Advances in Mechanisms of Activation and Deactivation of Environmental Chemicals

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Environmental chemicals are both activated and detoxified by phase I and phase II enzymes. The principal enzymes involved in phase I reactions are the cytochrome P-450s. The phase II enzymes include hydrolase and the conjugative enzymes such as glucuronyltransferases, glutathione transferases, *N*-acetyltransferase, and sulfotransferase. Although other phase I and phase II enzymes exist, the present review is limited to these enzymes. Once thought to be a single enzyme, multiple cytochrome P-450 enzymes have been purified and characterized from many different species across the evolutionary tree. The application of molecular biology techniques to this field has identified more than 150 cytochrome P-450 genes to date. At least 20–30 cytochrome P-450 enzymes appear to exist in each mammalian species, and many polymorphisms in these enzymes are being identified. The cytochrome P-450 enzymes can now be expressed in recombinant form using cDNA expression systems. The phase II conjugative enzymes add a hydrophilic moiety such as sulfate, glucuronide, or acetate to compounds, which increases their water solubility and facilitates their excretion. However, conjugates of a number of compounds also result in more reactive electrophilic species, which appear to be the ultimate carcinogens. Many of these phase II enzymes also represent families of enzymes, and polymorphisms can affect the ability of these enzymes to metabolize chemicals. Whenever possible, we have reviewed knowledge of the human enzymes involved in particular pathways.

Introduction

Organisms have always been exposed to environmental chemicals, and they have developed a variety of enzymes to transform these "xenobiotics." The majority of environmental chemicals are highly lipophilic and cannot readily be excreted from the body without metabolism to more hydrophilic, water-soluble species. Metabolic pathways have therefore evolved to convert these lipophilic compounds to water-soluble metabolites. The initial reaction(s) (phase I metabolism) usually involves the introduction of oxygen into a compound as the first step toward detoxification and elimination (*1*). The principal enzymes responsible for phase I reactions are the cytochrome P-450s, although other enzyme systems such as the flavin-containing monooxygenase, alcohol dehydrogenases, and prostaglandin synthetase may also serve this function for certain substrates. The hydrophilicity of the metabolite can then be further increased by phase II reactions. During phase II metabolism, various membrane-bound and cytosolic enzymes, including epoxide hydrolases (*2,3*), glutathione *S*-transferases (*4*), UDP-glucuronosyltransferases (*5,6*), acetyltransferases (*7,8*), and sulfotransferases (*9*), attach highly water-soluble moieties to polar groups (often those introduced by phase I reactions). The net result of the combined phase I and phase II reactions is generally to detoxify and eliminate environmental xenobiotics from the body. However, these same pathways can also carry out the activation of most toxic and carcinogenic chemicals to electrophilic forms which can react irreversibly with macromolecules such as proteins and nucleic acids. The importance of metabolism in the activation of carcinogens was first recognized by Miller and Miller (*10,11*) in their pioneering studies on the metabolism of the aminoazo dyes and 2-acetylaminofluorene. The majority of these electrophiles are formed by the cytochrome P-450-dependent enzymes, although some phase II conjugations produce the ultimate carcinogen for certain chemicals (*1*).

The microsomal cytochrome P-450 enzymes were first reported by Klingenberg in 1958 (*12*), who noted the characteristic carbon monoxide difference spectrum of microsomes with a maximum near 450 nm, which is now known to be due to the axial thiolate ligation of heme with a conserved cysteine residue in the P-450 apoprotein. Omura and Sato (*13*) provided evidence that the pigment was a b-type cytochrome and proposed the name "P-450" to denote a pigment with absorption at 450 nm. They also partially purified the pigment, determined it was a hemoprotein, and measured its extinction coefficient (*14,15*). A physiological function for this pigment was first reported by Estabrook and co-workers (*16,17*), who found that it participated in the C21 hydroxylation carried out by adrenal cortex micro-

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somes and the hydroxylation and dealkylations of various drugs by liver microsomes. Another important development was the finding of Conney and Burns (*18,19*) that administration of drugs to animals could induce drug-oxidizing activities of mammalian liver microsomes. This could be explained by increases in the cytochrome P-450 content of liver microsomes of drug-treated animals.

Cytochrome P-450 was originally thought to be one enzyme. However, when phenobarbital and 3-methylcholanthrene (3-MC) were administered to animals, they had different effects on the oxidation of various drugs by liver microsomes. In 1967, Conney (*19*) proposed that inducible cytochrome P-450s could be characterized as either phenobarbital- or 3-MC-like. Sladek and Mannering (*20*) also reported evidence for the presence of a different form of cytochrome P-450 in liver microsomes of 3-MC-treated rats than in control or phenobarbital-treated rats. The 3-MC-inducible cytochrome had an absorption maximum of 448 nm compared to 450 nm for the phenobarbital-inducible form and could be distinguished by differences in the pH dependency of its ethyl isocyanide spectrum. This cytochrome was called cytochrome P_1-450 or P-448. However, there was a controversy for several years as to whether there was a single form of cytochrome P-450 or multiple forms of this enzyme.

Initial attempts to purify catalytically active cytochrome P-450 were unsuccessful due to the instability of this enzyme in the presence of the detergents used for solubilization. However, in a milestone study, Lu and Coon (*21*) were able to solubilize and fractionate cytochrome P-450, NADPH-dependent cytochrome P-450 reductase, and a lipid fraction using DEAE-cellulose chromatography in the presence of glycerol and subsequently reconstitute catalytic activity. With the availability of techniques for purification, it soon became apparent that multiple forms of cytochrome P-450 existed in each species (*22,23*). The cytochromes P-450 are now known to comprise a superfamily of proteins, each with a characteristic spectrum of substrate specificities (see below). Some of these forms are constitutive, others are inducible by phenobarbital, 3-methylcholanthrene, certain steroids, ethanol, or peroxisome proliferators such as clofibrate (*23,24*). The cDNA and gene structure of many of these P-450s have been elucidated (*25*). These proteins have been grouped into families and subfamilies based on similarities in protein structure. Individual cytochrome P-450 enzymes are defined as belonging to the same family if they are more than 40% similar in amino acid sequence, and within a subfamily are >59% identical. The cytochrome P-450s are named with the root CYP followed by an arabic numeral for the family, a capital letter for the subfamily, and an arabic numeral for the particular gene. More than 150 cytochrome P-450 genes have been characterized to date, and 10 families exist in mammalian species. Some of these families are responsible for the metabolism of endogenous compounds, including the stereo-specific synthesis of steroids from cholesterol and the subsequent metabolism of steroids. Three families (CYP1, 2, and 3) represent the hepatic mixed-function oxidases, which metabolize primarily xenobiotics and may have evolved in response to environmental stresses such as plant toxins and environmental compounds derived from combustion.

Phase I Reactions

As discussed above, the phase I monooxygenase system has been resolved into three main components: cytochrome P-450, NADPH-cytochrome P-450 reductase, and lipid. Cytochrome P-450 acts as the terminal oxidase in this system and catalyzes the insertion of one atom of molecular oxygen into a substrate (monooxygenase) to convert the substrate to a more polar compound, while converting the other atom of oxygen into water, using electrons donated by NADPH via NADPH cytochrome P-450 reductase (Fig. 1). In the general reaction of cytochrome P-450-mediated oxidation, the oxidized ferric form of the P-450 first interacts with a specific substrate. Subsequently, an electron is transferred from NADPH via the reductase to produce the

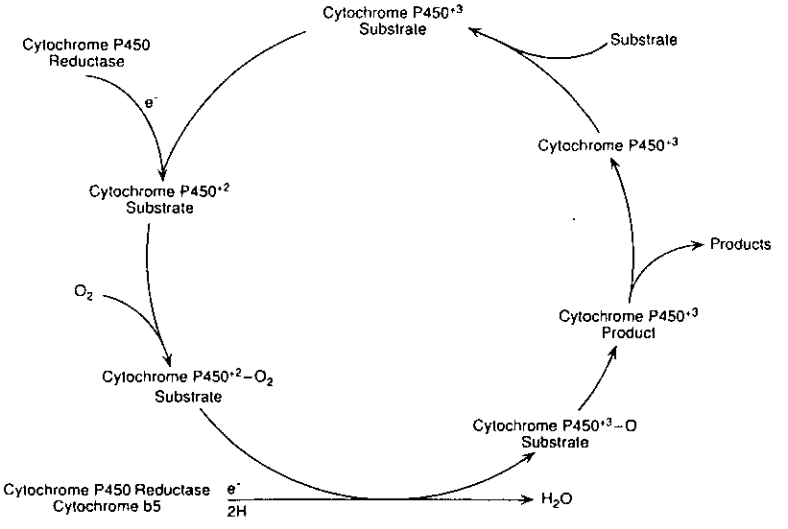

FIGURE 1. General scheme for P-450-mediated oxidation of substrates.

ferrous-P-450–substrate complex, which then binds molecular oxygen. After the oxidation of the ferrous cytochrome back to the ferric form, the oxygen becomes "activated." Another electron is then transferred from NADPH via the reductase, and this results in the stereo-specific oxidation of the substrate, formation of water, and regeneration of the ferric form of cytochrome P-450. The substrate-free oxidized cytochrome P-450 can then reenter the catalytic cycle.

Although a wide variety of oxidative reactions are mediated by the cytochrome P-450-dependent monooxygenase system, the majority of these reactions can be visualized essentially as hydroxylations. Some examples of types of reactions catalyzed by this system are shown in Figure 2 and are discussed below.

C-*Hydroxylation.* Aromatic groups can be hydroxylated or epoxidized, sometimes resulting in the production of toxic metabolites [reviewed by Guengerich and Shimada (*1*) and Nelson and Harvison (*26*)]. Examples are metabolites of benzene and polycyclic aromatic hydrocarbons. Aliphatic compounds can also be hydroxylated. For example, *n*-hexane is metabolized to the ultimate neurotoxin, 2,5-hexanedione by this process. The *C*-hydroxylation of *N*-nitrosodialkylamines produces unstable intermediates that rearrange to release reactive carbonium ions, which are the ultimate carcinogens. The carbonium ions can methylate at oxygen or nitrogen atoms in the purines and pyrimidines of DNA.

N-*Hydroxylation.* The *N*-hydroxylation of arylamines and acetylamines (such as benzidine, 4-aminobiphenyl, 2-acetylaminofluorene [2-AAF], and heterocyclic arylamines formed by food pyrolysis) is a critical initial step in the production of the ultimate carcinogens. The *N*-hydroxylamines are considered proximate carcinogens because they are generally more mutagenic and carcinogenic than the parent compounds (*27*). Further conjugative processes described later in this review lead to the production of more electrophilic species, which are the ultimate carcinogens.

S-*Oxidation.* As an example of *S*-oxidation of thionosulfur compounds, the insecticide phorate is oxidized to the *S*-sulfoxide, which can be further metabolized to the sulfone and the phorate oxon sulfone (*28*). Many of these sulfoxide metabolites are cytotoxic (*26*).

Dealkylation. Dealkylations at nitrogen, oxygen, or sulfur are also catalyzed by the cytochrome P-450 enzymes. Examples of these reactions include the *O*-dealkylation of various drugs such as phenacetin to acetaminophen (reaction shown in Fig. 2), the *N*-demethylation of aminopyrine, and the *S*-dealkylation of 6-methylthiopurine to 6-mercaptopurine. Amines can also be oxidatively deaminated by a similar process (not shown), which is primarily a detoxification process.

Epoxidation at Olefinic Centers. Olefinic compounds such as styrene, haloalkenes such as vinylidene chloride and trichloroethylene, and dihydrofurans such as aflatoxin are activated at the carbon–carbon π-bond by epoxidation (*26*). Many of these epoxides can react with cellular macromolecules and are genotoxic. These reactions can also result in the alkylation and suicide-based inactivation of the heme group of cytochrome P-450.

Oxidative and Reductive Dehalogenation. A variety of halogenated alkanes, alkenes, and alkynes are oxidized at the carbon–hydrogen bond to produce unstable intermediates that dehalogenate to form aldehydes or ketones (*26*). Examples are

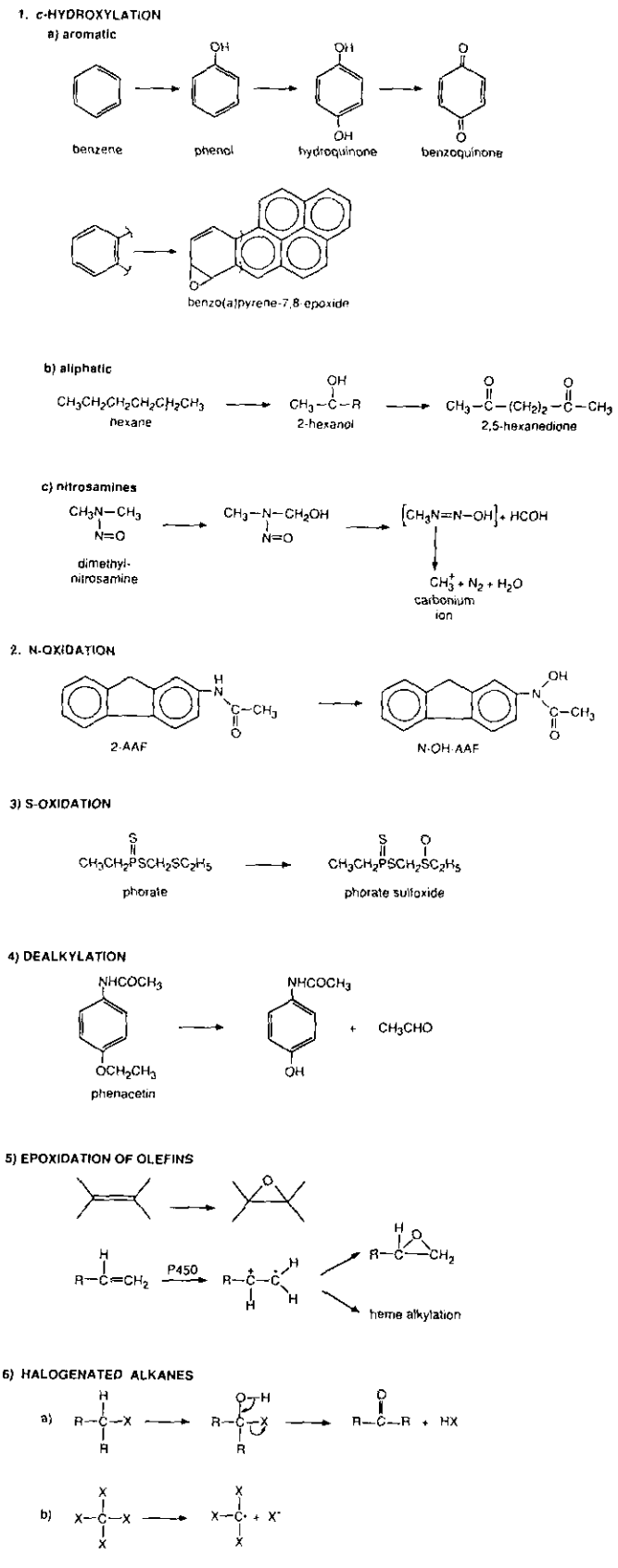

FIGURE 2. Examples of activation and deactivation pathways for xenobiotic metabolism by cytochrome P-450 enzymes.

the oxidation of ethylene dibromide to 2-bromacetaldehyde and the oxidation of chloroform to phosgene. The metabolites of the mono- and dihalomethanes are only weakly cytotoxic. However, trihalomethane metabolites such as phosgene produce hepatotoxicity and renal toxicity. The tetrahalogenated methanes produce toxicity by a different mechanism. These compounds undergo reductive dehalogenation to produce a free radical. Carbon tetrachloride produces both hepatic and renal damage via this mechanism.

Human Cytochrome P-450 Enzymes Involved in the Activation of Chemicals

Many of the human P-450 enzymes were first isolated in the laboratory of Guengerich and co-workers (*1*). The advent of cloning has allowed comparison of the deduced amino acid structures of the various enzymes (*25*). It is possible to estimate the point in the evolutionary scale that proteins diverged based on their percent homology. Some cytochrome P-450 enzymes are very old and apparently evolved before the divergence of humans and rodents. In this respect, analogs for cytochromes P-450 1A1 and P-450 1A2 have been found in all mammalian species studied. These cytochromes are inducible by chemicals such as 3-MC and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) via a receptor-mediated mechanism [the Ah receptor; reviewed in Gonzalez (*24*)]. P-450 1A1 appears to be important in the metabolic activation of polycyclic aromatic amines in all species examined (*1*). P-450 1A1 is not found or is only present in small concentrations in human liver (*29,30*), but it is inducible by polycyclic hydrocarbons in extrahepatic tissues such as lung (*31*). In contrast, P-450 1A2 is present in human liver but not extrahepatic tissues and appears to be more active in the *N*-hydroxylation of arylamines in both humans and rodents (*29,32*).

Many other forms of human cytochrome P-450 involved in the oxidation of xenobiotics do not appear to have exact counterparts in laboratory animals (the CYP2A, 2B, 2C, 2D, 3A, and 4A subfamilies). Therefore, the different species may have diverged before the evolution of the subfamilies from ancestral forms. The various cytochrome P-450 genes are assigned to subfamilies based on their amino acid sequence homologies; however, the catalytic specificity determined for apparent P-450 homologs across species are not always identical. In addition, recent studies by Negishi and co-workers (*33*) have shown that a single amino acid substitution converts the substrate specificity of P-450 coh to P-450 15a (from coumarin to steroid 15α-hydroxylase). These studies clearly indicate that it is impossible to predict substrate specificity across species.

More than 20 cytochrome P-450s have been identified in humans. Many members of the CYP2C and CYP2D families appear to be involved in the metabolism of clinically important drugs (*24*). These P-450s also exhibit a large degree of polymorphism in expression, which may be important for interindividual differences in susceptibility to exposure to environmental toxicants or therapeutic agents metabolized by these P-450s. For example, P-450 2D6 metabolizes the antihypertensive drug debrisoquine. A polymorphism in the expression of this gene has been described (*34*). There are multiple mechanisms for this polymorphism, but a predominant mechanism is an abberant splice recognition site (*35*). A correlation between susceptibility to lung cancer and the poor debrisoquine metabolizer phenotype has been suggested (*36*). However, this association has been questioned in other studies (*37*), and there is no known example of a carcinogen metabolized appreciably by P-450 2D6 (*1*). It is not yet clear whether the CYP2C or CYP2D subfamilies are important in the activation of chemical carcinogens in humans (*1*).

P-450 2E1 is an ethanol-inducible form that catalyzes the oxidation of ethanol to acetaldehyde. It also appears to be important in the activation of methyl substituted *N*-nitrosamines in rodents and humans (*1*). However, it does not appear to contribute to the oxidation of tobacco-specific nitrosamines or a number of other nitrosamines. Recently Guengerich et al. (*38*) have provided evidence that human P-450 2E1 is important in the metabolism of low molecular weight suspect carcinogens such as vinyl chloride, vinyl bromide, benzene, styrene, carbon tetrachloride, ethylene dibromide, trichloroethylene, ethylene dichloride, methylene dichloride, 1,2-dichloropropane and 1,1,1-trichloropropane.

In humans, the P-450 3A subfamily contains three members. The predominant form, P-450 3A4, appears to play a major role in the oxidation of many steroids including testosterone, androstenedione, progesterone, and cortisol (*1*). These are substrates which are metabolized by other subfamilies (P-450 2B, P-450 2C) in rodents (*23*). Human P-450 3A4 also appears to play a major role in the activation of aflatoxin B_1, the fire retardant *tris*(2,3-dibromopropyl)phosphate, 6-aminochrysene, and a number of polycyclic hydrocarbon dihydrodiols that can be converted to bay-region diol epoxides (*1*). This enzyme can also deactivate certain substrates, including alternate oxidative detoxification pathways for aflatoxin B_1.

Phase II Reactions

Epoxide Hydrolases

Epoxides are reactive metabolites that are generated principally by the cytochrome P-450 dependent pathways. The epoxide hydrolases are considered to be protective enzymes that hydrate the epoxides to form *trans*-1,2-dihydrodiols. These less reactive dihydrodiols can then be conjugated with glucuronic acid, sulfate, or glutathione and excreted. The metabolism of benzene via these pathways is shown in Figure 3. However, the activity of epoxide hydrolase can also result in the production of more reactive species, as in the hydrolysis of benzo[*a*]pyrene-7,8-oxide to its dihydrodiol, which is then metabolized by P-450 to the ultimate carcinogen, benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (*39*). There are multiple forms of epoxide hydrolase (*3*). The two forms that metabolize xenobiotics are microsomal epoxide hydrolase (mEH) and cytosolic epoxide hydrolase (cEH). The mEH metabolizes a wide variety of substrates, including both K-region and non-K region arene oxides of polycyclic hydrocarbons, butadiene, vinyl chloride, epoxides derived from herbicides and insecticides, and epoxides derived from therapeutic drugs such as phenytoin. Bay region diol-epoxides of polycyclic hydrocarbons, on the other hand, are poor substrates. Microsomal epoxide hydrolase also has a small role in the detoxification of aflatoxin B_1 8,9-oxide, the major species responsible for the genotoxicity of aflatoxin B_1. These compounds are detoxified primarily by glutathione conjugation. Cytosolic epoxide hydrolase is found primarily in the cytosol and peroxisomes. It

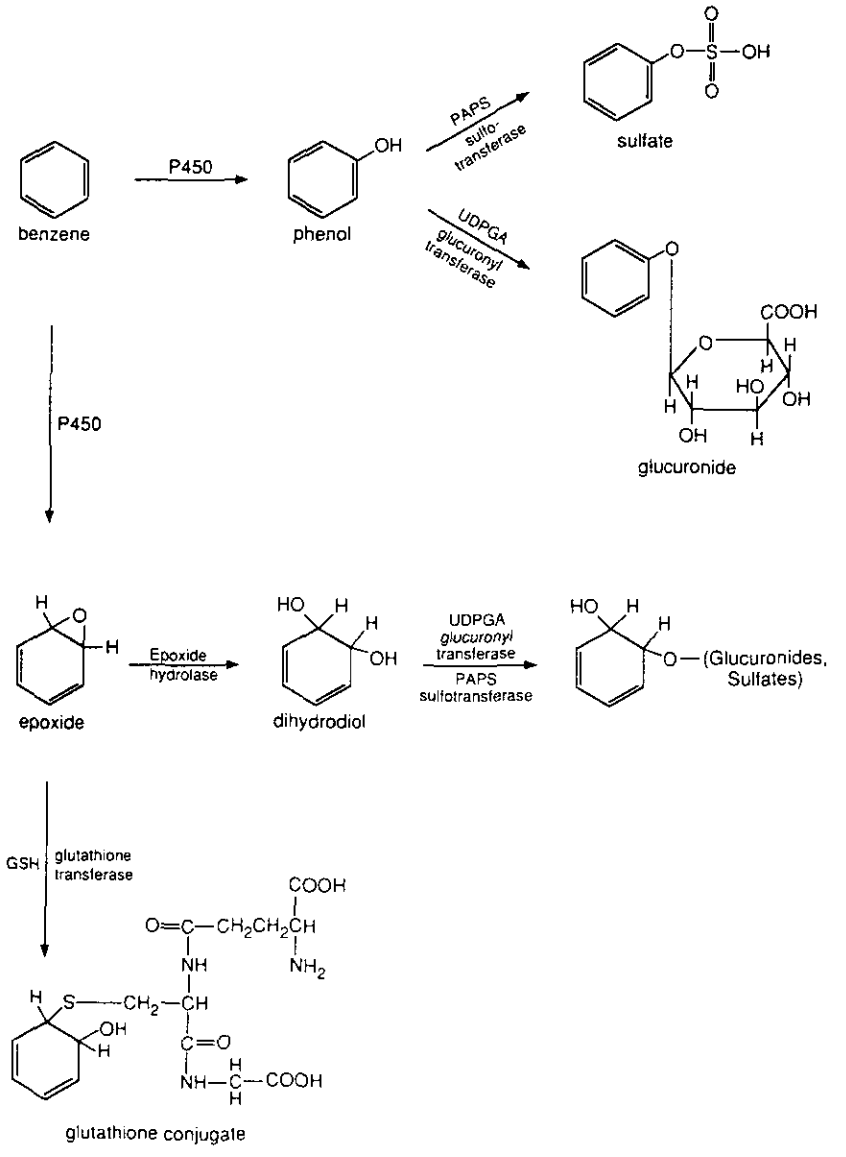

FIGURE 3. Summary of some of the phase II routes of metabolism of benzene including epoxide hydrolase, sulfotransferase, glucuronidation, and glutathione transferase. UDPGA (uridine diphosphoglucuronic acid) is the cofactor for glucuronosyltransferase. PAPS is the cofactor (adenosine 3′-phosphate 5′-phosphosulfate) for sulfotransferase.

specifically catalyzes the hydrolysis of mono- and disubstituted oxiranes, but does not metabolize arene oxides appreciably. Substrates include *trans*-substituted styrene oxides, stilbene oxides, and lipid epoxides (e.g., epoxide derivatives of arachidonic acid).

Epoxide hydrolase has been found in all organisms examined to date, including fungi. However, mEH and cEH can be classified only in higher organisms. The enzymes have been purified from a number of species including humans. Although the highest activity of both mEH and cEH is found in the liver, the organ distribution of mEH is quite broad. Oesch and co-workers (*40*) found considerable interindividual variability in the activities of these two enzymes in human liver (from 63-fold for mEH to 539-fold for cEH). cDNAs for rat and human mEH have been isolated, and hybridization studies indicate that there is only one gene copy of mEH in the human and the rat (*2*). Hepatic mEH is inducible in rats by both phenobarbital and 3-MC type inducers, while cEH is inducible by peroxisome proliferators (*3*).

Glutathione *S*-Transferases

There are multiple forms of the soluble glutathione *S*-transferases, which can be divided into four gene families based on their electrophoretic mobilities (*4,41*). The α-ϵ group consists of basic proteins, μ is a neutral class, and the π enzyme is acidic. The microsomal membrane-bound glutathione *S*-transferase represents an unrelated enzyme. Glutathione *S*-transferases are found in almost all tissues examined. These enzymes transfer glutathione (the tripeptide γ-glutamyl cysteinyl glycine) to com-

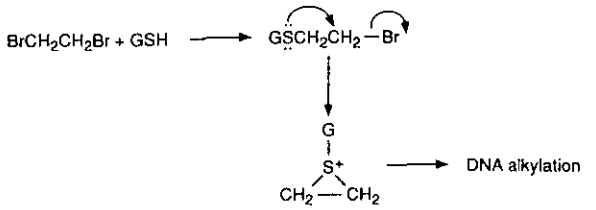

FIGURE 4. Activation of ethylene dibromide by glutathione transferase.

pounds that are electrophilic per se as well as those generated by phase I reactions. Thus, glutathione transferases can play a protective role by covalently binding and removing reactive electrophiles. Examples are the detoxification of benzene epoxide (Fig. 3), aflatoxin B_1-8,9-oxide, and benzo[*a*]pyrene-7,8-dihydrodiol-9,10-oxide. However, some procarcinogens are activated by conjugation with glutathione to produce unstable reactive metabolites (*41*). Examples of such genotoxic metabolites are the glutathione conjugates of ethylene dibromide and methylene chloride (Fig. 4). The μ form is found in the livers of only 60% of the human population (*4*). This form is efficient in conjugating epoxides of benzo[*a*]pyrene 4,5- oxide. It has been suggested that a hereditary polymorphism of the μ form may be related to susceptibility to lung cancer among cigarette smokers (*42*).

UDP-Glucuronosyltransferases

Uridine diphosphate glucuronsyltransferases represent a major phase II family of endoplasmic reticulum-bound enzymes that utilize the cofactor uridine diphosphoglucuronic acid (UDPGA) to conjugate glucuronic acid to a large number of potentially reactive compounds including phenols, dihydrodiols, quinones, and quinols (*5,6*). The addition of glucuronic acid renders these compounds more water soluble and enables the body to excrete them more readily. Examples of substrates that are conjugated with glucuronic acid by this system include the phenols, dihydrodiols, quinones, and quinols of numerous compounds such as benzene (Fig. 3), benzo[a]pyrene, and the chlorinated benzenes. Conjugates can include ether *O*-glucuronides, ester *O*-glucuronides (glucuronic acid groups), *N*-glucuronides, *S*-glucuronides, and *C*-glucuronides. Although glucuronidation is a major route of detoxification for many compounds, glucuronidation is involved in the etiology of human colorectal cancer from heterocyclic amines. These compounds are first *N*-oxidated and then *N*-glucuronidated. The *N*-hydroxy metabolites can be transported to the colon where they are hydrolyzed by β-glucuronidases. The *N*-hydroxy derivatives can then be *O*-acetylated in the colon mucosa, which results in formation of *N*-acetoxyarylamines which can react with DNA to form covalent adducts (*43*).

Acetylation

Acetylation involves the enzymatic or nonenzymatic transfer of the acetyl group from acetyl coenzyme A to molecules containing a primary amine, hydroxyl, or sulfhydryl group (*7*). These enzymes are distributed in many organs, but the liver is a major site of *N*-acetylation. Acetylation of the primary amine group of arylamines and hydrazines is a major route of biotransformation of these compounds. Arylamides and primary arylamines are interconverted by *N*-acetyltransferases and

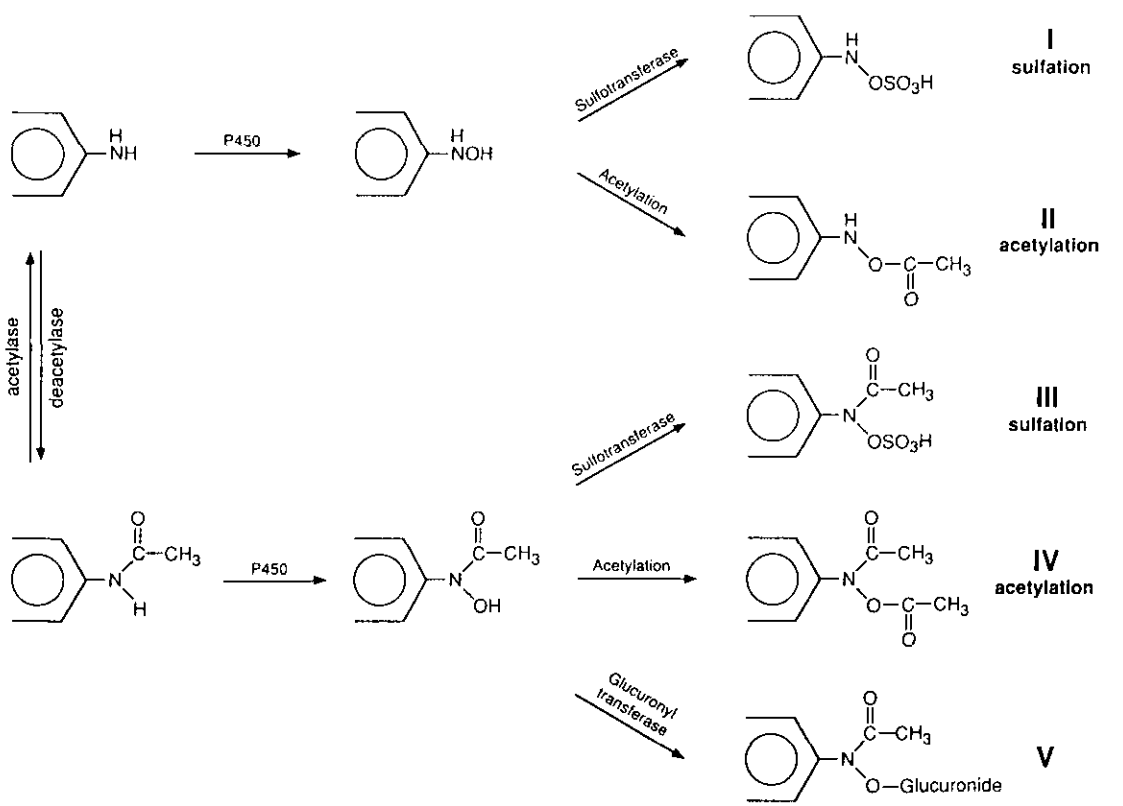

FIGURE 5. Acetylation and sulfation of arylamines to putative mutagenic and carcinogenic metabolites (metabolites I–V).

deacetylases (27). They are initially activated by the cytochrome P-450s and flavin monooxygenase enzymes to form *N*-hydroxyarylamides and *N*-hydroxyarylamines that can be interconverted by enzymatic *N*-deacetylation/*N*-acetylation (Fig. 5). These compounds are considered proximate carcinogens because they are more mutagenic and carcinogenic than the parent compounds. *N*-Hydroxyarylamides are converted to the ultimate carcinogens through conjugation with sulfuric, acetic, or glucuronic acids. *N*-acetoxyarylamines (metabolite II in Fig. 5) are highly reactive and appear to have an important role as ultimate carcinogens. Therefore, *O*-acetylation appears to play an important role in the activation of aromatic amines and food pyrolysates classified as human carcinogens, including 2-aminofluorene, benzidine, 4-aminobiphenyl, and β-naphthylamine.

There is a polymorphism in acetylation in humans, with the population being divided into slow and rapid acetylators. Meyer and co-workers (*44*) have cloned the genes *NAT*1 and *NAT*2, that encode two proteins with *N*-acetyltransferase activity when expressed in cultured mammalian cells. The *NAT*2 gene is the gene which shows the polymorphism. There appear to be three major mutant alleles in Caucasian populations, which consist of single amino acid mutations.

Acetylation may affect the carcinogenicity of aromatic amines in two ways. In bladder cancer, acetylation appears to be a detoxification mechanism for aromatic amines such as 2-naphthylamine and benzidine. Epidemiological studies in various populations including workers in the dye industry have found that bladder cancer is more common in the slow acetylator phenotype than the rapid acetylator phenotype (*45,46*). On the other hand, epidemiological studies indicate that slow acetylators have a decreased risk for colorectal cancer (*47*), presumably because the glucuronides of aromatic amines are hydrolyzed in the colon, and then acetylated to reactive *N*-acetoxyarylamines in colon mucosa as discussed earlier. Studies of the aromatic amine 2-aminofluorene indicate that acetylation and sulfation yield more reactive electrophiles which react with nucleic acids to produce adducts (*8*).

Sulfation

Sulfate conjugation improves water solubility and excretion. The sulfotransferases are a family of enzymes that transfer the sulfuryl group (SO_3) from adenosine 3′-phosphate 5′-phosphosulfate (PAPS) to hydroxyl or amino groups (Figs. 3 and 5) (*9,27*). Sulfotransferases are widespread in tissues, with high activities found in platelets, erythrocytes, the jejunum, brain, liver, and placenta. They are found in both the cytosol and endoplasmic reticulum. Conjugation with sulfate is generally considered a detoxification mechanism. However, the addition of sulfate also introduces a good leaving group on unstable compounds such as hydroxylamines (Fig. 5). The *N*-sulfonyloxy derivatives are considered to be among the predominant metabolites of 2-acetylaminofluorene responsible for the formation of DNA adducts and its carcinogenicity.

Future Studies

Originally, only a single form of cytochrome P-450 was thought to exist. However, it has become apparent that the cytochrome P-450 enzymes represent a vast superfamily of enzymes. In recent years, a number of approaches to studying the role of these enzymes in the metabolism of various substrates have been particularly useful. Techniques for purification of the P-450 enzymes and reconstitution of the complete enzyme systems have been developed. Antibodies have been utilized to quantitate isozymes and to inhibit specific catalytic pathways. Antibody inhibition studies using microsomes from particular human tissues are particularly useful for assessing the contribution of a P-450 form to a particular pathway.

Molecular biology techniques have clarified some of the confusion concerning the multiplicity of the cytochrome P-450 family, and the use of cDNA expression systems allows investigators to examine the catalytic activity of specific isozymes. Minor changes in amino acid sequence can produce dramatic effects on the catalytic activities or even the substrate specificities of individual P-450 enzymes. Therefore, the substrate specificity of human enzymes cannot be simply inferred from animal studies or studies with rodent P-450s. cDNA expression studies are particularly useful in determining the intrinsic ability of particular cytochrome P-450 enzymes to activate mutagens, and the availability of cDNAs for multiple human forms provides a means for studying the ability of a particular human P-450 (or conjugative enzyme) to activate specific chemicals. Site-directed mutagenesis studies allow scientists to probe the active sites and determine which portions of the enzyme are important in substrate binding and catalytic activity. However, activity in a recombinant cell line may not duplicate the enzymatic activity in human liver; therefore, studies with human liver microsomes and antibodies to human cytochromes remain necessary. Bacterial cDNA expression systems will also be useful in the future for purifying large amounts of recombinant human proteins that were previously difficult to isolate.

Many of the cytochrome P-450 enzymes are polymorphic and the relationship between polymorphisms and cancer is an area of active research between molecular biologists and epidemiologists. The molecular biology of some of the conjugative enzymes has not been studied as extensively as that of the P-450 enzymes, but recent studies have begun to address these families. There is clear evidence for polymorphisms in one of the enzymes involved in acetylation in humans. Information regarding the ability of specific metabolites to form DNA adducts and the relationship of these adducts to cancer is vital to understanding the role of metabolism in the etiology of environmentally induced cancer.

The authors are grateful to L. Tom Burka, National Institute of Environmental Health Sciences, Ernest Hodgson, North Carolina State University, and Fred Kadlubar, National Center for Toxicological Research, for reviewing this manuscript and for helpful suggestions. We also thank F. P. Guengerich, Vanderbilt University, for sharing preprints of recent reviews.

REFERENCES

1. Guengerich, F. P., and Shimada, T. Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem. Res. Toxicol. 4: 391–407 (1991).
2. Thomas, H., Timms, C. W., and Oesch, F. Epoxide hydrolases: molecular properties, induction, polymorphisms and function. In: Frontiers in Biotransformation, Vol. 2. Principles, Mechanisms and Biological Consequences of Induction (K. Ruckpaul and H. Rein, Eds.). Taylor and Francis, London, 1990, pp. 278–337.

3. Guenthner, T. M. Epoxide hydrolases. In: Conjugation Reactions in Drug Metabolism (G. J. Mulder, Ed.), Taylor and Francis, London, 1990, pp. 365–404.
4. Ketterer, B., and Taylor, J. B. Glutathione transferases. In: Frontiers in Biotransformation, Vol. 2. Principles, Mechanisms and Biological Consequences of Induction (K. Ruckpaul and H. Rein, Eds.), Taylor and Francis, London, 1990, pp. 244–277.
5. Mulder, G. J., Coughtrie, M. W. H., and Burchell, B. Glucuronidation. In: Conjugation Reactions in Drug Metabolism (G. J. Mulder, Ed.), Taylor and Francis, London, 1990, pp. 51–105.
6. Mackenzie, P. I. Structure and Regulation of UDP glucuronosyltransferases. In: Frontiers in Biotransformation, Vol. 2. Principles, Mechanisms and Biological Consequences of Induction (K. Ruckpaul and H. Rein, Eds.), Taylor and Francis, London, 1990, pp. 213–243.
7. Weber, W. W., Levy, G. N., and Hein, D. W. Acetylation. In: Conjugation Reactions in Drug Metabolism (G. J. Mulder, Ed.), Taylor and Francis, London, 1990, pp. 163–191.
8. Timbrell, J. A. Acetylation and its toxicological significance. In: Metabolism of Xenobiotics (J. W. Gorrod, H. Oelschlager, and J. Caldwell, Eds), Taylor and Francis, London, 1988, pp. 259–266.
9. Langner, A., Borchert, H.-H. and Pfeifer, S. Postoxidation Enzymes. In: Frontiers in Biotransformation, Vol 2. Principles, Mechanisms and Biological Consequences of Induction (K. Ruckpaul and H. Rein, Eds.), Taylor and Francis, London, 1990, pp. 150–210.
10. Miller, E. C., and Miller, J. A. Mechanisms of chemical carcinogenesis: nature of proximate carcinogens and interactions with macromolecules. Pharmacol. Rev. 18: 805–838 (1966).
11. Miller, E. C., and Miller, J. A. Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. Cancer 47: 2327–2345 (1981).
12. Klingenberg, M. Pigments of rat liver microsomes. Arch. Biochem. Biophys. 75: 376–386 (1958).
13. Omura, T., and Sato, R. A new cytochrome in liver microsomes. J. Biol. Chem. 237: 1375–1376 (1962).
14. Omura, T., and Sato, R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem. 239: 2370–2378 (1964).
15. Omura, T., and Sato, R. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. J. Biol. Chem. 239: 2379–2385 (1964).
16. Estabrook, R. W., Cooper, D. Y., and Rosenthal, O. The light reversible carbon monoxide inhibition of the steroid C-21 hydroxylase system of the adrenal cortex. Biochem. Z. 338: 741–755 (1963).
17. Cooper, D. Y., Levine, S., Narasimhulu, S., Rosenthal, O., and Estabrook, R. W. Photochemical action spectrum of the terminal oxidase of mixed function oxidase systems. Science 147: 400–402 (1965).
18. Conney, A. H., and Burns, J. J. Stimulatory effect of foreign compounds on ascorbic acid biosynthesis and on drug-metabolizing enzymes. Nature 184: 363–364, (1959).
19. Conney, A. H. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19: 317–366 (1967).
20. Sladek, N. E., and Mannering, G. J. Evidence for a new P-450 hemoprotein in hepatic microsomes from methylcholanthrene-treated rats. Biochem. Biophys. Res. Commun. 24: 668–674 (1966).
21. Lu, A. Y. H., and Coon, M. J. Role of hemoprotein P-450 in fatty acid ω-hydroxylation in a soluble enzyme system from liver microsomes. J. Biol. Chem. 243: 1331-1332 (1968).
22. Lu, A. Y. H., and West, S. B. Multiplicity of mammalian microsomal cytochromes P-450. Pharmacol. Rev. 31: 277–295 (1980).
23. Ryan, D. E., and Levin, W. Purification and characterization of hepatic microsomal cytochrome P450. Pharmacol. Ther. 45: 153–239 (1990).
24. Gonzalez, F. J. The molecular biology of cytochrome P450s. Pharmacol. Rev. 40: 244–288 (1989).
25. Nebert, D. W., Nelson, D. R., Coon, M. J., Estabrook, R. W., Feyereisen, R., Fujii-Kuriyama, Y., Gonzalez, F. J., Guengerich, F. P., Gunsalus, C., Johnson, E. F., Loper, J. C., Sato, R., Waterman, M. R., and Waxman, D. J. The P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. DNA Cell Biol. 10: 1–14 (1991).
26. Nelson, S. D., and Harvison, P. J. Roles of cytochromes P-450 in chemically induced cytotoxicity. In: Mammalian Cytochromes P450, Vol. 2 (F. P. Guengerich, Ed.), CRC Press, Boca Raton, FL, 1987, pp. 19–79.
27. Kadlubar, F. F., and Beland, F. A. Chemical properties of ultimate carcinogenic metabolites of arylamines and arylamide. In: Polycyclic Hydrocarbons and Carcinogenesis (R. G. Harvey, Ed.), American Chemical Society, Washington, D.C., 1985, pp. 341–370.
28. Levi, P. E., and Hodgson, E. Stereospecificity in the oxidation of phorate and phorate sulphoxide by purified FAD-containing monooxygenase and cytochrome P-450 isozymes. Xenobiotica 18: 29–39 (1988).
29. Butler, M. A., Iwasaki, M., Guengerich, F. P., and Kadlubar, F. F. Human cytochrome P-450$_{PA}$, the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic amines. Proc. Natl. Acad. Sci. USA 86: 7696–7700 (1989).
30. McNanus, M. E., Burgess, W. M., Veronese, M. E., Huggett, A., Quattrochi, L. C., and Tukey, R. H. Metabolism of 2-acetylaminofluorene and benzo(a)pyrene and activation of food-derived heterocyclic amine mutagens by human cytochromes P-450. Cancer Res. 50: 3367–3376 (1990).
31. Petruzelli, S., Camus, A.-M., Carrozzi, L., Ghelarducci, L., Rindi, M., Menconi, G., Angeletti, C. A., Ahotupa, M., Hietanene, E., Aitio, A., Saracci, R., Bartsch, H., and Giuntin, C. Long-lasting effects of tobacco smoking on pulmonary drug-metabolizing enzymes: a case-control study on lung cancer patients. Cancer Res. 48: 4695–4700 (1988).
32. Shimada, T., Iwasaki, M., Martin, M. V., and Guengerich, F. P. Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by *umu* gene response in *Salmonella typhimurium* TA 1535/pSK1002. Cancer Res. 49: 3218–3228 (1989).
33. Lindberg, R. L. P., and Negishi, M. Alteration of mouse cytochrome P450$_{coh}$ substrate specificity by mutation of a single amino-acid residue. Nature 339: 632–634 (1989).
34. Mahgoub, A., Idle, J. R., Dring, L. G., Lancaster, R., and Smith, R. L. Polymorphic hydroxylation of debrisoquine in man. Lancet ii: 584–586 (1977).
35. Hanioka, N., Kimura, S., Meyer, U. A., and Gonzalez, F. J. The human CYP2D locus associated with a common genetic defect in drug oxidation: a $G_{1934} \rightarrow$ A base change in intron 3 or a mutant cyp2D6 allele results in an abberant 3′ splice recognition site. Am. J. Hum. Genet. 47: 994–1001 (1990).
36. Ayesh, R., Idle, J. R., Ritchie, J. C., Crothers, M. J. and Hetzel, M. R. Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. Nature 312: 169–170 (1984).
37. Speirs, C. J., Murray, S., Davies D. S., Biolamabadeje, A. F., and Boobis, A. R. Debrisoquine oxidation phenotype and susceptibility to lung cancer. Br. J. Clin. Pharmacol. 29: 101–109 (1990).
38. Guengerich, F. P., Kim, D.-H., and Iwasaki, M. Role of human cytochrome P-450 IIE1 in the oxidation of several low molecular weight cancer suspects. Chem. Res. Toxicol. 4: 168–179 (1991).
39. Wood, A. W., Wislocki, P. G., Chang, R. L., Levin, W., Lu, A. Y. H., Yagi, H., Hernandez, O., Jerina, D. M., and Conney, A. H. Mutagenicity and cytotoxicity of benzo(a)pyrene benzo-ring epoxides. Cancer Res. 36: 3358–3366 (1976).
40. Mertes, I., Fleischmann, R., Glatt, H. R., and Oesch, F. Interindividual variations in the activities of cytosolic and microsomal epoxide hydrolase in human liver. Carcinogenesis 6: 219–223 (1985).
41. Guengerich, F. P. Interindividual variation in biotransformation of carcinogens: basis and relevance. In: Molecular Dosimetry and Human Cancer: Analytical, Epidemiological and Social Considerations (J. D. Groopman and P. L. Skipper, Eds.), CRC Press, Boca Raton, FL, 1991, pp. 27–52.
42. Seidegard, J., Pero, R. W., Markowitz, M. M., Roush, G., Miller, D. G., and Beattie, E. J. Isoenzyme(s) of glutathione transferase (class mu) as a marker for the susceptibility to lung cancer: a follow up study. Carcinogenesis 11: 33–36 (1990).
43. Turesky, R. J., Lang, N. P., Butler, M. A., Teitel, C. H., and Kadlubar, F. F. Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. Carcinogenesis 12: 1839–1845 (1991).
44. Grant, D. M., Blum, M., Beer, M., and Meyer, U. A. Monomorphic and polymorphic human arylamine N-acetyltransferases: a comparison of liver isozymes and expressed products of two cloned genes. Mol. Pharmacol. 39: 184–191 (1991).
45. Mommsen, S., Barfod, N. M., and Aagaard, J. N-Acetyltransferase phenotypes in the urinary bladder carcinogenesis of a low-risk population. Carcinogenesis 6: 199–201 (1985).
46. Cartwright, R. A., Glashan, R. W., Rogers, H. J., Ahmad, R. A., Barham-Hall, D., Higgins, E., and Kahn, M. A. Role of N-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. Lancet ii: 842–846 (1982).
47. Ilett, K. F., David, B. M., Detchon, P., Castleden, W. M., and Kwa, R. Acetylation phenotype in colorectal carcinoma. Cancer Res. 47: 1466-1469 (1987).