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THE PULMONARY UPTAKE, ACCUMULATION, AND METABOLISM OF XENOBIOTICS[1]

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INTRODUCTION

Researchers now recognize that the lungs are a site for the uptake, accumulation, and/or metabolism of numerous endogenous and exogenous, or xenobiotic, chemicals and various aspects of these pulmonary processes have recently been reviewed (1–8). Much of the impetus for studying the uptake and metabolism of xenobiotics by lungs is due to their intimate contact with both blood and the external environment and their sensitivity to many chemicals that selectively cause pulmonary damage (9). The lungs have two important characteristics that facilitate the absorption of airborne or blood-borne chemicals. First, the venous drainage from virtually the entire body perfuses through the alveolar-capillary unit, which has an extensive capillary endothelium. Second, the alveolar epithelial and endothelial layers are very thin to facilitate gas exchange. Such an architectural arrangement, which places the epithelial layers in close contact with the blood, allows rapid absorption of unionized lipophilic xenobiotics and metabolism and/or transfer to the circulation.

One important function of lung is to regulate the systemic concentration of biologically active endogenous compounds by selective removal or metabolism. Certain polypeptides (6), prostaglandins (7), and vasoactive amines (8) in venous blood are handled in this manner. Pulmonary endothelial cells are important in the regulation of circulating hormones (10), and consequently it is not surprising that certain xenobiotics, which have physicochemical properties

similar to those of the endogenous substrates, also serve as substrates or ligands for the specialized enzymes, receptors, binding sites, and transport mechanisms localized on or in endothelial cells. Similar considerations are relevant for the uptake of chemicals into or transport through the epithelial cells lining the airways of lungs; certain xenobiotics are able to enter these cells by carrier-mediated mechanisms, others enter by nonionic diffusion.

In this review, we describe the classes of xenobiotics known to be taken up by lung and delineate the relative roles, if known, of the endothelium and epithelium. We also discuss the importance of chemical-metabolizing enzymes and their cellular localization in relationship to the clearance of chemicals by the lung and target-cell toxicity. We have taken this particular approach for several reasons. First, we feel it complements several excellent existing reviews in this research area. Second, it emphasizes the structural, functional, and cellular heterogeneity of lungs and the differential contribution of various cell types to the pulmonary uptake, accumulation, and metabolism of chemicals. Finally, it provides a convenient framework for the critical evaluation of recent research in this field, while allowing identification of subject areas that merit additional attention.

HISTORICAL AND GENERAL CONSIDERATIONS

Certain biogenic amines, including 5-hydroxytryptamine (5-HT, serotonin), *l*-norepinephrine (NE), and β-phenylethylamine (PEA), are removed from pulmonary circulation whereas others, such as histamine, dopamine, and epinephrine, generally are not (11). In perfused lungs, the removal of 5-HT and NE occurs via carrier-mediated transport processes that are saturable and sodium-, energy-, and temperature-dependent. Rapid intracellular metabolism of 5-HT and NE by monoamine oxidases and/or catechol-0-methyltransferases follow, and the metabolites are released into the circulation (12–14). Histochemical localization studies have shown that both 5-HT and NE are transported into pulmonary endothelial cells (15). 5-HT and NE are also known to be removed by saturable processes in vivo, and the endothelial sites for removal of these two amines are distinct (16). On the other hand, PEA enters the lung by passive diffusion and is inactivated by monoamine oxidase activity in lung (17, 18). Although the uptake and metabolism of endogenous compounds by lung remains an active research area, similar studies have been initiated with xenobiotics because many chemicals are concentrated preferentially in lung (19) or cause selective toxicity to the lung (4, 9) after in vivo administration.

Types of Xenobiotics Cleared by the Lungs

BASIC AMINES Extensive reviews of the types of drugs concentrated in lung are available (3, 19). Although many pharmacological classes are represented

(e.g. antihistamines, antimalarials, morphine-like analgesics, anorectics, tricyclic antidepressants, anesthetics), many of the compounds concentrated in the lung are basic amines. Detailed studies of the mechanisms of accumulation of several xenobiotic amines by perfused lungs performed by Eling, Anderson, and collaborators at NIEHS (10–23) have been previously reviewed (3, 24). Similar experiments were also performed by others (e.g. 25). Briefly, the conclusions drawn from these investigations are:

1. To be efficiently removed from the circulation, amines must have a pKa greater than eight and substantial lipophilic character. (Many of the amines removed are amphiphilic in nature, containing both a large hydrophobic region and a charged group, normally cationic, at physiological pH. Examples of such amines include chlorcyclizine (CHLORCY), chlorphentermine (CHLORPH), chlorpromazine (CHLORPR), cyclizine, desipramine, diphenhydramine, fluphenazine, imipramine (IMP), iprindole, methadone (METH), promazine, propranolol, and tripelennamine.
2. The mechanism for the steady-state uptake of basic amines into lung consists of two components, one saturable and one non-saturable. For most basic amines the saturable component is attributed to intracellular binding by facilitated diffusion and not to carrier-mediated transport or metabolism. However, amphetamine appears to be removed by a carrier-mediated transport process (24), and it is conceivable, because of structural similarities, that this compound is a substrate for the NE transport system of pulmonary endothelium. The magnitude of the non-saturable component of uptake is too large to be a reflection of diffusion into the extracellular space.
3. Results of efflux studies of IMP or METH from perfused lungs into drug-free perfusion medium are consistent with at least three different pools of accumulated amine (22, 23). The linear component of lung uptake corresponds to the two components of efflux that have the shortest half-lives, whereas the saturable component of uptake corresponds to the efflux component of longest half-life (18 seconds, 58 seconds, and approximately 8 minutes for IMP efflux respectively). The linear component of uptake is believed to represent the partitioning of amine into membranes in contact with perfusion medium; the saturable component is attributed to intracellular binding to at least two distinct sites, although the cell type or types where this occurs is not precisely known. There is also one pool of IMP or METH that does not efflux during the normal perfusion period (60 minutes). This latter pool, which accounts for approximately 30% of the IMP taken up by lung, is termed the slowly effluxing pool (SEP), and this pool appears to account for the persistence of amphiphilic amines in lung.

A more recent study with IMP in vivo demonstrated that the half-life for the disappearance of the SEP is approximately four hours (26). Subsequent auto-

radiographic evidence of Wilson et al (27) suggests that intracellular (non-covalent) binding in the alveolar macrophage is the major component of the SEP. This observation has led to the assumption that macrophage removal via the mucociliary clearance mechanism accounts for drug removal from the SEP.

The persistence of certain amines in lung appears important in drug-induced pulmonary phospholipidosis. This condition, characterized by an increased presence of phospholipids in bronchiolar epithelium, type I and type II alveolar epithelial cells, alveolar macrophages, vascular endothelial cells, and pulmonary smooth muscle cells, results after chronic treatment with one of more than twenty drugs, although not all animal species are susceptible (28). It is probable that amphiphilic cationic compounds interact physicochemically with phospholipid (29), leading to decreased catabolism of phospholipid and its accumulation in lung cells (30). Wilson et al (26) have demonstrated that compounds known to induce pulmonary phospholipidosis, such as IMP and CHLORPH, form much larger SEP in vivo than basic amines such as amphetamine, phentermine, and 5-HT, which do not cause this condition.

It follows that drug-endogenous substrate and drug-drug interactions of clinical importance are possible in lung, and this area of research has attracted considerable attention. Most of this work has been performed with perfused lung preparations (3, 24, 31, 32), but recently Gillis and his colleagues (33, 34) adapted the single injection, double or triple indicator-dilution technique (35) for in vivo studies and determined the pulmonary clearance of 5-HT in man (33). They also investigated the effects of IMP or cocaine treatment on the pulmonary clearance of 5-HT and NE in the rabbit (34). With cardiogreen as the reference compound, extraction of 5-HT is decreased significantly when measured 15 minutes, 45 minutes, 75 minutes, and 165 minutes after the administration of IMP (0.5 mg/kg, iv) relative to the 0-hour control value; NE extraction is also decreased by this dose of IMP between 15 minutes (the first time sampled) and 135 minutes after treatment. However, treatment with cocaine (0.5 mg/kg) did not affect NE clearance in vivo, although earlier studies showed that cocaine inhibits the clearance of both 5-HT and NE in perfused rabbit lungs (36, 37). Collectively, these data emphasize that extrapolation of in vitro data to the in vivo situation, particularly where complex interactions are involved, requires caution. For this reason it is encouraging that other authors are now using in vivo approaches (38–40) for investigating drug-endogenous substrate interactions in lung.

Another drug-drug interaction studied recently is the effect of CHLORPH-induced pulmonary phospholipidosis on the clearance of biogenic and non-biogenic amines by perfused rat lungs. Mehendale and his colleagues have shown that, although phospholipidosis decreases the uptake and metabolism of 5-HT (42), it enhances the uptake and accumulation of CHLORPR, IMP, and CHLORPH itself (43, 44). Interactions of these drugs with pulmonary phos-

pholipid, which is increased 60% by CHLORPH, may limit their access to the metabolic sites and thus retard both metabolism (via N-oxidation) and efflux while concomitantly increasing accumulation of amphiphilic amines in perfused lungs. The facts that drug-induced phospholipidosis in rats is accompanied by marked changes in mitochondrial structure and cellular bioenergetics (45) and that the pulmonary clearance mediated by alveolar macrophages is almost totally suppressed in vivo (46) suggest that similar interaction studies should also be conducted in intact animals.

XENOBIOTICS METABOLIZED BY LUNGS Although lipophilic basic amines are removed from pulmonary circulation, this frequently occurs in the absence of metabolism. There are also many chemicals that are cleared from the circulation by lungs due to metabolism mediated by enzymes present in this tissue. The lung contains, although generally at lower concentrations, virtually all of the hepatic pathways required for the biotransformation of exogenous chemicals. Not all hepatic isozymes are present in lung, however (47). Xenobiotic metabolism by lungs is the subject of several extensive recent reviews (4, 5, 24, 47, 48) and only a few relevant points are repeated here. First, the cytochrome P-450-dependent monooxygenase system is distributed heterogeneously among various pulmonary cell types. Immunochemical localization studies (49), enzymatic studies with freshly isolated lung cells (50, 51), and autoradiographic studies of 4-ipomeanol-derived radioactivity (52) have shown that the nonciliated bronchiolar epithelial (Clara) cell is a major site of cytochrome P-450-dependent activity. However, the alveolar type II cell also contains considerable amounts of monooxygenase activity (50, 51), whereas the alveolar macrophage, at least in the rabbit, is virtually devoid of such activity (53). Recent immunochemical studies in our laboratory have also shown that rabbit aorta and/or its underlying smooth muscle layer contain small amounts of cytochrome P-450 isozymes and their associated enzymatic activity (54). Obviously, these data represent information on only a few of the more than forty cell types found in lung, but they are at least a start in correlating xenobiotic metabolizing activity with pulmonary cell type, a difficult task with such a complex organ.

It must be emphasized that the degree of cellular integrity of the experimental system used for testing can qualitatively and quantitatively affect the results obtained. For example, studies in our laboratory showed (55) that CHLORCY and IMP are not degraded over 60 minutes in a recirculating rabbit lung preparation, whereas these drugs are readily metabolized by rabbit lung homogenate or microsomes. Similarly, Minchin et al (56) found that the kinetically estimated binding capacity for CHLORPH is 0.109 μg/g in perfused rat lungs but 8.3 μg/g in rat lung homogenate (57). It is likely that these experimental differences between the perfused organ and whole homogenate

are due to limited substrate access to the sites of metabolism and binding in intact tissue. In any case, we have found that an integrated experimental approach, which incorporates both broken *and* intact cellular preparations, is best suited for the study of xenobiotic metabolism by lung (5). It should be remembered in this context that all chemicals metabolized by lung in vitro have the potential to be cleared from the circulation by this tissue in vivo.

Relationships between xenobiotic metabolism and target cell toxicity in lung, and the use of kinetic parameters of drug-metabolizing enzymes determined in vitro to predict pulmonary clearance of chemicals will be discussed in subsequent sections of this review.

PARAQUAT Paraquat (PQ) is a widely used quaternary ammonium bipyridyl herbicide (1,1'-dimethyl-4,4-bipyridylium dichloride). The most characteristic feature of PQ toxicity is its pulmonary involvement, and lung damage is observed in rats following systemic (58) or intrabronchial (59) doses. The iv administration of PQ to rats results in the accumulation of unchanged compound in the lung (60), and a recent autoradiographic study showed that PQ is concentrated in discrete areas of mouse lung, presumably in alveolar type II cells, at 3, 9, or 24 hours after iv injection (61). This is interesting because the first phase of PQ-mediated toxicity in the lung is extenstive damage to alveolar epithelial cells (62). Other studies showed that PQ is taken up by lung slices by an energy-dependent mechanism (63, 64), and it is now generally accepted that the selective toxicity of this herbicide to lungs is related to the compound accumulated. The characteristics of this transport process, which is believed to be localized in alveolar type I and type II cells (65), is discussed later.

PULMONARY VASCULATURE: ROLE IN XENOBIOTIC UPTAKE, METABOLISM, AND TOXICITY

General Characteristics of the Pulmonary Vasculature

In considering the contribution of this tissue to xenobiotic metabolism and toxicity, one must appreciate the heterogeneity of the vasculature of various organs of an individual as well as differences among species, because relevant data are often derived from tissue homogenates or slices, homogeneous cultures, or isolated perfused organs. Structurally, blood vessels differ in the configuration and proportion of endothelium and smooth muscle between veins and arteries as well as among species (66). Biochemically, differences between veins and arteries and among capillary beds of various organs have been shown: the hexose monophosphate pathway functions in arteries but not in veins (67); receptor and carrier-mediated transport mechanisms of endothelium for endogenous substrates (sugars, amino acids, lipids) are organ specific (68). Given this diversity, the potential for overinterpreting simple qualitative parameters

exists. Thus, we shall discuss the uses of various parameters, the suggested mechanisms of toxicity or detoxication, and the limitations of certain approaches in studies of the pulmonary vasculature. In addition, our recent biochemical and immunochemical analyses of rabbit aorta (54) are included because of their potential relevance to the clearance of xenobiotics by the pulmonary vasculature. There are several informative reviews on studies of the uptake and metabolism of biogenic amines, the production of vasoactive peptides, prostaglandins, and hyperoxic or hypoxic response relevant to this tissue (8, 11, 69–73).

Briefly, some previously reviewed characteristics of the pulmonary vasculature are:

1. The lung regulates the concentration of circulating vasoactive peptides. The caveolae of the plasma membrane of endothelial cells (EC's) contain a carboxypeptidase, angiotensin-converting enzyme (ACE) that converts the decapeptide angiotensin I to the potent vasopressor angiotensin II. Although ACE is widely distributed, other tissues significantly degrade angiotensin II, whereas the undamaged lung does not. Bradykinin, a vasoactive peptide, is rapidly degraded during passage through the lungs due to the carboxypeptidase activity (ACE or other peptidases) of the vasculature.
2. The control of vasoactive amines is also a major pulmonary function. The uptake of amines such as 5-HT by the endothelium is a rapid and energy-dependent process, especially in the small vessels, and monoamine oxidases or catechol-0-methyltransferases mediate the deactivation. Thus, monoamine oxidase inhibitors such as tricyclic antidepressants diminish the pulmonary clearance of these vasoactive amines with effects on the cardiovasculature; poor clearance of 5-HT has been associated with carcinoid heart disease, and cardiovascular complications arise from overdoses of antidepressants. The clearance of 5-HT by the lung relative to other tissues may be a reflection of the relative endothelial surface areas rather than a tissue-selective uptake mechanism (74). The uptake sites for all vasoactive amines are not identical, as indicated by the lack of competition between 5-HT and NE and by the differential effects of inhibitors. The specificity of the 5-HT uptake mechanism is further indicated by the lack of competition with polyamines (e.g. spermidine, spermine), which are competitors of the pulmonary uptake of PQ (65).
3. Another aspect of the regulation of vasoactive substances by the lung is the endothelial production of prostacyclin or PGI_2, a potent inhibitor of platelet aggregation and a vasodilator. Although the lung effectively degrades other prostaglandins that stimulate vasoconstriction or platelet aggregation, the role of the vasculature in this process is not clearly established. Prostaglandin synthesis has been indirectly localized in situ to the perivascular cells,

and autoradiographic studies with cultured EC's indicate compartmentalization of prostaglandin synthetase on endoplasmic reticulum. Formation of atherosclerotic plaques is associated with diminished production of PGI_2 (75). The capacity of vascular smooth muscle to produce PGI_2 may correspond to a lower susceptibility to atherosclerosis; thus, the resistance of the rat and the susceptibility of the pig to atherosclerosis. Diminished production of PGI_2 may also facilitate metastasis of tumors that form platelet emboli. Another factor that contributes to the unhindered passage of blood through the lungs appears to be the lack of receptors that lead to immune complex deposition, i.e. bovine EC's in culture lack receptors for the fragment crystallizable (Fc) region of immunoglobulin G and for the 3b component of complement (76, 77). However, injury unmasks these receptors and may lead to deposition of immune complexes, as in the case of certain pulmonary inflammatory diseases.

4. Exposure to inhaled oxidants, oxygen more than ozone or nitrogen dioxide, damages the endothelium with subsequent edema and possibly death. The active species is presumed to be the superoxide anion, and the induction of superoxide dismutase (SOD) by exposure to sublethal doses is associated with the development of tolerance to acute exposures. Production of superoxide radicals and induction of SOD are also associated with exposure to PQ, which during the initial stages of toxicity selectively damages the epithelium. Synergistic acute toxicity of hyperoxia and PQ has been reported (78).

Effects of Xenobiotics on the Pulmonary Vasculature

The biochemical response of the pulmonary vasculature to environmental or therapeutic exposure has been an area of limited study, in spite of the obvious importance of the special functions and size of this vascular bed. Current methods of study include the culturing of EC's from lung or other tissues (79), isolated perfused lungs (31), morphological and immunohistochemical techniques (10), as well as in vivo determinations (74, 80–82). The consequence of exposure to pulmonary toxicants or drugs such as bleomycin, monocrotaline, PQ, oxygen, ozone, IMP, and diesel emissions (9, 24, 80a, 83–90) (Table 1) on the vasculature has been investigated.

Clearly, many physiologically significant functions beside oxygen/carbon dioxide exchange depend on the integrity of the vasculature. Thus, an index of early endothelial damage or disruption may be useful for monitoring the effects of drug therapy or exposure to pollutants. The uptake of 5-HT and NE or ACE activity measured in vivo for this purpose has been proposed (80–82). A radioassay of ACE activity for the diagnosis of sarcoidosis and monitoring the subsequent corticosteroid therapy is a suggested application (82); however, the range of activity in 62 normal subjects varied fourfold, and many clinically

Table 1 Pulmonary vascular toxins

Compound	Source or use	Risk to humans	Mechanism of toxicity	Some parameters of injury				References
				5-HT[a]	NE[a]	ACE[a]	morphology[b]	
Monocrotaline	Plant *(Crotalaria spectabilis);* honey and milk	+	Formation of pyrrole (in liver)	↓	→↓		+	89–92
3-Methylindole	Ruminal fermentation of tryptophan	■	Microsomal mono-pyrrolooxygenase	↓			+	93, 94
α-Naphthylthio-urea (other aryl-thioureas)	Rat poison	+	Microsomal flavin monooxygenase; cytochrome P-450	↓			+	95–97
Bleomycin	Anti-neoplastic drug	+	Free radical formation/ lipid peroxidation	↓	→↓	→↓	+	80a, 98, 99
Oxygen (hyperoxia)	Therapy for respiratory deficiency	+	Free radical formation/ lipid peroxidation	↓ ↑	↓	→↓	+	85, 100–102
Hypoxia	High altitude	+	Vascular hyperplasia				+	103, 104
Ozone[c]	Air pollutant	+	Free radical formation/ lipid peroxidation	↓			+	85
Nitrogen dioxide[c]	Air pollutant	+	Free radical formation/ lipid peroxidation	↓			+	85
Paraquat[c]	Herbicide	+	Free radical formation/ lipid peroxidation	↓			+	84

[a] 5-Hydroxytryptamine, 1-norepinephrine, uptake or accumulation, and angiotensin-converting enzyme activity: (→■ no change, (↑) increase, (↓) decrease
[b] + denotes vascular damage/edema
[c] Effect on endothelium secondary to damage of epithelium

diagnosed patients had ACE activities within that range. Thus, a low-serum ACE does not preclude the presence of the disease.

Many types of chemical insult to the endothelium are reflected in diminished uptake of 5-HT, NE, or decreased ACE activity (Table 1) and occur prior to any apparent morphological damage. An example of this is bleomycin, an antineoplastic agent that causes pulmonary fibrosis. A subacute dose in rabbits (i.e. with no morphological evidence or elevated hydroxyproline content indicative of fibrosis) resulted in a sustained decrease in 5-HT uptake and serum ACE activity (80a, 83). The consequence of subnormal enzymatic activity or the critical threshold values of these parameters is not known.

In addition to bleomycin, monocrotaline (a pyrrolizidine alkaloid) and hypoxia induce pulmonary hypertension and have been used for investigating the vascular changes associated with the clinically significant condition (89, 103, 104). Thickening of smooth muscle and EC's concomitant with increased vascular resistance was observed. Further, this research may be directly relevant because of the opportunity for human exposure to pyrrolizidine alkaloids (herbal teas, honey, or milk from bees or cattle that consume the plants that produce these compounds) or hypoxia (high altitude).

Oxidant-Induced Alterations of the Pulmonary Vasculature

The potential for human exposure to oxygen, ozone, or nitrogen dioxide is also recognized, and the resulting endothelial damage and induction of detoxication pathways undoubtedly alter the pulmonary disposition of xenobiotics. Subacute exposure to oxygen, for instance, potentiates the effects of PQ yet provides protection against previously lethal concentrations of oxygen. Increased levels of SOD, glucose-6-phosphate dehydrogenase, glutathione peroxidase, glutathione reductase, or non-protein sulfhydryl groups are associated with exposure to these gases (31, 105). However, the mechanism for development of tolerance and/or toxicity is not yet clear. The lack of complete cross-tolerance, as well as the poor correlation between the time courses of elevated enzyme concentrations and maintenance of tolerance, indicate that the increase in overall levels of deactivating components is not the explanation for tolerance. At least in the case of oxygen toxicity, tolerance does not appear to involve an enhanced rate of induction of these components during exposure (105). Tolerance to acute levels of oxygen appears to provide more protection against acute levels of nitrogen dioxide (106, 107) and other oxidants than vice versa (108). Perhaps the adaptive responses to oxygen occur in more cell types than in the case of ozone or nitrogen dioxide. As changes in content of enzymes or antioxidants have generally been monitored in whole tissue and not in the revelant cell types, the biochemical mechanisms of toxicity and tolerance are not discernable.

The effect of exposure to various levels of oxygen on cultured EC's has been

determined, but not with regard to understanding tolerance or mechanisms for xenobiotic uptake and metabolism. Under hypoxic conditions, glycolysis may be favored over oxidative phosphorylation and vice versa under normoxia; the effects of in vitro exposure appear to mimic differences observed in EC's freshly isolated from regions exposed in vivo to different oxygen tensions (109). However, many cautions against artifactual changes have been published. The conditions of isolation (explants or enzymatic digests) and culture (culture medium and cell density) of EC's or smooth muscle cells greatly affect prostaglandin synthesis (110). Although it is tempting to extrapolate from cellular adaptations in vitro to responsiveness to in vivo physiological or pathological changes, restraint is advisable. The dynamic nature of the vasculature presents challenges and obstacles to research; even the method of killing can influence the uptake of 5-HT (111), and the uptake efficiency of the isolated perfused lung is dependent on perfusion flow rates that unfortunately have often been less than the physiological flow rate (112). Correlation of endothelial damage with decreased uptake of 5-HT in the isolated perfused lung (101) excludes the role of platelets in vivo. Platelets contain most of the circulating 5-HT that may be released from platelet thrombi formed in microvascular injury and so increase the pulmonary 5-HT content; thus, the murine pulmonary 5-HT content increases with increasing time of exposure to 100% oxygen (100). It has been suggested that increased 5-HT content may potentiate vascular injury by enhancing interactions between the endothelium and polymorphonuclear leukocytes, which can release harmful oxidants. And, of course, the differences among species are also an important consideration, e.g. the difference between the dog and rabbit lung in their hemodynamic response to acute hypoxia (113). However, species difference in susceptibility to atherosclerosis may be useful for identifying relevant factors in the development of plaques. As previously mentioned, the susceptibility of the pig versus the rat may perhaps be explained by the relative low capacity of porcine vascular smooth muscle cells in culture to produce PGI_2, which in vivo serves to inhibit platelet aggregation at sites of endothelial damage (101).

Vasculature-Associated Xenobiotic Metabolism

The monoclonal proliferation of smooth muscle in the development of atherosclerosis is proposed to be the result of a mutational event. While the ability of vascular tissue to activate potential mutagens/carcinogens has not been studied with pulmonary tissue, the information derived from the aorta is likely relevant. The mitochondrial supernatant fraction of aortal tissue can metabolize benzo(a)pyrene (BP) or 7,12-dimethylbenz(a)anthracene to give a positive reaction in the Ames test or to form metabolite-DNA adducts (114). The increase in these activities following exposure to hydrocarbons such as 3-methylcholanthrene (3-MC) further indicates that a cytochrome P-450

monooxygenase system mediates these reactions. Cytohistochemical studies of pulmonary vasculature suggest that the endothelium may be the cellular site for this enzyme system (115). However, it is possible to recover the majority of the cytochrome P-450, NADPH cytochrome P-450 reductase, and enzymatic activity in microsomes from rabbit aorta after the removal of the endothelium (C. J. Serabjit-Singh, J. R. Bend, R. M. Philpot, unpublished observations). Therefore, whether or not the endothelium contains components of the monooxygenase system, vascular smooth muscle has the necessary enzymes for activation of promutagens that may enhance the development of atherosclerosis. The isozymes of cytochrome P-450 forms 2-, 5-, 6-, and P-450 reductase were detected immunochemically (Western blotting) and enzymatically (activity toward 7-ethoxyresorufin, BP, or cytochrome *c*) in the microsomal fractions of both intact rabbit aorta (54) and aorta after removing the endothelium (C. J. Serabjit-Singh, J. R. Bend, R. M. Philpot, unpublished observations). The sensitivity of the immunochemical techniques now available provides the means for examining the role of detoxifying or activating enzyme systems in the development of toxicity or disease in the vasculature of various organs. Thus, the contribution of the pulmonary vasculature to the intrinsic metabolic clearance of drugs and pollutants by lung may soon be delineated.

Lymphatic Vasculature

The lymphatic system of the lung plays a major role in alveolar clearance of fluid and particulate matter that may accumulate in the lung (116). Very little data pertaining to mechanisms of cellular damage or biochemical profile of the lymphatic vasculature are available. In α-naphthylthiourea-induced edema, pulmonary lymph flow was shown to increase with increases in extravascular water (117). However, it is difficult to correlate chemically induced edema with lymphatic dysfunction, which is perhaps an indirect effect the study of which would provide little insight into toxic mechanisms.

PULMONARY EPITHELIUM: ROLE IN XENOBIOTIC UPTAKE, METABOLISM, AND TOXICITY

The epithelial lining of the airways and its protective layer of mucus or pulmonary surfactant is in intimate contact with the environment and represents a tremendous surface area for xenobiotic absorption. The organization of the airways is complex, consisting of the trachea, bronchi, and bronchioles, the so-called conducting airways, in addition to the alveolar-capillary unit, the major area for gas exchange. In mammals, the surface area of the alveoli is approximately tenfold greater than that of the conducting airways. There is a heterogeneous distribution of cell types in the pulmonary epithelium (118,

119). For example, the tracheobronchial lining consists of up to eleven epithelial and two mesenchymal cell types, depending upon species; these include the ciliated cell, the goblet cell, the epithelial serous cell, the brush cell, the intermediate cell, the oncocyte, the Kultschitzky-like or K cell, and the nonciliated bronchiolar epithelial (Clara) cell. The epithelial lining layer of the alveoli consists mostly of squamous epithelial, or type I cells, and granular cuboidal cells, called type II cells. There are also a very few brush cells, termed type III cells, present and free alveolar macrophages occur in the alveolar spaces. Because lungs function as organs of gas exchange, their epithelial cells are generally in intimate contact with the vasculature. This is especially true in the alveolar-capillary area. Consequently, it is not surprising that xenobiotics transit the respiratory epithelium, whether exposure is via the air-space or the vasculature. Absorption of drugs such as sulfanilic acid and *p*-aminohippuric acid through the pulmonary epithelium of the rat is much faster (at least eight to forty-two times faster) than from the rat small intestine (120). Absorption is also more rapid from the alveolar than from the tracheobronchial area (121). Since alveolar type II cells, and especially Clara cells, contain endoplasmic reticulum and its associated xenobiotic-metabolizing enzymes (50), transport through these cell types is likely to be accompanied by partial biotransformation. Volatile xenobiotics and/or their metabolites are also exhaled from the lung across the epithelial lining layer. As a result of recent experiments, quite a bit is known about the transport mechanisms for xenobiotic chemicals in respiratory epithelium.

Diffusion of Xenobiotics Across the Pulmonary Epithelium

Much of the quantitative information concerning xenobiotic absorption from pulmonary airways has come from Schanker's laboratory (122). Due to the complex structure of the lung, it is difficult to evaluate directly the individual permeability of the alveolar and tracheobronchial epithelia in vivo. Routinely, a test compound is added to the airspace in solution or via an aerosol, and passage of the material into blood, lymph, or vascular perfusion fluid follows. Alternatively, unabsorbed chemical remaining in the lungs is assayed, an approach used by Schanker and his colleagues (120). In this case, absorption is equated with passage of the compound across both the epithelial lining layer of the airways and the pulmonary endothelium to enter the circulation. Small volumes (100 μl) of xenobiotic in solution are injected 1 or 2 mm above the bifurcation of the trachea of rats or guinea pigs. A tight-fitting tracheal cannula is employed to prevent clearance of the compound via the mucociliary mechanism. The major advantages of this procedure are that known amounts of chemical are introduced directly into lungs and that there is no interruption of the bronchial circulation or lymph flow, such as occurs in perfused lung preparations. Major disadvantages include the nonuniform distribution of

solute throughout the lungs, the fact that the site of solute transport (tracheobronchial versus alveolar epithelium) is unknown, and the inability to detect metabolites formed during passage of a compound through the air-blood barrier.

In spite of these limitations, this methodology has provided valuable information about the permeability of the pulmonary epithelium to xenobiotics (Table 2). For the non-lipid soluble compounds listed, the rate of absorption varies in inverse order with the size of the molecule, and uptake occurs by a non-saturable diffusion process (120, 122). These hydrophilic chemicals pass through paracellular aqueous paths, or pores, in the airway epithelium, and Schanker has suggested that there are at least three populations of different-sized pores in rat lung. It is generally accepted that pores of the alveolar epithelium have a radius of 0.5 to 1.5 nm (127, 128). However, recent studies with canine trachea and bronchi (128, 129) indicate the presence of larger pores (>4 nm) in epithelium of the conducting airways. Thus, there may be a differential distribution of the various-sized pores attributed to rat pulmonary epithelium. In any event, many hydrophilic chemicals are able to enter the circulation from the airways. Of some interest is the observation that pulmonary epithelium of the guinea pig is more permeable to non-lipid soluble compounds than that of the rat. When hydrophilic or lipophilic compounds are administered as a liquid aerosol, absorption is approximately twice as rapid. This phenomenon is presumably due to the delivery of more of the chemical to the alveolar spaces subsequent to administration by aerosol. In rats less than twelve days of age, certain lipid-insoluble xenobiotics are absorbed about twice as fast as in older rats (eighteen days of age to adult). Compounds with a molecular weight of 60 or less, and those of molecular weight 5,250–20,000, are absorbed at similar rates in neonatal and adult animals, but compounds of molecular weight 122–1,355 are absorbed more rapidly in neonatal rats (126). The administration of cortisone (130) or thyroxine (131) accelerates the development of the pulmonary epithelial permeability properties characteristic of adult rats.

However, because many airborne pollutants, toxicants, and drugs that might be administered via the airways are lipophilic in nature, the study of their absorption is more relevant. These lipid-soluble chemicals cross the pulmonary epithelium more rapidly in rats and guinea pigs than hydrophilic compounds of the same molecular weight. Absorption is generally by a non-saturable, passive diffusion process. The higher the lipid/water partition coefficient, the more rapid is the disappearance of compound from the lung. These data are consistent with those illustrating passage through lipid portions of epithelial (and endothelial) cell membranes. As shown in Table 2, there is no significant difference in the rate of absorption of lipid soluble compounds from the airways of neonatal and of adult rat lungs.

An experimental approach that could be used to delineate the alveolar

Table 2 Absorption of several xenobiotics from the airways of lungs of guinea pigs and neonatal and adult rats after drug administration by intratracheal injection or liquid aerosol exposure

Compound	Rate of absorption (half-time, minutes)			
	Guinea pig[a]	Neonatal rat[a] (6 days of age)	Adult rat[a]	Adult rat[b]
Lipid-soluble				
Salicyclic acid	—[c]	—	1.0 (120)[d]	0.67 (121)
Barbital	—	—	1.4 (123)	0.93 (121)
Sulfisoxazole	2.9 (124)	3.2 (125)	3.4 (125)	—
Procainamide	4.5 (124)	5.1 (125)	5.7 (125)	2.3 (121)
Non-Lipid Soluble				
Urea	—	4.1 (126)	4.7 (126)	1.4 (121)
Guanidine	—	5.1 (126)	6.3 (126)	3.1 (121)
p-Aminohippuric acid	22.2 (124)	22 (125)	41 (125)	21.7 (121)
Mannitol	25.8 (124)	32 (125)	60 (125)	26.5 (121)
Tetraethylammonium	22.5 (124)	28 (125)	63 (125)	—
Inulin	—	211 (126)	220 (126)	—
Dextran	—	635 (126)	688 (126)	—

[a] Administered intratracheally (120)
[b] Administered by liquid aerosol (121)
[c] Data not reported
[d] Reference in parentheses

contribution to epithelial transport and to determine the amount of metabolism that occurs during transit through the air-blood barrier is non-recirculating perfused lungs in which known amounts of chemical are administered to the air spaces. In the perfused organ, the bronchial circulation is not functional, so only absorption/metabolism occurring within the alveolar-capillary unit is measured. Such experiments would best be done at short times after the introduction of chemicals into the lung, and this is possible with lipophilic compounds because of their rapid rate of absorption.

There is one metabolically controlled mechanism for the accumulation of xenobiotic metabolites in pulmonary epithelium. Several chemicals are converted to electrophilic products that react covalently with cellular macromolecules, including protein and DNA. The parent chemical reaches the epithelium by diffusion from the vasculature. Metabolic activation is generally catalyzed by the cytochrome P-450-dependent monooxygenase system, known to be concentrated in Clara cells but also present in other epithelial cells (50). For compounds converted to unstable products which then alkylate macromolecules in the cells where they are formed, selective or specific Clara cell toxicity is anticipated. This is observed experimentally with several chemicals, including 4-ipomeanol (132), 3-methylfuran (132), napthalene (133), 2-

methylnapthalene (134), carbon tetrachloride (135), and 1,1-dichloroethylene (136). Other compounds, such as butylated hydroxytoluene (137) and *O,S,S,*-trimethylphosphorothioate (138), also require cytochrome P-450-dependent metabolism, but they cause selective toxicity in the alveolar type I cell. Probable explanations for the latter observation are that the reactive metabolites formed are stable enough to migrate from the site of formation to the site of alkylation, and/or the type I cell is deficient in detoxication enzymes so that only a small amount of intracellular metabolism is required to initiate the deleterious response. It is important to remember that enzyme systems other than the P-450-associated monooxygenases are present in lung and are capable of metabolically activating certain substrates. Moreover, these systems can be distributed unevenly among pulmonary epithelial cells. A recent study from Eling's group (139), for example, demonstrated that freshly isolated rat alveolar type II cells are more efficient than Clara cells at converting, BP 7,8-dihydrodiol to 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetra-hydrobenzo(a)pyrene (BPDEI) by the prostaglandin H synthase pathway.

Pulmonary epithelium is an important target for chemical carcinogenesis by some polycyclic aromatic hydrocarbons (PAH), and DNA alkylation by specific PAH metabolites is a required step for the initiation of tumorigenesis. Consequently, relationships between the cellular localization of metabolic activation systems and cell-specific DNA alkylation in tissues, like lung, that are susceptible or resistant to PAH-mediated neoplasia are of interest. A recent study from Anderson's laboratory at NIEHS (140) demonstrated that similar amounts of (+)−BPDEI-DNA adducts are present in tissues of widely divergent monooxygenase activity twenty-four hours after the iv administration of BP (1 mg/kg) to rabbits in vivo. The lung contained the greatest amount (0.06 ± 0.01 pmol/mg DNA, mean ± S.D., N = 5) of (+)−BPDEI-deoxyguanosine, the major DNA adduct formed in each tissue, but tissues deficient in monooxygenase activity such as the muscle (0.03 ± 0.02) and blood (0.03 ± 0.01) had as much of this adduct as liver (0.02 ± 0.002). Subsequently, we conducted a collaborative in vivo-in vitro study to determine the degree of specific DNA alkylation in individual populations of lung and liver cells (141). Treatment conditions identical to those described above (140) were used, except that various cells were isolated from lung and liver twenty-four hours after treatment with BP. BP metabolite-DNA adducts were assayed by HPLC and the values obtained were: alveolar type II cells 0.026 pmol (+)−BPDEI-deoxyguanosine/mg DNA, Clara cells <0.02, alveolar macrophages 0.044, hepatocytes 0.024, and liver non-parenchymal cells 0.030. Collectively, these data demonstrate that there is no obvious correlation between cytochrome P-450-dependent monooxygenase activity and the amount of specific DNA alkylation observed in the various cell types, and this raises the

possibility that transport of ultimate carcinogenic metabolites of PAH from one tissue or cell type to another occurs in vivo. Caution must also be exercised with this interpretation, however. Muscle does contain cytochrome P-450, but at a very low concentration (C. J. Serabjit-Singh, J. R. Bend, R. M. Philpot, unpublished observations). Yet low levels of a P-450 isozyme efficient at forming BPDEI could account for the DNA binding observed in muscle. Thus, it is possible that there is a correlation between the relevant parameters, and sensitive immunochemical quantitation techniques are now available to establish this. These recent experiments illustrate how existing technology can be used to study relationships between xenobiotic metabolism and alkylation in lung at the cellular level, but only in a limited number of cell types.

Energy-Dependent Epithelial Transport Systems

PARAQUAT An interesting recent development is the demonstration by Smith and his colleagues (65, 142, 143) of a high affinity, energy-dependent, sodium-independent, saturable transport system for endogenous diamines and polyamines in rat lung slices. Due to its structural similarity with these diamines, PQ is also transported by this pathway (65, 144), accounting for its accumulation in lung. The diamines putrescine and cadaverine and the polyamines spermine and spermidine are substrates for this transport system, and the apparent K_m values of this first-order process for these compounds are 13.1, 19.0, 14.8, and 10.9 μM respectively (143); the comparable K_m for PQ is 70 μM (63). This transport system is also present in human peripheral lung, where K_m values for putrescine accumulation in four individuals varied from 2–11 μM (145).

The site for PQ uptake has been attributed, at least in part, to the pulmonary epithelium by both autoradiographic (61) and indirect methods. Smith noted that putrescine and PQ accumulation are markedly decreased in lung slices from rats that displayed selective alveolar type I and type II cell damage subsequent to treatment with PQ. In this experiment, 5-HT uptake was the same in lung slices from paraquat-treated and control animals, suggesting that endothelial function is not compromised (62, 142). Further, the administration of PQ to a perfused rat lung preparation via the vasculature did not cause long-term storage, whereas introduction into the airways did (146); the slow component of PQ efflux was about six hours. Some recent experiments with rat alveolar type II cells (granular pneumocytes) cultured for twenty-four hours and alveolar macrophages demonstrated that the type II cells accumulated PQ by an energy-dependent process, presumably carrier-mediated and by diffusion, but the macrophages took up PQ only by diffusion (147). Some experiments performed in our laboratory with Clara cells, alveolar type II cells, and macrophages isolated from rabbit lungs gave complementary information (R.

Brigelius, J. K. Horton, R. P. Mason, J. R. Bend, unpublished observations). Intact Clara cells and type II cells incubated with PQ (1 mM) in an anaerobic ESR sample tube formed PQ radical signal at the earliest times we could scan (4–5 minutes), whereas alveolar macrophages did not produce a detectable ESR signal for this radical even after sixty minutes of repetitive scanning. More ESR signal was generated per Clara cell than per type II cell, consistent with the greater amount of cytochrome P-450 reductase activity present. Subsequent to the lysis of cells by sonication and the addition of NADPH, intense ESR signals of the PQ radical were produced by all three cell types. Collectively, these data are consistent with the facile uptake of PQ by Clara and type II cells but not by alveolar macrophages.

Other biogenic and nonbiogenic amines, including 5-HT (64) and IMP (148), interfere with PQ uptake by lung slices, although they are not substrates for the polyamine transport system. This strongly suggests that the binding sites for various mono-, di-, and polyamines on the plasma membranes of different types of lung cells must have several features in common, even though the processes for substrate transport into the cell or the intracellular binding sites can be quite specific, even demonstrating stereoselectivity (149). Consequently, it seems advisable to conduct interaction studies between PQ and amines, both bio- and nonbiogenic, in highly enriched populations of freshly isolated or cultured lung cells. The use of both broken and intact cells should allow mechanistic studies of extracellular and intracellular transport, binding, and metabolism phenomena.

OTHER XENOBIOTICS The presence of other energy-dependent transport processes in pulmonary epithelium has also been described. Schanker and his colleagues (150, 151) have shown that phenol red and disodium chromoglycate, organic anions, are transported by a carrier-mediated transport pathway that is saturable and selectively inhibited by low-temperature, anaerobic conditions and metabolic inhibitors (152). Similarly, the nonmetabolized amino acid 1-aminocyclopentanecarboxylic acid is transported across the pulmonary epithelium of the rat by a combination of at least two processes, one a saturable, energy-dependent carrier-type system and the other nonsaturable diffusion (153). Lung epithelia of the mouse, hamster, and guinea pig also transport this amino acid by an active process (154). However, lung epithelium does not actively take up quaternary ammonium compounds, apparently lacking a carrier-mediated system for the absorption of organic cations (155); these compounds are absorbed by diffusion through aqueous membrane channels in the epithelial membranes of lung. We hope these studies will be extended to elucidate the anatomical and cellular localization of the carrier-mediated processes identified and to determine the luminal versus basolateral distribution of these transport systems.

PREDICTION OF CLEARANCE OF XENOBIOTICS BY LUNG

The contribution of the lung to the metabolic clearance of chemicals from the blood has only recently been investigated in detail. Practical and theoretical considerations by Collins & Dedrick (156) and Gillette (157) suggest that in some cases the relative participation of the lung in clearance may be substantially greater than predicted solely on the basis of whole-organ metabolic potentials. In fact, with some chemicals pulmonary clearance may equal or exceed hepatic clearance, even though the metabolic capacity of the liver may be much greater than that of the lung. Two factors make important contributions to this possibility: first, the location of the lung between the arterial and venous branches of the circulatory system, where it is perfused by the entire blood supply; second, limitation of hepatic clearance due to the flow rate of the blood, not metabolic capacity. Attempts to elucidate the precise role of the lung in clearance are being made through the application of predictive mathematical models that can be rationalized by readily obtainable experimental values, by direct measurements of clearance in isolated organs, and by indirect determinations made with intact animals.

In 1973, Rowland et al (158) concluded that clearance (CL) of chemicals from the blood could be described in terms of blood flow (Q), intrinsic clearance (CL_i), and the fraction of the chemical free in the blood (f_b) by the relationship: $CL = Qf_bCL_i/Q + f_bCL_i$. For chemicals whose clearance is a function of metabolism, the results of Rane et al (159) indicate that CL_i can be calculated from the kinetic constants (V_{max} and K_m) of the enzyme(s) involved: $CL_i = V_{max}/K_m$. Limitations on the applicability of the clearance equation include: (*a*) uptake of the chemical from the blood must be passive (it is assumed that the concentrations of unbound chemical in the blood and tissue are equal); (*b*) the effective substrate concentration must be low enough to insure that metabolism is a first-order process.

The isolated, perfused lung has been used for the assessment of pulmonary clearance predicted for a number of chemicals. Early studies with this preparation were compromised by flow rates that were much less than physiological. The importance of flow rate, as well as the mode of uptake, is shown by the results of Wiersma & Roth (160), who studied the uptake of 5-HT by isolated rat liver and lung. The predicted clearances at normal blood flows indicated that the efficiency of the liver should exceed that of the lung by about fivefold (7.6 versus 1.34 ml per minute). The predicted relationship between clearance and flow rate was observed with the isolated liver system; at normal flow (11 ml per minute) observed clearance was 6.6 ml per minute. In contrast, predicted pulmonary clearance was substantially less than that observed. At normal flow (44.8 ml per minute) the observed clearance was 19.1 ml per minute. This

discrepancy likely reflects a much greater involvement of specific uptake processes in the clearance of 5-HT by lung as compared to liver. The possibility of complete removal by the lung had been suggested by several investigators (161–163) who had examined 5-HT uptake by isolated lungs perfused at low flow rates. Wiersma & Roth (160) did in fact observe nearly complete pulmonary clearance of 5-HT at a flow rate of 10 ml per minute. The effect of flow rate on the pulmonary uptake of 5-HT confirmed earlier findings of Pickett et al (13), who investigated the roles of active transport, flow rate, and concentration on the uptake of 5-HT in the isolated rabbit lung. It should be noted that the effect of flow rate on the clearance of passively transported chemicals ranges from negligible (when CL_i is low with respect to Q) to absolute (when CL_i is much greater than Q, so that the clearance is essentially equal to the flow rate).

In contrast to results with 5-HT, the predicted and observed pulmonary clearances of BP are in agreement (164). For lungs from rats treated with 3-MC, which induces the synthesis of a cytochrome P-450 isozyme that efficiently metabolizes BP, the predicted CL was 7.0 ml per minute and the observed CL was 8.9 ml per minute. The corresponding values for lungs from untreated rats were 0.97 and 0.99 ml per minute. In both cases the major factor in the predicted clearance was CL_i. In contrast, the predominant factor in the prediction of hepatic clearance was flow rate. With livers from treated rats, the predicted clearance was actually equal to the flow rate; however, the observed clearance was significantly less (6.7 versus 10.0 ml per minute). These findings suggest two things: first, the pulmonary clearance of BP in rats treated with 3-MC may be nearly equal to the hepatic clearance; second, values predicted for the hepatic clearance are not realized with the isolated liver. A number of factors associated with binding, distribution, and metabolism could contribute to the discrepancy between the predicted and observed hepatic clearances. In any case, it is not clear that Wiersma & Roth (164) are correct in their conclusion that the differences between the hepatic values are minor. The CL_i calculated from the V_{max} and K_m for the hepatic (3-MC) metabolism of BP was reported to be 28,257 ml per minute; CL_i calculated from the observed clearance is about 15 ml per minute. Therefore, if the inaccuracy of the prediction is a function of an incorrect value for CL_i, the error exceeds a factor of 10^3, an error not likely due to shortcomings in the determinations of the kinetic constants. Unlike the results obtained with livers from treated rats, the observed clearance with liver from untreated rats was reported to be greater than the predicted clearance (5.9 versus 4.6 ml per minute). In this case, however, a value of 0.14 was used for f_b compared to the values of 1.4 used for liver (3-MC), 1.0 for lung (3-MC), and 1.1 for lung (untreated). These values were determined in vitro from the ratios of the rates of microsomal metabolism obtained with 32 or 1 mg BSA/ml in the incubations (the binding of BP could not be measured directly). The relationship between these determinations and

the binding of BP to plasma components seems obscure, and the use of different values of f_b for liver and lung is difficult to justify. The predicted clearance by liver, using a value of 1.0 for f_b, is 8.8 ml per minute, which is greater than the observed clearance. In spite of the possible shortcomings in the application of the clearance model to hepatic systems, several important conclusions reached by Wiersma & Roth are strongly supported by their findings. First, the clearance of BP by the isolated, perfused lung can be predicted with a high degree of accuracy. Second, increases in CL_i may have a major effect on pulmonary, but not hepatic, clearance. Third, pulmonary clearance of BP may be equal to or greater than hepatic clearance in rats treated with 3-MC. The results of in vivo experiments appear to confirm the last conclusion, although the observed pulmonary and hepatic clearances appear to be less than those obtained with the isolated organs (165).

Prediction of clearance when metabolism is catalyzed by more than one enzyme requires calculations for each pathway, may be compromised by undetermined competitive effects, and is inherently more prone to error than predictions based on a single enzyme. However, Smith & Bend (166) have examined the pulmonary clearance of BP 4,5-oxide with reasonable success. This arene oxide is metabolized by epoxide hydrolase, a microsomal enzyme, and the glutathione S-transferases, a group of cytosolic enzymes. The predicted extraction ratios ($E = CL/Q$) were 0.25 and 0.74 for the hydrolase and transferase pathways respectively. Therefore, it was predicted that the clearance (20 ml/minute/g) of the oxide would be essentially 100%. A total extraction ratio of 0.64 was calculated from the observed clearance. The difference between the predicted and observed values are accounted for almost entirely by the transferase pathway (observed $E = 0.44$); observed E for the hydrolase pathway (0.20) was in close agreement with the predicted value. The possibility that the transferase activity was limited by the concentration of glutathione in the isolated lung (saturating levels were used for the determination of the kinetic constants) has been suggested. It should be noted that an increase in the flow rate that was used in this study (20 ml/minute/g) to the estimated normal rate (35 ml/minute/g) would be expected to increase the clearance and decrease the extraction efficiency. The predicted values with $Q = 35$ ml/minute/g are 28 and 0.80 ml/minute/g for CL and E respectively.

Because the clearance model appears to estimate pulmonary clearance with reasonable accuracy, it may be of use in predicting the pulmonary toxicity of some chemicals. Therefore, we have analyzed data from a number of studies on the pulmonary toxin, 4-ipomeanol, to determine if predicted clearances are consistent with the observation that covalent binding of metabolites of 4-ipomeanol is about five times greater (per mg tissue protein) in lung than in liver of rats and rabbits (167). The available data include K_m and V_{max} constants for the metabolism of 4-ipomeanol in microsomal preparations from

liver and lung of both species (168, 169). The total organ V_{max} and K_m for rat liver are both higher than for lung (twenty and fifteen times respectively). Therefore, the predicted intrinsic clearances for the two tissues are similar, 0.29 ml per minute for liver and 0.20 for lung. These low values relative to Q result in predicted clearances (0.28 and 0.20 ml per minute) that are essentially the same as the intrinsic clearances. Based on these rates, total covalent binding is predicted to be greater in the liver, and binding per mg tissue protein is predicted to be six to eight times greater in the lung.

The difference between the K_m values for the metabolism of 4-ipomeanol in rat liver and lung partially explains why the CL_i values for the two tissues are fairly similar. This difference also suggests that the relevant enzyme(s) in rat liver and lung is dissimilar, which is not the case for the rabbit. The results of antibody inhibition and kinetic studies indicate that the same isozymes of cytochrome P-450 metabolize 4-ipomeanol in rabbit liver and lung (169, 170). However, the concentrations of these enzymes are higher in lung than in liver, and the V_{max} (nmol product/minute/mg microsomal protein) for the lung is about three times that of the liver. The predicted clearances, 7.2 ml per minute for liver and 2.1 ml per minute for lung, are, as with the rat, essentially the same as those predicted for CL_i. These values suggest that binding in the lung (per mg tissue protein) should be three to five times that of the liver. Therefore, we conclude that reasonable estimates of the relative covalent binding of 4-ipomeanol in liver and lung of rats and rabbits can be obtained from predicted clearances.

CONCLUSION

The lung is anatomically complex and contains more than forty different cell types. The macromolecules that are responsible for xenobiotic uptake, accumulation, and/or biotransformation include enzymes for oxidative, reductive, hydrolytic, and biosynthetic metabolism, carriers for energy-dependent transport systems, extracellular and intracellular binding sites of low to high specificity, and receptors. Each of these processes also functions with endogenous chemicals. One of the major problems in pulmonary research is assigning biological activity to a specific anatomical region (tracheobronchiolar, bronchiolar, or alveolar) and/or cell type(s). The integrated experimental approach that is being used by us and others includes immunochemical, biochemical, and metabolic studies in vivo, with perfused lung preparations, whole lung homogenate and its subcellular fractions, homogeneous enzymes purified from lung, and freshly prepared and cultured cells isolated from lungs following mild proteolytic digestion. Each of these procedures used alone has its limitations (5); however, in concert they allow the elucidation of chemical and

biochemical mechanisms involved in the uptake and metabolism of chemicals by lungs.

Over the last few years our knowledge of the lungs' contribution to the clearance of xenobiotics from the circulation and their metabolism has increased dramatically, primarily as a result of advances in in vivo and in vitro techniques. Further progress in understanding the contribution of individual pulmonary cell types to these processes is anticipated, especially as methodology for the isolation and culture of lung cells becomes more standardized and widely used, and as highly enriched populations of additional tracheobronchial, bronchiolar, and alveolar cells become available.

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Literature Cited

1. Bakhle, Y. S., Vane, J. R., eds. 1977. *Metabolic Functions of the Lung*, Vol. 4. New York: Dekker. 353 pp.
2. Witschi, H., Nettesheim, P., eds. 1982. *Mechanisms in Respiratory Toxicology*, Vols. 1, 2. Boca Raton: CRC. 286 pp., 230 pp.
3. Wilson, A. G. E. 1982. Toxicokinetics of uptake, accumulation and metabolism of chemicals by the lung. See Ref. 2, 1:161–85
4. Minchin, R. F., Boyd, M. R. 1983. Localization of metabolic activation and deactivation systems in the lung: Significance to the pulmonary toxicity of xenobiotics. *Ann. Rev. Pharmacol. Toxicol.* 23:217–38
5. Bend, J. R., Serabjit-Singh, C. J. 1984. Xenobiotic metabolism by extrahepatic tissues: Relationship to target organ and cell toxicity. In *Drug Metabolism and Drug Toxicity*, ed. J. R. Mitchell, M. G. Horning, pp. 99–136. New York: Raven. 436 pp.
6. Ryan, J. W. 1982. Processing of endogenous polypeptides by the lungs. *Ann. Rev. Physiol.* 44:241–55
7. Said, S. I. 1982. Pulmonary metabolism of prostaglandins and vasoactive peptides. *Ann. Rev. Physiol.* 44:257–68
8. Gillis, C. N., Pitt, B. R. 1982. The fate of circulating amines within the pulmonary circulation. *Ann. Rev. Physiol.* 44:269–81
9. Witschi, H., Côté, M. G. 1977. Primary pulmonary responses to toxic agents. *CRC Crit. Rev. Toxicol.* 5:23–66
10. Ryan, U. S. 1982. Structural bases for metabolic activity. *Ann. Rev. Physiol.* 44:223–39
11. Gillis, C. N., Greene, N. M. 1977. Possible implications of metabolism of bloodborne substrates by the human lung. See Ref. 1, pp. 173–93
12. Hughes, J., Gillis, C. N., Bloom, F. E. 1969. The uptake and disposition of DL-noradrenaline in perfused rat lung. *J. Pharmacol. Exp. Ther.* 169: 237–48
13. Pickett, R. D., Anderson, M. W., Orton, T. C., Eling, T. E. 1975. The pharmacodynamics of 5-hydroxytryptamine uptake and metabolism by the isolated perfused rabbit lung. *J. Pharmacol. Exp. Ther.* 194:545–53
14. Alabaster, V. A. 1977. Inactivation of endogenous amines in the lungs. See Ref. 1, pp. 3–31
15. Iwasawa, Y., Gillis, C. N., Aghajanian, G. 1973. Hypothermic inhibition of 5-hydroxytryptamine and nonepinephrine uptake by lung: Cellular location of amines after uptake. *J. Pharmacol. Exp. Ther.* 186:498–507
16. Catravas, J. D., Gillis, C. N. 1983. Single pass removal of [^{14}C]-5-hydroxytryptamine and [^{3}H]-norepinephrine by rabbit lung, in vivo: Kinetics and

sites of removal. *J. Pharmacol. Exp. Ther.* 224:28–33
17. Bakhle, Y. S., Youdim, M. B. H. 1976. Metabolism of phenylethylamine in rat isolated perfused lung: Evidence for monoamine oxidase "type B" in lung. *Br. J. Pharmacol.* 56:125–27
18. Bakhle, Y. S., Youdim, M. B. H. 1979. The metabolism of 5-hydroxytryptamine and β-phenylethylamine in perfused rat lung and in vitro. *Br. J. Pharmacol.* 65:147–54
19. Brown, E. A. B. 1974. The localization, metabolism and effects of drugs and toxicants in lung. *Drug Metab. Rev.* 3:33–87
20. Orton, T. C., Anderson, M. W., Pickett, R. D., Eling, T. E., Fouts, J. R. 1973. Xenobiotic accumulation and metabolism by isolated perfused rabbit lungs. *J. Pharmacol. Exp. Ther.* 186:482–97
21. Anderson, M. W., Orton, T. C., Pickett, R. D., Eling, T. E. 1974. Accumulation of amines in the isolated perfused rabbit lung. *J. Pharmacol. Exp. Ther.* 189:456–66
22. Eling, T. E., Pickett, R. D., Orton, T. C. Anderson, M. W. 1975. A study of the dynamics of imipramine accumulation in the isolated perfused rabbit lung. *Drug Metab. Disp.* 3:389–400
23. Wilson, A. G. E., Law, F. C. P., Eling, T. E., Anderson, M. W. 1976. Uptake, metabolism, and efflux of methadone in "single pass" isolated perfused rabbit lungs. *J. Pharmacol. Exp. Ther.* 199:360–67
24. Philpot, R. M., Anderson, M. W., Eling, T. E. 1977. Uptake, accumulation and metabolism of chemicals by the lung. See Ref. 1, pp. 123–71
25. Junod, A. F. 1972. Accumulation of ^{14}C-imipramine in isolated perfused rat lungs. *J. Pharmacol. Exp. Ther.* 183:182–87
26. Wilson, A. G. E., Pickett, R. D., Eling, T. E., Anderson, M. W. 1979. Studies on the persistence of basic amines in the rabbit lung. *Drug Metab. Disp.* 7:420–24
27. Wilson, A. G. E., Sar, M., Stumpf, W. E. 1982. Autoradiographic study of imipramine localization in the isolated perfused rabbit lung. *Drug Metab. Disp.* 10:281–83
28. Lüllmann, H., Lüllmann-Rauch, R., Wassermann, O. 1975. Drug-induced phospholipidoses. *CRC Crit. Rev. Toxicol.* 4:185–218
29. Seydel, J. K., Wassermann, O. 1973. NMR studies on the molecular basis of drug-induced phospholipidosis. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 297:207–10
30. Mitchell, R. H., Allan, D., Bowler, M., Brindley, D. N., 1976. A possible metabolic explanation for drug-induced phospholipidosis. *J. Pharm. Pharmacol.* 28:331–32
31. Mehendale, H. M., Angevine, L. S., Ohmiya, Y. 1981. The isolated perfused lung—A critical evaluation. *Toxicology* 21:1–36
32. Bakhle, Y. S., Vane, J. R. 1974. Pharmacokinetic function of the pulmonary circulation. *Physiol. Rev.* 54:1007–45
33. Gillis, C. N., Cronau, L. H., Mandel, S., Hammond, G. L. 1979. Indicator dilution measurement of 5-hydroxytryptamine clearance by human lung. *J. Appl. Physiol.* 46:1178–83
34. Catravas, J. D., Gillis, C. N. 1980. Pulmonary clearance of [^{14}C]-5-hydroxytryptamine and [^{3}H]norepinephrine in vivo: Effects of pretreatment with imipramine or cocaine. *J. Pharmacol. Exp. Ther.* 213:120–27
35. Crone, C. 1963. The permeability of capillaries in various organs as determined by the use of the indicator diffusion method. *Acta Physiol. Scand.* 58:292–305
36. Gillis, C. N., Iwasawa, Y. 1972. Technique for measurement of noradrenaline and 5-hydroxytryptamine uptake by rabbit lung. *J. Appl. Physiol.* 33:404–8
37. Iwasawa, Y., Gillis, C. N. 1974. Pharmacological analysis of norepinephrine and 5-hydroxytryptamine removal from the pulmonary circulation: Differentiation of uptake sites for each amine. *J. Pharmacol. Exp. Ther.* 188:386–93
38. Minchin, R. F., Barber, H. E., Ilett, K. F. 1982. Effect of prolonged desmethylimipramine administration on the pulmonary clearance of 5-hydroxytryptamine and β-phenylethylamine in rats. *Drug Metab. Disp.* 10:356–60
39. Mehendale, H. M., Morita, T., Angevine, L. S. 1983. Effect of chlorphentermine on the pulmonary clearance of 5-hydroxytryptamine in rabbits in vivo. *Pharmacology* 26:274–83
40. Morita, T., Mehendale, H. M. 1983. Effects of chlorphentermine and phentermine on the pulmonary disposition of 5-hydroxytryptamine in the rat *in vivo*. *Am. Rev. Respir. Dis.* 127:747–50
41. Deleted in proof
42. Angevine, L. S., Mehendale, H. M. 1982. Effect of chlorphentermine pretreatment on 5-hydroxytryptamine disposition in the isolated perfused rat lung. *Fund. Appl. Toxicol.* 2:306–12
43. Ohyima, Y., Angevine, L. S., Mehendale, H. M. 1983. Effect of drug-induced

phospholipidosis on pulmonary disposition of pneumophilic drugs. *Drug Metab. Disp.* 11:25–30
44. Angevine, L. S., Lockhard, V. G., Mehendale, H. M. 1984. Effect of chlorphentermine pretreatment on the distribution of chlorphentermine in isolated perfused rabbit lungs. *Fund. Appl. Toxicol.* 4:202–9
45. Zychlinski, L., Montgomery, M. R., Shamblin, P. B., Reasor, M. J. 1983. Impairment in pulmonary bioenergetics following chlorphentermine administration to rats. *Fund. Appl. Toxicol.* 3:192–98
46. Ferin, J. 1982. Alveolar macrophage mediated pulmonary clearance suppressed by drug-induced phospholipidosis. *Exp. Lung Res.* 4:1–10
47. Philpot, R. M., Wolf, C. R. 1981. The properties and distribution of the enzymes of pulmonary cytochrome P-450-dependent monooxygenase systems. *Rev. Biochem. Toxicol.* 3:51–76
48. Smith, B. R., Bend, J. R. 1981. Metabolic interactions of hydrocarbons with mammalian lung. *Rev. Biochem. Toxicol.* 3:77–122
49. Serabjit-Singh, C. J., Wolf, C. R., Philpot, R. M., Plopper, C. G. 1980. Cytochrome P-450: Localization in rabbit lung. *Science* 207:1469–70
50. Devereux, T. R., Fouts, J. R. 1981. Isolation of pulmonary cells and use in studies of xenobiotic metabolism. *Methods Enzymol.* 77:147–54
51. Jones, K. G., Holland, J. F., Foureman, G. L., Bend, J. R., Fouts, J. R. 1983. Induction of xenobiotic metabolism in Clara cells and alveolar type II cells isolated from rat lungs. *J. Pharmacol. Exp. Ther.* 225:316–19
52. Boyd, M. R. 1977. Evidence for the Clara cell as a site of cytochrome P-450-dependent mixed-function oxidase activity in lung. *Nature* 269:713–14
53. Hook, G. E. R., Bend, J. R., Fouts, J. R. 1972. Mixed-function oxidases and the alveolar macrophage. *Biochem. Pharmacol.* 21:3267–77
54. Serabjit-Singh, C. J., Domin, B. A., Bend, J. R., Philpot, R. M. 1983. Immunochemical and biochemical evidence for the presence of cytochrome P-450 monooxygenase components in rabbit heart and aorta. In *Extraheptic Drug Metabolism and Chemical Carcinogenesis,* ed. J. Rydström, J. Montelius, M. Bengtsson, pp. 253–55. Amsterdam: Elsevier. 630 pp.
55. Law, F. C. P., Eling, T. E., Bend, J. R., Fouts, J. R. 1974. Metabolism of xenobiotics by the isolated perfused lung: Comparison with in vitro incubations. *Drug Metab. Disp.* 2:433–43
56. Minchin, R. F., Ilett, K. F., Madsen, B. W. 1981. A compartmental model for the uptake of chlorphentermine in isolated rat lung. *Eur. J. Drug Metab. Pharmacokin.* 6:127–33
57. Minchin, R. F., Ilett, K. F., Madsen, B. W. 1979. Chlorphentermine binding in rat lung subcellular fractions and its displacement by desmethylimipramine. *Biochem. Pharmacol.* 28:2273–78
58. Murray, R. E., Gibson, J. E. 1972. A comparative study of paraquat intoxication in rats, guinea-pigs and monkeys. *Exp. Mol. Pathol.* 17:317–25
59. Wyatt, I., Doss, A. W., Zavala, D. C., Smith, L. L. 1981. Intrabronchial instillation of parquat in rats: Lung morphology and retention study. *Br. J. Ind. Med.* 38:42–48
60. Sharp, C. W., Ottolenghi, A., Posner, H. S. 1972. Correlation of paraquat toxicity with tissue concentrations and weight loss of the rat. *Toxicol. Appl. Pharmacol.* 22:241–51
61. Waddell, W. J., Marlowe, C. 1980. Tissue and cellular distribution of paraquat in mice. *Toxicol. Appl. Pharmacol.* 56:127–40
62. Sykes, B. I., Purchase, I. F. H., Smith, L. L. 1977. Pulmonary ultrastructure after oral and intravenous dosage of paraquat to rats. *J. Pathol.* 121:233–41
63. Rose, M. S., Smith, L. L., Wyatt, I. 1974. Evidence for the energy dependent accumulation of paraquat into rat lung. *Nature* 252:314–15
64. Lock, E. A., Smith, L. L., Rose, M. S. 1976. Inhibition of paraquat accumulation in rat lung slices by a component of rat plasma and a variety of drugs and endogenous amines. *Biochem. Pharmacol.* 25:1769–72
65. Smith, L. L. 1982. The identification of an accumulation system for diamines and polyamines into the lung and its relevance for paraquat toxicity. *Arch. Toxicol.* 5:1–14 (Suppl.)
66. Tucker, A., McMurtry, I. F., Reeves, J. T., Alexander, A. F., Will, D. H., et al. 1975. Lung vascular smooth muscle as a determinant of pulmonary hypertension at high altitude. *Am. J. Physiol.* 228:762–67
67. Beaconsfield, P. 1962. Metabolism of the normal cardiovascular wall: 2. The pentose phosphate pathway. *Experentia* 18:276–77
68. Syrota, A., Girault, M., Pocidalo, J.-J., Yudilevich, D. L. 1982. Endothelial up-

take of amino acids, sugars, lipids, and prostaglandins in rat lung. *Am. J. Physiol.* 243:C20–26
69. Longnecker, G. L., Huggins, C. G. 1977. Biochemistry of the pulmonary angiotensin-converting enzyme. See Ref. 1, pp. 55–83
70. Gillis, C. N., Catravas, J. D. 1982. Altered removal of vasoactive substances in the injured lung: Detection of lung microvascular injury. *Ann. NY Acad. Sci.* 384:458–74
71. Flower, R. J. 1977. Prostaglandin metabolism in the lung. See Ref. 1, pp. 85–122
72. Weksler, B. B. 1982. Prostacyclin. In *Progress in Hemostasis and Thrombosis,* ed. T. H. Spaet, 6:113–38. New York: Grune & Stratten. 368 pp.
73. Eling, T. E., Alley, A. I. 1984. Pulmonary biosynthesis and metabolism of prostaglandins and related substances. *Environ. Health Perspect.* 55:159–68
74. Pitt, B. R., Hammond, G. L., Gillis, C. N. 1982. Comparison of pulmonary and extrapulmonary extraction of biogenic amines. *J. Appl. Physiol.* 52:545–51
75. Neichi, T., Chang, W.-C., Mitsui, Y., Murota, S. 1982. Comparison of prostaglandin biosynthetic activity between porcine aortic endothelial and smooth muscle cells in culture. *Artery* 11:47–63
76. Ryan, U. S., Schultz, D. R., Del Vecchio, P., Ryan, J. W. 1980. Endothelial cells of bovine pulmonary artery lack receptors for C3b and for the Fc portion of IgG. *Science* 208:748–49
77. Ryan, U. S., Schultz, D. R., Ryan, J. W. 1981. Fc and C3b receptors on pulmonary endothelial cells: Induction by injury. *Science* 214:557–58
78. Wyatt, I. S., Keeling, P. L., Smith, L. L. 1980. The effect of high concentrations of oxygen on paraquat and diquat toxicity in rats. *Arch. Toxicol.* 4:415–18 (Suppl.)
79. Thilo-Körner, D. G. S., Freshney, R. I., eds. 1984. *The Endothelial Cell—A Pluripotent Control Cell of the Vessel Wall.* Basel: Karger. 206 pp.
80. Ryan, J. W. 1983. Assay of peptidase and protease enzymes in vivo. *Biochem. Pharmacol.* 32:2127–37
80a. Catravas, J. D., Lazo, J. S., Dobuler, K. J., Mills, L. R., Gillis, C. N. 1983. Pulmonary endothelial dysfunction in the presence or absence of interstitial injury induced by intratracheally injected bleomycin in rabbits. *Am. Rev. Respir. Dis.* 128:740–46
81. Friedland, J., Silverstein, E. 1976. A sensitive fluorimetric assay for serum angiotensin-converting enzyme. *Am. J. Clin. Pathol.* 66:416–27
82. Rohatgi, F. K., Ryan, J. W. 1980. Simple radioassay for measuring serum activity of angiotensin-converting enzyme in sarcoidosis. *Chest* 78:69–76
83. Lazo, J. S., Catravas, J. D., Dobuler, K. J., Gillis, C. N. 1983. Prolonged reduction in serum angiotensin converting enzyme activity after treatment of rabbits with bleomycin. *Toxicol. Appl. Pharmacol.* 69:276–82
84. Roth, R. A., Wallace, K. B., Alper, R. H., Bailie, M. D. 1979. Effect of paraquat treatment of rats on disposition of 5-hydroxytryptamine and angiotensin I by pefused lung. *Biochem. Pharmacol.* 28:2349–55
85. Mustafa, M. G., Tierney, D. F. 1978. Biochemical and metabolic changes in the lung with oxygen, ozone, and nitrogen dioxide toxicity. *Am. Rev. Resp. Dis.* 118:1061–90
86. McCord, J. M. 1979. Superoxide, superoxide dismutase and oxygen toxicity. *Rev. Biochem. Toxicol.* 1:109–24
87. Stokinger, H. E. 1965. Ozone toxicology. *Arch. Environ. Health* 10:719–31
88. Vostal, J. J., Chan, T. L., Garg, B. D., Lee, P. S., Strom, K. A. 1981. Lymphatic transport in lungs of rats and guinea pigs exposed to diesel exhaust. *Environ. Int.* 5:339–48
89. Huxtable, R. J. 1979. New aspects of the toxicology and pharmacology of pyrrolizidine alkaloids. *Gen. Pharmacol.* 10:159–67
90. Hilliker, K. S., Bell, T. G., Roth, R. A. 1982. Pneumotoxicity and thrombocytopenia after single injection of monocrotaline. *Am. J. Physiol.* 242:H573–79
91. Gillis, C. N., Huxtable, R. J., Roth, R. A. 1978. Effects of monocrotaline pretreatment of rats on removal of 5-hydroxytryptamine and noradrenaline by perfused lung. *Br. J. Pharmacol.* 63:435–43
92. Huxtable, R., Ciaramitaro, D., Eisenstein, D. 1978. The effect of a pyrrolizidine alkaloid, monocrotaline, and a pyrrole, dehydroretronecine, on the biochemical functions of the pulmonary endothelium. *Mol. Pharmacol.* 14:1189–203
93. Atwal, O. S., Persofsky, M. S. 1984. Ultrastructural changes in intraacinar pulmonary veins. Relationship to 3-methylindole-induced acute pulmonary edema and pulmonary arterial changes in cattle. *Am. J. Pathol.* 114:472–86
94. Hammond, A. C., Carlson, J. R., Willett, J. D. 1979. The metabolism and

disposition of 3-methylindole in goats. *Life Sci.* 25:1301–6
95. Block, E. R., Schoen, F. J. 1981. Effect of alpha napthylthiourea on uptake of 5-hydroxytryptamine from the pulmonary circulation. *Am. Rev. Respir. Dis.* 123:69–73
96. Cashman, J. R., Traiger, G. J., Hanzlik, R. P. 1982. Pneumotoxic effects of thiobenzamide derivatives. *Toxicology* 23:85–93
97. Boyd, M. R., Neal, R. A. 1976. Studies on the mechanism of toxicity and of development of tolerance to the pulmonary toxin α-napthylthiourea (ANTU). *Drug Metab. Disp.* 4:314–22
98. Tom, W.-M., Montgomery, M. R. 1980. Bleomycin toxicity: Alteration in oxidative metabolism in lung and liver microsomal fractions. *Biochem. Pharmacol.* 29:3239–44
99. Sugiura, U., Kikuchi, T. 1978. Formation of superoxide and hydroxy radicals in iron (II)-bleomycin-oxygen system: Electron spin resonance detection by spin trapping. *J. Antibiot.* 31:1310–12
100. Mais, D. E., Lahr, P. D., Bosin, T. R. 1982. Oxygen-induced lung toxicity: Effect on serotonin disposition and metabolism. *Toxicol. Appl. Pharmacol.* 64:221–29
101. Block, E. R., Fisher, A. B. 1977. Depression of serotonin clearance by rat lungs during oxygen exposure. *J. Appl. Physiol.* 42:33–38
102. Block, E. R., Cannon, J. K. 1978. Effect of oxygen exposure on lung clearance of amines. *Lung* 155:287–95
103. Meyrick, B., Reid, L. 1978. The effect of continued hypoxia on rat pulmonary arterial circulation: An ultrastructural study. *Lab Invest.* 38:188–200
104. Meyrick, B., Reid, L. 1979. Hypoxia and incorporation of ^{3}H-thymidine by cells of rat pulmonary arteries and alveolar wall. *Am. J. Pathol.* 96:51–70
105. Ospital, J. J., Kasuyama, R. S., Tierney, D. F. 1983. Poor correlation between oxygen toxicity and activity of glutathione peroxidase. *Exp. Lung Res.* 5:193–99
106. Crapo, J. D., Sjostrom, K., Drew, R. T. 1978. Tolerance and cross-tolerance using NO_2 and O_2. I. Toxicology and biochemistry. *J. Appl. Physiol.* 44:364–69
107. Crapo, J. D., Marsh-Salin, J., Ingram, P., Pratt, P. C. 1978. Tolerance and cross-tolerance using NO_2 and O_2. II. Pulmonary morphology and morphometry. *J. Appl. Physiol.* 44:370–79
108. Douglas, J. S., Curry, G., Geffkin, S. A. 1977. Superoxide dismutase and pulmonary ozone toxicity. *Life Sci.* 20: 1187–92
109. Cummiskey, J. M., Simon, L. M., Theodore, J., Ryan, U. S., Robin, E. D. 1981. Bioenergetic alterations in cultivated pulmonary artery and aortic endothelial cells exposed to normoxia and hypoxia. *Exp. Lung Res.* 2:155–63
110. Ager, A., Gordon, J. L., Moncada, S., Pearson, J. D., Salmon, J. A., et al. 1982. Effects of isolation and culture on prostaglandin synthesis by porcine aortic endothelial and smooth muscle cells. *J. Cell. Physiol.* 110:9–16
111. Bosin, T. R., Lahr, P. D. 1981. Mechanisms influencing the disposition of serotonin in mouse lung. *Biochem. Pharmacol.* 30:3187–93
112. Roth, R. A. 1982. Flow dependence of norepinephrine extraction by isolated perfused rat lungs. *Am. J. Physiol.* 242:H844–48
113. Catravas, J. D., Gillis, C. N. 1981. Metabolism of [^{3}H]benzoylphenylalanyl-alanyl-proline by pulmonary angiotensin converting enzyme *in vivo:* Effects of bradykinin, SQ 14225 or acute hypoxia. *J. Pharmacol. Exp. Ther.* 217:263–70
114. Bond, J. A., Yang, H.-Y. L., Majesky, M. W., Benditt, E. P., Juchau, M. R. 1980. Metabolism of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene in chicken aortas: Monooxygenation, bioactivation to mutagens, and covalent binding to DNA in vitro. *Toxicol. Appl. Pharmacol.* 52:323–35
115. Dees, J. A., Masters, B. S. S., Muller-Eberhard, U., Johnson, E. F. 1982. Effect of 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin and phenobarbital on the occurrence and distribution of four cytochrome P-450 isozymes in rabbit kidney, lung, and liver. *Cancer Res.* 42:1423–32
116. Lauweryns, J. M., Baert, J. H. 1977. Alveolar clearance and the role of the pulmonary lymphatics. *Am. Rev. Resp. Dis.* 115:625–83
117. Pine, M. B., Beach, P. M., Cottrell, T. S., Scola, M., Turino, G. M. 1976. The relationship between right duct lymph flow and extravascular lung water in dogs given α-napthylthiourea. *J. Clin. Invest.* 58:482–92
118. Gil, J. 1982. Comparative morphology and ultrastructure of the airways. See Ref. 2, 1: pp. 3-25
119. Breezer, R., Turk, M. 1984. Cellular structure, function and organization in the lower respiratory tract. *Environ. Health Perspect.* 55:3–24
120. Enna, S. J., Schanker, L. S. 1972.

Absorption of drugs from the rat lung. *Am. J. Physiol.* 223:1227–31
121. Brown, R. A., Schanker, L. S. 1983. Absorption of aerosolized drugs from the rat lung. *Drug Metab. Disp.* 11:355–60
122. Schanker, L. S. 1978. Drug absorption from the lung. *Biochem. Pharmacol.* 27:381–85
123. Brown, R. A. Jr., Schanker, L. S. 1983. Sex comparison of pulmonary absorption of drugs in the rat. *Drug Metab. Disp.* 11:392–93
124. Hemberger, J. A., Schanker, L. S. 1983. Pulmonary absorption of drugs in the neonatal and adult guinea pig. *Drug Metab. Disp.* 11:615–16
125. Hemberger, J. A., Schanker, L. S. 1978. Pulmonary absorption of drugs in the neonatal rat. *Am. J. Physiol.* 234:C191–97
126. Hemberger, J. A., Schanker, L. S. 1983. Relation between molecular weight and pulmonary absorption rate of lipid insoluble compounds in neonatal and adult rats. *Biochem. Pharmacol.* 32:2599–601
127. Wangensteen, D., Yankovich, R. 1979. Alveolar epithelium transport of albumin and sucrose: Concentration difference effect. *J. Appl. Physiol.* 47:846–50
128. Gatzy, J. T. 1982. Paths of hydrophilic solute flow across excised bullfrog lung. *Exp. Lung Res.* 3:147–61
129. Boucher, R. C., Stutts, M. J., Gatzy, J. T. 1981. Regional differences in canine airway epithelial ion transport. *J. Appl. Physiol.* 51:706–14
130. Hemberger, J. A., Schanker, L. S. 1981. Effect of cortisone on permeability of the neonatal rat lung to drugs. *Biol. Neon.* 40:99–104
131. Hemberger, J. A., Schanker, L. S. 1978. Effect of thyroxine on permeability of the neonatal rat lung to drugs. *Biol. Neon.* 34:299–303
132. Boyd, M. R. 1980. Biochemical mechanisms in pulmonary toxicity of furan derivatives. *Rev. Biochem. Toxicol.* 2:71–102
133. Warren, D. L., Brown, D. L., Buckpitt, A. R. 1982. Evidence for cytochrome P-450 mediated metabolism in the bronchiolar damage by naphthalene. *Chem.-Biol. Interact.* 40:287–303
134. Griffin, K. A., Johnson, C. B., Breger, R. K., Franklin, R. B. 1981. Pulmonary toxicity, hepatic and extrahepatic metabolism of 2-methylnaphthalene in mice. *Toxicol. Appl. Pharmacol.* 61:185–96
135. Boyd, M. R., Statham, C. N., Longo, N. S. 1980. The pulmonary Clara cell as a target for toxic chemicals requiring metabolic activation; studies with carbon tetrachloride. *J. Pharmacol. Exp. Ther.* 212:109–14
136. Forkert, P.-G., Reynolds, E. S. 1982. 1,1-Dichlorethylene-induced pulmonary toxicity. *Exp. Lung Res.* 3:57–68
137. Adamson, I. Y. R., Bowden, D. H., Côté, M. G., Witschi, H. 1977. Lung injury induced by butylated hydroxytoluene. Cytodynamic and biochemical studies in mice. *Lab Invest.* 36:26–32
138. Aldridge, W. N., Nemery, B. 1984. Toxicology of trialkylphosphorothioates with particular reference to lung toxicity. *Fund. Appl. Toxicol.* 4:S215–23
139. Sivarajah, K., Jones, K. G., Fouts, J. R., Devereux, T., Shirley, J. E., et al. 1983. Prostaglandin synthetase and cytochrome P-450-dependent metabolism of (±)benzo(α)pyrene 7,8-dihydrodiol by enriched populations of rat Clara cells and alveolar type II cells. *Cancer Res.* 43:2632–36
140. Stowers, S. J., Anderson, M. W. 1984. Ubiquitous binding of benzo(a)pyrene metabolites to DNA and protein in tissues of the mouse and rabbit. *Chem.-Biol. Interact.* 51:151–66
141. Horton, J. K., Rosenoir, J., White, C., Brier, D., Bend, J. R. et al. 1984. Quantitation of metabolite-DNA adducts in selected pulmonary and hepatic cell types isolated from rabbits treated with benzo(a)pyrene. *Pharmacologist* 26:204 (Abstr.)
142. Smith, L. L., Wyatt, I. 1981. The accumulation of putrescine into slices of rat lung and brain and its relationship to the accumulation of paraquat. *Biochem. Pharmacol.* 30:1053–58
143. Smith, L. L., Wyatt, I., Cohen, G. M. 1982. The accumulation of diamines and polyamines into rat lung slices. *Biochem. Pharmacol.* 31:3029–33
144. Ross, J. H., Krieger, R. I. 1981. Structure-activity correlations of amines inhibiting active uptake of paraquat (methyl viologen) into rat lung slices. *Toxicol. Appl. Pharmacol.* 59:238–49
145. Brooke-Taylor, S., Smith, L. L., Cohen, G. M. 1983. The accumulation of polyamines and paraquat by human peripheral lung. *Biochem. Pharmacol.* 32:717–20
146. Charles, J. M., Abou-Donia, M. B., Menzel, D. B. 1978. Absorption of paraquat and diquat from the airways of the perfused rat lung. *Toxicol.* 9:59–67
147. Forman, H. J., Aldrich, T. K., Posner, M. A., Fisher, A. B. 1982. Differential paraquat uptake and redox kinetics of rat granular pneumocytes and alveolar mac-

rophages. *J. Pharmacol. Exp. Ther.* 221:428–33
148. Drew, R., Siddik, Z., Gram, T. E. 1979. The uptake and efflux of ^{14}C-paraquat by rat lung slices: The effect of imipramine and other drugs. *Toxicol. Appl. Pharmacol.* 49:473–78
149. Chi, C. H., Dixit, B. N. 1977. Characterization of (±)-methadone uptake by rat lung. *Br. J. Pharmacol.* 59:539–49
150. Enna, S. J., Schanker, L. S. 1973. Phenol red absorption from the rat lung: Evidence of carrier transport. *Life Sci.* 12:231–39
151. Gardiner, T. H., Schanker, L. S. 1974. Absorption of disodium cromoglycate from the rat lung: Evidence of carrier transport. *Xenobiotica* 4:725–31
152. Gardiner, T. H., Schanker, L. S. 1976. Active transport of phenol red by rat lung slices. *J. Pharmacol. Exp. Ther.* 196:455–62
153. Lin, Y.-J., Schanker, L. S. 1981. Pulmonary absorption of amino acids in the rat: Evidence of carrier transport. *Am. J. Physiol.* 240:C215–21
154. Lin, Y.-J., Schanker, L. S. 1983. Pulmonary absorption and lung slice uptake of a foreign amino acid—species comparison. *Drug Metab. Disp.* 11:75–76
155. Hemberger, J. A., Schanker, L. S. 1983. Mechanism of pulmonary absorption of quaternary ammonium compounds in the rat. *Drug Metab. Disp.* 11:73–74
156. Collins, J. N., Dedrick, R. L. 1982. Contribution of lungs to total body clearance: Linear and nonlinear effects. *J. Pharm Sci.* 71:66–70
157. Gillette, J. R. 1982. Sequential organ first-pass effects: Simple methods for constructing compartmental pharmacokinetic models from physiological models of drug disposition by several organs. *J. Pharm. Sci.* 71:673–77
158. Rowland, M., Benet, L. Z., Graham, G. G. 1973. Clearance concepts in pharmacokinetics. *J. Pharmacokinet. Biopharm.* 1:123–36
159. Rane, A., Wilkinson, G. R., Shand, D. G. 1977. Prediction of hepatic extraction ratio from in vitro measurement of intrinsic clearance. *J. Pharmacol. Exp. Ther.* 200:420–24
160. Wiersma, D. A., Roth, R. A. 1980. Clearance of 5-hydroxytryptamine by rat lung and liver: The importance of relative perfusion and intrinsic clearance. *J. Pharmacol. Exp. Ther.* 212:97–102
161. Iwasawa, Y., Gillis, C. N. 1974. Pharmacological analysis of norepinephrine and 5-hydroxytryptamine removal from the pulmonary circulation: Differentiation of uptake sites for each amine. *J. Pharmacol. Exp. Ther.* 188:386–93
162. Junod, A. F. 1975. Metabolism, production and release of hormones and mediators in the lung. *Am. Rev. Respir. Dis.* 112:93–108
163. Alabaster, V. A., Bakhle, Y. S. 1970. Removal of 5-hydroxytryptamine in the pulmonary circulation of rat isolated lungs. *Br. J. Pharmacol.* 40:468–82
164. Wiersma, D. A., Roth, R. A. 1983. The prediction of benzo[α]pyrene clearance by rat liver and lung from enzyme kinetic data. *Mol. Pharmacol.* 24:300–8
165. Wiersma, D. A., Roth, R. A. 1983. Total body clearance of circulating benzo(α)pyrene in conscious rats: Effect of pretreatment with 3-methylcholanthrene and the role of liver and lung. *J. Pharmacol. Exp. Ther.* 226:661–67
166. Smith, B. R., Bend, J. R. 1980. Prediction of pulmonary benzo(α)pyrene 4,5-oxide clearance: A pharmacokinetic analysis of epoxide-metabolizing enzymes in rabbit lung. *J. Pharmacol. Exp. Ther.* 214:478–82
167. Dutcher, J. S., Boyd, M. R. 1979. Species and strain differences in target organ alkylation and toxicity by 4-ipomeanol: Predictive value of covalent binding in studies of target organ toxicities by reactive metabolites. *Biochem. Pharmacol.* 28:3367–72
168. Boyd, M. R. 1980. Biochemical mechanisms in pulmonary toxicity of furan derivatives. In *Reviews in Biochemical Toxicology*, ed. E. Hodgson, J. Bend, R. Philpot, pp. 71–102. New York: Elsevier/North Holland. 300 pp.
169. Wolf, C. R., Statham, C. N., McMenamin, M. K., Bend, J. R., Boyd, M. R., Philpot, R. M. 1982. The relationship between the catalytic activities of rabbit pulmonary cytochrome P-450 isozymes and the lung-specific toxicity of the furan derivative, 4-ipomeanol. *Mol. Pharmacol.* 22:738–44
170. Slaughter, S. R., Statham, C. N., Philpot, R. M., Boyd, M. R. 1983. Covalent binding of metabolites of 4-ipomeanol to rabbit pulmonary and hepatic microsomal monooxygenase system. *J. Pharmacol. Exp. Ther.* 224:252–57