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# Genetic Differences in Susceptibility to Chemically Induced Myelotoxicity and Leukemia

## by Daniel W. Nebert*

The *Ah* locus represents a complex "cluster" of genese controlling the induction of numerous drug-metabolizing enzyme "activities" by polycyclic aromatic compounds. Allelic differences at the *Ah* locus are reflected in the large differences in inducibility of cytochrome $P_1$-450 and benzo[a]pyrene metabolism in numerous tissues when the mice receive the chemical daily in their diet. This experimental model system offers to the hematologist and clinical pharmacologist a means to study genetic differences in toxic chemical depression of the bone marrow, as well as a potential model to study aplastic anemia and leukemia explainable on a single-gene basis.

The genetically "responsive" individual who is at increased risk for cancer caused by subcutaneous or topical or intratracheal polycyclic hydrocarbons is at decreased risk for toxicity of the bone marrow and leukemia caused by oral benzo[a]pyrene (when compared with the genetically "nonresponsive" individual receiving the same dose of the same xenobiotic). In other words, tissue sites in direct contact with the carcinogen develop cancer in responsive *animals because of induced $P_1$-450; tissues in distant sites of the body may develop malignancy* in nonresponsive animals because more carcinogen reaches that tissue due to decreased $P_1$-450 induction all over the body and therefore decreased detoxication. Not only the dose but the route of administration and the tissue in which the malignancy or toxicity develops are therefore very important in the interpretation of data from tumorigenesis or toxicity experiments involving $P_1$-450 inducers such as polycyclic hydrocarbons.

There exists sufficient evidence that heritable variation of the *Ah* locus occurs in man. Growing evidence indicates that persons with higher aryl hydrocarbon hydroxylase inducibility in their cultured mitogen-activated lymphocytes may have a statistically significantly increased risk for certain types of cancer and drug toxicity. It remains to be determined at the present time, however, whether this genotype can be used as a biochemical marker in the individual patient for predicting increased susceptibility to certain types of environmentally caused cancers or toxicity in man.

## Introduction

To study the genetic control of drug metabolism is often called pharmacogenetics. In a single sentence, pharmacogenetics may be defined as the attempt to understand why the same dose of the same drug given to two different individuals (with the possible exception of identical twins) may cause widely varying responses. These responses include therapeutic effects of a drug, e.g., anticoagulation or control of seizures, but also unwanted deleterious effects such as cancer or drug toxicity. The experimental system to be examined in detail in this chapter represents principally a genetic difference in receptor concentration; because of this defect, there are large genetic differences in the biotransformation and pharmacokinetics of certain drugs and other environmental pollutants, resulting in important differences in risk toward cancer, drug toxicity, mutation, and birth defects.

The general characteristics of the P-450-mediated monooxygenases and their coordinated enzymes

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ences in this model system in mice are examined. How these differences are associated with increased risk toward myelotoxicity and leukemia are then shown as examples. Numerous other conditions in mice associated with this genetic system are also listed. Lastly, current evidence for this genetic difference in man is briefly assessed.

## Cytochrome P-450 Monooxygenases and Coordinated Enzymes

Many environmental pollutants and other foreign compounds are chemicals that are so hydrophobic they would remain in the body indefinitely were it not for the metabolism resulting in more polar derivatives. These drug-metabolizing enzyme systems, which are localized principally in the liver, are usually divided into two groups: phase I and phase II. During phase I metabolism, one or more polar groups (such as hydroxyl) are introduced into the hydrophobic parent molecule, thus allowing a handle, or position, for the phase II conjugating enzymes (such as UDP glucuronosyltransferase) to attack. The conjugated products are sufficiently polar, so that these detoxified chemicals are now excreted from the cell and from the body (*1*).

One of the most interesting of the phase I enzyme systems is a group of enzymes known collectively as the cytochrome P-450-mediated monooxygenases.* The genetic relationship between these inducible enzymes and cancer or toxicity has been reviewed recently (*3*). These membrane-bound enzyme systems are known to metabolize: polycyclic aromatic hydrocarbons such as benzo[a]pyrene (BP) (ubiquitous in city smog, cigarette smoke and charcoal-cooked foods) and biphenyl; halogenated hydrocarbons such as polychlorinated and polybrominated biphenyls, insecticides, and ingredients in soaps and deodorants; strong mutagens such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and nitrosamines; aminoazo dyes and diazo compounds; *N*-acetylarylamines and nitrofurans; numerous aromatic amines, such as those found in hair dyes; nitro aromatics, and heterocyclics; wood terpenes; epoxides; carbamates; alkyl halides; safrole derivatives; certain fungal toxins and antibiotics; many of the chemotherapeutic drugs; small chemicals such as benzene, thiocyanate, or ethanol; both endogenous and synthetic steroids; and other endogenous compounds such as biogenic amines, indoles, thyroxine, and fatty acids.

Evidence is growing that metabolism to reactive intermediates by cytochrome P-450-mediated monooxygenases is a prerequisite for mutagenesis, carcinogenesis, and toxicity caused by numerous drugs, polycyclic hydrocarbons, and other environmental pollutants. These reactive intermediates probably bind covalently to numerous cellular macromolecules. Most of this binding is probably random, but some may be nonrandom, i.e., specific binding dependent upon the chemical structures of the reactive intermediate and the cellular macromolecule. Among these various types of covalent binding, there probably exists a very small amount of important binding of the ultimate carcinogen to its critical subcellular target, thereby initiating tumorigenesis. Two examples of apparent specific binding include the binding of BP 7,8-diol-9,10-epoxide to the $^2N$-amino of guanine (*4*) and of aflatoxin $B_1$ 2,3-oxide to the $^7N$ of guanine (*5*).

The steady-state levels of these reactive electrophilic intermediates and, consequently, the rates at which they interact with the critical nucleophilic target are dependent upon a delicate balance between their generation and detoxication (Fig. 1). Changes in the balance between toxification and detoxication in any particular tissue of an individual may therefore affect his risk of tumorigenesis or toxicity.

## The *Ah* Locus: Genetic Expression of Induced AHH Activity and Cytochrome $P_1$-450 Induction

The *Ah* locus is an experimental model system that has provided several good examples of a delicate balance between genetic and environmental factors in the etiology of cancer, drug toxicity, and birth defects (*2*). The *Ah* locus of the mouse regulates the induction (by polycyclic aromatic compounds such as 3-methylcholanthrene, BP, or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) of numerous drug-metabolizing enzyme "activities" associated with several new induced forms of cytochrome $P_1$-450. The induction of aryl hydrocarbon hydroxylase (AHH) activity and more than 20 other monooxygenase activities and associated $P_1$-450 occurs in 3-methylcholanthrene-treated B6 (the inbred C57BL/6N mouse strain) and other genetically "responsive" inbred strains and is absent or always

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*Cytochrome P-450 is defined as all forms of CO-binding hemoproteins associated with membrane-bound NADPH-dependent monooxygenase activities. We define cytochrome $P_1$-450 as all forms of CO-binding hemoprotein that increase in amount concomitantly with rises in induced AHH activity following polycyclic aromatic inducer treatment. In view of more than one such form of $P_1$-450 (*2*), it is emphasized that this definition of $P_1$-450 is simplistic.

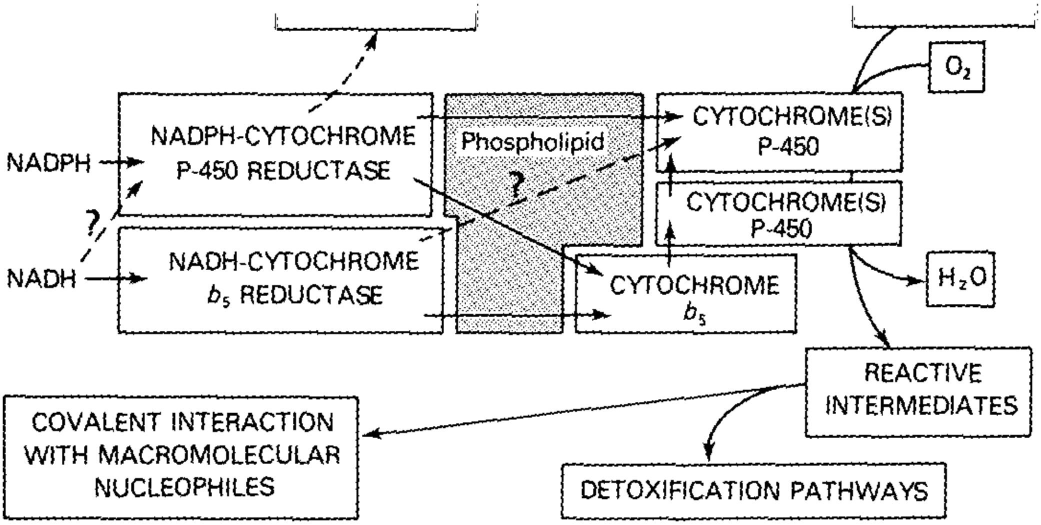

FIGURE 1. Scheme for the membrane-bound multicomponent monooxygenase system(s) and the various possibly important pathways for hydrophobic substrates. For any given substrate, the relative balance between metabolic activation and detoxication likely would differ among different tissues, strains, and species. Age, genetic expression, nutrition, hormone concentration, diurnal rhythm, pH, saturating versus nonsaturating conditions of the substrate, $K_m$ and $V_{max}$ for each enzyme, subcellular compartmentalization of each enzyme, efficiency of DNA repair, and the immunological competence of the animal may all be important factors affecting this balance.

much lower in 3-methylcholanthrene-treated D2 (the inbred DBA/2N mouse strain) and other genetically "nonresponsive" strains (at any given dose of inducer). Besides the liver, this genetic expression is seen in such tissues as lung, kidney, intestine, lymph nodes, skin, bone marrow, pigmented epithelium of the retina, brain, mammary gland, uterus, ovary, and testis. The genetic response is therefore called "systemic," or occurring throughout virtually all tissues of the animal. Responsiveness to aromatic hydrocarbons has been designated the *Ah* complex: $Ah^b$ is the dominant allele; $Ah^d$ is the recessive allele; the $Ah^b/Ah^d$ heterozgote is phenotypically similar to the $Ah^b/Ah^b$ mouse in terms of degree of responsiveness (Fig. 2).

Several studies indicate that the fundamental genetic difference is in the regulatory *Ah* gene, which encodes for a cytosolic receptor (Fig. 3) capable of binding to inducers such as 3-methylcholanthrene, BP, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. To our knowledge, only foreign chemicals bind to this receptor with high affinity (less than 1 n*M*). The B6 mouse appears to have at least 50 times more receptor (and/or increased affinity toward inducers of $P_1$-450) than the $Ah^d/Ah^d$ mouse; translocation of the inducer-receptor complex into the nucleus has now been demonstrated in the phenotypically responsive heterozygote and homozygote (*8*). What happens in the nucleus is not yet known, but somehow the "message" (that these inducers of $P_1$-450 exist in the cell's microenvironment) is received; the response is transcription of specific mRNA's, translation of these mRNA's into specific enzymes such as $P_1$-450, and incorporation of $P_1$-450 into cellular membranes. These induced enzymes may aid in detoxication or they may generate increased amounts of reactive intermediates.

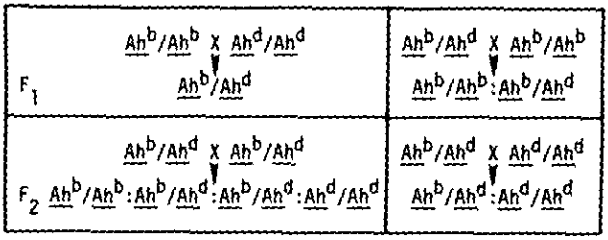

FIGURE 2. Simplified genetic scheme for aromatic hydrocarbon "responsiveness" in the mouse (*6*). Reproduced with permission from Plenum Press.

## Genetic Differences in Myelotoxicity

Large doses of oral BP (100 to 125 mg/kg/day) produce bone marrow toxicity in $Ah^d/Ah^d$ mice, whereas the $Ah^b/Ah^b$ and $Ah^b/Ah^d$ individuals are extremely resistant to oral BP-induced marrow

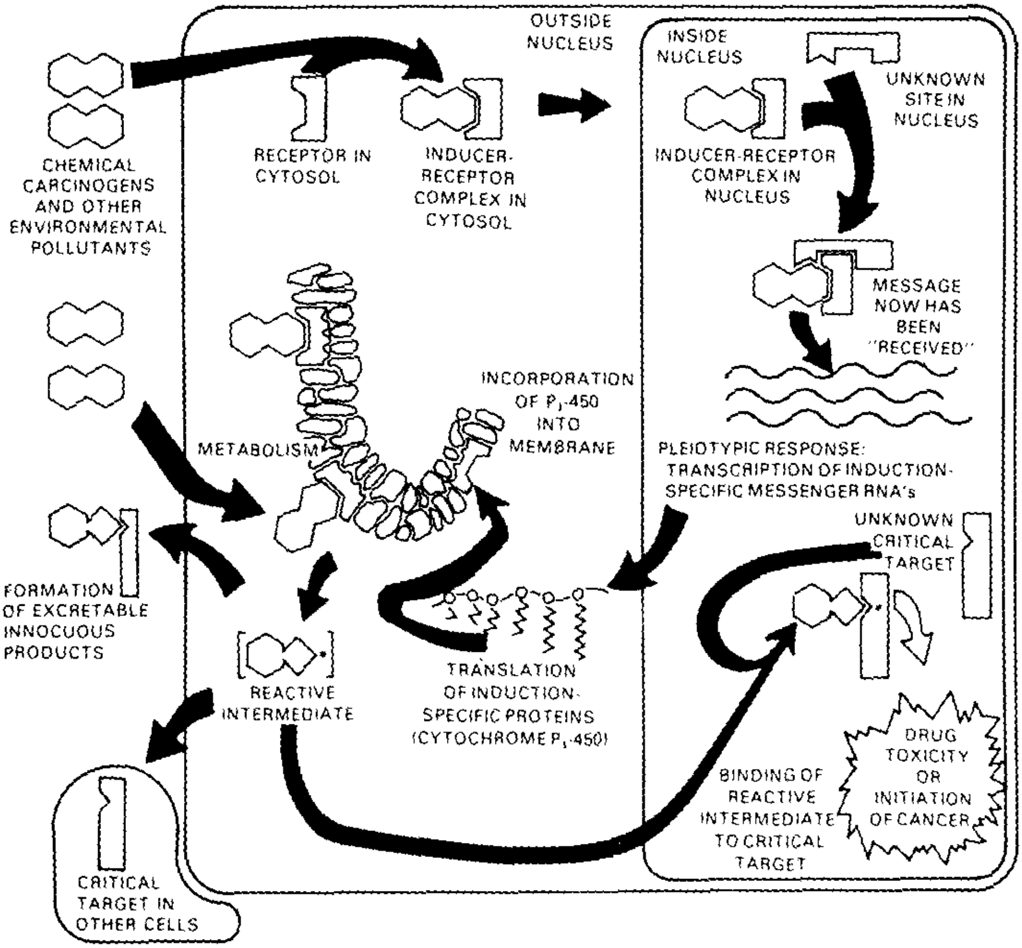

FIGURE 3. Diagram of a cell and the hypothetical scheme by which a cytosolic receptor, product of the regulatory *Ah* gene, binds to inducer (*7*). Depending upon the half-life of the reactive intermediate, the rate of formation of the intermediate, and the rate of conjugation and other means to detoxify the intermediate—important covalent binding may occur in the same cell in which metabolism took place, or in some distant cell. Although the "unknown critical target" is illustrated here in the nucleus, there is presently no experimental evidence demonstrating unequivocally the subcellular location of a "critical target(s)" required for the initiation of drug toxicity or cancer or, for that matter, whether the "target" is nucleic acid or protein. Reproduced with permission from Dr. W. Junk Publishers.

toxicity (*9*). Figure 4 illustrates the lethal effects of high doses of oral BP in $Ah^d/Ah^d$ mice. Concomitant oral phenobarbital treatment protects the $Ah^d/Ah^d$ individual from oral BP toxicity, probably by inducing various drug-conjugating enzyme activities. Concomitant oral α-naphthoflavone treatment protects the $Ah^d/Ah^d$ individual, presumably by inhibiting $P_1$-450-mediated metabolism (in the myeloid precursor cells of the marrow) so that a decreased amount of toxic BP intermediates can be generated. These observations are supported by the markedly greater amount of radiolabeled BP (Fig. 5) which enters the marrow and which becomes metabolized and covalently bound in the marrow of the $Ah^d/Ah^d$ mouse, compared with that of the $Ah^b/Ah^d$ mouse receiving the same diet.

## Effect of Oral BP on Induced BP Metabolism

Table 1 shows that daily doses of oral BP induced AHH activity in the $Ah^b/Ah^d$ heterozygote more than 800-fold in bowel, approximately 3-fold in liver, and more than 16-fold in bone marrow. Daily doses of oral BP induced AHH activity in the $Ah^d/Ah^d$ mouse about 50-fold in bowel and more than 7-fold in marrow, but a decrease in AHH activity was seen in liver. Further, the rate of increase in AHH activity as a function of days in mice receiving the BP diet was much slower in the nonresponsive $Ah^b/Ah^d$ than in the responsive $Ah^b/Ah^d$ mouse.

Concomitant phenobarbital treatment induced

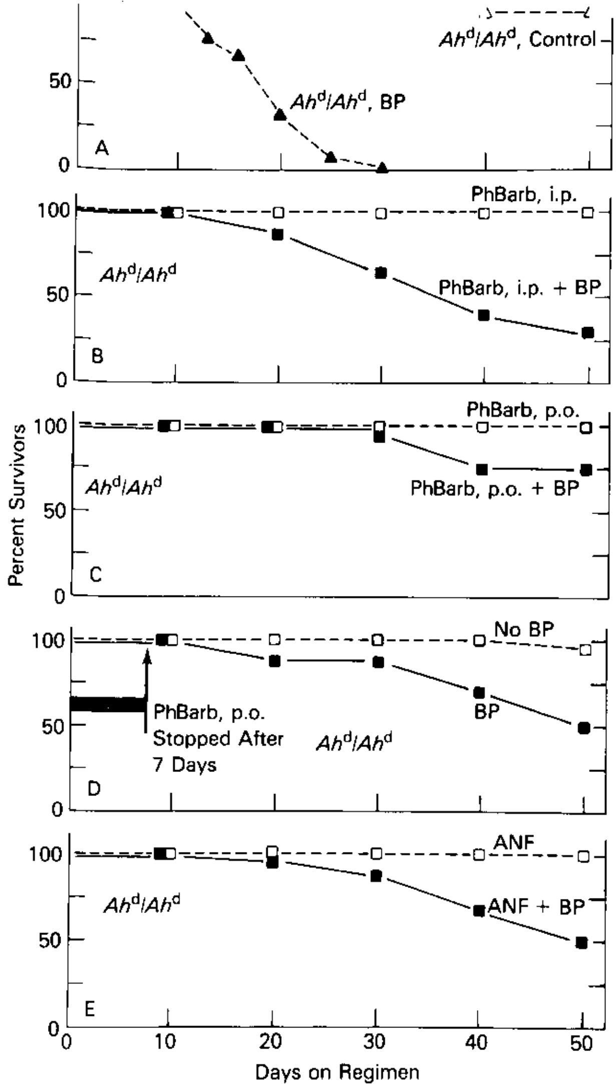

FIGURE 4. Plots of (*A*) toxicity of BP ingested daily among the $Ah^b/Ah^d$ heterozygotes and $Ah^d/Ah^d$ homozygotes of the B6D2F$_1$ × D2 backcross and effects of (*B*) intraperitoneal phenobarbital (PhBarb), (*C*) oral phenobarbital continuously for 50 days, (*D*) oral PhBarb for only 7 days, and (*E*) oral α-naphthoflavone (ANF) on the oral BP toxicity in $Ah^d/Ah^d$ homozygotes. Groups of 24 to 30 mice were started on each study, and survival rates were recorded over the 50-day period (*10*). Reproduced with permission from Marcel Dekker, Inc.

BP metabolism in the bowel, liver, and marrow of both $Ah^b/Ah^d$ and $Ah^d/Ah^d$ mice. Concomitant α-naphthoflavone treatment, on the other hand, did not increase AHH activity in any of these tissues. The data suggest than phenobarbital protection of oral BP toxicity is caused by enzyme induction, inhibition [illegible] metabolism.

## Length of BP Exposure and Subsequent Appearance of Myelotoxicity

Between 3 and 5 days of continuous oral BP (120 mg/kg/day) was required to cause aplastic anemia (Fig. 6): none died when exposed for only 2 days; 20% died when exposed for 3 days; about 83% died when exposed for 4 days; and 100% died when exposed for 5 or more days. Development of the aplastic anemia and therefore the mean survival time was longer, the shorter the length of oral BP exposure; all deaths occurred within 32 days following the completion of the oral BP regimen. Hence, if the toxic insult can be repaired within 32 days on a regular diet, the damage to the bone marrow is no longer irreversible (*9*).

In $Ah^d/Ah^d$ mice receiving oral BP for 2 days and then the regular diet for 8 days, the bone marrow was only slightly hypocellular; in $Ah^d/Ah^d$ mice receiving oral BP for 5 days and then the regular diet for 5 days, the marrow was considerably more hypocellular. The bone marrow in $Ah^d/Ah^d$ mice after 5 days of oral BP was extremely hypocellular. In those few surviving $Ah^d/Ah^d$ mice receiving oral BP for 4 days and then the regular diet for 30 days, the histological appearance of bone marrow was normal. This experimental picture is similar to that reported in total-body irradiation of anemic mice (*11*): the marrow cellularity reaches a nadir between 2 and 8 days after the irradiation insult and recovers fully by 12 to 30 days. In other words, the chemical toxicity produced by oral BP looks similar and quite closely parallels the nonspecific marrow toxicity produced by physical damage (x-ray). However, there exists an underlying genetic predisposition for the chemical toxicity to occur, and this genetic difference might not be expected to occur with the x-irradiation-caused toxicity. [A genetic difference in radiosensitivity between normal (*w*/*w*) and anemic (*W*/*W*) mice, however, has been reported (*11*) and is caused by differences in regeneration capability of erythropoietic tissue.]

This type of latent effect (Fig. 6) is therefore distinctly different from that seen with chloramphenicol-induced aplastic anemia in man (*12*). To date, the calf is the only experimental animal in which aplastic anemia can be consistently produced by a chemical after a latency period. In this case, the agent is *S*-(1,2-dichlorovinyl)-L-cysteine (*13*).

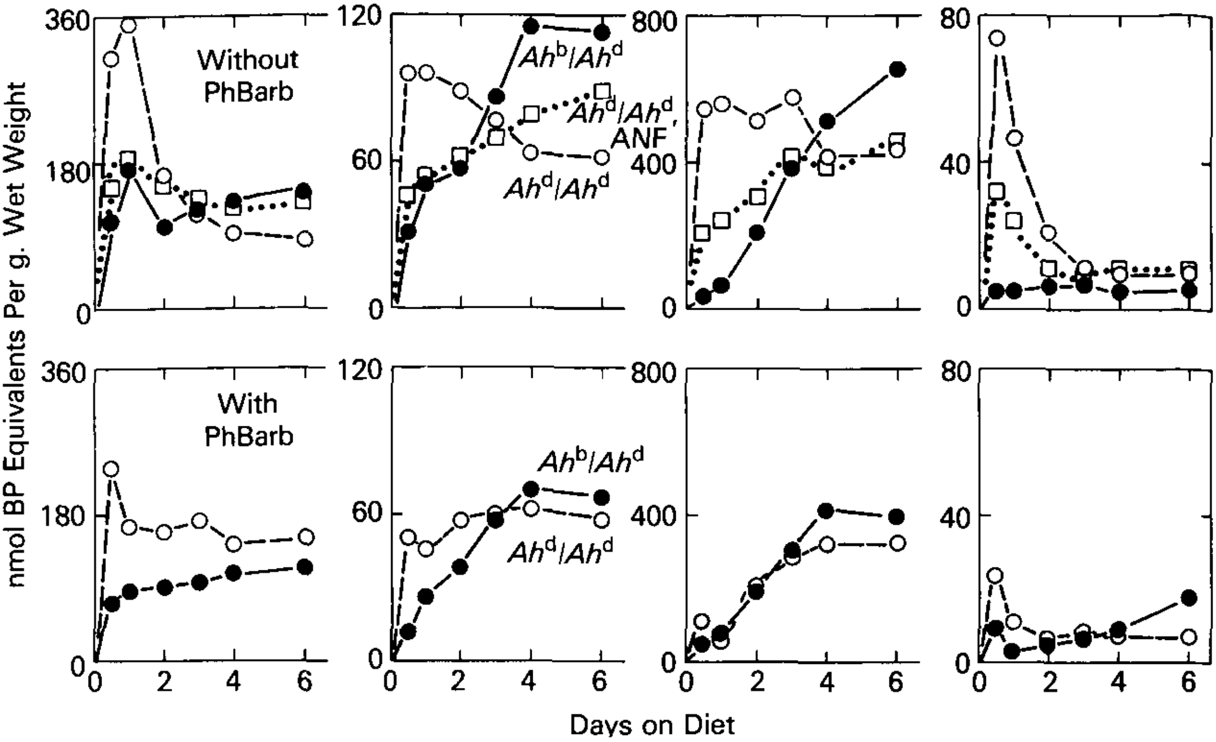

FIGURE 5. Pharmacokinetic uptake of BP administered in the diet (approximately 120 mg/kg/day) in (●) $Ah^b/Ah^d$ heterozygotes and (O) $Ah^d/Ah^d$ homozygotes in the absence (top four graphs) or presence (bottom four graphs) of oral phenobarbital (PhBarb). The effect of oral α-naphthoflavone (ANF) on uptake of BP in $Ah^d/Ah^d$ individuals (☐) is also shown in the top four graphs. The "nmol BP equivalents" comprise some metabolites of BP, but more than 90% represents the nonmetabolized parent drug. Tissue samples were combined from groups of five or six mice (*10*). Reproduced with permission from Marcel Dekker, Inc.

## Size of Oral BP Dose and Onset of $P_1$-450-Mediated Leukemogenesis

Although massive doses of 100 or 125 mg BP ingested/kg/day produce bone marrow toxicity and death in 100% of $Ah^d/Ah^d$ mice in less than 4 weeks, no responsive $Ah^b/Ah^b$ or $Ah^b/Ah^d$ mouse develops aplastic anemia even when this dose is continued for 6 months (*14*). Because these are such large doses of BP, we wondered how small a dose of oral BP would still cause an effect associated with the *Ah* locus.

Figure 7 shows the results of groups of 30 $Ah^d/Ah^d$ or $Ah^b/Ah^d$ mice which received estimated doses of 12 or 6 mg BP/kg/day. Differences in weight gain attributed to allelic differences at the *Ah* locus were detectable. To our surprise, however, the mice that became ill and began dying did not have hypoplastic or aplastic bone marrow but rather developed hematopoietic neoplasms, especially of the lymph nodes, spleen and thymus. No increased incidence of leukemia or differences in weight gain between $Ah^d/Ah^d$ and $Ah^b/Ah^d$ mice were found at estimated doses of 1.2 mg of BP/kg/day in the diet for 240 days (data not illustrated).

When α-naphthoflavone was added to the diet at a dose 20 times greater than that of BP (Fig. 7), the incidence of leukemia was prevented almost completely, and the general health of the $Ah^d/Ah^d$ mice remained as good as that of $Ah^b/Ah^d$ mice receiving 12 mg BP/kg/day. These data suggest that α-naphthoflavone-sensitive metabolism of BP— presumably cytochrome $P_1$-450—in the bone marrow of $Ah^d/Ah^d$ individuals is responsible for producing the reticuloendothelial malignancies.

$Ah^d/Ah^d$ mice are more susceptible than $Ah^b/Ah^d$ mice to leukemia produced by percutaneously applied 3-methylcholanthrene (*18*). Obviously the presence or absence of murine leukemia virus expressed by the various inbred strains (*19*) will modify the response elicited by $P_1$-450-mediated metabolism of polycyclic hydrocarbons under the control of the *Ah* locus.

| Genotype | Diet | Days on diet | Microsomal specific AHH activity, units/mg protein | | |
|---|---|---|---|---|---|
| | | | Bowel | Liver | Bone marrow |
| $Ah^b/Ah^d$ | Control | 12 | <1 | 510 | <1 |
| $Ah^d/Ah^d$ | | 12 | <1 | 470 | <1 |
| $Ah^b/Ah^d$ | BP | 0 | <1 | 480 | <1 |
| | | 2 | 410 | 1180 | 12 |
| | | 6 | 810 | 1380 | 18 |
| | | 9 | 640 | 1340 | 16 |
| | | 12 | 360 | 1420 | 14 |
| $Ah^d/Ah^d$ | | 0 | <1 | 500 | <1 |
| | | 2 | 5 | 460 | 6 |
| | | 6 | 18 | 370 | 7 |
| | | 9 | 22 | 310 | 6 |
| | | 12 | 48 | 280 | 2 |
| $Ah^b/Ah^d$ | BP + phenobarbital | 2 | 440 | 1600 | 15 |
| | | 12 | 380 | 1890 | 19 |
| $Ah^d/Ah^d$ | | 2 | 11 | 1020 | 1 |
| | | 12 | 60 | 980 | 4 |
| $Ah^b/Ah^d$ | BP + α-naphthoflavone | 2 | 180 | 1100 | 4 |
| | | 12 | 220 | 1190 | 8 |
| $Ah^d/Ah^d$ | | 2 | 2 | 480 | <1 |
| | | 12 | 21 | 400 | 3 |

[a] Groups of $Ah^b/Ah^d$ and $Ah^d/Ah^d$ mice were placed on control, BP, BP plus phenobarbital, or BP plus α-naphthoflavone regimens for the indicated number of days (*10*). Microsomal fractions were prepared from the indicated tissues combined from groups of five or six mice, and AHH activity was determined.

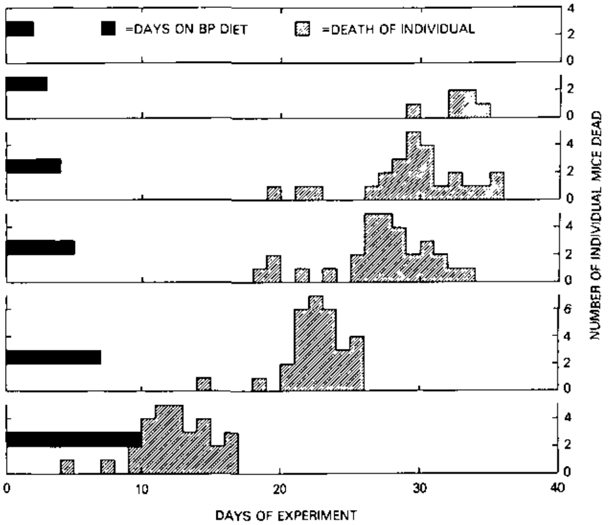

FIGURE 6. Effect of the length of time of oral BP treatment on the occurrence or lack of occurrence of terminal aplastic anemia occurring several weeks later in $Ah^d/Ah^d$ mice (*9*). From top to bottom, groups of 30 mice each received oral BP 2, 3, 4, 5, 7, and 10 days, respectively, following which normal diet was reinstated. Deaths, histologically confirmed to be associated with hypoplastic bone marrow, occurred in 0/30, 6/30, 25/30, 30/30, 30/30, and 30/30, respectively. Following cessation of the oral BP and return to the regular diet, the mean time for the mouse to die from aplastic anemia was about 23, 16, and 3 days for the groups exposed to 5, 7, and 10 days of oral BP, respectively. All mice that were alive on day 37 of the experiment remained alive at 60 days, at which time the experiment was stopped. Reproduced with permission from Springer-Verlag.

## Protective Barrier by the *Ah*-Responsive Intestinal Epithelium

BP treatment (30 μg/ml of growth medium) is much more toxic to $Ah^b/Ah^d$ marrow cells than $Ah^d/Ad^d$ marrow cells in culture (unpublished data). When $Ah^d/Ah^d$ mice having transplanted $Ah^b/Ah^b$ marrow are given oral BP (100 mg/kg/day), their death rate is similar to sham-treated $Ah^d/Ah^d$ mice with $Ah^d/Ah^d$ marrow; $Ah^b/Ah^b$ mice having transplanted $Ah^d/Ah^d$ marrow are just as resistant to oral BP daily as sham-treated $Ah^b/Ah^b$ mice with $Ah^b/Ah^b$ marrow (*20*). We therefore conclude that the *Ah*-responsive intestine (and/or liver) is important in protecting the individual. If the target marrow cells are exposed directly to BP in culture, the cells having the higher levels of induced $P_1$-450 are more prone to BP toxicity. Despite the genetic origin of the bone marrow, the mice having the $Ah^d/Ah^d$ intestine and liver are more prone to develop aplastic anemia following oral BP.

## Importance of the Route of Administration

In sum, the picture which has begun to emerge from numerous studies is categorized in Table 2. When the carcinogen (or other toxic drug) is placed in relatively direct contact with the tissue being studied, the genetically responsive $Ah^b/Ah^b$ or

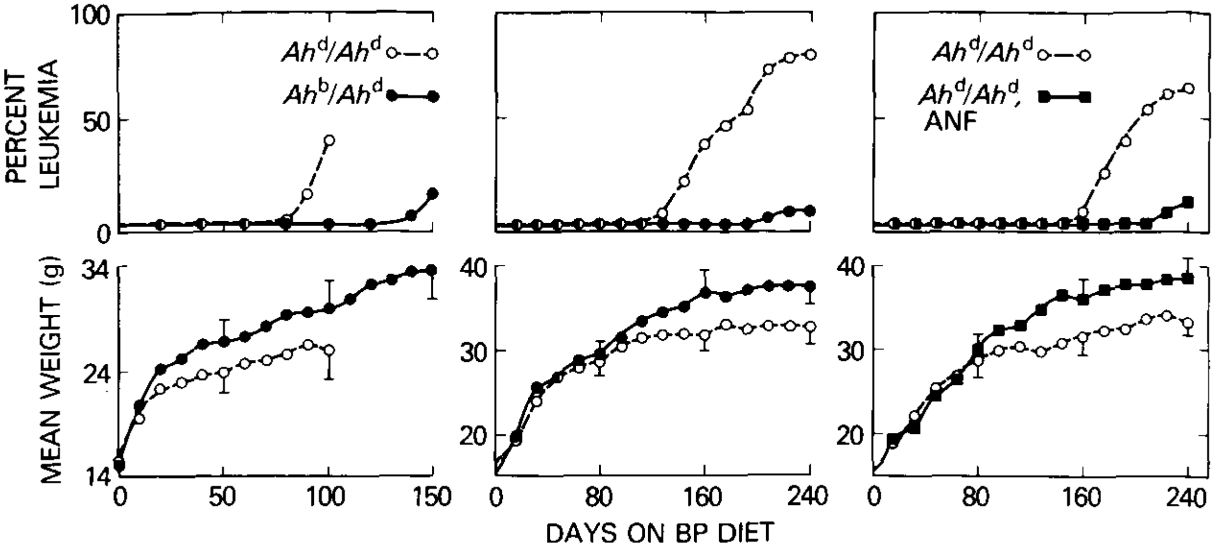

FIGURE 7. Incidence of leukemia and mean weight gain for groups of 30 $Ah^{d}/Ah^{d}$ or $Ah^{b}/Ah^{d}$ mice receiving oral BP (left) at about 12 mg/kg/day or (middle) 6 mg/kg/day (*15*). Each symbol represents the mean of 30 (or less, if some had died) mice in the group; the I-bars represent standard deviations. Weanlings from the B6D2F$_1$ × D2 backcross were phenotyped by the zoxazolamine paralysis test, as described previously (*16*). Ten days later, the BP diet, prepared as described previously (*14*), was begun. In the case of α-naphthoflavone (ANF) (right), approximately 120 mg of α-naphthoflavone/kg/day was included with the 6 mg of BP/kg/day. Wasted animals were killed and studied when it was judged that they probably would not live more than 1-2 days longer. We are grateful to Drs. Lawrence, Corash, Michael M. Orlando and Alan S. Rabson for their advice about performing autopsies and examining histological sections of lymph nodes, spleen, thymus, bone marrow, kidney and liver. Whole blood counts were not especially helpful in the diagnosis of hematopoietic tumors. Lymphocytic leukemias, apparent stem-cell leukemias, and reticulum-cell neoplasms were all scored as "leukemia," according to the classification and description by Murphy (*17*). At an estimated 12 mg of BP/kg/day (left), all $Ah^{d}/Ah^{d}$ mice died before 110 days on the diet; none of the starting 30 $Ah^{b}/Ah^{d}$ had died by day 100, and three had died after 150 days. At an estimated 6 mg of BP/kg/day (center), 24 of the starting 30 $Ah^{d}/Ah^{d}$ mice and two of the starting 30 $Ah^{b}/Ah^{d}$ mice had died after 240 days on the diet. At an estimated 6 mg of BP/kg/day (right), 19 of the starting 30 not receiving α-naphthoflavone had died, and four of the starting 30 receiving α-naphthoflavone had died after 240 days on the diet. Reproduced with permission from Pergamon Press Ltd.

$Ah^{b}/Ah^{d}$ mouse is at increased risk for developing a tumor or toxicity in that tissue, compared with the nonresponsive $Ah^{d}/Ah^{d}$ receiving the same dose of xenobiotic (Fig. 8). On the other hand, if the malignancy or toxicity is found at a site distant from the administered drug, the $Ah^{d}/Ah^{d}$ mouse is at increased risk, compared with the $Ah^{b}/Ah^{d}$ or $Ah^{d}/Ah^{d}$ individual receiving the same dose of xenobiotic. In this latter case, we believe the data are explainable by the "first-pass effect," also termed "presystemic drug elimination" (*31*). Fundamentally, presystemic elimination reflects the metabolism and excretion of a drug before the drug reaches its site of action. How BP metabolism in the intestine can be induced 400- to 800-fold by oral BP—yet not exhibit any apparent toxicity (*9*, *10*)—is not clear; an increase in conjugating enzymes or mechanism of efficient excretion of toxic metabolites must be involved. It will be of interest to see if the $Ah^{d}/Ah^{d}$ mouse is more susceptible than the $Ah^{b}/Ah^{d}$ mouse to *in utero* fetal toxicity or primordial oocyte depletion, when the polycyclic hydrocarbon is administered daily in the diet.

The data summarized in this report demonstrate that $P_1$-450 induction represents a double-edged sword. Therefore, in all cancer and toxicity experiments, the dose and especially the route of administration and the tissue in which the malignancy or toxicity develops are all very important factors in the interpretation of the observations.

## Evidence of the *Ah* Locus in the Human

Lindane (*32-34*), other insecticides (*32*, *33*), various anticancer chemotherapeutic agents (*35*), and chloramphenicol (*36*) have all been implicated in the cause of certain aplastic anemias in man. To prove that a drug or chemical is the direct cause of aplastic anemia has always been difficult in clinical medicine, and most cases remain categorized as

| Individual at increased risk | Tumor or toxicity | Route of administration | Chemical | References |
|---|---|---|---|---|
| $Ah^{b}/Ah^{b}$ and $Ah^{b}/Ah^{d}$ | Skin inflammation | Topical | 7,12-Dimethylbenzo[a]anthracene | (*21*) |
| | Fibrosarcomas | Subcutaneous | 3-Methylcholanthrene or BP | (*22*) |
| | Pulmonary tumors | Intratracheal | 3-Methylcholanthrene >> BP | (*23*) |
| | *In utero* fetal toxicity | Intraperitoneal | BP, 3-methylcholanthrene, 7,12-dimethylbenzo[a]anthracene | (*24*) |
| | Primordial oocyte depletion | Intraperitoneal | 7,12-Dimethylbenzo[a]anthracene, 3-methylcholanthrene, BP | (*25*) |
| | *Epidermal carcinoma* | *Topical* | *BP* | (*26*) |
| | Cleft palate in fetus | Intraperitoneal | 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin | (*27*) |
| | Experimental porphyria | Intraperitoneal | Chlorinated aromatic compounds | (*28*) |
| $Ah^{d}/Ah^{d}$ | Lymphoma, lymphosarcoma | Intraperitoneal | 7,12-Dimethylbenzo[a]anthracene | (*29*) |
| | Bone marrow toxicity | Oral | BP | (*14*) |
| | Leukemia | Subcutaneous | 3-Methylcholanthrene | (*18*) |
| | Leukemia | Oral | BP | (*15*) |

[a] Data from Nebert (*30*).

idiopathic. Almost all of these agents mentioned require P-450-mediated metabolism either for detoxication or for metabolic potentiation to attain the desired pharmacological effect. Chloramphenicol and *p,p'*-DDT toxicity are not associated with the *Ah* locus (unpublished data). We suggest that genetic differences between inbred strains of mice—with respect to marrow toxicity caused by these various agents known (or suspected) to cause aplastic anemia in man—might be developed successfully as a useful laboratory animal experimental model. Needless to say, such a model should help define the etiologic mechanisms, and thereby a better understanding about treatment and prevention, for certain human aplastic anemias.

With the use of 20 to 40 cc of drawn blood, peripheral lymphocytes have been cultured in the presence of mitogens and an inducer of AHH activity such as 3-methylcholanthrene, in order to assess the human *Ah* phenotype. In spite of the shortcomings with this assay method reviewed in ref. (*37*), a growing list of clinical disorders (Table 3) appears to be associated with the human *Ah* locus.

There clearly exists sufficient evidence that heritable variation of AHH inducibility occurs in man. Experimental difficulties, however, make it impossible at this time to be certain of whether AHH induction is controlled by a single genetic locus or by two or more loci (i.e., polygenic). Until one can increase the range of fold inducibility of AHH activity and/or decrease the magnitude of day-to-day

**Table 3. Human disorders that appear to be associated with the *Ah* locus.**

| Disorder | Association with high or low AHH inducibility | References |
|---|---|---|
| Malignancy | | |
| Bronchogenic carcinoma | High[a] | (*38-47*) |
| Bronchogenic carcinoma | No association found | (*48-53*) |
| Laryngeal carcinoma | High[b] | (*53*) |
| Cancer of oral cavity | High[b] | (*55, 56*) |
| Cancer of renal pelvis or ureter | No association found | (*57*) |
| Cancer of urinary bladder | No association found | (*58, 59*) |
| Acute leukemia of childhood | Low[a] | (*60*) |
| Toxicity | | |
| Zoxazolamine-induced fatal hepatic necrosis | Unknown | (*61*) |
| Earlier onset of menopause among cigarette smokers | Unknown[c] | (*62*) |
| Infertility among cigarette smokers | Unknown[c] | (*63-66*) |
| Acetaminophen-induced diffuse bilateral cataracts | Unknown[c] | (*67*) |

[a] Consistent with genetic data from inbred strains of mice (*3, 15, 22*).

[b] Studies of these disorders in mice have not been specifically carried out, but the human data are consistent with what is known (*30*) about environmental carcinogens and their effect on local and distant tissue sites in *Ah*-responsive and *Ah*-nonresponsive mice.

[c] *Genetically responsive mice are at increased risk for these disorders* (*3*). *In retrospect (or in studies to be designed in the future)*, it would have been (or would be) of interest to know the *Ah* phenotype of afflicted clinical patients.

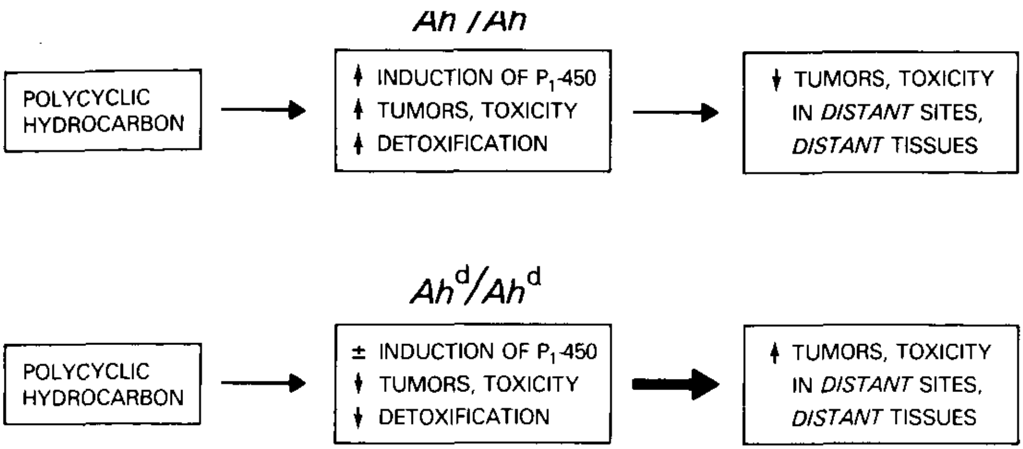

FIGURE 8. Illustrated scheme indicating that the genetically responsive $Ah^b/Ah^d$ individual (top) is at increased risk for tumors or toxicity at sites in direct contact with the xenobiotic (*30*). The nonresponsive $Ah^d/Ah^d$ individual (bottom) is at increased risk for tumors or toxicity at distant sites, due to decreased detoxication in many tissues of the body. Reproduced with permission from Academic Press, Inc.

variability of "control" AHH activity, however, AHH inducibility in cultured mitogen-activated lymphocytes or any other similar test system cannot be used as a promising biochemical marker for determining who is at risk for aplastic anemia, leukemia, bronchogenic carcinoma, or other various types of environmentally caused toxicity or malignancy. We believe that a high ratio of $P_1$-450 to other forms of P-450 exists in many, if not all, extrahepatic tissues *in vivo*, just as appears to be the case in cultured lymphocytes, monocytes, pulmonary macrophages, and even skin fibroblasts. An alternative assay for assessing the human *Ah* locus phenotype (such as a receptor assay or a radioimmunoassay for induced $P_1$-450) might be more successful than the existing commonly performed AHH inducibility assay.

The major emphasis of this report, however, has been to point out the importance of genetics in response to environmental stimuli. Such genetic heterogeneity in the human population undoubtedly reflects the large amount of "background noise," thereby making it difficult for the clinical investigator to discern distinct subgroups. If the genetic components eventually can be characterized among the clinical population, it should become easier to understand the etiology of environmentally caused aplastic anemia and/or leukemia.

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