-Methylcholanthrene—Tables V and VI show the hydroxylase-specific activities in the bowel and lung of the same groups of inbred and hybrid mice. In the bowel of control and PB-treated mice, the mean values for the constitutive enzyme ranged between 0.31 and 3.0. The higher constitutive levels were found in the inbred NZW/BLN or in hybrids involving the NZW/BLN strain, and one of these means (i.e. 3.0) was significantly (p < .05) different from the lowest means in the control groups (e.g. 0.21 and 0.31). MC treatment produced the same bimodal distribution, with one population not different from control levels and the other population inducible and highly variable. The magnitude of induction by MC was 30-fold to more than 80-fold; the means of the specific activities ranged from 31 to 81.

The genetic expression of the oxygenase induction in the lung by MC was the same. The mean specific activities in the control groups, MC-treated mice in which the enzyme was noninducible, and PB-treated mice were higher than that for kidney or bowel, ranging between 3.0 and 7.0. We found that means of the MC-inducible hydroxylase specific activities from lung ranged between 13 and 31. Similar studies with mouse skin demonstrated that the identical genetic expression also exists in that tissue, for control animals and MC-treated mice in which the enzyme is genetically noninducible the specific activities were less than 1.5, whereas MC-inducible hydroxylase activities were greater than 7 units per mg of skin homogenate protein.

DISCUSSION

In the neonatal C57BL/6N mouse the appearance of aryl hydrocarbon hydroxylase induction in response to polycyclic hydrocarbons occurs in all tissues which regularly contain the inducible enzyme. This response to a pharmacological, exogenous stimulus fails to develop in the inbred DBA/2N, NZW/BLN, or NZB/BLN strains. We found that the small differences which could have affected the enzyme assay (i.e. pH optima, substrate affinities, thermostability, or benzo(a)pyrene metabolism) are not re-
TABLE VI
Genetic variance of lung aryl hydrocarbon hydroxylase induction by MC or PB in inbred and hybrid C57BL/6N, DBA/2N, NZW/BLN, and NZB/BLN mouse strains

| Genetic cross | Theoretical genotype | Hydroxylase specific activitya (Units per mg of lung homogenate protein) |
|--------------|----------------------|------------------------------------------------------------------------|
|              |                      | Control                  | MC                   | PB                    |
|              |                      |                          |                      |                       |
| CC           | AhAh x AhAh          | 6.7 ± 3.8 (5)            | 31 ± 13 (11)         | 8.2 ± 5.6 (4)         |
| DD           | ahah x ahah          | 3.5 ± 3.1 (5)            | 4.7 ± 2.3 (5)        | 3.4 ± 2.2 (4)         |
| NN           | ahah x ahah          | 3.6 ± 0.78 (4)           | 6.8 ± 3.8 (6)        | 3.4 ± 1.2 (4)         |
| RR           | ahah x ahah          | 3.5 ± 0.93 (4)           | 4.1 ± 2.7 (8)        | 4.3 ± 1.1 (7)         |
| DM           | ahah x ahah          | 6.2 ± 4.0 (6)            | 6.8 ± 3.8 (9)        |                       |
| DB           | ahah x ahah          | 3.0 ± 2.2 (4)            | 3.6 ± 1.2 (9)        | 4.3 ± 3.0 (4)         |
| CD           | AhAh x ahah          | 6.4 ± 4.1 (6)            | 17 ± 11 (15)         | 7.0 ± 4.6 (9)         |
| CW           | AhAh x ahah          | 5.7 ± 4.7 (6)            | 25 ± 6.4 (15)        | 5.5 ± 2.0 (4)         |
| CB           | AhAh x ahah          | 3.9 ± 1.9 (5)            | 13 ± 4.4 (10)        | 5.9 ± 3.9 (5)         |
| C/CD         | AhAh x AhAh          | 7.0 ± 3.0 (7)            | 19 ± 8.2 (10)        | 6.9 ± 5.5 (4)         |
| CD/D         | ahah x ahah          | 6.9 ± 4.8 (6)            | 6.6 ± 4.0 (6)        | 13 ± 5.4 (9)          | 3.9 ± 3.0 (4)         |
| CD/W         | AhAh x ahah          | 3.1 ± 2.1 (6)            | 6.4 ± 4.2 (8)        | 14 ± 8.4 (7)          | 6.2 ± 7.6 (6)         |

a Inducible

|              |                      | Noninducible             | Inducible              |
|--------------|----------------------|--------------------------|------------------------|
|              |                      |                          |                        |
|              |                      |                          |                        |

Responsible for the genetically different response to MC in the C57BL/6N mouse, compared with that in the MC-nonresponsive strains.

What cellular mechanisms are involved in the genetic regulation of this hydroxylase induction by polycyclic hydrocarbons? Which steps in the sequence of events occurring during the induction process are genetically expressed in the MC-responsive strain or are genetically suppressed in the MC-nonresponsive strains? From our studies of microsomal enzyme induction (1-14), we can exclude the possibility that changes in aryl hydrocarbon hydroxylase activity are regulated solely by the post-translational activation or inhibition of pre-existing enzyme protein. Also, the quantity of active enzyme rather than the kinetics constants appears to be the main factor in determining the amount of polycyclic hydrocarbon hydroxylase. Therefore, all the steps involved in normal gene expression must be considered as possibilities for explaining the genetic difference in the regulation of polycyclic hydrocarbon-inducible hydroxylase activity described in this report. Hence, possible differences might occur during the (a) uptake and binding of inducer to receptor sites, (b) transcription of induction-specific RNA, (c) nucleocytoplasmic transport of this new RNA species and translation into induction-specific protein, or (d) assembly or degradation of the microsomal membrane components. We found that the enzyme induction by polycyclic hydrocarbons is expressed as an autosomal dominant trait and that when the individual mouse is genetically responsive to MC, induction of the hydroxylase activity is an all-or-none phenomenon occurring in every tissue regularly containing the polycyclic hydrocarbon-inducible enzyme.

To our knowledge this is the first example in mammalian genetics in which the induction of enzyme activity is regulated by one chromosome and perhaps by genes at a single locus. Our studies with clones of mouse 3T3 cells are also consistent with this concept that genetic control of the hydroxylase induction by polycyclic hydrocarbons is an all-or-none phenomenon occurring in every tissue regularly containing the polycyclic hydrocarbon-inducible enzyme.

A receptor site for either PB or a polycyclic hydrocarbon is presently hypothetical. Presumably, these inducers must bind to some cellular macromolecule so as to effect the sequence of events (i.e. transcription and translation) leading to the induction of aryl hydrocarbon hydroxylase activity. Whether such a binding involves a high affinity binding constant or is a fairly nonspecific event, and the subcellular location of such a phenomenon, all remain to be elucidated.

Induction-specific RNA involved in enzyme induction may be a single species of mRNA or several mRNA species coding for several specific proteins. On the other hand, the RNA may be of the ribosomal or transfer RNA type, which might control the translation of a pre-existing, stable mRNA template that is coded for the induction-specific protein. This latter possibility is perhaps less likely, since mRNA and tRNA species are generally thought to have longer half lives.

10 W. F. Benedict and D. W. Nebert, manuscript in preparation.
polycyclic hydrocarbons may reside on a single chromosome. The possibility of nearby genes at two or more loci on the same chromosome (i.e. coordinated genome) cannot be ruled out from these data. Single gene mutations which result in varying magnitudes of enzyme induction are known (43) to occur in microorganisms. The genetic expression of microsomal oxidase induction in houseflies by the insecticide dieldrin is apparently inherited dominantly (44); however, because the enzyme activity in individual houseflies could not be measured, the appropriate backcrosses were not done. The induction of \( \beta \)-aminolevulinic acid synthetase in mice by 3,5-dicarbethoxy-1,4-dihydro-2,4,6-trimethylpyridine requires genes at several loci (45). Cytoplasmic aldehyde dehydrogenase induction in rat liver by phenobarbital (46) is inherited as a codominant trait. In mice the activities of several constitutive enzymes are controlled by a single gene or genome: \( \beta \)-glucuronidase (47), several kidney esterases (48), hepatic \( \beta \)-aminolevulinic acid dehydratase (49), liver catalase (50), and \( \beta \)-ureidopropionase involved in pyrimidine catabolism (51).

The autosomal dominant trait in the C57BL/6N mouse may be the manifestation of either a structural or a regulatory gene. Because structural gene mutations may produce phenotypic effects similar to those expected for regulatory gene mutants, the problem of distinguishing between regulatory and structural gene mutations is difficult. Generally, the structural gene mutant is (a) inherited as a single factor, (b) codominant so that the enzyme content in heterozygotes is the arithmetic mean of the two homozygous parents, and (c) expressed equally in all tissues at all times. However, in many structural gene mutations secondary effects on enzyme concentration or activity that may be expressed to varying degrees (52). Thus, it is possible that a structural gene for the MC-inducible hydrolase differs from that for the constitutive enzyme. We did find a lower pH optimum for the control liver hydroxylase from strains nonresponsive to MC, compared with that for the constitutive C57BL/6N hepatic enzyme and the hydroxylase systems from the liver of MC-treated mice. There are direct correlations between polycyclic hydrocarbon-inducible oxygenase activity and (a) a loss of inhibition of 3-methyl-4-monomethylaminoazobenzene \( N \)-demethylation activity in vitro by 2-diethylaminomethyl-2,2-diphenylvalerate hydrochloride (53), (b) formation of a spectrally distinct CO-binding cytochrome (1, 7, 11, 54), (c) a loss of inhibition of polycyclic hydrocarbon metabolites bound covalently to cellular material by 2-diethylaminomethyl-2,2-diphenylvalerate hydrochloride in cell culture (8), (d) an increase in Type II binding sites (55), and (e) greater inhibition of aryl hydrocarbon hydroxylase in vitro by \( \alpha \)-naphthoflavone (56). Hence, the hydroxylase induction by polycyclic hydrocarbons may be associated with the observed (2) conversion of low spin iron-containing cytochrome \( P_{490} \) to the high spin form. Therefore, we conclude that the microsomal membrane and specifically the ligand environment of cytochrome \( P_{490} \) is altered during polycyclic hydrocarbon treatment of mice genetically responsive to MC; however, at the present time the question of whether or not there exists a difference in the structural gene and therefore a structural difference in the enzyme protein cannot be answered. For example, in C57BL/6N liver the amount of "substrate-specific protein" (11) may be merely increased: microsomal membrane containing 20% of its cytochrome \( P_{490} \) iron in the high spin state and having a hydroxylase specific activity of 700 may be changed by MC treatment to a membrane containing 80% high spin iron-containing hemoprotein and having an enzyme specific activity of 2800.

Differences in a regulatory gene would be reflected in alterations in the rates of enzyme synthesis or degradation, leading to the establishment of a new steady state level of hydroxylase activity. Thus, an increased rate of degradation for the induced oxygenase activity in the strains nonresponsive to MC may explain our data. Alternatively, because of an unstable induction-specific RNA, perhaps the rate of hydroxylase synthesis in the MC-nonresponsive strains cannot occur faster than the rate at which the constitutive enzyme is synthesized. Possible changes in the rates of enzyme synthesis or degradation in vivo are presently difficult to examine, since the multicomponent nature of the hydroxylase system (22) renders purification of an enzyme protein technically unsatisfactory. The first example of a genetically controlled alteration in the rate of mammalian enzyme degradation is the slower rate of hepatic catalase breakdown (50) in C57BL/Ho mice, compared with that in the C57BL/6 strain.

At least in microorganisms, gene regulation occurs primarily by attachment of the regulatory gene product to DNA. When a regulatory gene produces a repressor, the system is under negative control, as in the lactose operon; when the product of a regulatory gene stimulates an operon, as in the arabinose operon, the system is under positive control (57, 58). If the induction of hydroxylase activity by polycyclic hydrocarbons were under negative control, one would postulate that the inducer is able to bind to repressor in the C57BL/6N strain, thereby derepressing the genome for enzyme induction, whereas the inducer cannot bind to repressor in the MC-nonresponsive strains. The repressor may limit the synthesis, stability, or the efficiency of transcription of induction-specific RNA. However, because the freely diffusible repressor (which is unable to bind to the inducer) would remain bound to the operator regions, the hydroxylase induction by MC would be predicted to be recessive in heterozygotes (cf. Reference 58 concerning \( \beta \)/\( \alpha \)-diploid lac operons of Escherichia coli). This conclusion is not consistent with our observations. Conceivably, our results might be explainable on the basis of negative control if one supposes that an inactive enzyme (e.g. subunits) is formed by the combination of inducer with a repressor in the DBA/2N, NZW/BLN, and NZB/BLN strains and that this inactive form of enzyme in the presence of one allele from C57BL/6N in the F1 hybrid is converted to fully inducible enzyme activity in the heterozygote. On the other hand in the case of positive control, regulatory mutants which require the combination of inducer with regulatory macromolecules would be dominant if heterozygous or homozygous, and this is what we observe (cf. Reference 58 concerning \( C^+ / C^- \) diploid ara operons of E. coli). Most likely, negative control of the repressor type is not the common means of regulation in eukaryotic cells; rather, the properties of most apparently regulatory mutants favor positive control, i.e. that soluble regulatory gene products are required for enzyme realization to proceed (52).

A recent report (59) indicates that the metabolism of polycyclic hydrocarbons involves several steps, epoxide formation and subsequent conversions to dihydrodiols, phenols, glutathione conjugates, or products covalently bound to cellular macromolecules. The close linkage of functionally related genes commonly exists in bacteria (90), presumably to offer the organism a greater opportunity to pass advantageous coordinated genetic information.

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