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Genetic Differences in Drug Metabolism Associated with Ocular Toxicity*

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The tissue localization and subcellular distribution of drug-metabolizing enzymes in the eye are described. With the use of inbred strains of mice, the [*Ah*] complex is shown to be an important experimental system for probing genetic differences in drug metabolism and related drug toxicities. Although the genetic system described in detail here involves mice, there is ample evidence that the same system operates in man.

Genetic differences in acetaminophen- and naphthalene-induced cataract formation and other ocular degeneration are shown to be related to the [*Ah*] complex. Because this toxicity appears similar to senile cataracts, we propose that certain types of drug-induced cataracts might exist among clinical populations of senile cataracts but that any cause-and-effect relationship would be very difficult to determine because of underlying interindividual differences in genetic predisposition. It is therefore suggested that genetic differences in drug metabolism be an important consideration in the clinical assessment of ocular toxicity caused by drugs and other environmental pollutants.

Introduction

Other contributions in this symposium are concerned with drug toxicity and pathology related to tissues of the head and neck. This paper deals more directly with the drug-metabolizing enzymes involved. These enzymes are responsible for toxification (i.e., potentiation of the toxic response by forming a reactive drug intermediate), as well as detoxication (metabolism leading to the excretion of innocuous products). This paper also emphasizes genetic differences in these enzymes, whereas genetics is not addressed in the other contributions. Although the genetic system described in detail here involves mice, there is ample evidence that the same system operates in man. It therefore should be obvious that, due to differences in genetic predisposition among individuals in the human population, a particular dose of an ophthalmic drug might be toxic to one person but not to another (and these two persons might even be siblings). Similar genetic differences in toxicity caused by drugs and other environmental pollutants are expected to be manifest among other tissues of the body, as well as in the eye.

We first describe the tissue localization and subcellular distribution of drug-metabolizing enzymes in the eye. Next, the genetic system, called the [*Ah*] complex, is introduced. Finally, genetic differences in acetaminophen- and naphthalene-induced cataract formation (lens opacification) and other ocular degeneration are shown to be related to the [*Ah*] complex.

Concept of Extrahepatic Drug Metabolism

Until the last two decades, it was generally accepted that the liver was the only important organ for drug metabolism. More recently, however, it has become clear that virtually every cell of every plant and animal (and certain bacteria) contains drug-metabolizing capability. At least part of this appreciation reflects the development of much more sensitive assays for drug-metabolizing enzymes.

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Toxification and detoxication of drugs and other environmental pollutants in extrahepatic tissues therefore can be important (*1*).

The eye is no exception. For example, chloroquine retinopathy observed in malaria patients was attributed to an accumulation of unusually high levels of chloroquine in the eye (*2*). Unless the eye possesses a mechanism for removing such drugs that enter ocular tissues via the circulating blood, therefore, accumulated chemicals of all types might exert adverse effects on photoreceptor cells, leading to visual impairment. Shortly after Bernstein's report (*2*), cytochrome P-450, a principal component of the drug-metabolizing enzyme system,* was detected spectrally in the pigmented epithelium of bovine retina (*4*). Although much progress has been made in studies on absorption, distribution, and clinical usefulness of ophthalmic drugs (*5*), information on ocular drug metabolism and toxicity remains quite scanty, in spite of obvious clinical importance.

Futher, information about genetic differences in ocular drug toxicity is even more scarce. Steroid-induced glaucoma is a pharmacogenetic disorder, for example, although the etiology is unknown. About 5% of the United States population is homozygous for the recessive allele causing glaucoma induced by corticosteroid ophthalmic medications (*6*).

P-450-Mediated Monooxygenase Activities and Other Coordinated Drug-Metabolizing 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 body (*7*).

One of the most interesting of the phase I enzyme systems is a group of enzymes known collectively as the P-450-mediated monooxygenases (*3*). These membrane-bound enzyme systems metabolize polycyclic aromatic hydrocarbons such as benzo[a]pyrene (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*-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 agents used to treat human cancer; most 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. 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.

Subcellular Localization and Distribution of Drug-Metabolizing Enzymes in the Eye

The distribution (Table 1) and subcellular localization (Table 2) of several drug-metabolizing enzyme activities were studied in bovine eye. AHH activity† was chosen as an example of phase I metabolism (reflecting basal forms of P-450). UDP glucuronosyltransferase, glutathione transferase, and *N*-acetyltransferase activities are typical phase II enzymes (*9*). γ-Glutamyltranspeptidase and cystine

*In this paper, 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 (*3*), it is emphasized that this definition of P_1-450 is simplistic.

†Abbreviations used include: AHH, aryl hydrocarbon (benzo[a]pyrene) hydroxylase (EC 1.14.14.1); B6, the C57BL/6N inbred mouse strain; D2, the DBA/2N inbred mouse strain.

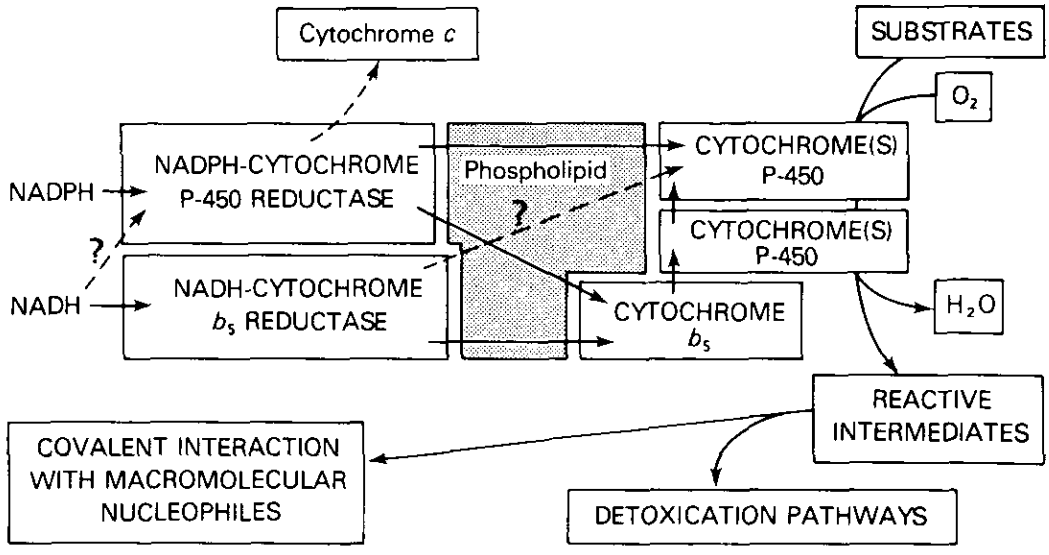

FIGURE 1. Scheme for the membrane-bound multicomponent monooxygenase system(s) and the various possibly important pathways for hydrophobic substrates (*8*). For any given substrate, the relative balance between metabolic activation and detoxication likely would differ among different tissues, strains, 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.

Table 1. Distribution of AHH, UDP glucuronosyltransferase and γ-glutamyltranspeptidase activities in various tissues of bovine eye.

	Enzyme activity, units/mg protein[a]		
Tissues	AHH	UDP Glucuronosyltransferase	γ-Glutamyltranspeptidase
Retina	0.03	6	400
Lens	Not detectable	5	3
Cornea	0.02	8	320
Iris	0.15	23	640
Pigmented epithelium-choroid	0.31	84	430
Ciliary body	5.74	396	3850

[a]Tissue homogenates were used for assay of enzymic activities. One unit of activity is the amount of enzyme protein that produces 1 pmole of product per minute. Enzyme activities given here are specific activities, i.e., units/mg of tissue protein/minute.

Table 2. Subcellular activities of enzymes involved in mercapturate synthesis in the bovine eye.[a]

	Glutathione transferase[b]		γ-Glutamyltranspeptidase		Cystine aminopeptidase		*N*-Acetyltransferase	
Subcellular fraction	Ciliary body	Pigmented epithelium-choroid	Ciliary body	Pigmented epithelium-choroid	Ciliary body	Pigmented epithelium-choroid	Ciliary body	Pigmented epithelium-choroid
Tissue homogenate	5210	480	3850	430	100	8	13	2
Nuclei	780	230	270	67	1	Not detectable	7	1
Mitochondria	1400	120	1620	310	73	7	64	2
Microsomes	3690	590	5120	570	1130	54	160	8
Supernatant	6340	870	1230	130	450	13	13	Not detectable

[a]Activities are expressed as units/mg protein. See Table 1 for definition of one unit of activity.
[b]With 1-chloro-2,4-dinitrobenzene as the substrate.

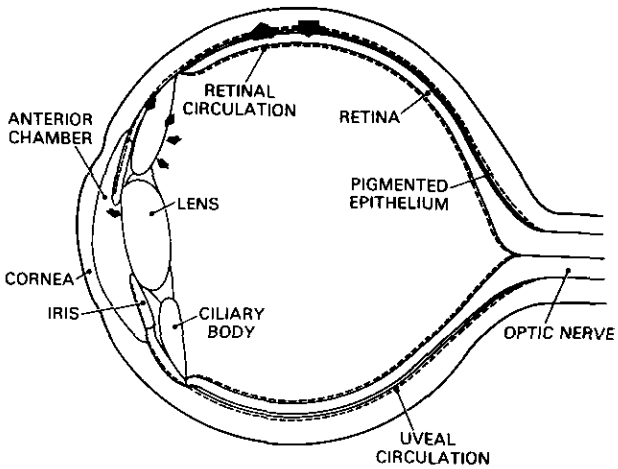

FIGURE 2. Retinal and uveal blood circulation systems (dashed lines). The retinal circulation provides nutrients for the retinal neurons, while the uveal circulation supplies nutrients for the pigmented epithelium (and visual cells), the ciliary body (and lens and cornea), and the iris. Large arrows denote entry of nutrients and exit of metabolic products between the uveal circulation (choroidal capillaries) and the pigmented epithelium. Small arrows indicate aqueous humor secretion by the ciliary body into the anterior chamber and aqueous humor.

aminopeptidase are important in the pathway from glutathione conjugates of aromatic hydrocarbons to mercapturate formation (*10*). Most, if not all, of these six enzymes (Tables 1 and 2) are induced by various drugs or other environmental pollutants (*3, 9, 10*). Low levels of γ-glutamyltranspeptidase (*11*) and glutathione transferase (*12*) have been reported in the lens.

We conclude from Tables 1 and 2 that virtually all ocular tissues have detectable drug-metabolizing capability (*13-15*). The ciliary body and pigmented epithelium-choroid are by far the richest in these activities (see Fig. 2 for the location of these tissues in the eye). In fact, the specific activities of AHH and UDP glucuronosyltransferase in the ciliary body are only about 10 to 20 times less than those in bovine liver.

The [*Ah*] Complex: Genetic Expression of Induced AHH Activity and P_1-450 Induction

The [*Ah*] complex 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 (*16*). The [*Ah*] complex of

Table 3. Induction of ocular AHH activity in B6 and D2 mice by polycyclic aromatic compounds.

Intraperitoneal treatment	Specific AHH activity, units/mg protein[a] B6	D2
Control	0.08	0.06
β-Naphthoflavone	0.36	0.07
3-Methylcholanthrene	0.39	0.08
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	0.47	0.10

[a]See Table 1. Tissue homogenates of the entire eye were prepared for the enzyme assay.

the mouse regulates the induction (by polycyclic aromatic compounds such as 3-methylcholanthrene, benzo[a]pyrene, or 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin) of numerous drug-metabolizing enzyme "activities" associated with several (or many) new induced forms of cytochrome P_1-450. The [*Ah*] complex comprises numerous regulatory, structural, and probably temporal genes that may or may not be on the same chromosome.

At any given dose of inducer, the induction of AHH activity and at least two dozen other monooxygenase activities and associated forms of P_1-450 occurs in 3-methylcholanthrene-treated B6 and other genetically "responsive" inbred strains and is always much lower in 3-methylcholanthrene-treated D2 and other genetically "nonresponsive" strains. 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 (Table 3), 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 heterozygote is phenotypically similar to the Ah^b/Ah^b mouse in terms of degree of responsiveness. The trait of *Ah*-responsiveness among crosses involving B6 and D2 mice is therefore autosomal dominant (*16*).

Several studies indicate that the fundamental genetic difference resides in the *Ah* regulatory gene (Fig. 3), which encodes the cytosolic receptor capable of binding to inducers such as 3-methylcholanthrene, benzo[a]yprene, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. To our knowledge, only certain foreign chemicals bind to this receptor with saturability and high affinity (less than 1 nmole). The B6 receptor appears to have at least 50 times better affinity toward inducers of P_1-450 than the Ah^d/Ah^d mouse (*18*). Translocation of the inducer-

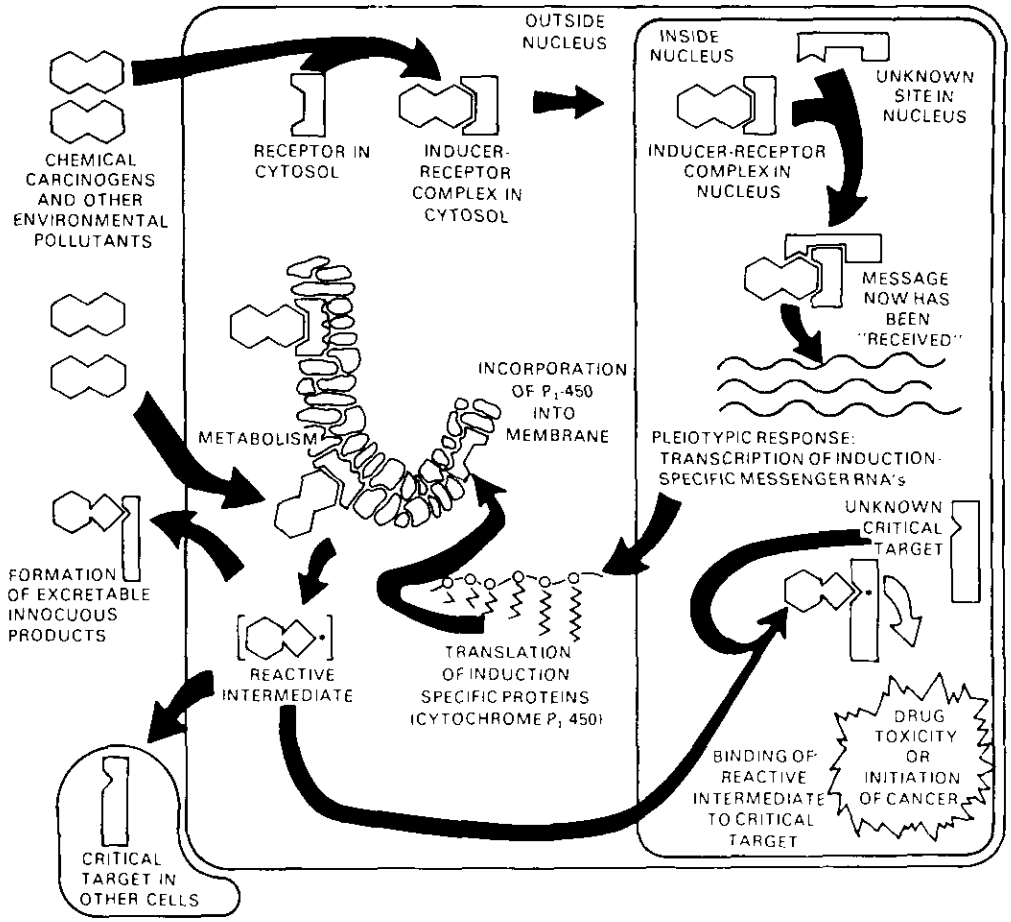

FIGURE 3. Diagram of a cell and the hypothetical scheme by which a cytosolic receptor, product of the regulatory *Ah* gene, binds to inducer (*17*). Depending upon the half-life of the reactive intermediate, the rate of formation of the intermediate, and the rate of conjugation and other means to detoxify 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. (Reproduced with permission).

receptor complex into the nucleus has been demonstrated in the *Ah*-responsive heterozygote and homozygote (*18*) and requires a temperature-dependent step (*19*). Discrepancies between the dextran-charcoal adsorption assay and the sucrose density gradient assay have been recently understood via chromatographic studies of the *Ah* receptor (*20*).

What happens in the nucleus is not yet known (Fig. 3), but somehow the "information" (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.

The *Ah* Complex in Human Populations

In spite of shortcomings with the AHH assay in human cultured lymphoblasts (*8*, *21*), a growing list of clinical disorders appears to be associated with the human [*Ah*] complex. 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 whether AHH induction is controlled by one or more genetic loci.

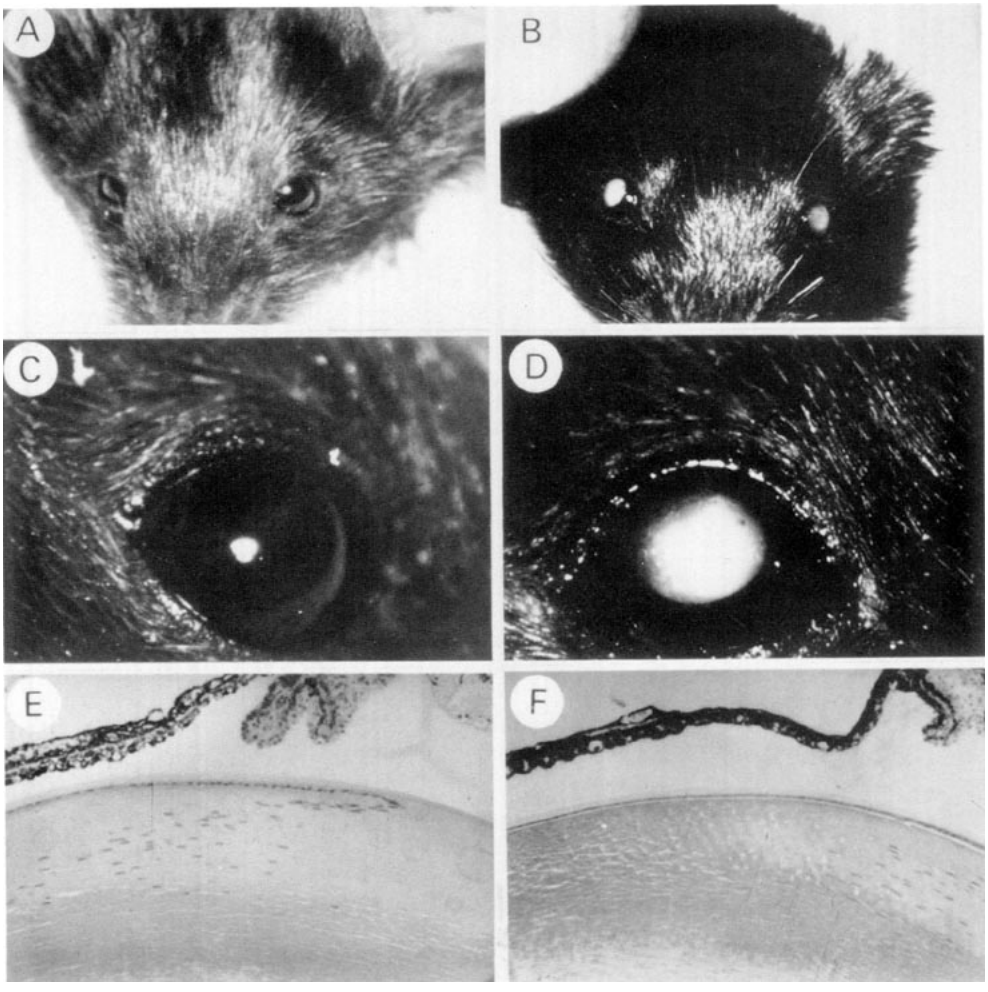

FIGURE 4. (*A*, *C*) Genetically nonresponsive Ah^d/Ah^d homozygote, and (*B*, *D*) genetically responsive Ah^b/Ah^d heterozygous sibling from the B6D2F$_1$ × D2 backcross (*23*). These photographs were taken 5 hr after the mice had received acetaminophen and 53 hr after the mice had received 3-methylcholanthrene, an inducer of P$_1$-450. (*E*, *F*) Hematoxylin- and eosin-stained sections of ocular tissue from mice in (*A*) and (*B*), respectively (× 500). The tissues from top to bottom are iris, cornea and lens. Vacuoles seen in the subepithelial layer of the lens in (*F*) are the result of hydration of the lens cells and are always associated with cataract formation (*24*). Doses of more than 1000 mg of acetaminophen/kg body weight were almost always fatal to Ah^b/Ah^d mice within the first 8 hr after acetaminophen administration, but these doses were not lethal, nor did they ever cause lens opacification to Ah^d/Ah^d mice. At lower doses of acetaminophen (400 to 800 mg/kg), the ocular opacity developed more slowly in Ah^b/Ah^d mice. If a cataract did not appear within 10 hr after acetaminophen administration, however, no cataract developed subsequently. The degree of opacification that had developed within these 10 hr was never reversible. (Reproduced with permission).

Correlation between the Ah^b Allele and Lens Opacification Caused by Intraperitoneal Acetaminophen

Acetaminophen, a widely used analgesic-antipyretic agent, is metabolized to a toxic intermediate largely by some form of polycyclic aromatic-induced P$_1$-450 (*16*). Therefore, we theorized that *Ah*-responsive mice—following treatment with polycyclic aromatic inducers to enhance P$_1$-450 levels—would display more acetaminophen toxicity than *Ah*-nonresponsive mice. This hypothesis turns out to be true not only in liver, where hepatic necrosis and covalent binding of acetaminophen metabolites occur (*22*), but also in the lens, where cataract formation was found (Fig. 4). An absolute correlation is found (Fig. 5) between lens opacification and the Ah^b allele among four *Ah*-responsive inbred mouse strains, five *Ah*-

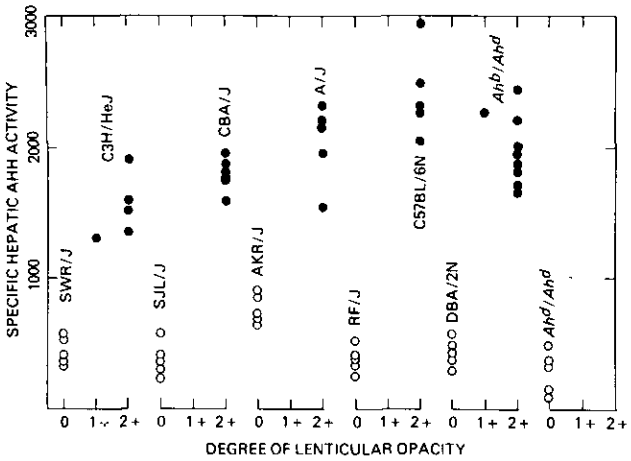

FIGURE 5. Correlation between the hepatic AHH inducibility and cataractogenesis among five nonresponsive inbred strains (○), four responsive inbred strains (●), and the (●) responsive (Ah^b/Ah^b) and (○) nonresponsive (Ah^d/Ah^d) progeny from the B6D2F_1 × D2 backcross (*25*). Each symbol represents an individual mouse. All mice had received 3-methylcholanthrene intraperitoneally 48 hr prior to intraperitoneal acetaminophen. The eyes were evaluated with an ophthalmic slit lamp 5 hr following acetaminophen treatment: ○ = no signs of opacification; 1+ = about 50% opacification; 2+ = complete opacification. The mice were then immediately killed, and the liver microsomal hydroxylase activity was determined.

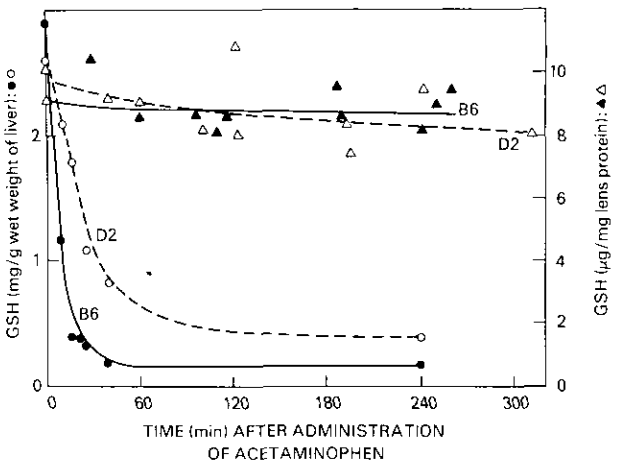

FIGURE 6. Total glutathione (GSH) levels in (○, ●) the liver and (Δ, Δ) lens of 3-methylcholanthrene-treated B6 mice (●, Δ) and D2 mice (○, Δ) following a large dose (1,000 mg/kg) of intraperitoneal acetaminophen (*25*).

nonresponsive inbred strains and among children of the B6D2F_1 × D2 backcross.

Figure 6 illustrates the effects of acetaminophen on glutathione concentrations in the liver and lens of 3-methylcholanthrene-pretreated B6 and D2 mice. As expected, hepatic glutathione depletion is more pronounced in B6 than in D2 mice. Quite unexpectedly, however, lenticular glutathione levels are not depleted in either B6 or D2 mice following the large intraperitoneal dose of acetaminophen.

One possibility is that acetaminophen and its metabolites do not reach the lens and therefore glutathione is not depleted. This possibility was ruled out with the use of radiolabeled acetaminophen (Fig. 7). Similar kinetics of covalent binding occurs in both liver and lens protein, and in each case B6 tissues exhibit two to five times greater covalent binding than the corresponding D2 tissues.

How can covalent binding of acetaminophen metabolites occur in the apparent absence of any glutathione depletion? At least two possible answers come to mind. First, assuming glutathione depletion in the lens is necessary for cataract formation, one might envision some sort of compartmentalization of glutathione. In other words, although total lens glutathione concentrations are similar in B6 and D2 mice, a subcellular depletion of glutathione (directly in the anterior subcapsular region of the lens where the cataracts develop) might occur in B6 but not in D2. Such a localized depletion might be augmented by a sudden influx of reactive intermediates (generated by the liver, ciliary body or whatever) so that local concentrations of the toxic agent far exceed local concentrations of glutathione in and near the anterior chamber. Alternatively, ocular toxicity may develop by a mechanism independent of glutathione levels. Electrophilic reactive intermediates could cause changes in cellular macromolecules in many ways: alterations in oxidations and reductions of endogenous molecules, free radical formation, permeability changes in the lens membrane, alterations in metabolism of endogenous substrates, etc.

Very large doses of acetaminophen were used for the experiments illustrated in Figures 4-7. The only clinical situation that such large doses might be achieved would be attempted suicide. Relevant observations, however, have been recently reported. Upon hearing a seminar about the data in this report, Cohen and Burk (*26*) examined ten patients showing evidence of acetaminophen-induced hepatotoxicity due to self-inflicted overdoses. The age range was 17 to 45 years old, with only two women more than 23 years of age. One of these patients, a 45-year-old alcoholic about whom no previous medical history was available and who died 1 day later, was found on admission to have bilateral diffuse cataracts; no previous history of cataracts was known. She also was the only fatality in the series of 10 cases (*26*).

Table 4. Effect of oral acetaminophen or naphthalene on cataract formation in B6 and D2 mice.

Inbred strain	Intraperitoneal P1-450 inducer treatment[a]	Oral treatment[b]	Drug concentration, mg/ml[c]	Mean body weight[d]			Incidence cataracts, %	Comments
				Day 0	Day 21	Day 60		
B6	3-Methylcholanthrene	Acetaminophen	5	15.7 (15)	17.1 (13)	(0)	13.3	Two developed bilateral cataracts about day 10 and both died 1 to 2 weeks thereafter
D2	"	"	5	14.6 (15)	16.9 (15)	(0)	0	No cataracts at time of death
B6	3-Methylcholanthrene	Acetaminophen	10	16.2 (15)	17.6 (12)	(0)	13.3	Two developed bilateral cataracts about day 3 and both died within the next 2 weeks thereafter
D2	"	"	10	15.9 (15)	17.7 (15)	(0)	0	No cataracts at time of death
B6	β-Naphthoflavone	Acetaminophen	5	16.4 (15)	19.9 (15)	21.5 (15)	6.7	One developed a unilateral cataract about day 14; this was examined when mouse was killed 7 months later
D2	"	"	5	15.4 (15)	20.5 (15)	22.8 (14)	0	No cataracts
B6	β-Naphthoflavone	Acetaminophen	10	15.9 (15)	20.8 (14)	22.3 (4)	13.3	One developed a unilateral cataract about day 11 and another developed bilateral cataracts about day 14; both died about day 36
D2	"	"	10	16.9 (15)	21.6 (14)	23.9 (14)	0	No cataracts
B6	β-Naphthoflavone	Naphthalene	5	15.4 (15)	19.4 (14)	20.7 (13)	6.7	One developed bilateral cataracts about day 27; these were examined when mouse was killed 6 months later
D2	"	"	5	16.5 (15)	20.3 (15)	21.3 (15)	0	No cataracts
B6	β-Naphthoflavone	Naphthalene	10	15.4 (15)	17.2 (13)	18.8 (12)	6.7	One developed a unilateral cataract about day 8; this was examined when mouse was killed 7 months later
D2	"	"	10	16.5 (15)	16.7 (15)	19.4 (15)	0	No cataracts

[a]Injections twice weekly (*25*).
[b]The drug was dissolved in corn oil and the food was soaked in this solution of corn oil.
[c]Estimated drug concentration in corn oil in which the food was soaked.
[d]Mean body weights of group during feeding of the drug. The number of survivors is shown in parentheses.

Effect of Daily Oral Acetaminophen or Naphthalene on Cataract Formation

In additional experiments, we chose oral doses of acetaminophen, lower doses that are used clinically (Table 4). Oral naphthalene was also chosen for study because its monooxygenation is also catalyzed by some form of P_1-450 and associated with the Ah^b allele (*16*). Naphthalene-induced cataracts have been described (*27*) in workers from dye and chemical factories. The cataractogenic agent is claimed (*28*) not to be naphthalene itself but rather 1,2-naphthoquinone, which is formed from naphthalene-1,2-dihydrodiol. This diol of naphthalene reaches the eye via the bloodstream. Pathways of naphthalene metabolism have been extensively investigated both *in vivo* (*29*) and with liver microsomes *in vivo* (*30-32*). The phenol and the reactive arene oxide of naphthalene and acetaminophen are conjugated with glucuronide, sulfate, and glutathione.

Relatively small numbers of B6 mice develop detectable cataracts when acetaminophen or naphthalene is ingested daily (Table 4). Phenobarbital induces these conjugating pathways, and this fact may explain our finding (*25*) that oral phenobarbital can completely prevent acetaminophen-induced cataract formation. It is possible that the reactive intermediate of acetaminophen is an arene oxide or a quinone. No D2 mouse ever developed acetaminophen- or naphthalene-induced cataracts. All mice treated with 3-methylcholanthrene died within 6 weeks, apparently due to the cytotoxicity of the

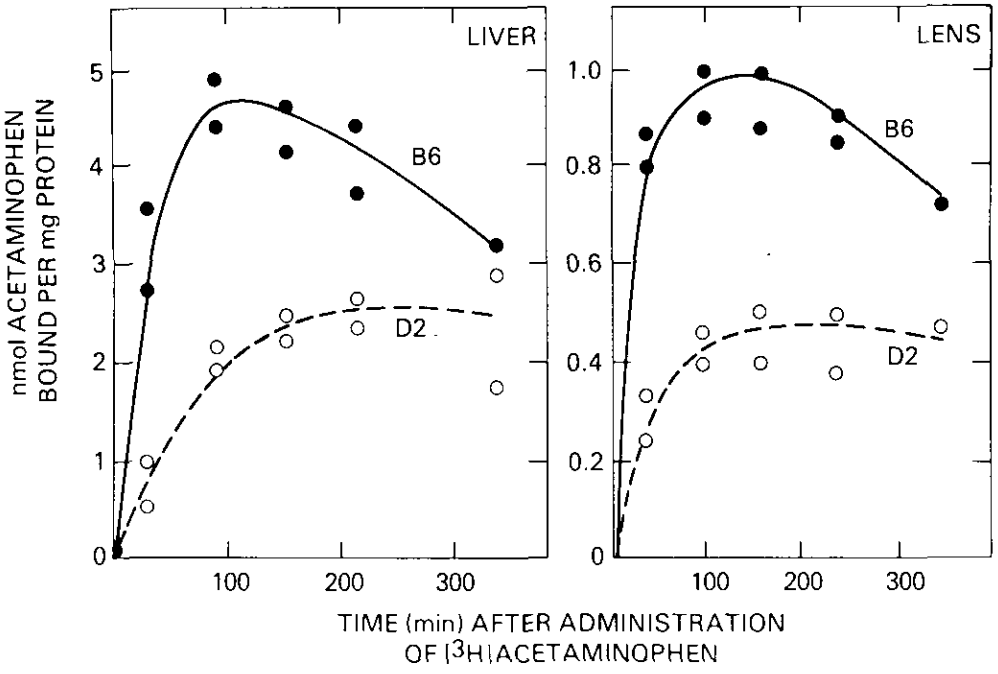

FIGURE 7. Covalent binding of [^{3}H]acetaminophen metabolite(s) to liver and lens proteins of 3-methylcholanthrene-treated (●) B6 and (O) D2 mice (*25*).

chemical when injected twice weekly. On the other hand, β-naphthoflavone treatment was not very toxic; relatively few animals died during 60 days of daily oral acetaminophen or naphthalene combined with intraperitoneal β-naphtoflavone twice weekly. Out of the nine B6 mice that developed cataracts on these various oral regimens, three remained otherwise healthy for 6 or 7 months, at which time the animals were killed in order to examine histologically the lenticular opacities.

Histology of Ocular Toxicity Induced by Oral Acetaminophen

Upon close microscopic examination, we found that tissue degeneration occurs not only in the anterior lens cortex but also in large areas of the uveal tract (Fig. *8a, c*). The retina and pigmented epithelium are normal (Fig. *8b*). The degeneration seen in the choroid, ciliary body and iris appears to be of a chronic nature. Anterior synechiae and inflammatory cells at the angle are frequently observed. The number of epithelial cells is usually decreased in cataracts that were young (several hours or several days old). Intermittent loss of cells from the lens epithelial layer is seen in cataracts that had been present for at least 1 week (Fig. *8c*). The gradual loss of the epithelial cells might result in metabolic derangements in the lens fibers, thereby leading to lens opacification. All lenses from D2 mice similarly treated do not show any evidence of ocular abnormalities.

Possible Interrelationship among Genetic Differences in Drug Metabolism, Drug-Induced Cataracts and Senile Cataracts in the Human Population

Cataracts induced by chemicals and drugs, especially naphthalene, have been extensively investigated because of their similarity to senile cataracts (*28, 33, 34*). The problem of trying to assess ocular toxicity in elderly patients receiving large daily doses of acetaminophen would be far more difficult than to study patients attempting suicide with acetaminophen, because of the combination of genetic factors (i.e., the [*Ah*] complex in man) and environmental factors (i.e., history of cigarette smoking, other drugs prescribed, dietary intake, etc.). Perhaps there exist other drugs in this same category: they may cause ocular toxicity in a

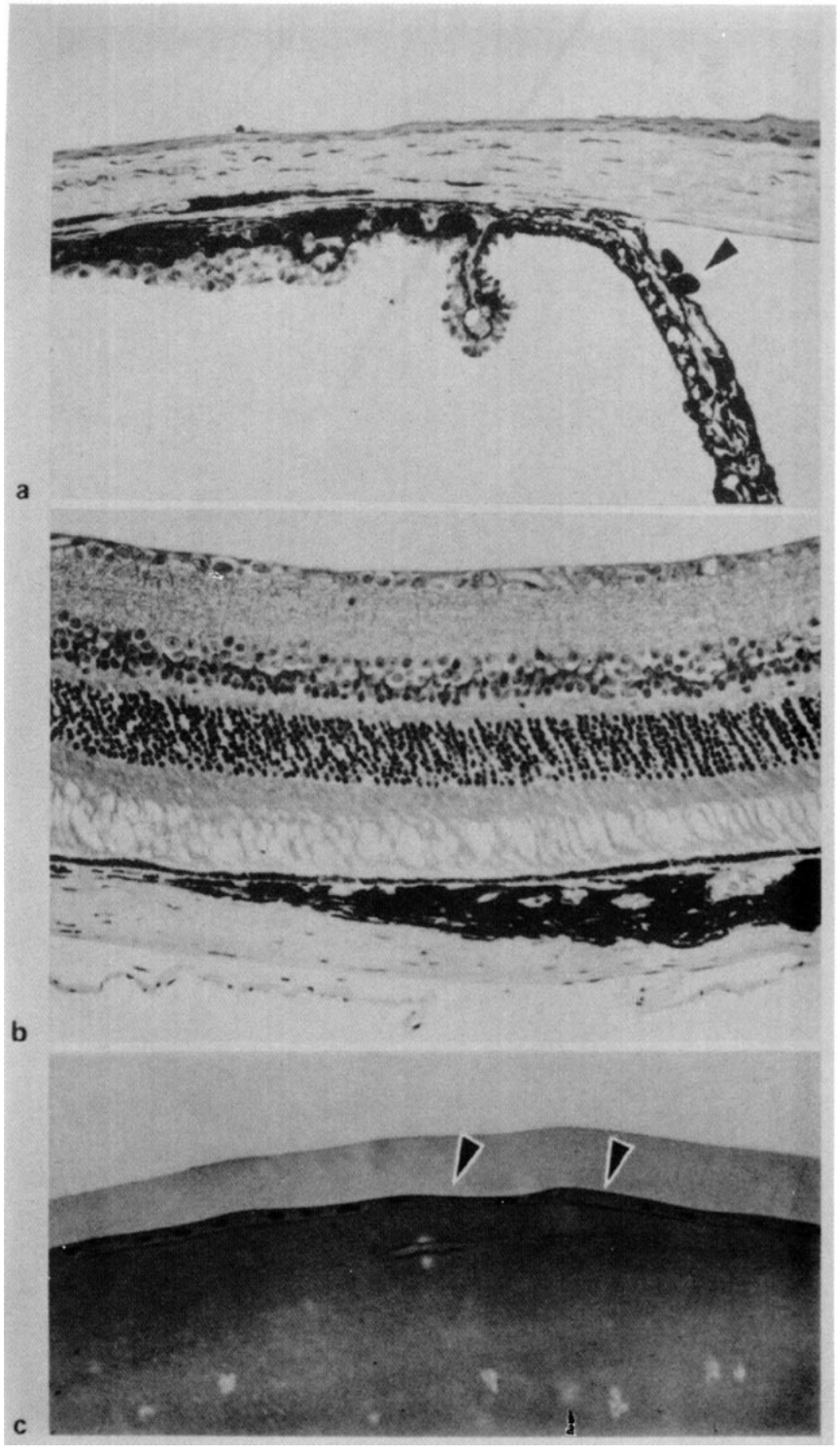

FIGURE 8. Hematoxylin- and eosin-stained sections of ocular tissues from B6 mice that had received acetaminophen daily in the diet for more than 1 month (*25*). (*a*) Light micrograph showing ciliary body, iris and trabecular meshwork. Note the presence of inflammatory cells in the angle (arrowhead) and the degenerative changes in the ciliary body. An atrophic iris and a certain amount of cellular infiltration of the episclera are observed. A peripheral anterior synechia is also seen. × 200. (*b*) Light micrograph showing the retina and choroid. Chronic degeneration of the choroid was frequently seen. The retina shows an almost normal appearance. × 200. (*c*) Light micrograph of the anterior cortical zone of the cataractous lens. Note the intermittent loss of epithelial cells from the epithelial cell layer (arrowheads). × 230.

genetically determined small proportion of the total human population. We do suggest, however, that the ocular toxicity data shown in this report may be clinically important to certain patients receiving either a single large overdose of acetaminophen or high doses over a long period of time.

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REFERENCES

1. Gram, T., Ed. Extrahepatic Metabolism of Drugs and Other Foreign Compounds, SP Medical and Scientific Books, New York, 1980.
2. Bernstein, H. N. Chloroquine ocular toxicity. Survey Ophthalmol. 12: 415-447 (1967).
3. Nebert, D. W., Eisen, H. J., Negishi, M., Lang, M. A., Hjelmeland, L. M., and Okey, A. B. Genetic mechanisms controlling the induction of polysubstrate monooxygenase (P-450) activities. Ann. Rev. Pharmacol. Toxicol. 21: 431-462 (1981).
4. Shichi, H. Microsomal electron transfer system of bovine retinal pigment epithelium. Exptl. Eye Res. 8: 60-68 (1969).
5. Zimmerman, T. J., Leader, B., and Kaufman, H. E. Advances in ocular pharmacology. Ann. Rev. Pharmacol. Toxicol. 20: 415-428 (1980).
6. Armaly, M. F. Genetic factors related to glaucoma. Ann. N. Y. Acad. Sci. 151: 861-875 (1968).
7. Williams, R. T., Ed. Detoxication Mechanisms, John Wiley & Sons, New York, 1959, 2nd ed.
8. Nebert, D. W. Genetic differences in susceptibility to chemically induced myelotoxicity and leukemia. Environ. Health Perspect. 39: 11-22 (1981).
9. Dutton, G. J. Developmental aspects of drug conjugation with special reference to glucuronidation. Ann. Rev. Pharmacol. Toxicol. 18: 17-35 (1978).
10. Chasseaud, L. F. Conjugation with glutathione and mercapturic acid. In: Glutathione: Metabolism and Function, I. M. Arias and W. B. Jacoby, Eds., Raven Press, New York, 1976, pp. 77-144.
11. Reddy, V. N., and Unakar, N. J. Localization of gamma-glutamyl transpeptidase in rabbit lens, ciliary process and cornea. Exptl. Eye Res. 17: 405-408 (1973).
12. Awasthi, Y. C., Saneto, R. P., and Srivastave, S. K. Purification and properties of bovine lens glutathione-S-transferase. Exptl. Eye Res. 30: 29-39 (1980).
13. Shichi, H., Tsunematsu, Y., and Nebert, D. W. Aryl hydrocarbon hydroxylase induction in retinal pigmented epithelium: possible association of genetic differences in a drug-metabolizing enzyme system with retinal degeneration. Exptl. Eye Res. 23: 165-167 (1976).
14. Shichi, H., and Nebert, D. W. Drug metabolism in ocular tissues. In: Extrahepatic Metabolism of Drugs and Other Foreign Compounds, T. E. Gram, Ed., SP Medical and Scientific Books, New York, 1980, pp. 333-363.
15. Das, N. D., and Shichi, H. Enzymes of mercapturate synthesis and other drug-metabolizing reactions-specific localization in the drug-metabolizing reactions-specific localization in the eye. Exptl. Eye Res. 33: 525-533 (1981).
16. Nebert, D. W., and Jensen, N. M. The *Ah* locus: genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450-mediated monooxygenases. In: CRC Critical Reviews in Biochemistry, G. D. Fasman, Ed., CRC Press, Inc., Cleveland, Ohio, 1979, Vol. 6, pp. 401-437.
17. Nebert, D. W. Multiple forms of inducible drug-metabolizing enzymes. A reasonable mechanism by which any organism can cope with adversity. Mol. Cell. Biochem. 27: 27-46 (1979).
18. Okey, A. B., Bondy, G. P., Mason, M. E., Kahl, G. F., Eisen, H. J., Guenthner, T. M., and Nebert, D. W. Regulatory gene product of the *Ah* locus. Characterization of the cytosolic inducer-receptor complex and evidence for its nuclear translocation. J. Biol. Chem. 254: 11636-11648 (1979).
19. Okey, A. B., Bondy, G. P., Mason, M. E., Nebert, D. W., Forster-Gibson, C., Muncan, J., and Dufresne, M. J. Temperature-dependent cytosol-to-nucleus translocation of the *Ah* receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in continuous cell culture lines. J. Biol. Chem. 255: 11415-11422 (1980).
20. Hannah, R. R., Nebert, D. W., and Eisen, H. J. Regulatory gene product of the *Ah* complex. Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3-methylcholanthrene binding to several moities in mouse liver cytosol. J. Biol. Chem. 256: 4584-4590 (1981).
21. Atlas, S. A., and Nebert, D. W. Pharmacogenetics: A possible pragmatic perspective in neoplasm predictability. Sem. Oncol. 5: 89-106 (1978).
22. Thorgeirsson, S. S., and Nebert, D. W. The *Ah* locus and the metabolism of chemical carcinogens and other foreign compounds. Adv. Cancer Res. 25: 149-193 (1977).
23. Shichi, H., Gaasterland, D. E., Jensen, N. M., and Nebert, D. W. *Ah* locus: genetic differences in susceptibility to cataract induced by acetaminophen. Science 200: 539-541 (1978).
24. van Heyningen, R., Ed. Cataract and Abnormalities of the Lens, Grune and Stratton, New York, 1975.
25. Shichi, H., Tanaka, M., Jensen, N. M., and Nebert, D. W. Genetic differences in cataract and other ocular abnormalities induced by paractetamol and naphthalene. Pharmacology 20: 229-241 (1980).
26. Cohen, S. B., and Burk, R. F. Acetaminophen overdoses at a county hospital: A year's experience, Southern Med. J. 71: 1359-1364 (1978).
27. Hollwich, F., Boateng, A., and Kilck, B. Toxic cataract. In: Cataract and Abnormalities of the Lens J. G. Bellow, Ed., Grune and Stratton, Inc., New York, 1975, pp. 230-243.
28. van Heyningen, R. Experimental studies on cataract. Invest. Ophthalmol. 15: 685-697 (1976).
29. Boyland, E., Ramsay, G. S., and Sims, P. Metabolism of polycyclic compounds. 18. The secretion of metabolites of naphthalene, 1: 2-dihydronaphthalene and 1:2-epoxy-1:2:3:4-tetrahydronaphthalene in rat bile. Biochem. J. 78: 376-384 (1961).
30. Jerina, D. M., Daly, J. W., Witkop, B., Zaltzman-Nirenberg, P., and Udenfriend, S. The role of the arene oxide oxepin system in the metabolism of aromatic substrates. Biochemistry 9: 147-156 (1970).
31. Oesch, F., and Daly, J. Conversion of naphthalene to *trans*-naphthalene dihydrodiol: evidence for the presence of coupled aryl monooxygenase-epoxide hydrase system in hepatic microsomes. Biochem. Biophys. Res. Commun. 46: 1713-1720 (1972).
32. Bock, K. W., Van Ackeren, G., Lorch, F., and Birke, F. W. Metabolism of naphthalene to naphthalene dihydrodiol glucuronide in isolated hepatocytes and in liver microsomes. Biochem. Pharmacol. 25: 2351-2356 (1976).
33. Adams, D. R. The nature of the ocular lesions produced experimentally by naphthalene. Brit. J. Ophthalmol. 14: 49-60 (1930).
34. Rees, J. R., and Pirie, A. Possible reactions of 1, 2-naphthaquinone in the eye. Biochem. J. 102: 853-863 (1967)