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Pulmonary Metabolism of Foreign Compounds: Its Role in Metabolic Activation

by Gerald M. Cohen*

The lung has the potential of metabolizing many foreign chemicals to a vast array of metabolites with different pharmacological and toxicological properties. Because many chemicals require metabolic activation in order to exert their toxicity, the cellular distribution of the drug-metabolizing enzymes in a heterogeneous tissue, such as the lung, and the balance of metabolic activation and deactivation pathways in any particular cell are key factors in determining the cellular specificity of many pulmonary toxins. Environmental factors such as air pollution, cigarette smoking, and diet markedly affect the pulmonary metabolism of some chemicals and, thereby, possibly affect their toxicity.

Introduction

The lung is the major portal of entry into the body of all inhaled compounds such as those contained in polluted city air, tobacco smoke, and medicinal and household aerosols. The very large surface area of the alveoli (about 70 m^2) besides being ideally suited for the function of gas exchange, also facilitates the penetration into the body of these potentially toxic agents. As the entire cardiac output passes through the lungs, all chemicals present in the circulation must also traverse the lungs. Not surprisingly, many of the foreign chemicals and biological agents to which the airways are exposed have been implicated in a wide variety of diseases of the respiratory tract, for example, lung cancer (tobacco smoke, benzo(a)pyrene, nitrosamines, polonium-210, chromates, nickel and arsenic); chronic bronchitis and emphysema (cigarette smoking and air pollution); diffuse pulmonary fibrosis (busulfan, bleomycin, nitrofurantoin, methysergide, paraquat, methylphenylethylhydantoin, and diphenylhydantoin); and phospholipidoses (chlorphentermine).

The lung is exposed to a wide variety of exogenous chemicals that may exert profound pharmacological, therapeutic, or toxicological actions. These chemicals may be metabolized by the lung, possibly increasing or decreasing their toxicity or pharmacological action (*1–4*). The enzymes responsible for the pulmonary metabolism of these foreign chemicals (xenobiotics) include both mixed-function oxidases and enzymes catalyzing conjugation reactions and are the subject of this review.

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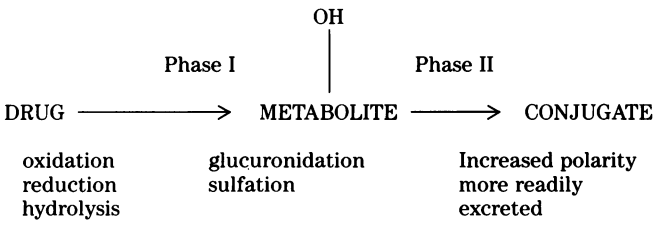
Pulmonary Drug-Metabolizing Enzymes

While the liver has been recognized as the major site of drug or xenobiotic (foreign compound) metabolism, recently much attention has also been paid to the metabolism of xenobiotics by extrahepatic tissues, in particular, at portals of entry into the body (i.e., the lung, intestine, and skin), which is markedly influenced by the environment, dietary status, and prior exposure to other chemicals (*5*). Owing to a deficiency of good data, it is difficult to assess the contribution of pulmonary metabolism to the overall metabolism of a compound. In most cases, *in vitro* enzymic activities of liver are significantly higher than those of lung, but these differences may be offset *in vivo* by other factors such as blood flow and distribution. Thus, with compounds such as certain amines that are concentrated in lung tissue (*2,6*), the contribution of the lung to the overall metabolism of the compound may be far greater than can be determined from *in vitro* enzymic activities.

The metabolic transformations, which most xenobiotics undergo in the body, are numerous and diverse, but they may be conveniently classified into two main phases (*7*). In phase I, compounds may undergo either oxidation, reduction, or hydrolysis reactions generally resulting in the addition or exposure of functional groups, such as -OH or COOH, which may then undergo phase II or synthetic (conjugation) reactions. The net result of such phase I and II metabolism is often the production of more polar, less lipid-soluble, more readi-

ly excretable, and less biologically active compounds. This overall scheme is illustrated below:



Many chemicals that already possess functional groups may undergo phase II reactions directly.

Phase I Reactions

Many phase I reactions, in particular oxidations, are carried out in the endoplasmic reticulum (microsomes) of the cell. These reactions are catalyzed by a microsomal mixed-function oxidase system, which requires NADPH and molecular oxygen. The terminal oxidase of this system is cytochrome P-450 (so called because of the absorption peak at 450 nm when the reduced microsomes are bubbled with carbon monoxide). This enzyme system appears to be unique in having such a broad substrate specificity. Its activity may also be markedly enhanced by prior administration of other xenobiotics. While much of what is known about the xenobiotic metabolizing enzyme systems comes from studies on the liver, comparable work on the lung emphasizes the similarities rather than the diversities of this enzyme system in different tissues. However, with some compounds important qualitative and quantitative differences are found in the metabolites formed and the inducibility of the enzymes. These differences may, in part, be due to the presence of different isozymes of cytochrome P-450; in rabbit lung at least two have been described with markedly different substrate specificities and biological activities (*8*). Thus, the intracellular distribution of different isozymes of cytochrome P-450 may play a key role in determining the cellular specificity of some toxins to the lung. Various reductases and esterases have also been found in different subcellular fractions of lung (Table 1), often in the cytosol.

Phase II Reactions

Phase II synthetic or conjugation reactions generally involve the addition of small endogenous compounds to the xenobiotic or its metabolite(s). Such endogenous compounds include uridine diphosphate glucuronic acid (UDPGA), 3′-phosphoadenosine 5′-phosphosulphate (PAPS), *S*-adenosylmethionine, acetyl-CoA and glutathione in glucuronidation, sulfation, methylation, acetylation, and glutathione conjugation reactions, respectively (*7*). All these conjugation reactions have been demonstrated in pulmonary tissues (Table 2).

Since much of the *in vitro* data have been obtained using various subcellular fractions, it should be emphasized that caution must be exercised in extrapolating this data to possible *in vivo* significance, since the loss of structural integrity on homogenization or damage caused by slicing the lung may result in enzyme activities *in vitro* that are not observed either *in vivo* or in the isolated perfused lung. This is well illustrated in some examples.

Histamine is metabolized by slices or homogenates of human, cat, guinea pig, and rat lung, but not by isolated perfused lungs of the dog or rat (*27*). It has been

Table 1. Phase I reactions catalyzed by pulmonary tissues.

Reaction		Reference
Oxidation		
Side-chain oxidation, Pentobarbitone →	5-Ethyl-5-(3′-hydroxyl-1′-methylbutyl) barbituric acid	(*9*)
Aromatic hydroxylation, Benzo(a)pyrene →	3-Hydroxybenzo(a)pyrene	(*10*)
N-Dealkylation, Nortriptyline →	Desmethylnortriptyline	(*11*)
N-Hydroxylation, *N*-Methylaniline →	Phenylhydroxylamine +aniline	(*12*)
O-Dealkylation, Ethoxyresorufin →	Resorufin	(*13*)
Epoxidation, Aldrin →	Dieldrin	(*14*)
Reduction		
Nitroreductase, Nitrazepam →	7-Aminonitrazepam	(*15*)
Ketone reductase, Acetophenone →	1-Phenylethanol	(*16*)
N-Oxide reductase, Imipramine *N*-oxide →	Imipramine	(*17*)
Hydrolysis		
Acetylcholinesterase, Acetylcholine →	Choline + acetate	(*18*)

Table 2. Phase II reactions catalyzed by pulmonary tissues.

Reaction		Reference
Glucuronide conjugation, 4-Methylumbelliferone	4-Methylumbelliferone glucuronide	(*19*)
Sulfate conjugation, 3-Hydroxybenzo(a)pyrene	Benzo(a)pyren-3-yl-hydrogen sulfate	(*20*)
Methyltransferases		
N-Methyltransferase, 5-Hydroxytryptamine	*N*-Methyl 5-hydroxytryptamine	(*21*)
O-Methyltransferase, Phenol	Anisole	(*22*)
S-Methyltransferase, Mercaptoethanol	*S*-Methylmercaptoethanol	(*23*)
Catechol *O*-methyltransferase, Isoprenaline	3-*O*-Methylisoprenaline	(*24*)
Acetylation, Sulfanilamide	N^4-Acetylsulfanilamide	(*25*)
Glutathione conjugation, Benz(a)anthracene 5,6-oxide	*S*-(5,6-dihydro-6-hydroxybenz(a)anthracen-5-yl)-glutathione	(*26*)

suggested that in the perfused lung a permeability barrier prevents the histamine from reaching its inactivating enzyme. Similar results have also been obtained showing metabolism of angiotensin II in lung homogenates but not in isolated perfused lungs (*27*). While the previously mentioned examples apply to relatively polar endogenous compounds where one may expect permeability barriers, similar problems have also been observed with some lipophilic compounds.

In a comparative study of the metabolism of xenobiotics by isolated perfused rabbit lung and lung microsomes, no detectable metabolism of either chlorcyclizine and imipramine was observed using isolated perfused lung, whereas significant metabolism was observed using lung microsomes (*9*). In the same study other substrates such as methadone, pentobarbitone, and parathion were metabolized to qualitatively similar metabolites by isolated perfused lungs or microsomes. The absence of detectable metabolism of chlorcyclizine and imipramine in the isolated perfused lungs may be very significant.

As both of these compounds are concentrated in lung tissue (*6*), the results suggest that their sites of uptake and storage are different from the pulmonary metabolizing enzymes, and also that these compounds cannot apparently gain access to the enzymes. Some amines that are stored in the lungs are metabolized, such as nortriptyline (*11*). This raises the possibility that there may be discrete storage sites in the lung for these compounds.

From the previous discussion, the problems inherent in extrapolating *in vitro* data from subcellular fractions to *in vivo* significance are only too apparent. For this reason, *in vitro* data obtained using different preparations of lung are more reliable than data obtained using a single preparation such as a homogenate, particularly when one of these methods involves using an isolated perfused lung preparation. As seen in Tables 1 and 2, the lung has the inherent capability of metabolizing chemicals by a large number of diverse chemical pathways, and the extent to which this metabolic potential is realized *in vivo* is very complex.

Pulmonary Metabolism of Chemicals in Relation to Their Toxicity

Because of the pioneering work of the Millers and many other groups, it is now recognized that many chemicals require metabolic activation before exerting their toxicity (*1,28,29*). This activation may take place in the lung or in another tissue such as liver. The possible contribution of extrapulmonary metabolic activation of chemicals to metabolites that are toxic to the lung will depend, in part, on the relative stability of such metabolites and the inherent susceptibility to toxicity of the lung compared to other tissues. Alternatively a stable, proximate, toxic metabolite may be formed in an extrapulmonary site, transported to the lung, and then metabolized to the ultimate toxic form. Finally, the lung may be responsible for the formation of the toxic metabolite from the parent chemical (Fig. 1). These potential mechanisms have been reviewed elsewhere (*1,30,31*).

In considering the generation of reactive metabolites in the lung, it is essential to consider both the activating and deactivating enzymes. The balance of the activity of these enzymes and their respective cofactors and their cellular distribution ultimately determines how much of a reactive metabolite(s) may be present in the lung and, therefore, available for interaction with critical cellular targets leading to toxicity (*1,30,32*). Some of these enzymes and their cofactors are shown in Table 3. Obvi-

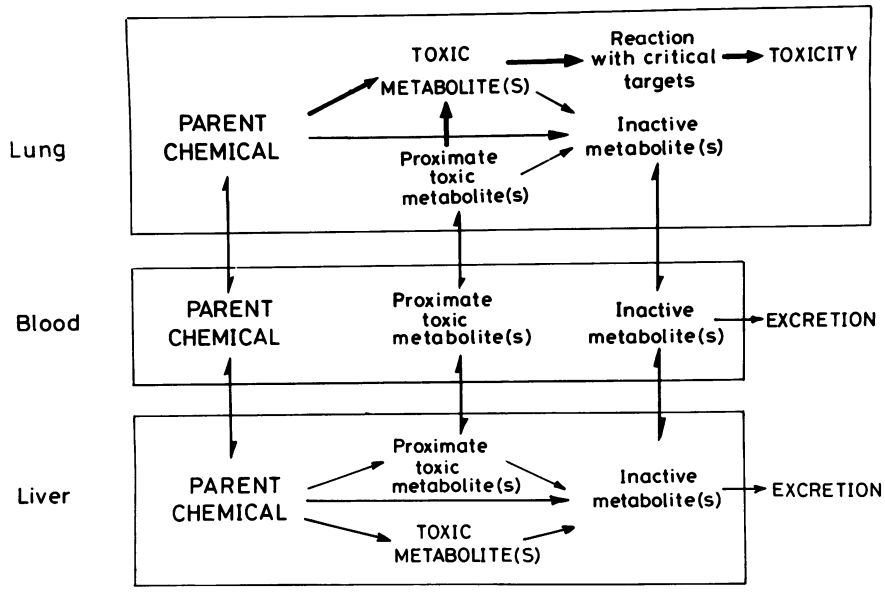


FIGURE 1. Toxicity mediated by reactive metabolites formed in the lung either from the parent chemical or from proximate toxic metabolite. If the toxic metabolite is chemically very reactive, it may have to be generated *in situ* in the lung in order to exert toxicity. Depending on the metabolic capability of the tissue, the metabolic activation by the parent chemical may be completely carried out in the lung. Alternatively, part of the metabolic activation may be carried out in other tissues, such as the liver, but the ultimate toxic metabolites are formed *in situ* in the lung.

Table 3. Balance of activating and deactivating enzymes and their cofactors.

Cytochrome P-450	NADH-cytochrome b_5 reductase
Prostaglandin synthetase	Xanthine oxidase
Flavin-containing monooxygenase	Glutathione
	Glutathione peroxidase
	Glutathione *S*-transferase
NADPH-cytochrome P-450 reductase	UDP-glucuronosyltransferase
Catalase	Vitamin E
Superoxide dismutase	Ascorbic acid
NAD(P)H	

ously, the relative importance of these will differ for different chemicals. It is also important to note that many of these enzyme activities may be altered by various environmental agents, thus potentially affecting the pulmonary toxicity of some chemicals.

Pulmonary Cell Types Responsible for Metabolism of Xenobiotics

The lung is a very complex organ composed of at least 40 different cell types (*33*). The balance of activating and deactivating enzymes and their cofactors in a particular cell type in the lung may, in part, determine the cellular specificity of the toxic chemical (*34*). Early histochemical studies demonstrated mixed-function oxidase activity (benzo(a)pyrene 3-monooxygenase) in the alveolar walls, but not in the tracheal or bronchial mucosa (*5*). By using a different histochemical technique, aniline 4-hydroxylase was demonstrated in both alveolar tissue and bronchial epithelium, and the enzymic activity appeared greatest in the latter (*35*). One group of cells easily obtained from lung are alveolar macrophages. Rabbit alveolar macrophages possessed high levels of acetyl transferase, but they lacked detectable activity of benzo(a)pyrene 3-monooxygenase, even in animals pretreated with 3-methylcholanthrene (*36*). However, important species differences are also observed in pulmonary metabolism, e.g., human alveolar macrophages have been shown to metabolize benzo(a)pyrene to reactive metabolites (*37*).

By using autoradiographic and immunohistochemical methods, it was shown that much of the total lung content of cytochrome P-450, the mixed-function oxidase responsible for the activation of many chemicals to reactive metabolites, was localized in the nonciliated bronchiolar epithelial (Clara) cells (*38,39*). This provided a metabolic basis for the susceptibility of this cell type to many pulmonary toxins including 4-ipomeanol, 3-methylfuran, and naphthalene (Table 4). The nature of the isozyme of cytochrome P-450 (*8*) present in a particular cell type may also be of importance. However, other factors must also be of importance in determining the marked susceptibility of the Clara cells to damage because some chemicals in Table 4, e.g., methylcyclopentadienyl manganese tricarbonyl (*40*), do not appear to require metabolic activation by Clara cells in order to exert their toxicity. The specific example of 4-ipomeanol is considered in more detail later.

Table 4. Chemicals causing damage to Clara cells.

Chemical	Occurrence
4-Ipomeanol	Moldy sweet potatoes
3-Methylfuran	Atmospheric pollutant
Naphthalene	Industrial chemical, moth balls
2-Methylnaphthalene	Food, cigarette smoke
1-Nitronaphthalene	Diesel exhaust
3-Methylindole	Ruminal fermentation product of tryptophan, cigarette smoke
1,1-Dichloroethylene	Copolymer in plastics industry, food and water
Bromobenzene	Industrial chemical
CCl_4	Organic solvent
O,O,S-Trimethyl phosphorothioate	Impurity in organophosphorous insecticides
O_3 and NO_2	Environmental pollutants
Organometallic carbonyls Methylcyclopentadienyl Manganese tricarbonyl	Gasoline

More recently, work from Devereux and Fouts (*41*) and a number of groups has concentrated on isolating different lung cell types—in particular, Clara cells and alveolar Type II cells—and determining their metabolic capabilities. Despite the limitations of such methods such as the loss of enzyme activities or cofactors during isolation, much valuable information may be obtained from isolated cells. These studies have demonstrated the presence of a number of drug-metabolizing enzymes including mixed-function oxidase, UDP-glucuronosyltransferase, epoxide hydrolase, and glutathione *S*-transferase in both isolated Type II cells and Clara cells. In general, higher levels of mixed-function oxidase activity are present in Clara cells, compared to levels in Type II cells (*41*). However, differences are observed dependent on the substrate chosen for study, implying a possible differential distribution of different isozymes of cytochrome P-450 in different cell types in agreement with immunohistochemical studies (*39*).

While most attention has been devoted to Type II and Clara cells, mixed-function oxidase activity is also found in other cell types in the respiratory tract including tracheal and bronchial epithelial cells, ciliated cells, and pulmonary alveolar macrophages from some species. The cellular heterogeneity of the lung presents enormous difficulties to the isolation of all but a few of the many cell types in sufficient yield and viability required for metabolic studies. Thus, the most recent advances on the distribution of the drug-metabolizing enzymes in the respiratory tract have been provided by immunohistochemical and immunofluoresence methods in conjunction with fluorescence histochemical methods (*42*). These results demonstrate that there are numerous sites within the respiratory tract where xenobiotics may be metabolized and that the enzymes are not distributed uniformly. In untreated rat lungs benzo(a)pyrene was hydroxylated by bronchial, Clara, and ciliated bronchiolar epithelial cells, as well as Type II pneumocytes and other cells in the alveolar wall. These studies also showed that the bronchus, bronchiole, and alveolar walls contained antigens related to NADPH-cytochrome P-450 reductase; cytochromes P-450, phenobarbitone (PB-B), and pregnanalone-16α-carbonitrile (PCN-E); epoxide hydrolase; and glutathione *S*-transferases B, C, and E; but only very low levels of cytochrome P-450, β-naphthoflavone (BNF-B) (*42*). On induction with Aroclor 1254, benzo(a)pyrene hydroxylase, but not cytochromes P-450, BNF-B, or 3-methylcholanthrene (MC-B), were induced throughout the bronchial and bronchiolar epithelia, suggesting that other immunochemically unrelated cytochrome P-450 isozymes were induced (*42*).

Very recently, an ultrastructural immunocytochemical characterization of cytochrome P-450 in rabbit lung has further increased our knowledge of its distribution in the respiratory tract (*43*). Antisera against the major pulmonary microsomal cytochrome P-450 isozymes 2 and 5 and NADPH-cytochrome P-450 reductase bound specifically to regions rich in agranular endoplasmic reticulum in the cytoplasm of Clara cells. The plasma membranes of bronchiolar Clara cells, the tips of microvillae of ciliated cells, secretory granules of goblet cells, and the cell membrane and pinocytotic vesicles of endothelial cells were intensely labeled with antisera from isozymes 2 and 5, but not to the reductase. Differences in the distribution of P-450 isozymes were also noted with the Golgi membranes of ciliated cells being labeled intensely by antisera to isozyme 5 only (*43*). The physiological and toxicological significance of these findings is not yet clear, but further studies of this nature should prove to be of great value.

Specific Examples

There are many examples of chemicals where pulmonary metabolic activation has been implicated in their pulmonary toxicity. Some of these are summarized in Table 5, but rather than give a detailed review of all these chemicals, I have chosen to illustrate the gen eral principles involved with two well-documented examples, i.e., benzo(a)pyrene and 4-ipomeanol.

Benzo(a)pyrene

Reviews of the metabolism of carcinogens, in particular, polycyclic aromatic hydrocarbons by respiratory tissues have been published (*44,45*). Polycyclic aromatic hydrocarbons such as benzo(a)pyrene are ubiquitous environmental carcinogens. Hundreds of tons of benzo(a)pyrene are emitted annually into the atmosphere. Much of these emissions arise from heat and power generation, the burning of rubbish, and car exhausts. As benzo(a)pyrene is also found in cigarette smoke, much effort has been devoted to elucidating the possible role of this environmental carcinogen in the etiology of lung cancer.

The pulmonary metabolism of benzo(a)pyrene is considered in detail because it illustrates important general principles such as *a*) the versatility of the pulmonary enzymes as reflected by the large number of metabolites obtained from the parent compound; *b*) the activities of

Table 5. Enzymes involved in the metabolic activation of various pulmonary toxins.

Compound	Source	Toxicity	Enzymes involved
Benzo(a)pyrene	Cigarette smoke	Lung cancer	P-448, epoxide hydrolase, PES
4-Ipomeanol	Moldy sweet potatoes	Clara cell necrosis	P-450
3-Methylfuran	Environmental pollutant	Clara cell necrosis	P-450
Parathion	Insecticide	Respiratory edema and failure	P-450, amine oxidase
α-Naphthylthiourea	Rodenticide	Edema	P-450, amine oxidase
Carbon tetrachloride	Organic solvent	Clara and Type II cell damage	P-450
Nitrofurantoin	Antibacterial	Fibrosis	P-450 reductase, xanthine oxidase
Paraquat	Herbicide	Type I and II cell damage	P-450 reductase
Naphthalene	Moth balls	Clara cell necrosis	P-450, glutathione *S*-transferase
Butylated hydroxytoluene	Food additive	Type I cell damage (mice)	P-450
3-Methylindole	Bacterial metabolite of trypotophan	Edema, Clara cell necrosis	P-450
p-Xylene	Solvent	Decrease in pulmonary mixed-function oxidases	P-450, deficiency of alcohol and aldehyde dehydrogenase
4-(*N*)Methyl-*N*-nitrosoamino)-1-(3-pyridyl)-1-butanone	Tobacco	Carcinogen	P-450

these enzymes may be dramatically altered by exposure to a wide variety of environmental chemicals; *c*) the possible importance of studying the metabolism of a compound in its target tissue; and *d*) the metabolism of benzo(a)pyrene is representative of other environmental polycyclic hydrocarbons such as dibenz(a,h)anthracene, dibenzo(a,i)pyrene, and benzo(c)phenanthrene.

It is well known, primarily due to the work of the Millers, that many chemical carcinogens have to be metabolically activated before exerting their carcinogenic actions (*28*). Although active metabolites may be formed in the liver and then transported to the lung, it is more likely that highly reactive metabolites are formed in susceptible tissues. Thus, much attention has recently been focused both on the pulmonary metabolism of benzo(a)pyrene and the many environmental factors that by affecting this metabolism may alter its carcinogenicity.

Polycyclic aromatic hydrocarbons are metabolized by the microsomal mixed-function oxidase system (E.C.1.14.14.2, aryl hydrocarbon hydroxylase [AHH], benzo(a)pyrene 3-monooxygenase) found in many tissues of different species including the lungs of man, rat, hamster, mouse, guinea pig, and rabbit. Many important environmental agents such as cigarette and marijuana smoke and ozone, as well as compounds such as 3-methylcholanthrene alter pulmonary benzo(a)pyrene 3-monooxygenase activity. Rat pulmonary benzo(a)-pyrene 3-monooxygenase activity was decreased by 27% after 1 hr of exposure to cigarette smoke, but increased after 4 hr of exposure (*46*). This increase was apparently due to enzyme induction, since it was abolished by prior administration of either actinomycin D or puromycin. The time course of induction was studied after exposure to cigarette smoke for 4 hr. Some induction of enzymic activity was observed immediately after exposure; this increased to a maximum of 28-fold at 24 hr and remained high until day 6 when it returned to almost control level. These studies suggested the presence of potent inducers of rat lung benzo(a)pyrene 3-monooxygenase activity in cigarette smoke that were retained in the lung after smoking. It has also been shown that benzo(a)pyrene 3-monooxygenase activity present in human pulmonary alveolar macrophages is significantly induced by an inhalation of cigarette smoke (*47*).

Other factors such as diet also affect pulmonary benzo-(a)pyrene 3-monooxygenase activity, which was reduced almost to zero when rats were fed a purified diet (*5,48*). In these studies potent inducers of pulmonary benzo(a)-pyrene 3-monooxygenase activity were found both in members of the Brassicae family (e.g., brussel sprouts, cabbage, and cauliflower) and in naturally occurring compounds such as flavone and tangeritin. Also, certain pollutants such as ozone, carbon monoxide, and cadmium, but not NO_2 decrease pulmonary benzo(a)pyrene 3-monooxygenase activity (*49*). Thus, numerous factors present in our environment and diet may modify the pulmonary metabolism of inhaled carcinogens such as benzo(a)pyrene and therefore alter their carcinogenicity. The complexity of the problem is increased by the large number of metabolites with differing biological activities that may be formed from benzo(a)pyrene by respiratory and other tissues.

Benzo(a)pyrene is metabolized to a variety of products including phenols, dihydrodiols, and glutathione conjugates. Studies by Sims and coworkers in the UK and Jerina and Conney and coworkers in the US recognized that the ultimate carcinogens from polycyclic aromatic hydrocarbons were the bay region diol-epoxides formed from dihydrodiols (*50,51*). The general scheme for the metabolic activation of polycyclic aromatic hydrocarbons is shown in Figure 2 and illustrated specifically with benzo(a)pyrene in Figure 3. All the metabolites in Figure 3 are formed by respiratory tract tissues (*44*). These figures demonstrate the large number of metabolites that may be formed within the respiratory tract. The polycyclic aromatic hydrocarbon is first metabolized to an epoxide by the pulmonary microsomal or nuclear mixed-function oxidase enzymes (Fig. 2). The epoxides then either: *a*) rearrange spontaneously to form the phenol; *b*) react with glutathione to form a glutathione conjugate either spontaneously or in a reaction catalyzed by glutathione *S*-transferase: *c*) are hydrated to form a dihydrodiol in a reaction catalyzed by microsomal or cytosolic epoxide hydrolase; or *d*) react with cellular macro-

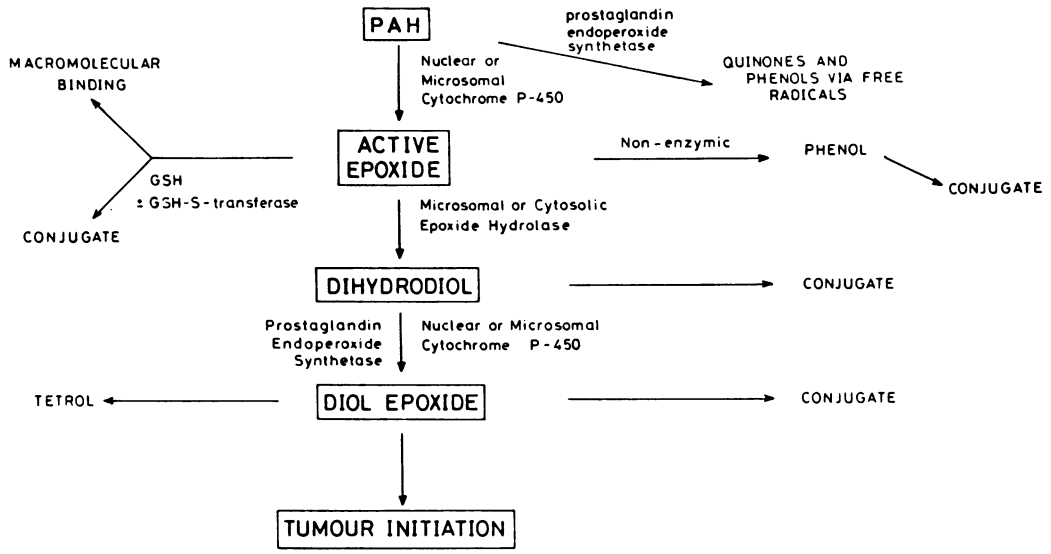


FIGURE 2. General scheme for the metabolic activation of polycyclic aromatic hydrocarbons illustrating the complexity of metabolism, the diversity of enzymes involved, and their different subcellular localizations.

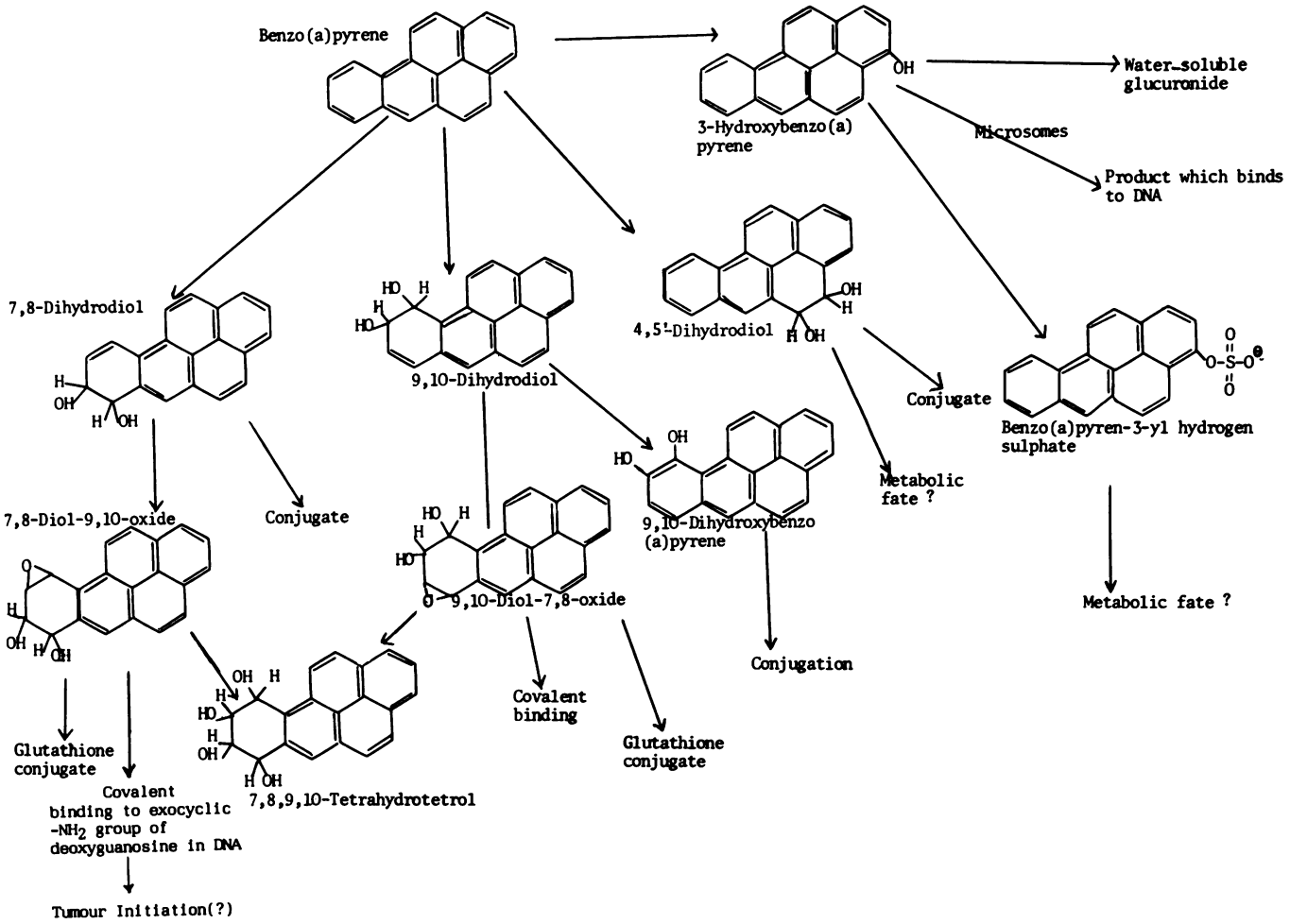


FIGURE 3. Metabolism of benzo(a)pyrene by tissues from the respiratory tract illustrating the wide array of metabolites of differing biological activities that may be formed by the drug-metabolizing enzymes in the respiratory tract.

molecules (Fig. 2) (*52*). Initially, the first three reactions were thought to be detoxication reactions while the last was considered to be the toxic reaction (*52*). However, more recent evidence shows that dihydrodiols may be further metabolized to diol-epoxides, e.g., 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (7,8-dihydrodiol) is metabolized to 7,8-dihydro-7,8-dihydroxybenzo(a)-pyrene-9,10-oxide (Fig. 3); it is this diol-epoxide that is bound to DNA when intact cells or tissues are exposed to benzo(a)pyrene (*50*). These diol-epoxides, but not the parent dihydrodiols, do not require metabolic activation in order to be potent mutagens.

With rat lung homogenates and microsomes, benzo(a)pyrene was metabolized to 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene (4,5-dihydrodiol), 7,8-dihydrodiol, and 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene-(9,10-dihydrodiol) (Fig. 3), as well as 3-hydroxybenzo(a)pyrene (*53*). Similar metabolites were obtained from benzo(a)pyrene using isolated perfused rat lung (*10*). Cigarette smoke exposure caused significant increases in 9,10-, 7,8-, and 4,5-dihydrodiols, in 3-hydroxybenzo(a)pyrene, and in the amount of covalently bound radioactivity in isolated perfused lungs and intratracheally administered [^{3}H]-benzo(a)pyrene (*54*).

Grover (*55*) demonstrated the formation of benzo(a)pyrene 4,5-oxide and the presence of epoxide hydrolase and glutathione *S*-epoxide transferase using rat lung microsomes from animals pretreated with the microsomal enzyme inducer 3-methylcholanthrene. Of interest was the finding that the epoxide hydrolase activity of lung was much lower than that of liver, while the transferase activity in the lung was higher. While such differences in enzymic activities may, in part, determine the susceptibility of tissues to tumor induction, many other factors such as DNA repair are also important.

The majority of lung cancers in man arise from a squamous metaplastic differentiation of the bronchial epithelium. In the animal model, which gives a similar histological picture to that obtained in man, many of the tumors arise in the trachea. Thus, several studies have also been carried out on the metabolism of benzo(a)pyrene and related polycyclics by short-term organ cultures of trachea (*56,57*). Of interest was the observation that the major metabolites obtained from either human bronchial epithelium or rat or hamster trachea were dihydrodiols (*10,57*). The formation of the proximate carcinogens in the area of the respiratory tract most susceptible to tumor formation may be of particular significance.

We have also shown striking differences between routes of conjugation of benzo(a)pyrene and 1-naphthol, a model phenolic substrate, by short-term explant cultures of peripheral lung from rats and humans (*58*). In rat tissue the major route of conjugation was glucuronidation, while sulfation was the predominant route with normal human tissue. Sulfation was a major route of conjugation during the metabolism of benzo(a)pyrene by cultured human bronchus; in the rodent and bovine species, sulfation and glucuronic acid conjugation of benzo(a)pyrene metabolites were approximately equal. In the human tissues, such as trachea, main stem bronchus, or lower bronchi, sulfation occurred to a much greater extent than glucuronidation. Significant amounts of glutathione conjugates were also observed in the different species (*55*). In this study, a higher ratio of organic solvent-extractable metabolites to water-soluble metabolites of benzo(a)pyrene was observed in species known to be susceptible to benzo(a)pyrene-induced carcinogenesis. This ratio suggested that possible deficiencies in the conjugating enzymes may be an important factor in determining species susceptibility.

Human bronchial mucosa was able to activate benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, 3-methylcholanthrene, and dibenz(a,h)anthracene into metabolites that covalently bound to DNA. In a study of 37 patients, a 75-fold interindividual variation was found in the binding of benzo(a)pyrene to cultured human bronchus (*59*). Following the culture of human bronchus with benzo(a)pyrene, the product bound to DNA was indistinguishable from that formed when the diol-epoxide 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide reacted with DNA. From these studies, it is clear that the area of the respiratory tract most susceptible to develop lung cancer (i.e., bronchial epithelium) is capable of metabolically activating benzo(a)pyrene to a metabolite that covalently binds to DNA. The rates of formation and removal of reactive metabolites in these cells may well be critical factors in determining individual susceptibilities to lung cancer.

Most of the data discussed above implicate the cytochrome P-450-dependent mixed-function oxidase system in the pulmonary metabolism of benzo(a)pyrene and other polycyclic aromatic hydrocarbons. Some recent evidence suggests a possible involvement of cooxygenation of polycyclic aromatic hydrocarbons during the oxidation of polyunsaturated fatty acids (*60*). Benzo(a)pyrene and its 7,8-dihydrodiol are cooxidized to quinones and to tetrols, respectively, following incubation with lung microsomes and arachidonic acid (*61*). Thus, a possible role for prostaglandin endoperoxide synthetase in the metabolic activation of carcinogens and other xenobiotics in the lung has been implicated but not proven.

Thus, in summary, the metabolism of benzo(a)pyrene by different portions of the respiratory tract has been described. Human bronchus, a prime target for the action of polycyclic aromatic hydrocarbons and for the development of lung cancer in man, metabolizes benzo(a)pyrene into various metabolites, in particular, the proximate carcinogen 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene, which may then be further metabolized to the reactive diol-epoxide 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide that can bind to DNA of the bronchus and possibly initiate tumor formation.

A large variety of environmental factors such as air pollution, diet, drugs, and cigarette smoking by modifying the pulmonary metabolism of benzo(a)pyrene may either increase or decrease its carcinogenicity. This alteration in pulmonary enzyme activities by environmental factors will result in interindividual differences in pulmonary metabolism and in responses to pulmonary toxins.

4-Ipomeanol

It is apparent from the work of Boyd (*1*) that 4-ipomeanol, (1-(3-furyl)-4 hydroxypentanone), a furan found on moldy sweet potatoes, produces a striking pulmonary toxicity in a number of species. The characteristic pulmonary toxicity observed is necrosis of the nonciliated bronchiolar epithelial or Clara cells (*1,38*). Following *in vivo* administration of [^{14}C]ipomeanol to rats, significantly more covalently bound radioactivity was observed in lungs than any other tissues. Autoradiography revealed that the covalently bound radioactivity was predominantly associated with Clara cells, and *in vitro* studies suggested that a cytochrome P-450-dependent monooxygenase was required to activate 4-ipomeanol to a reactive metabolite(s) that bound covalently to macromolecules. While much of the early data suggested that the reactive metabolite of 4-ipomeanol was the furan epoxide, studies with 2- and 3-methylfuran identified the unsaturated aldehydes acetylacrolein and methylbutenedial, respectively, as the principal reactive metabolites (*62*). Thus, in the absence of further data we cannot yet conclude the nature of the reactive metabolites of 4-ipomeanol.

Both rat lung and liver were capable of activating 4-ipomeanol to a binding species. However, when the results were expressed on the basis of covalent binding per molecule of cytochrome P-450, the lung appeared approximately 8-fold more active than the liver. Boyd (*38*) and Serabjit-Singh (*39*) have independently shown that at least one type of cytochrome P-450 is specifically localized in the Clara cells. These observations demonstrate the importance of the qualitative nature of the cytochrome P-450 and its tissue distribution in determining which cells in a specific organ may be predisposed to toxicity. The pulmonary toxicity of 4-ipomeanol is apparently mediated by reactive metabolites generated in specific cells in the lung — that is, the mechanism shown in Figure 1. Recent work with isolated rabbit lung cells showed that isolated Clara cells activated [^{14}C] ipomeanol to covalently bound species at a much higher rate than isolated alveolar Type II cells (*63*).

Further support for the key role of pulmonary metabolism of 4-ipomeanol in determining its pulmonary toxicity came from a series of studies with enzyme inducers and inhibitors. Three inhibitors (pyrazole, piperonyl butoxide, and cobaltous chloride) decreased both hepatic and pulmonary binding of 4-ipomeanol both *in vitro* and *in vivo*, correlating well with the decreased pulmonary toxicity *in vivo* (*1*). Pretreatment of rats with the inducing agents phenobarbitone and 3-methylcholanthrene caused a marked increase in the *in vitro* covalent binding of 4-ipomeanol to liver, but not to lung microsomes. These alterations were accompanied by decreased covalent binding to lung *in vivo* and decreased pulmonary toxicity. Treatment with 3-methylcholanthrene, however, caused an increase in *in vivo* covalent binding in the liver that correlated with centrilobular hepatic necrosis. Thus, differential alterations in the balance of the enzymes in the lung and liver caused by the 3-methylcholanthrene caused a shift in target organ toxicity. The observation that pretreatment with 3-methylcholanthrene increased both the toxicity and the covalent binding of 4-ipomeanol in the liver, but decreased these actions in the lung, supports the hypothesis that reactive metabolites of 4-ipomeanol will bind in those cells and tissues in which they are generated — that is, the mechanism in Figure 1.

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