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Role of Cytochrome P-450 and Related Enzymes in the Pulmonary Metabolism of Xenobiotics

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The lung metabolizes a wide variety of xenobiotics and, in the process, forms products that may be more or less toxic than the parent compound. The consequence of metabolism, activation or detoxication, is a function of the nature of the substrate and of the characteristics and concentrations of the enzymes involved. As a result, the biotransformation of xenobiotics can lead to their excretion or to the formation of reactive products that produce deleterious effects by binding covalently to tissue macromolecules. Among the enzymes that metabolize xenobiotics, those associated with the cytochrome P-450-dependent monooxygenase system are probably the most important. The route by which a given substrate is metabolized in a tissue or cell is, to a great extent, determined by the types and concentrations of cytochrome P-450 isozymes present. We are just beginning to understand the distribution of these enzymes in lung and to appreciate the species and cellular differences that exist.

Introduction

The metabolism of xenobiotics in the lung, as in other organs, generally results in the formation of products that can be processed via the excretory pathways. In this way, the lung contributes to the clearance of drugs and other foreign chemicals from the body. In addition to aiding in clearance, and probably more important, metabolism can greatly alter the toxic properties of many xenobiotics. In cases where toxicity is decreased, the advantages of these metabolic systems are obvious and we may optimistically, if somewhat naively, employ the phrase "detoxication pathways." However, modification of numerous innocuous compounds by the same enzyme systems can produce highly reactive and potentially deleterious metabolites. In these cases, the phrase "toxication pathways" is used. While these labels may tell us something about the nature of the products formed, they do not necessarily describe different metabolic pathways.

While the actions of many compounds, particularly some drugs and pesticides, are diminished or eliminated by metabolism, it is not surprising that the biological activity of some xenobiotics is increased by the same process. The persistence of numerous chemicals in the environment, where they may be exposed to sunlight, bacteria, extremes of pH, moisture, etc., is a measure of their chemical stability and, in many cases, is also indicative of a lack of direct biological reactivity. Oxidative metabolism can, however, form highly reactive products from many of these biologically "inert" substances. The reactive intermediates may then combine with cellular macromolecules such as proteins (*1*) or nucleic acids (*2,3*) and disrupt vital cellular functions. The importance of metabolic activation in chemically induced lung injury has been clearly established (*4*).

A number of different enzymes and enzyme systems are involved in the metabolism of xenobiotics, but the majority of these reactions are catalyzed by the cytochrome P-450–dependent monooxygenase systems (P-450 systems). Polycyclic aromatic hydrocarbons, including several pulmonary carcinogens, aromatic amines, fatty acids and steroids, are among the myriad of chemical classes that provide substrates for cytochrome P-450–mediated oxidative metabolism.

Cytochrome P-450 catalyzes monooxygenase reactions in which one atom of molecular oxygen is introduced into the substrate and the other goes to form water. The chemical modifications brought about by this reaction include *N*- and *O*-dealkylation, aromatic and aliphatic hydroxylation, epoxidation, *N*-hydroxylation, and oxidation of thioethers and phosphorothioates. P-450 systems reside primarily in the endoplasmic reticulum of cells in a number of tissues. These systems are comprised of NADPH-cytochrome P-450 reductase, a flavoprotein, and an undetermined number of isozymes of cytochrome P-450. The substrate specificities of the P-450 isozymes that have been isolated and characterized overlap somewhat but, in general, vary

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considerably. This variability and the relative concentrations of individual isozymes may determine that a tissue or cell can defend itself against certain toxic chemicals but produce critical concentrations of reactive metabolites from others.

Although the exact complement of P-450 isozymes in a tissue or cell type cannot be determined by existing techniques, the occurrence of species, age and tissue differences, in both the number of isozymes and their concentrations, is evident. In addition, populations of P-450 isozymes can be drastically altered by the inductive, repressive or destructive effects of various exogenous chemicals. As a consequence of these factors and others, the catalytic properties of P-450 systems from various sources can exhibit marked differences.

Pulmonary Cytochrome P-450-Dependent Monooxygenase Systems

General Considerations

Cytochrome P-450 systems are present in the lungs of mammals (*5*) including humans (*6,7*). That these systems form reactive metabolites from certain xenobiotics can be inferred from the many pulmonary toxicoses that result from the activation of exogenous chemicals and from the ability of pulmonary enzymes to form reactive products from a number of carcinogens, mutagens and other toxic compounds *in vitro* (*5*). Therefore, it can be said that P-450 systems play a requisite role in the initiation of a number of pulmonary diseases including some cancers. The more important question, however, is whether P-450 systems play any role in the determination of tissue- or cell-specific responses to various toxins. Several lines of evidence suggest that for the lung the answer is no. Cytochrome P-450-catalyzed metabolism of benzo(a)pyrene (BP), for example, is much more extensive in the liver, a nontarget organ for BP-induced carcinogenesis, than in the lung. Also, the amounts of adducts formed *in vivo* between DNA and the proposed ultimate carcinogens derived from BP (*8*) are the same in liver and lung of A/HeJ and ICR/Ha mice, strains that are highly susceptible to BP-induced pulmonary carcinogenesis (*9, 10*). Other comparisons of pulmonary and hepatic P-450 systems have produced similar findings. For most substrates, monooxygenase activity in lung, whether expressed as total activity or activity per milligram microsomal protein, is substantially less than that of the liver. In general, these differences reflect the 6 to 20 times lower concentrations of monooxygenase enzymes in the lung. From these observations it appears reasonable to conclude that the specificities of toxic reactions initiated by P-450-catalyzed metabolism and confined toally or partially to the lung cannot be explained by unique properties of pulmonary P-450 systems. This conclusion is, perhaps, not difficult to accept when other potential determinants of tissue specificity for toxic reactions—distribution, uptake, cell division, DNA repair—are considered. On the other hand, comparisons of P-450 systems from different tissues, whether they are based on studies of intact organs, microsomal fractions or purified enzymes may not be relevant to the question of tissue specificity. Arguments derived from these comparisons assume that the monooxygenase systems are somewhat evenly distributed throughout the tissues being compared; an assumption that pertains to both the qualitative and quantitative properties of the P-450 enzymes. While this may be at least partially true for the liver, we now know that the complement of P-450 enzymes in the lung is quite different from that of the liver and that the concentrations of pulmonary P-450 systems vary considerably among the pulmonary cell types that have been examined.

Rabbit Pulmonary Cytochrome P-450 Monooxygenase System

Enzymes and Their Distribution. The best understood pulmonary P-450 system is that of the rabbit. Cytochrome P-450 isozymes, forms 2 and 5,* make up greater than 70% of the rabbit pulmonary cytochrome P-450 (*11*). In contrast, these isozymes are minor components of the hepatic system except in rabbits that have been treated with phenobarbital, an inducer that has little or no effect on the pulmonary P-450 system. The identity of the pulmonary isozymes as forms 2 and 5 has been established by direct comparison with the hepatic enzymes. No structural (molecular weights, amino acid compositions, N- and C-terminal sequences, peptide maps), immunochemical (Ouchterlony immunodiffusion, Western blotting, inhibition of activity), or catalytic (substrate specificities in purified and microsomal systems) differences between the sets of isozymes from liver and lung have been detected (*11–16*). Whether the microheterogeneous populations of these isozymes from liver and lung are identical remains to be resolved. A third P-450 isozyme, form 6, has been detected in rabbit lung but at very low concentrations relative to forms 2 and 5 (*17*). The synthesis of form 6, however, can be induced by treatment of rabbits with tetrachlorodibenzo-*p*-dioxin (TCDD) (*18*), polychlorinated biphenyls (PCBs) (*17*), or benzo(a)pyrene (BP). Although a number of isozymes in addition to forms 2, 5, and 6 have been isolated from rabbit liver (*19,20*), no direct evidence for the existence of other isozymes in the lung is available.

Apparent high concentrations of isozymes 2 and 5 have been detected in the nonciliated bronchiolar

*These forms of cytochrome P-450 were termed $P\text{-}450_I$ and $P\text{-}450_{II}$ when they were isolated from rabbit lung. $P\text{-}450_I$ has since been determined to be the same as form 2 from the liver. $P\text{-}450_{II}$ has been identified in liver, and has a monomeric molecular weight that falls between those of form 4 and form 6. Therefore, in keeping with the nomenclature used for isozymes of cytochrome P-450 in the rabbit, $P\text{-}450_{II}$ is now called form 5.

epithelial (Clara) cells of the lower airways of rabbit lung in tissue sections (*21,22*) and isolated cells (*23*). Positive identification of these isozymes in other cell types, using immunochemical methods and light microscopy in tissue sections, is difficult. However, both isozymes have been detected in isolated alveolar Type II cells, whereas efforts to identify P-450 systems in the alveolar macrophage have been unsuccessful (*23*). Cytochrome P-450-dependent monooxygenase activity is much greater in preparations from isolated Clara cells as compared to isolated Type II cells; an apparent reflection of the difference in enzyme concentrations (*24*). Isozyme 6 has been detected in the pulmonary endothelium in sections of lung from rabbits treated with TCDD, but not in preparations from untreated rabbits (*25*). Similar results were reported for isozyme 4 (*25*), but we have been unable to detect this isozyme in pulmonary preparations from rabbits treated with TCDD, BP or PCBs by techniques that clearly identify isozyme 6 in pulmonary preparations from untreated rabbits (unpublished results).

Modulation of Cytochrome P-450 Isozymes. One of the most studied aspects of P-450 systems is the ability of numerous exogenous chemicals to induce the synthesis of cytochrome P-450 isozymes. In general, these inducers are divided into two major classes that are commonly represented by phenobarbital (PB) and 3-methylcholanthrene (3-MC). PB is thought of as a more general inducer than 3-MC and, in general, it is. A number of effects related to treatment of animals with PB—proliferation of smooth endoplasmic reticulum and increases in NADPH-cytochrome P-450 reductase, for example—are not seen with 3-MC. However, both compounds alter the profile of P-450 isozymes in the same general way; they induce the synthesis of two or more isozymes in the liver.

Treatment of adult rabbits with TCDD results in an increase in the hepatic concentrations of cytochrome P-450 isozymes, forms 4 and 6 (*26*). In the lung, however, only the concentration of isozyme 6 is increased by TCDD (*18*). In fact, we have been unable to detect isozyme 4 in pulmonary preparations from either untreated or TCDD-treated rabbits. Treatment of several animal species with 3-MC-type inducers, including 3-MC, TCDD, and BP, brings about at least three cytochrome P-450-related changes in the properties of pulmonary microsomal preparations: first, the metabolism of BP is markedly increased; second, the cytochrome P-450 content is increased; third, the maximum of the spectrum of cytochrome P-450 complexed with carbon monoxide shifts from 450 to 448 nm. These changes are not detected in pulmonary microsomal preparations from rabbit and, because of this, we concluded some time ago that 3-MC and BP had no effect on the P-450 system of rabbit lung (*5*). The finding that treatment of rabbits with TCDD increases the pulmonary concentration of isozyme 6, lead us to reinvestigate the effects of BP and it is now clear that BP, like TCDD, induces the synthesis of isozyme 6 in rabbit lung and that "P-450$_{III}$," reported to be a unique isozyme induced in rabbit lung by 3-MC (*27*), is also isozyme 6 (unpublished results).

In contrast to the 3-MC-type inducers, PB has not been shown to have any notable effect on pulmonary P-450 systems. (We have observed some increase in the pulmonary concentration of isozyme 2 following treatment of rabbits with PB, but the increases are marginal and becomes statistically significant only when the sample size reaches about thirty.) The lack of effect of PB on rabbit lung is quite interesting because PB does increase the hepatic concentrations of isozymes 2 and 5 (*28,29*). Although PB has no major inductive effect on the P-450 system of rabbit lung, it does alter the pulmonary profile of P-450 isozymes. The pulmonary content of isozyme 6, which is low in untreated rabbits, is even lower (less than 50% of control) in rabbits treated with PB. More important, PB blocks the inductive effective of coplanar isomers of PCBs on isozyme 6 (*17*). PCB mixtures (Aroclor 1248, 1254 and 1260) modify the rabbit pulmonary P-450 system in two ways: first, as mentioned above, the coplanar isomers, which are 3-MC-type inducers, increase the microsomal concentration of isozyme 6 (*17*); second, other isomers (unidentified at present) markedly decrease the microsomal concentration of isozyme 2 (*17,30*). The decrease in isozyme 2 can be detected by several immunochemical methods as well as by loss of activity specific for isozyme 2.

Pulmonary P-450 systems can also be altered by the destructive effects of several compounds. For example, lung-specific destruction of cytochrome P-450 follows treatment of rats or rabbits with *p*-xylene (*31*). The mechanism of this specific effect (relative to the liver) is not clear, but the inability of the lung to convert *p*-methylbenzaldehyde to *p*-methylbenzoic acid appears to be involved (*32*). *p*-Xylene is converted to *p*-methylbenzylalcohol by a P-450-catalyzed reaction and is further metabolized to the aldehyde by alcohol dehydrogenase. The results of *in vitro* experiments show that *p*-methylbenzaldehyde, NADPH and O_2 are required for the destruction of P-450. Destruction can be brought about in systems composed of purified NADPH-cytochrome P-450 reductase and cytochrome P-450, a result that demonstrates the participation of the P-450 system in the critical reaction. The addition of aldehyde dehydrogenase to microsomal or purified systems effectively blocks the destruction of cytochrome P-450 by *p*-methylbenzaldehyde (*32*).

Substrate Specificities of Rabbit Pulmonary Isozymes of Cytochrome P-450. A number of differences between the activities of the rabbit hepatic and pulmonary P-450 systems can now be explained by the substrate specificities and concentrations of P-450 isozymes in the two tissues. The capacity of the pulmonary P-450 system to metabolize xenobiotics and differences between the activities of liver and lung are, for the most part, functions of isozymes 2 and 5. The substrate specificities of isozymes 2 and 5 have been

determined in purified systems and confirmed in microsomal preparations by antibody-inhibition studies (*12,14,33,34*). For example, purified isozyme 2 is highly active in the *N*-demethylation of benzphetamine, whereas this activity cannot be detected with isozyme 5. In pulmonary microsomal preparations, over 90% of the benzphetamine *N*-demethylation activity is inhibited by antibodies to isozyme 2; antibodies to isozyme 5 have no effect on this reaction. Even though isozyme 2 is a minor form of P-450 in rabbit liver, antibodies to isozyme 2 inhibit about 50% of the hepatic *N*-demethylation of benzphetamine. Thus, the high rate (per nmole P-450) of benzphetamine metabolism in pulmonary versus hepatic microsomal preparations is a function of the difference between the relative proportions of isozyme 2 in liver and lung and the high activity of isozyme 2, in comparison with the bulk of the hepatic cytochrome P-450, with benzphetamine. Treatment of rabbits with PB results in an increase in hepatic *N*-demethylation activity that is consistent with the induction of isozyme 2. Benzphetamine, along with ethylmorphine, aminopyrene, 7-ethoxycoumarin and *p*-nitroanisole, are examples of substrates that are metabolized by isozyme 2, but not by isozyme 5. Several compounds, like 4-ipomeanol and *p*-xylene, are substrates for both isozymes (*35,36*). In the case of 4-ipomeanol, this appears to be important in the pulmonary-specific effects of this toxin (*13,34,36*). (The importance of the substrate specificities and localization of isozymes 2 and 5 in the pulmonary toxicity of 4–ipomeanol is discussed in detail by Boyd elsewhere in this issue (*37*). As is the case for isozyme 2, the metabolism of several xenobiotics by rabbit pulmonary microsomal preparations is catalyzed only by isozyme 5. Among these substrates are several aromatic amines whose metabolism in rabbit pulmonary and hepatic microsomal preparations has been studied in some detail.

Differences between rabbit liver and lung in the metabolism of aromatic amines to mutagenic products *in vitro* point out the marked effect that the substrate specificities and relative concentrations of various isozymes of cytochrome P-450 can have on metabolic capacity. At present, these differences cannot be associated with any pulmonary-specific carcinogenesis caused by aromatic amines. However, the effect of aromatic amines on rabbit lung should be thoroughly investigated before such an association is ruled out.

Microsomal preparations from rabbit lung are 20 to 30 times more active (per nmole cytochrome P-450; about five times per milligram microsomal protein) than those from liver in the metabolism of 2-aminofluorene (2-AF) and 2-aminoanthracene (2-AA) to mutagenic products (*33*)., With 2-acetylaminofluorene (2-AAF) as the substrate, the formation of mutagenic products (per mg protein) is about the same with either preparation. The metabolism of all three substrates in lung preparations is nearly completely inhibited by antibodies to cytochrome P-450, isozyme 5; inhibition in the liver preparations varies between 50 and 70%. The remaining hepatic activity is probably catalyzed by isozyme 4 (*38, 39*). In purified systems, isozyme 5 is 15 to 25 times more active than isozyme 4 with 2-AA or 2-AF as the substrate (*33*). The relative activities of isozymes 4 and 5 are consistent with the observed hepatic activity given that isozyme 5 makes up only about 2% of the total hepatic P-450 (*28*), whereas isozyme 4 has been reported to make up 30% of the total (*40*). That is to say that about half of the hepatic activity is catalyzed by a very small amount of highly active cytochrome. The high activity of isozyme 5 also explains why the lung preparations are so much more active than those from the liver; per mg microsomal protein, there is 5 to 10 times more isozyme 5 in the lung. If these findings provide an explanation for the difference between liver and lung for the activation of 2-AF and 2-AA, what accounts for the results obtained with 2-AAF? Arguing that isozyme 5 is less active with 2-AAF than with either 2-AA or 2-AF is not convincing; antibodies to isozyme 5 inhibit about 50% of the hepatic microsomal metabolism of 2-AAF to mutagenic products. In spite of this, it turns out that isozyme 5-catalyzed metabolism of 2-AAF to the product (*N*-hydroxy AAF) that leads to a mutagenic metabolite cannot be detected (*41*).

The generally accepted pathway for the metabolism of 2-AAF to mutagenic products is the formation of *N*-hydroxy AAF, catalyzed by cytochrome P-450, followed by deacetylation to form *N*-hydroxy AF (*42*). In fact, in the rabbit at least, the major pathway is deacetylation of 2-AAF to form 2-AF followed by cytochrome P-450-catalyzed *N*-hydroxylation to form *N*-hydroxy AF. The substrate for isozyme 5-mediated activation of a 2-AAF is actually 2-AF. The mutagenic activity of 2-AAF, as compared to 2-AA and 2-AF, in rabbit pulmonary microsomal preparations is limited by the deacetylase activity which is only about one-sixth that of the liver.

Activation vs. Detoxication: Pulmonary Metabolism of Polycyclic Aromatic Hydrocarbons

The overall biotransformation of many xenobiotics is a complicated process that may involve three or more stages of metabolism. Several enzymes, including multiple isozymes of cytochrome P-450, epoxide hydratase, various conjugating enzymes and transferases, may be involved with the metabolism of a single substrate and its products. The order in which these enzymes catalyze reactions is dependent upon the substrate and can be quite variable. While both toxic and nontoxic metabolites may be produced by some enzymes, either with the same or different substrates, other enzymes form primarily those of the nontoxic variety. The latter, however, may produce metabolites that can be activated by further metabolism. Thus, the outcome of metabolism cannot be readily predicted by dividing enzymes

into two groups, "activators and deactivators," and determining their relative concentrations and distribution in a tissue or cell type. The metabolism of polycyclic aromatic hydrocarbons (PAH) provides a good example of the complex interactions that can take place among the various enzymes involved with the biotransformation of xenobiotics. For polycyclic aromatic hydrocarbons and other substrates, the metabolic process may be further complicated by the involvement of prostaglandin synthase.

In 1950 Boyland (*43*) predicted that epoxides were formed during the metabolism of aromatic hydrocarbons. This insight came from an analysis of the stable metabolites produced from aromatic hydrocarbons and provided an explanation for the alkylation of tissue macromolecules by these relatively unreactive substances. Since then, indirect evidence has implicated epoxides as obligatory intermediates in the formation of phenolic derivatives from aromatic compounds (*44*). More recently, epoxides formed from polycyclic aromatic hydrocarbons (PAH) have been isolated and characterized (*45,46*).

The metabolism of aliphatic or aromatic carbon-carbon double bonds, such as those in BP, has been characterized largely with hepatic preparations; however, most of the enzyme pathways involved (see Fig. 1) have also been identified in the lung. Oxidative metabolism of BP by the cytochrome P-450-dependent monooxygenase system results in the formation of epoxides (*44*), which may spontaneously rearrange to form hydroxylated products (*44*) or may be converted to dihydrodiols by epoxide hydratase (*47*). The epoxide metabolites also undergo conjugation with glutathione, a reaction catalyzed by glutathione transferases (*48,49*). The conjugates can then be modified by a process known as mercapturic acid biosynthesis, the sequential removal of amino acid residues from the glutathione tripeptide, followed by *N*-acetylation of the residual cysteine derivative (*50*). The disposition of the dihydrodiol metabolites is of particular importance because of the competition between conjugation reactions with sulfuric (*44*) or glucuronic (*51*) acid and metabolism catalyzed by the P-450 system to form dihydrodiol

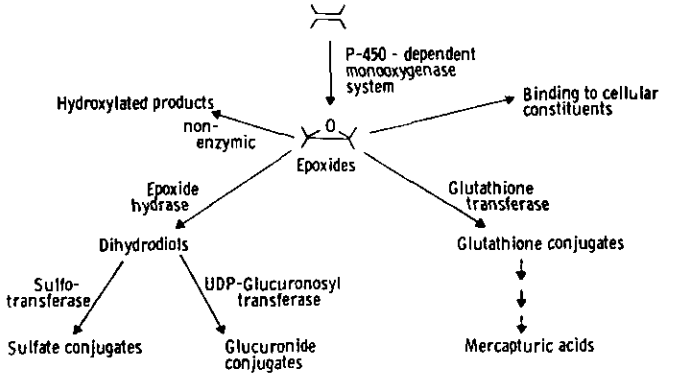

FIGURE 1. Pathways that contribute to the biotransformation of unsaturated or aromatic hydrocarbons.

epoxides such as the BP 7,8-dihydrodiol-9,10-oxide derivatives, which are believed to be the ultimate carcinogens derived from BP (*52,53*).

Because the lungs are a target organ for many airborne PAH, it was anticipated that a relationship between pulmonary metabolism of these chemicals and the lung's susceptibility to their carcinogenic effects might exist. The results of early investigations of this possible relationship demonstrated that rat pulmonary microsomal preparations metabolize BP and that the rate of metabolism is increased nearly 30-fold by prior exposure to cigarette smoke (*54–56*). Many other and more detailed studies of the metabolism of BP by rodent and human lung microsomal preparations followed, and these have been summarized in a recent review (*57*).

Results of investigations of the metabolism of xenobiotics by subcellular fractions of rabbit lung demonstrated a high potential in the microsomal fraction for the formation of reactive intermediates, but little epoxide metabolizing capacity. Metabolism of compounds, such as biphenyl or aniline, is mediated by rabbit pulmonary preparations at similar or even greater rates than by hepatic preparations (*58,59*). On the other hand, epoxide hydratase and glutathione transferase activities in rabbit lung are substantially lower than in rabbit liver (*60*). Because distal or peripheral lung (i.e., the terminal airways and alveoli) constitutes the bulk of whole lung homogenates, these findings are consistent with the report that peripheral rabbit lung is susceptible to the carcinogenic effects of PAH (*61*).

The above observations may be relevant to carcinogenesis in the rabbit lung, but most human lung tumors associated with smoking originate from the bronchiolar epithelium. Therefore, characterization of PAH metabolism in the bronchiolar epithelium is of obvious importance. Although microsomes have been prepared from the bronchiolar epithelium (*62,63*), the majority of the studies of PAH metabolism by this fraction of the lung have been carried out with short-term organ cultures, which have the advantage of allowing for extended periods of exposure to the substrate. Rat and hamster tracheae, which are histologically similar to human bronchus and are susceptible to PAH-induced tumors, are ideal for such studies (*64,65*). The usual experimental approach is to compare BP metabolism in a susceptible tissue (bronchus or trachea) versus a nonsusceptible tissue (peripheral lung or liver). Although many detailed studies, with both rodent and human lung tissue, have been carried out, no clear relationship between pulmonary metabolism of BP and the susceptibility of lung to PAH-induced carcinogenesis has materialized. Results of a study by Cohen and Moore (*66*) indicated that cultured rat and hamster peripheral lung tissue convert BP to water-soluble derivatives to a much greater extent than does trachea, but that trachea forms greater quantities of BP-dihydrodiols. Lung tissue, but not trachea, was also shown to form phenolic BP derivatives conjugated with sulfuric acid (*67*). Similar results were found with tissue cultures of

human lung (*67*). Increased dihydrodiol production might enhance susceptibility of a tissue to PAH-induced carcinogenesis because some BP-dihydrodiols are procarcinogens (*68*), and these findings provided a possible biochemical basis for PAH sensitivity in the upper airways. However, it was later demonstrated that either cultured hamster lung or trachea could convert BP 7,8-dihydrodiol to water-soluble conjugates (*69,70*). Furthermore, the conversion of BP to water-soluble derivatives by cultured rat trachea was increased when the animals were exposed to cigarette smoke (*71*).

An alternative approach is to measure the extent to which BP derivatives covalently bind to DNA. This binding is believed to be related to the formation of epoxide metabolites, and it probably plays an important role in the initiation of malignant transformations (*2,3*). Several investigators have demonstrated that BP is metabolized to products that covalently bind to macromolecules in bronchial (*72–75*) and peripheral lung (*76,77*) and that a relationship exists between metabolism and binding. Covalent binding of BP derivatives to bronchial samples, cultured in the presence of benz(a)anthracene (an inducer of monooxygenase activity), was greater than in bronchial samples that had not been treated with the inducer (*72,73*). Covalent binding of BP derivatives in cultured peripheral lung samples with high monooxygenase activity was also greater than in samples with low monooxygenase activity (*76,78*). Furthermore, covalent binding of BP to cultured bronchial mucosa was diminished by 7,8-benzoflavone, an inhibitor of the metabolism of BP. The products covalently bound to DNA in cultured bronchus were formed primarily from the metabolism of BP 7,8-dihydrodiol (*79,80*); metabolic data indicated that this was also the case in peripheral lung (*76,78,81*). These findings implicated the 7,8-dihydrodiol-9,10-oxides as the ultimate carcinogens formed by the metabolism of BP by human bronchi and peripheral lung, a conclusion that was strengthened by the identification of BP diol–epoxide–DNA adducts isolated from peripheral lung (*77*) and bronchi (*75*) cultured in the presence of BP. These findings, coupled with the observation that BP metabolism is much more rapid in bronchus than in peripheral lung (*78*), might be construed as an indication that the bronchus is a site for tumor formation because it generates larger quantities of reactive metabolites and, consequently, forms greater numbers of DNA adducts. However, it has been shown that covalent binding of metabolites of BP to DNA in bronchus and peripheral lung is quantitatively similar (*78*). It is also noteworthy that extensive formation of DNA–BP diol–epoxide adducts occurs in tissues, such as liver, that are not usually sites for PAH-induced tumor formation.

Although metabolism clearly plays an important role in the overall process by which some malignant transformations are initiated, it may not be a deciding factor in tissue- or cell-specific responses. However, a critical analysis of the available data shows that this is far from certain and that a great deal of work remains to be done. For example, metabolism and adduct formation in individual cell types has not been determined. It is quite possible that significant changes in a small population of cells can be masked by minor changes in a much larger population. Results of autoradiographic studies indicate that BP becomes covalently bound to most cell types (with the exception of stromal and mesenchymal cells) in a nonselective manner (*73,78*). Unfortunately, such techniques are only marginally quantitative, and they reveal little about the nature of the macromolecules that have been assaulted and nothing about the structure of the adducts (*73,78*). Determination of the activities of the enzymatic systems that catalyze the formation and further metabolism of epoxides in specific pulmonary cell types may result in some highly relevant findings, since the monooxygenase and other activities of different pneumocytes vary considerably (*21,23,24*).

The metabolism of polycyclic aromatic hydrocarbons in the lung may be further complicated by prostaglandin synthase-catalyzed co-oxidation. Prostaglandin synthase is a bifunctional enzyme that converts arachidonic acid to a hydroperoxy endoperoxide which it then metabolizes to a hydroxy endoperoxide (*82*). It is the hydroperoxidase activity of the enzyme that is involved with the co-oxidation of xenobiotics. The co-oxidation of BP by prostaglandin synthase was first demonstrated with microsomal preparations from ram seminal vesicles (*83,84*). The activity depends upon the addition of arachidonic acid to the microsomal incubations. It has now been shown that pulmonary microsomal preparations from guinea pig mediate prostaglandin synthase-dependent co-oxidation of BP, BP 7,8-dihydrodiol and 7,12-dimethylbenzanthracene to products that covalently bind to protein (*85*). More important, it has been found that the major product of the co-oxidation of BP 7,8-dihydrodiol by prostaglandin synthase in microsomal preparations from ram seminal vesicles or human lung is BP 7,8,9,10-tetrahydrotetrol (*86,87*). The production of this metabolite is indicative of the formation of BP 7,8-dihydrodiol 9,10-oxide as an intermediate in the co-oxidation pathway. The occurrence of this reaction *in vivo* could contribute significantly to the metabolism of BP to carcinogenic products, particularly in tissues or cells that have little or no cytochrome P-450-dependent monooxygenase activity.

Perspective

Without the ability to process and excrete xenobiotics, most organisms would quickly become stockrooms for a myriad of natural and synthetic chemicals. Cytochrome P-450-dependent metabolism is a major part of the overall process by which this potential is minimized. With some highly toxic chemicals, the protective effect of metabolism is more immediate; for example, the basis for the selection of many pesticide-resistant strains

of insects is more efficient cytochrome P-450–mediated "detoxication."

In higher animals, P-450 systems are found in most organs, but the liver is by far the primary site for the metabolism of xenobiotics. Extrahepatic metabolism may serve to minimize storage of exogenous chemicals and to detoxify others, possibilities that are difficult to prove. On the other hand, the adverse consequences of extrahepatic P-450 systems, particularly in lung, are evident.

At present, our knowledge concerning the biological functions of pulmonary P-450 systems is quite limited. We can explain why the Clara cell in some species is a target for the toxic effects of 4–ipomeanol, but not why the Clara cells in these species contain relatively high concentrations of cytochrome P-450. Recent technical advances will now allow us to classify cytochrome P-450 isozymes in lung, map their distribution, and determine their potential metabolic consequences. Perhaps this information will help provide some answers to the questions of why P-450 systems are present in the lung and why such great cellular and species diversity exists among pulmonary cytochrome P-450 systems.

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