Initially, lung injury caused by chemicals was primarily associated with certain professions and occupations. In his classic treatise of 1713, the Italian physician Bernardino Ramazzini provided detailed and harrowing accounts of the sufferings of miners, whose ailments had been known and described since antiquity. Two of Ramazzini’s quotations are noteworthy. With regard to miners of metals, he stated that “the lungs and brains of that class of workers are badly affected, the lungs especially, since they take in with the air mineral spirits and are the first to be keenly aware of injury.” Ramazzini also was aware of the important concept of exposure: “They (workers who shovel, melt, and cast and refine mined material) are liable of the same diseases, though in less acute form, because they perform their tasks in open air.” Thus, exposure to chemicals by inhalation can have two effects: on the lung tissues and on distant organs that are reached after chemicals enter the body by means of inhalation. Indeed, the term inhalation toxicology refers to target-organ toxicity, in this case abnormal changes in the respiratory tract produced by airborne (and on occasion blood-borne) agents. We now know of numerous lung diseases prompted by occupational exposures, many crippling and some fatal. Examples include black lung in coal miners, silicosis and silicotuberculosis in sandblasters and tunnel miners, and asbestosis in shipyard workers and asbestos miners. Occupational exposures to asbestos or metals such as nickel, beryllium, and cadmium can also cause lung cancer. In the twentieth century, it has become obvious that disease caused by airborne agents may not be limited to certain trades. The ubiquitous presence of airborne chemicals is a matter of concern, since “air pollution” adversely affects human health and may be an important contributor to mortality (Zemp et al., 1999).

To better understand environmental lung disease, we need more precise knowledge about the doses of toxic inhalants delivered to specific sites in the respiratory tract and an understanding
of the extent to which repeated and often intermittent low-level exposures eventually may initiate and propagate chronic lung disease. Lung tissue can be injured directly or secondarily by metabolic products from organic compounds. However, the most important effect of many toxic inhalants is to place an undue oxidative burden on the lungs. Observations made in humans and animals provide strong evidence that the sequelae of oxidative stress may be instrumental in initiating and propagating ailments such as chronic bronchitis, emphysema, interstitial disorders (fibrosis), and cancer (Crapo et al., 1992).

Respiratory tract toxicology is a field in which collaboration involving epidemiologists, physiologists studying human lung function, toxicologists, and cell and molecular biologists has become close and fruitful. Epidemiologists now use a variety of pulmonary function tests to assess decrements in lung function in workers and populations exposed to air pollutants. These tests have been adapted for animal studies and are used to examine the mechanisms responsible for the pulmonary effects of air pollutants. When similar data can be obtained in both experimental animals and human subjects (for example, studies of mucociliary clearance of particles or responsiveness to bronchoconstrictive agents), these direct comparisons assist in extrapolating from animals to humans. Progress has been made in understanding some of the mechanisms that underlie the response of the lungs to toxic agents. In response to toxic insult, pulmonary cells are known to release a variety of potent chemical mediators that may critically affect lung function. Biochemical data from the study of cells taken from exposed animals and in vitro exposure of cells in culture are also useful in assessing the toxic potential of many agents. Recently, molecular techniques, such as in situ hybridization and immunohistostains, have been applied to analyze production of chemical mediators and other important macromolecules produced by specific cell types in response to inhaled toxicants. Bronchoalveolar lavage is now widely exploited in experimental animals and human subjects to examine respiratory airways’ contents (cellular and acellular) after exposure. This chapter discusses how pulmonary toxicologists profit from these methods to study the biochemical, structural, and functional changes produced by the inhalation of pollutant gases and particles.

LUNG STRUCTURE AND FUNCTION

Nasal Passages

Figure 15-1 shows a schematic overview of the different regions of the respiratory tract. Air enters the respiratory tract through the nasal and oral regions. Many species, particularly small laboratory rodents, are obligatory nose breathers, in whom air passes almost exclusively through the nasal passages. Other species, including humans, monkeys, and dogs, can inhale air both through the nose and through the mouth (oronasal breathers). Air is warmed and humidified while passing through the nose. The nasal passages function as a filter for particles, which may be collected by diffusion or impaction on the nasal mucosa. Highly water-soluble gases are absorbed efficiently in the nasal passages, which reach from the nostril to the pharynx. The nasal turbinate thus form a first defensive barrier against many toxic inhalants.

The nasal passages are lined by distinctive epithelia: stratified squamous epithelium in the vestibule, nonciliated cuboidal/columnar epithelium in the anterior chamber, ciliated pseudostratified respiratory epithelium, and olfactory epithelium. The greater part of the internal nasal passages is covered by respiratory epithelium containing goblet cells, ciliated cells, nonciliated columnar cells, cuboidal cells, brush cells, and basal cells. Located in the superior part is the olfactory epithelium, which contains sensory cells. Nerve endings in the nasal passages are associated mostly with the fifth cranial (trigeminal) nerve.

Nasal epithelia are competent to metabolize foreign compounds (Fanucchi et al., 1999). Nasal tissue has been found to activate nitrosamines to mutagenic compounds. P-450 isozymes 1A1, 2B1, and 4B1 have been localized in the nose of several species by immunohistochemical procedures. The nasal cavity is thus a ready target site for metabolite-induced lesions. The olfactory epithelium appears to be particularly vulnerable. Metabolism by the olfactory epithelium may play a role in providing or preventing access of inhalants directly to the brain; for example, inhaled xylene may be converted to metabolites that move to the brain by axonal transport.

Conducting Airways

The proximal airways—the trachea and bronchi—have a pseudostratified epithelium containing ciliated cells and two types of nonciliated cells: mucous and serous cells. Mucous cells (and glandular structures) produce respiratory tract mucus, a family of high-molecular-weight glycoproteins with a sugar content of 80 percent or more that coat the epithelium with a viscoelastic sticky protective layer that traps pollutants and cell debris. Serous cells produce a fluid in which mucus may be dissolved. The action of the respiratory tract cilia, which beat in synchrony under the control of the central nervous system (CNS), continuously drives the mucous layer toward the pharynx, where it is removed from the respiratory system by swallowing or expectoration. The mucous layer is also thought to have antioxidant, acid-neutralizing, and free radical–scavenging functions that protect the epithelial cells (Cross et al., 1998).

Conducting airways have a characteristic branched bifurcating structure, with successive airway generations containing approximately twice the number of bronchi with a progressively decreasing internal diameter. Thus, the conducting airways contain a continuously increasing total surface area from the trachea to the distal airways. Bifurcations have flow dividers at branch points that serve as sites of impaction for particles, and successively narrower diameters also favor the collection of gases and particles on airway walls. Eventually a transition zone is reached where cartilaginous airways (bronchi) give way to noncartilaginous airways (bronchioles), which in turn give way to gas-exchange regions, respiratory bronchioles, and alveoli. Mucus-producing cells and glands give way to Clara cells in the bronchiolar epithelium. There are important structural and cellular differences between the conductive airways of humans and these of many commonly studied laboratory animals, as discussed later in this chapter.

Gas-Exchange Region

Human lungs are divided into five lobes: the superior and inferior left lobes and the superior, middle, and inferior right lobes. In small laboratory animals such as rats, mice, and hamsters, the left lung consists of a single lobe, whereas the right lung is divided into four lobes: cranial, middle, caudal, and ancillary. In the guinea pig and
rabbit, the left lung is divided into two lobes. Dogs have two left and four right lobes. The lung can be further subdivided at the periphery of the bronchial tree into distinct anatomic bronchopulmonary segments, then into lobules, and finally into acini. An acinus includes a terminal bronchiole and all its respiratory bronchioles, alveolar ducts, and alveolar sacs. An acinus may be made up of two to eight ventilatory units. A ventilatory unit is defined as an anatomic region that includes all alveolar ducts and alveoli distal to each bronchiolar-alveolar duct junction (Mercer and Crapo, 1991). The ventilatory unit is important because it represents the smallest common denominator when the distribution of inhaled gases to the gas-exchanging surface of the lung is modeled (Fig. 15-2).

Gas exchange occurs in the alveoli, which represent approximately 80 to 90 percent of the total parenchymal lung volume; adult human lungs contain an estimated 300 million alveoli. The ratio of total capillary surface to total alveolar surface is slightly less than 1. Within the alveolar septum, capillaries are organized
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in a single sheet. Capillaries, blood plasma, and formed blood elements are separated from the air space by a thin layer of tissue formed by epithelial, interstitial, and endothelial components (Pinkerton et al., 1991).

Type I and type II alveolar cells represent approximately 25 percent of all the cells in the alveolar septum (Fig. 15-3). Type III epithelial cells, also called brush cells, are relatively rare. Type I cells cover a large surface area (approximately 90 percent of the alveolar surface). They have an attenuated cytoplasm and appear to be poor in organelles but probably are as metabolically competent as are the more compact type II cells. Preferential damage to type I cells by various agents may be explained by the fact that they constitute a large percentage of the total target (surface of the epithelium). Type II cells are cuboidal and show abundant perinuclear cytoplasm. They produce surfactant and, in case of damage to the type I epithelium, may undergo mitotic division and replace damaged cells. The shape of type I and type II cells is independent of alveolar size and is remarkably similar in different species. A typical rat alveolus (14,000 \( \mu \text{m}^2 \) surface) contains an average of two type I cells and three type II cells, whereas a human alveolus with a surface of 200,000 to 300,000 \( \mu \text{m}^2 \) contains 32 type I cells and 51 type II cells (Pinkerton et al., 1991).

The mesenchymal interstitial cell population consists of fibroblasts that produce collagen and elastin, as well as other cell matrix components and various effector molecules. Pericytes, monocytes, and lymphocytes also reside in the interstitium and so do macrophages before they enter the alveoli. Endothelial cells have a thin cytoplasm and cover about one-fourth of the area covered by type I cells. Clara cells are located in the terminal bronchioles and have a high content of xenobiotic metabolizing enzymes.

Gas Exchange

The principal function of the lung is gas exchange, which consists of ventilation, perfusion, and diffusion. The lung is superbly equipped to handle its main task: bringing essential oxygen to the organs and tissues of the body and eliminating its most abundant waste product, CO2 (Weibel, 1983).

Ventilation During inhalation, fresh air is moved into the lung through the upper respiratory tract and conducting airways and into the terminal respiratory units when the thoracic cage enlarges and the diaphragm moves downward; the lung passively follows this expansion. After diffusion of oxygen into the blood and that of CO2 from the blood into the alveolar spaces, the air (now enriched in CO2) is expelled by exhalation. Relaxation of the chest wall and diaphragm diminishes the internal volume of the thoracic cage, the elastic fibers of the lung parenchyma contract, and air is expelled from the alveolar zone through the airways. Any interference with the elastic properties of the lung, for example, the decrease in elastic fibers that occurs in emphysema, adversely affects ventilation, as do decreases in the diameters of or blockage of the conducting airways, as in asthma.

The total volume of air in an inflated human lung, approximately 5700 cm\(^3\), represents the total lung capacity (TLC). After
a maximum expiration, the lung retains approximately 1200 cm$^3$ of air, the residual volume (RV). The air volume moved into and out of the lung with a maximum inspiratory and expiratory movement, which is called the vital capacity (VC), is thus approximately 4500 cm$^3$. Under resting conditions, only a fraction of the vital capacity, the tidal volume (TV), is moved into and out of the lung. In resting humans, the TV measures approximately 500 cm$^3$ with each breath (Fig. 15-4). The respiratory frequency, or the number of breaths per minute, is approximately 12 to 20. If an augmented metabolic demand of the body requires the delivery of increased amounts of oxygen—for example, during heavy and prolonged exercise—both the TV and the respiratory rate can be greatly increased. The amount of air moved into and out of the human lung may increase to up to 60 L/min. Increased ventilation in a polluted atmosphere increases the deposition of inhaled toxic material. For this reason, it is often stated that people, particularly children, should not exercise during episodes of heavy air pollution.

The TLC, as well as the ratio of RV to VC, changes when the lung is diseased. In emphysema, the alveoli overextend and more air is trapped. While the TLC may stay the same or even increase, the volume of air that is actually moved during breathing is diminished. This results in decreased VC with a concomitant increase in RV. If part of the lung collapses or becomes filled with edema fluid, TLC and VC are reduced. Pulmonary function tests give quantitative information on such changes.

**Perfusion** The lung receives the entire output from the right ventricle, approximately 70 to 80 cm$^3$ of blood per heartbeat, and thus may be exposed to substantial amounts of toxic agents carried in the blood. An agent placed onto or deposited under the skin (subcutaneous injection) or introduced directly into a peripheral vein (intravenous injection) travels through the venous system to the right ventricle and then comes into contact with the pulmonary capillary bed before distribution to other organs or tissues in the body.

**Diffusion** Gas exchange takes place across the entire alveolar surface. Contact to airborne toxic agent thus occurs over a surface (approximately 140 m$^2$) that is second only to the small intestine (approximately 250 m$^2$) and considerably larger than the skin (approximately 1.75 m$^2$), two other organs that are in direct contact with the outside world. A variety of abnormal processes may thicken the alveolar septum and adversely affect the diffusion of oxygen to the erythrocytes. Such processes may include collection of liquid in the alveolar space and an abnormal thickening of the pulmonary epithelium. It is often seen as a result of chronic toxicity because of an abnormal accumulation of tissue constituents in the interstitial space through proliferation of interstitial cells. Increased formation and deposition of extracellular substances such as collagen or because of the interstitial accumulation of edema fluid has similar consequences.

**GENERAL PRINCIPLES IN THE PATHOGENESIS OF LUNG DAMAGE CAUSED BY CHEMICALS**

**Oxidative Burden**

An important type of injury to the lung is thought to be caused by an undue oxidative burden that often is mediated by free radicals, such as those generated by ozone, NO$_2$, tobacco smoke, and lung defense cells (Witschi, 1997a). Evidence for the role of free radicals in lung damage includes a wide variety of observations. Numerous studies have reported increases in the activity of free radical–scavenging enzymes in the lungs of animals exposed to O$_3$, NO$_2$, and other toxicants, indirectly supporting this hypothesis. Treatment with various hydroxyl radical scavengers can protect rats from pulmonary edema induced by high doses of thiourrea and otherwise lethal levels of gamma irradiation.

Theories of lung oxidant toxicity relate to the formation of reactive and unstable free radicals, with subsequent chain reactions leading to uncontrolled destructive oxidation. Recent work has emphasized the pivotal roles of superoxide, nitric oxide, peroxy-nitrate, hydroxyl radicals, and perhaps singlet oxygen in mediating tissue damage. Reduction of O$_2$ to active O$_2$ metabolites normally occurs as a by-product of cellular metabolism during both microsomal and mitochondrial electron transfer reactions; considerable amounts of superoxide anion are generated by NADPH cytochrome P450 reductase reactions. Because these oxidant species are potentially cytotoxic, they may mediate or promote the actions of various pneumotoxins. Such mechanisms have been proposed for paraquat- and nitrofurantoin-induced lung injury. When cellular injury of any type occurs, the release of otherwise contained cellular constituents such as microsomes and flavoproteins into the extracellular space may lead to extracellular generation of deleterious reactive O$_2$ species.

Among mammalian cells, neutrophils, monocytes, and macrophages seem particularly adept at converting molecular O$_2$ to reactive O$_2$ metabolites; this probably is related to their phagocytosis and antimicrobial activities. As a by-product of this capability, toxic O$_2$ species are released (possibly by the plasmalemma itself) into surrounding tissues. As most forms of toxic pulmonary edema are accompanied by phagocyte accumulation in the lung microcirculation (pulmonary leukostasis) and parenchyma, oxidative damage may represent a significant component of all types of pneumotoxic lung injury accompanied by a phagocyte-mediated inflammatory component.

Chemotactic and phagocytic “activation” processes result in a substantial increase in the release of potent oxidants by stimulated phagocytes; these radicals cause oxidative damage to the sur-
rounding tissues. A key role of hydrogen peroxide as the mediator of the extracellular cytotoxic mechanism of “activated” phagocytes has been well documented. Phenomena occurring at the phagocyte surface, such as those which may occur in endogenous lung phagocytes after exposure to dusts and toxic gases, or in circulating phagocytes before their accumulation in the lung or after their attachment to normal or damaged lung endothelium seem to be important in determining their degree of enhanced oxidative activity, which is otherwise at a much lower basal level in the unstimulated cell. It also has been long appreciated that phagocytes may cause lysosomal enzyme release and tissue damage.

The fact that oxidative processes are complex is suggested by the finding that phagocytic production of active oxygen species causes inactivation of proteinase inhibitors and degranulation of mast cells. The production of oxygen radicals by phagocytes is enhanced not only by interactions of cell surface membranes with various appropriate stimuli but also by hyperoxia. Platelets (and platelet microthrombi) also have the ability to generate activated O₂ species.

The lung can respond with specific defense mechanisms that may be acquired over time and may be stimulated by constant exposure to numerous species of airborne microorganisms as well as by a variety of low- and high-molecular-weight antigenic materials. The immune system can mount either cellular or humorally mediated responses to these inhaled antigens. Direct immunologic effects occur when inhaled foreign material sensitizes the respiratory system to further exposure to the same material. The mammalian lung has a well-developed immune system. Lymphocytes reside in the hilar or mediastinal lymph nodes, lymphoid aggregates, and lymphoepithelial nodules as well as in aggregates or as single cells throughout the airways. Bronchoconstriction and chronic pulmonary disease can result from the inhalation of materials that appear to act wholly or partly through an allergic response. In some instances, these reactions are caused by spores of molds or bacterial contaminants. Frequently, chemical components of the sensitizing dusts or gases are responsible for the allergic response. Low-molecular-weight compounds can act as haptenics that combine with native proteins to form a complex that is recognized as an antigen by the immune system. Further exposure to the sensitizing compound can result in an allergic reaction that is characterized by the release of various inflammatory mediators that produce an early and/or a late bronchoconstrictor response. Such a response is observed in sensitized workers exposed to toluene diisocyanate (TDI), a chemical widely used in the manufacture of polyurethane plastics (Karol et al., 1994).

Indirect immune effects occur when exposure to air pollutants either suppresses or enhances the immune response to other materials. Both sulfur dioxide (SO₂) and ozone can boost the response of the respiratory system to inhaled foreign material, at least in experimental animals (guinea pigs). It is not known whether these effects occur in humans, but they form the bases for concerns about increased susceptibility of asthmatic individuals to air pollutants such as ozone and sulfur dioxide.

**Toxic Inhalants, Gases, and Dosimetry**

The sites of deposition of gases in the respiratory tract define the pattern of toxicity of those gases. Water solubility is the critical factor in determining how deeply a given gas penetrates into the lung. Highly soluble gases such as SO₂ do not penetrate farther than the nose and are therefore relatively nontoxic to animals, especially obligatory nose breathers such as the rat. Relatively insoluble gases such as ozone and NO₂ penetrate deeply into the lung and reach the smallest airways and the alveoli (centriacinar region), where they can elicit toxic responses. Mathematical models of gas entry and deposition in the lung that are based solely on the aqueous solubility of a gas predict sites of lung lesions fairly accurately. These models may be useful for extrapolating findings made in laboratory animals to humans (Kimbell and Miller, 1999; Medinsky et al., 1999). Very insoluble gases such as CO and H₂S efficiently pass through the respiratory tract and are taken up by the pulmonary blood supply to be distributed throughout the body.

**Particle Deposition and Clearance**

The site of deposition of solid particles or droplets in the respiratory tract, along with their chemical makeup, is important. Particle size is usually the critical factor that determines the region of the respiratory tract in which a particle or an aerosol will be deposited. Deposition of particles on the surface of the lung and airways is brought about by a combination of lung anatomy and the patterns of airflow in the respiratory system (Raabe, 1999; Miller, 1999) (Fig. 15–5).

**Particle Size** Inhaled aerosols are most frequently polydisperse in regard to size. The size distribution of many aerosols approximates a log-normal distribution that may be described by the median or geometric mean and the geometric standard deviation. A plot of the frequency of occurrence of a given size against the log of the size produces a bell-shaped probability curve. Particle data frequently are handled by plotting the cumulative percentage of particles smaller than a stated size on log-probability paper. This results in a straight line that may be fitted by eye or mathematically. In actual practice, it is not unusual to have some deviation.
from a straight line at the largest or smallest particle sizes measured. The geometric mean is the 50 percent size as the mean bisects the curve. The geometric standard deviation ($\sigma_g$) is calculated as

$$\sigma_g = \frac{84.1\% \text{ size}}{50\% \text{ size}}$$

The $\sigma_g$ of the particle size distribution is a measure of the polydispersity of the aerosol. In the laboratory, values for $\sigma_g$ of 1.8 to 3.0 are also encountered frequently. In the field, values for $\sigma_g$ may range up to 4.5. An aerosol with a $\sigma_g$ below 1.2 may be considered monodisperse.

The median diameter that is determined may reflect the number of particles, as in the count median diameter (CMD), or reflect mass, as in the mass median aerodynamic diameter (MMAD). The larger the number and mass of particles capable of penetrating the lung, the greater the probability of a toxic effect. The size distribution in relation to other factors, such as particle shape and surface area, also may be of interest. Surface area is of special importance when toxic materials are adsorbed on the surfaces of particles and thus are carried to the lung.

Particles that are nonspherical in shape are frequently characterized in terms of equivalent spheres on the basis of equal mass, volume, or aerodynamic drag. The MMAD takes into account both the density of the particle and aerodynamic drag. It represents the diameter of a unit density sphere with the same terminal settling velocity as the particle, regardless of its size, shape, and density. Aerodynamic diameter is the proper measurement for particles that are deposited by impaction and sedimentation. For very small particles, which are deposited primarily by diffusion, the critical factor is particle size, not density. It must be kept in mind that the size of a particle may change before its deposition in the respiratory tract. Materials that are hygroscopic, such as sodium chloride, sulfuric acid, and glycerol, take on water and grow in size in the warm, saturated atmosphere of the lower respiratory tract.

Deposition Mechanisms Deposition of particles occurs primarily by interception, impaction, sedimentation, and diffusion (Brownian movement). Interception occurs only when the trajectory of a particle brings it near enough to a surface so that an edge of the particle contacts the airway surface. Interception is important for the deposition of fibers. Whereas fiber diameter determines the probability of deposition by impaction and sedimentation, interception is dependent on fiber length. Thus, a fiber with a diameter of 1 $\mu$m and a length of 200 $\mu$m will be deposited in the bronchial tree primarily by interception rather than impaction.

As a result of inertia, particles suspended in air tend to continue to travel along their original path. In a bending airstream, such as at an airway bifurcation, a particle may be impacted on the surface. At relatively symmetrical bifurcations, which typically occur in the human lung, the deposition rate is likely to be high for particles that move in the center of the airway. Generalizations regarding the site of deposition of particles of a given size are problematic. However, in the average adult, most particles larger than 10 $\mu$m in aerodynamic diameter are deposited in the nose or oral pharynx and cannot penetrate to tissues distal to the larynx. Recent data have shown that very fine particles (0.01 $\mu$m and smaller) are also trapped relatively efficiently in the upper airways by diffusion. Particles that penetrate beyond the upper airways are available to be deposited in the bronchial region and the deeper-lying airways. Therefore, the alveolar region has significant deposition efficiencies for particles smaller than 5 $\mu$m and larger than 0.003 $\mu$m.

Sedimentation brings about deposition in the smaller bronchi, the bronchioles, and the alveolar spaces, where the airways are small and the velocity of airflow is low. As a particle moves downward through air, buoyancy and the resistance of air act on the particle in an upward direction while gravitational force acts on the particle in a downward direction. Eventually, the gravitational force equilibrates with the sum of the buoyancy and the air resistance, and the particle continues to settle with a constant velocity known as the terminal settling velocity. Sedimentation is not a significant route of particle deposition when the aerodynamic diameter is below 0.5 $\mu$m.

Diffusion is an important factor in the deposition of submicrometer particles. A random motion is imparted to these particles by the impact of gas molecules. This Brownian motion increases with decreasing particle size, and so diffusion is an important deposition mechanism in the nose and in other airways and alveoli for particles smaller than about 0.5 $\mu$m.

An important factor in particle deposition is the pattern of breathing. During quiet breathing, in which the TV is only two to three times the volume of the anatomic dead space (i.e., the volume of the conducting airways where gas exchange does not occur), a large proportion of the inhaled particles may be exhaled. During exercise, when larger volumes are inhaled at higher velocities, impaction in the large airways and sedimentation and diffusion in the smaller airways and alveoli increase. Breath holding also increases deposition from sedimentation and diffusion. Factors that modify the diameter of the conducting airways can alter particle deposition. In patients with chronic bronchitis, the mucociliary layer is greatly thickened and extended peripherally and may partially block the airways in some areas. Jets formed by air flowing through such partially occluded airways have the potential to increase the deposition of particles by impaction and diffusion in the small airways. Irritant materials that produce bronchoconstriction tend to increase the tracheobronchial deposition of particles. Cigarette smoking has been shown experimentally to produce such an effect.

Particle Clearance The clearance of deposited particles is an important aspect of lung defense. Rapid removal lessens the time available to cause damage to the pulmonary tissues or permit local absorption. The specific mechanisms available for the removal of particles from the respiratory tract vary with the site of the deposition. It is important to emphasize that clearance of particles from the respiratory tract is not synonymous with clearance from the body. Depending on the specific clearance mechanism used, particles are cleared to (1) the stomach and gastrointestinal (GI) tract; (2) the lymphatics and lymph nodes, where they may be dissolved and enter the venous circulation; or (3) the pulmonary vasculature. The only mechanisms by which the respiratory system can truly remove deposited particles from the body are coughing and blowing the nose.

Nasal Clearance Particles deposited in the nose are cleared by various mechanisms, depending on their site of deposition and solubility in mucus. The anterior portion of the nose is lined with relatively dry squamous epithelium, and so particles deposited there are removed by extrinsic actions such as wiping and blowing. The other regions of the nose are largely covered by a mucociliary epithelium that propels mucus toward the glottis, where it is swallowed. Insoluble particles generally are cleared from this region in
healthy adults and swallowed within an hour of deposition. Particles that are soluble in mucus may dissolve and enter the epithelium and/or blood before they can be mechanically removed. Uncertainties still remain about the clearance of particles that are deposited on olfactory regions or areas that are damaged by acute infection, chronic illnesses, or toxic injury.

**Tracheobronchial Clearance** The mucous layer covering the tracheobronchial tree is moved upward by the beating of the underlying cilia. This mucociliary escalator transports deposited particles and particle-laden macrophages upward to the oropharynx, where they are swallowed and pass through the GI tract. Mucociliary clearance is relatively rapid in healthy individuals and is completed within 24 to 48 h for particles deposited in the lower airways. Infection and other injuries can greatly impair clearance.

**Pulmonary Clearance** There are several primary ways by which particulate material is removed from the lower respiratory tract once it has been deposited:

1. Particles may be directly trapped on the fluid layer of the conducting airways by impaction and cleared upward, in the tracheobronchial tree via the mucociliary escalator.
2. Particles may be phagocytized by macrophages and cleared via the mucociliary escalator.
3. Particles may be phagocytized by alveolar macrophages and removed via the lymphatic drainage.
4. Material may dissolve from the surfaces of particles and be removed via the bloodstream or lymphatics.
5. Small particles may directly penetrate epithelial membranes.

Minutes after particles are inhaled, they may be found in alveolar macrophages. Many alveolar macrophages are ultimately transported to the mucociliary escalator. It is possible that macrophages are carried to the bronchioles with the alveolar fluid that contributes to the fluid layer in the airways. Other particles may be sequestered in the lung for very long periods, often in macrophages located in the interstitium.

### ACUTE RESPONSES OF THE LUNG TO INJURY

**Airway Reactivity**

Large airways are surrounded by bronchial smooth muscles, which help maintain airway tone and diameter during expansion and contraction of the lung. Bronchial smooth muscle tone is normally regulated by the autonomic nervous system. Reflex contraction occurs when receptors in the trachea and large bronchi are stimulated by irritants such as cigarette smoke and air pollutants. Bronchoconstriction can be provoked by cholinergic drugs such as acetylcholine, a phenomenon that serves as the basis for a sensitive measure of whether a toxicant can cause bronchoconstriction in animals or humans primed by a prior dose of an acetylcholinelike agent (bronchoprovocation testing). These agents bind to cell surface receptors (cholinergic receptors) and trigger an increase in the intracellular concentration of cyclic guanosine monophosphate (cGMP), which in turn facilitates smooth muscle contraction. The actions of cGMP can be antagonized by cyclic adenosine monophosphate (cAMP), which has bronchodilatory activity, and can be increased by agents that bind to beta-adrenergic receptors on the cell surface. Other important mediators of airway smooth muscle tone include histamine, various prostaglandins and leukotrienes, substance P, and nitric oxide. The bronchial smooth muscles of individuals with asthma contract with much less provocation than do those of normal subjects. Bronchoconstriction causes a decrease in airway diameter and a corresponding increase in resistance to airflow. Characteristic associated symptoms include wheezing, coughing, a sensation of chest tightness, and dyspnea. Exercise potentiates these problems. A major cause of concern about ambient air pollution is whether asthmatic individuals represent a population that is particularly susceptible to the adverse health effects of sulfur dioxide, ozone, nitrogen dioxide, other respiratory irritant gases, and respirable particles. Since the major component of airway resistance usually is contributed by large bronchi, inhaled agents that cause reflex bronchoconstriction are generally irritant gases with moderate solubility. Demonstrations of the bronchoconstrictive effects of gases in laboratory animals often are performed in guinea pigs, which seem to represent a natural animal model of asthmatic humans with respect to innate airway reactivity.

**Pulmonary Edema**

Toxic pulmonary edema represents an acute, exudative phase of lung injury that generally produces a thickening of the alveolar-capillary barrier. Edema fluid, when present, alters ventilation-perfusion relationships and limits diffusive transfer of O2 and CO2 even in otherwise structurally normal alveoli. Edema is often a sign of acute lung injury.

The biological consequences of toxic pulmonary edema not only induce acute compromise of lung structure and function but also may include abnormalities that remain after resolution of the edematous process. After exposure to some toxic agents in which the alveolar-capillary surface is denuded (such as alloxan), recovery is unlikely, whereas in situations of more modest injury (such as histamine administration), full recovery is readily achievable. Between these two extremes there are forms of severe lung injury accompanied by amplified inflammatory damage and/or exaggerated restorative-reparative processes (e.g., after paraquat ingestion). In these severe forms, the extensive interstitial and intraalveolar inflammatory exudate resolves via fibrogenesis, an outcome that may be beneficial or damaging to the lung. Accumulation and turnover of inflammatory cells and related immune responses in an edematous lung probably play a role in eliciting both mitogenic activity and fibrogenic responses.

Pulmonary edema is customarily quantified in experimental animals by some form of gravimetric measurement of lung water content. Very commonly, the wet (undesiccated) weight of the whole lung or that of a single lung lobe is determined. This value is often normalized to the weight of the animal from which the lung was taken. Alternatively, some investigators determine lung water content by weighing whole lungs or lung slices before and after complete drying in an oven or desiccator. Commonly used methods for expressing such data include (1) percentage water content [(wet weight − dry weight)/wet weight], (2) percentage dry weight [(100 × (dry weight)/wet weight)], and (3) water content ([milliliters of water]/[dry weight]).

**Mechanisms of Respiratory Tract Injury**

Airborne agents can contact cells lining the respiratory tract from the nostrils to the gas-exchanging region. The sites of interaction...
of toxicants in the respiratory tract have important implications for evaluation of the risk to humans posed by inhalants. For example, rats have much more nasal surface on a per body weight basis than do humans. Measurement of DNA-protein cross-links formed in nasal tissue by the highly reactive gas formaldehyde has demonstrated that rats, which readily develop nasal tumors, have many more DNA cross-links per unit of exposure (concentration of formaldehyde \( \times \) duration of exposure) than do monkeys. Because the breathing pattern of humans resembles that of monkeys more than that of rats, it was concluded that extrapolation of tumor data from rats to humans on the basis of formaldehyde concentration may overestimate doses of formaldehyde to humans. Patterns of animal activity can affect dose to the lung; nocturnally active animals such as rats receive a greater dose per unit of exposure at night than during the day, whereas humans show the opposite diurnal relationships of exposure concentration to dose.

Certain gases and vapors stimulate nerve endings in the nose, particularly those of the trigeminal nerve (Alarie et al., 1998). The result is holding of the breath or changes in breathing patterns, to avoid or reduce further exposure. If continued exposure cannot be avoided, many acidic or alkaline irritants produce cell necrosis and increased permeability of the alveolar walls. Other inhaled agents can be more insidious; inhalation of HCl, NO\(_2\), NH\(_3\), or phosgene may at first produce very little apparent damage in the respiratory tract. The epithelial barrier in the alveolar zone, after a latency period of several hours, begins to leak, flooding the alveoli and producing a delayed pulmonary edema that is often fatal.

A different pathogenetic mechanism is typical of highly reactive molecules such as ozone. It is unlikely that ozone as such can penetrate beyond the layer of fluid covering the cells of the lung. Instead, ozone lesions are propagated by a cascade of secondary reaction products, such as aldehydes and hydroperoxides produced by ozonolysis of fatty acids and other substrates in the lung’s lining fluid, and by reactive oxygen species arising from free radical reactions. Reactive oxygen species also have been implicated in pulmonary bleomycin toxicity, pulmonary oxygen toxicity, paraparotitis, and the development of chronic lesions such as the fibrogenic and carcinogenic effects of asbestos fibers.

Metabolism of foreign compounds can be involved in the pathogenesis of lung injury. The balance of activation and detoxification plays a key role in determining whether a given chemical ultimately will cause damage. The lung contains most of the enzymes involved in xenobiotic metabolism that have been identified in other tissues, such as the liver (Buckpitt et al., 1997). While the overall levels of these enzymes tend to be lower in lung than in liver, they often are highly concentrated in specific cell populations of the respiratory tract. Moreover, their specific content of particular cytochrome P450 isozymes may be much higher in lung. Thus, the turnover of a substrate for a lung P450 may be far more rapid than occurs in liver. Many isozymes of the cytochrome P450 complex have been identified in and isolated from the lungs of rabbits, rats, hamsters and humans. Cytochrome P450 1A1 is present in low amounts in normal rat and rabbit lungs but is highly inducible by polycyclic aromatic hydrocarbons, flavones, and mixtures of polyhalogenated biphenyls. This isozyme also is present in human lungs and is thought to be involved in the metabolic activation of the polycyclic aromatic hydrocarbons that are present in cigarette smoke. By inference, this P450 isozyme may play a role in the pathogenesis of lung cancer. Attempts have been made to use the expression of cytochrome P450 1A1 as a biomarker of exposure and sensitivity to cigarette smoke in humans, although the precise relationships remain unclear. Cytochrome P450 2B1, which is readily inducible in rat liver by phenobarbital, is not inducible in lung tissue. Other isozymes identified in human lung are cytochrome P450 2F1, 4B1, and 3A4. Further microsomal enzymes found in the lung include NADPH cytochrome P450 reductase, epoxide hydrolase, and flavin-containing monooxygenases. Finally, two important cytosolic enzymes involved in lung xenobiotic metabolism are glutathione-S-transferase and glutathione peroxidase. Adult human lungs appear to contain several forms of glutathione-S-transferase.

**Mediators of Lung Toxicity**

Advances in cell culture techniques (Leikauf and Driscoll, 1993) have allowed investigators to examine the role of specific signal molecules in toxicant-induced lung damage; this is a very active area of research. Such studies are often guided by results obtained by analysis of cytokines and other mediators in lung lavage fluid from animals or human volunteers exposed to inhaled toxic agents.

For example, interleukin 1 beta (IL-1\(\beta\)), transforming growth factor beta (TG\(\beta\)) and tumor necrosis factor alpha (TNF-\(\alpha\)) have all been implicated in the cascade of reactions that is thought to be responsible for the pathogenesis of pulmonary fibrosis (Zhang and Phan, 1999). Similarly, several of the nine described members of the interleukin family, especially IL-1, IL-2, IL-5 and IL-8, are thought to be essential components of the lung’s response to epithelial cell injury. Various specific prostaglandins, especially PG\(E_2\), and leukotrienes have been implicated in intracellular signaling pathways in the lung. The roles of cell surface adhesion molecules and their interaction with cell matrix components and with control of inflammatory cell migration (particularly neutrophil influx to the lung) have been studied intensively.

Analysis of normal lung homogenates suggests that the lung contains large amounts of endogenous cytokines and inflammatory mediators, far more than enough for these potent compounds to elicit effects. Thus, these agents must be compartmentalized in a healthy lung to control their potent bioactivity. How these processes are regulated normally, what exactly goes wrong with homeostasis in a damaged lung, the temporal and geographic relationship of different cytokines in the amplification of an initial injurious event, and detailed mechanisms of resolution of lung injury are not well understood and represent the current focus of much research on mechanisms of lung injury by toxic agents. The reader is referred to reviews of these topics (Massague, 1998; Barnes et al., 1998) for more details on specific mediators and toxic agents in this rapidly changing research area.

**Cell Proliferation**

The effects of toxicants on the lung may be reversible or irreversible. Postexposure progression of lung fibrosis has been demonstrated in rats exposed to ozone, mice exposed to cyclophosphamide, and hamsters exposed to bleomycin or bleomycin plus oxygen. The mechanisms for exacerbating lung damage or repairing such damage during a postexposure period in which filtered air alone is inhaled are not obvious. Examination of the time course and cellular components of reepithelialization of the alveolar ducts and walls during the period of postexposure would be especially important in this regard. Research on the postexposure effects of inhaled toxicants is an important area for further study.
The normal adult lung is an organ for which under normal circumstances very few cells appear to die and to be replaced. When damaged by a toxic insult, the lung parenchyma is capable to repair itself in an efficient manner. Type I cell damage is followed by proliferation of type II epithelial cells which eventually transform into new type I cells; in the airways, the Clara cells proliferate and divide following injury. The migration of mobile blood cells such as leukocytes across the pulmonary capillaries into the alveolar lumen may also trigger a mitotic response. Other cells in the alveolar zone, such as capillary endothelial cells, interstitial cells, and alveolar macrophages, also proliferate. The result is a normal looking organ again although on occasion excessive proliferation of fibroblasts may result in lung disease. In general, however, the lung appears to have a high capacity to repair itself and thus to deal with the many toxic insults presented by the environment (Witschi, 1997b).

**CHRONIC RESPONSES OF THE LUNG TO INJURY**

**Fibrosis**

Defined clinically, lung fibrosis refers to the type of interstitial fibrosis that is seen in the later stages of idiopathic pulmonary fibrosis (also called cryptogenic fibrosing alveolitis in the United Kingdom). In this disease, the hallmark of pulmonary fibrosis seen by the pathologist is increased focal staining of collagen fibers in the alveolar interstitium. Fibrotic lungs from humans with acute or chronic pulmonary fibrosis contain increased amounts of collagen as evaluated biochemically, in agreement with the histological findings.

In lungs damaged by toxicants, the response resembles adult or infant respiratory distress syndrome more closely than it resembles chronic interstitial fibrosis. Excess lung collagen is usually observed not only in the alveolar interstitium but also throughout the centriacinar region, including the alveolar ducts and respiratory bronchioles. The relationship between increased collagen deposition around small airways and lung mechanics is not understood either theoretically or empirically.

At least 19 genetically distinct collagen types are known to occur in all mammals, most of which have been found in normal lungs or to be synthesized by isolated lung cells. Two types predominate in the lung, representing about 90 percent or more of the total lung collagen. Type I and type III collagen are major interstitial components and are found in the normal lungs of all mammals in an approximate ratio of 2:1. Type I collagen is the material that stains histologically as “collagen,” whereas type III collagen is appreciated histologically as reticulin. Some types of toxicant-induced pulmonary fibrosis, including that induced by O₃, involve abnormalities in the type of collagen made. For example, there is an increase in type I collagen relative to type III collagen in patients with idiopathic pulmonary fibrosis. Similar shifts have been demonstrated in the lungs of adults and infants dying of acute respiratory distress syndrome. It is not known whether shifts in collagen types, compared with absolute increases in collagen content, account for the increased stiffness of fibrotic lungs. Type III collagen is much more compliant than is type I; thus, an increasing proportion of type I relative to type III collagen may result in a stiffer lung, as is observed in pulmonary fibrosis. Changes in collagen cross-linking in fibrotic lungs also may contribute to the increased stiffness. It is unclear whether the observed increase in

**Emphysema**

In many ways emphysema can be viewed as the opposite of fibrosis in terms of the response of the lungs to an insult: the lungs become larger and too compliant rather than becoming smaller and stiffer. Destruction of the gas-exchanging surface area results in a distended, hyperinflated lung that no longer effectively exchanges oxygen and carbon dioxide as a result of both loss of tissue and air trapping. The currently accepted pathological definition of emphysema is “a condition of the lung characterized by abnormal enlargement of the airspaces distal to the terminal bronchiole, accompanied by destruction of the walls, without obvious fibrosis” (Snider et al., 1985). The major cause of human emphysema is, by far, cigarette smoke inhalation, although other toxicants also can elicit this response. A feature of toxicant-induced emphysema is severe or recurrent inflammation, especially alveolitis with release of proteolytic enzymes by participating leukocytes.

A unifying hypothesis that explains the pathogenesis of emphysema has emerged from studies by several investigators. Early clinical research on screening blood protein phenotypes identified a rare mutation giving rise to a hereditary deficiency of the serum globulin alpha₁-antitrypsin. Homozygotes for this mutation had no circulating levels of this protein, which can prevent the proteolytic activity of serine proteases such as trypsin. Thus, alpha₁-antitrypsin (now called alpha₁-antiprotease) is one of the body’s main defenses against uncontrolled proteolytic digestion by this class of enzymes, which includes elastase. There is a clinical association between the genetic lack of this important inhibitor of elastase and the development of emphysema at an extraordinarily young age. Further studies in smokers led to the hypothesis that neutrophil (and perhaps alveolar macrophage) elastases can break down lung elastin and thus cause emphysema; these elastases usually are kept in check by alpha₁-antiprotease that diffuses into the lung from the blood. As the individual ages, an accumulation of random elastolytic events can cause the emphysematous changes in the lungs that are normally associated with aging. Toxicants that cause inflammatory cell influx and thus increase the burden of neutrophil elastase can accelerate this process. In accordance with this hy-
Asthma

Asthma is becoming increasingly prevalent in the United States and Europe, especially in crowded urban areas. This disease is characterized clinically by attacks of shortness of breath, which may be mild or severe. It is caused by narrowing of the large conducting airways (bronchi) either upon inhalation of provoking agents or for unknown causes. There are well-established links between occupational and environmental exposure to antigens or to chemicals that can act as hapten in and the pathogenesis of asthma. There are histopathologic components that are common between asthma and pulmonary fibrosis, but in this case the disease is centered in and around the large conducting airways rather than the centriacinar region of the lung parenchyma. There may be common mechanisms, especially with regard to the role of inflammatory cells and the cytokines and growth factors they secrete (Barnes et al., 1998). The clinical hallmark of asthma is increased airway reactivity: the smooth muscle around the large airways contract in response to exposure to irritants. The extreme sensitivity of guinea pigs (as opposed to rats or mice) to inhaled irritants such as ozone or SO2 may be an example of an animal model of the human asthmatic subject (Barnes et al., 1998).

Lung Cancer

Lung cancer, an extremely rare disease around the turn of the century, is now the leading cause of death from cancer among men and women. Retrospective and, more conclusively, prospective epidemiologic studies unequivocally show an association between tobacco smoking and lung cancer. It has been estimated that approximately 80 to 90 percent of lung cancers (and several other cancers, such as cancer of the bladder, esophagus, oral cavity, and pancreas) are caused by cigarette smoking. Average smokers have a 10-fold and heavy smokers a 20-fold increased risk of developing lung cancer compared with nonsmokers. Quitting the habit will reduce the risk (Wingo et al., 1999).

Inhalation of asbestos fibers and metallic dusts or fumes—such as arsenic, beryllium, cadmium, chromium, and nickel, encountered in smelting and manufacturing operations—has been associated with cancer of the respiratory tract. Workers who manufacture chloromethyl ether or mustard gas also have an increased risk of developing lung cancers, as do workers exposed to effluent gases from coke ovens. Radon gas is a known human lung carcinogen. Formaldehyde is a probable human respiratory carcinogen. Silica, human-made fibers, and welding fumes are suspected carcinogens (International Agency for Research on Cancer, 1987, 1993). Smokers who inhale radon or asbestos fibers increase their risk of developing lung cancer severalfold, suggesting a synergistic interaction between the carcinogens. To what extent common air pollutants such as ozone, nitrogen dioxide, sulfur dioxide, and fumes emanating from power plants, oil refineries, and Diesel fuel-powered trucks and cars contribute to the development of lung cancer in the general population remains an open question. Some evidence suggests that respirable particulates suspended in polluted air are a risk factor (Beeson et al., 1998). Indoor air pollution, including environmental tobacco smoke, increases the risk of developing lung cancer in nonsmokers (National Cancer Institute, 1999).

Human lung cancers may have a latency period of 20 to 40 years, making the relationship to specific exposures difficult to establish. Many lung cancers in humans originate from the cells lining the airways (lung cancer originating from such sites is often referred to as bronchogenic carcinoma), but during the last two decades a significant increase in peripheral adenocarcinomas has occurred. Compared with cancer in the lung, cancer in the upper respiratory tract is less common. Malignant lesions of the nasal passages, which are seen frequently in experimental animals, are comparatively rare in humans. They are associated with certain occupations, including work with chrome, nickel, mustard gas, isopropyl alcohol, the manufacture of wooden furniture, and boot and shoe manufacture. Possible carcinogens include hexavalent chromium compounds, metallic nickel and nickel subsulfide, nickel oxide, formaldehyde, and certain wood and leather dusts.

The potential mechanisms of lung carcinogenesis have been studied extensively by means of analysis of tumor material and in studies of human bronchial cells maintained in culture. Damage to DNA is thought to be a key mechanism. An activated carcinogen or its metabolic product, such as alkyl diazonium ions derived from N-nitrosamines, may interact with DNA. Persistence of O6-alkyldeoxyguanosine in DNA appears to correlate with carcinogenicity (Hecht, 1999). However, tumors do not always develop when adducts are present, and adduct formation may be a necessary but not sufficient condition for carcinogenesis. DNA damage caused by active oxygen species is another potentially important mechanism. Ionizing radiation leads to the formation of superoxide, which is converted through the action of superoxide dismutase to hydrogen peroxide. In the presence of Fe and other transition metals, hydroxyl radicals may be formed which then cause DNA strand breaks. Cigarette smoke contains high quantities of active oxygen species and other free radicals. Additional oxidative stress may be placed on the lung tissue of smokers by the release of superoxide anions and hydrogen peroxide by activated macrophages, metabolism of carcinogens, and lipid peroxidation caused by reactive aldehydes.

In laboratory animals, spontaneously occurring malignant lung tumors are uncommon unless the animals reach a very advanced age. Exposure to carcinogens by the inhalation route or by intratracheal instillation or systemic administration readily produces lung tumors in many laboratory species, such as mice, rats, hamsters, and dogs. There are several differences between lung tumors in animals and bronchogenic cancer in humans. In animals, particularly rodents, most tumors are in the periphery rather than arising from the bronchi. The incidence of benign lung tumors such as adenomas is often very high, and carcinomas seem to require more time to develop. Lung tumors in animals do not metastasize as aggressively, if they do so at all, as do human lung cancers (Hahn, 1997). Cancer of the nasal passages is readily induced in experimental animals in inhalation studies.
Because lung tumors in mice and rats are often seen in carcinogenesis bioassays, they deserve special mention. Murine lung tumors are mostly benign-appearing adenosomas originating from alveolar type II cells or bronchiolar Clara cells. They can progress to adenocarcinomas and invade lymphatics and blood vessels. Certain mouse strains, such as strain A and the Swiss-Webster mouse, have a high incidence of spontaneously occurring lung tumors. These animals respond with increased numbers of tumors to the inhalation or injection of many carcinogens. Other strains are much more resistant. Lung tumors in strain A mice have become valuable tools for studying the genetic factors that determine susceptibility (Malkinson, 1998). They contain frequent mutations in the K-ras gene, a mutation also found frequently in human lung cancers (Graziano et al., 1999). Methylating nitrosamines (NNK and DMN) produce mutations consistent with the formation of O⁶-methylguanine and ethylating nitrosamines (ENU and DEN) and mutations consistent with the formation of O⁷-ethylthymidine. In strains less susceptible than the A/J mouse, chemicals such as tetratinromethane, 1,3-butaedine, DMN, and NNK generate tumors with mutations consistent with the result of DNA adduct formation, whereas other chemicals (acetylaminofluorene, methylene chloride) do not produce tumors with carcino-gen-specific mutations.

Lung tumors in rats exposed to airborne carcinogens consist mostly of peripheral adenocarcinomas and squamous cell carcinomas. In addition, rat lungs on occasion contain lesions that are characterized by an epithelium surrounding a space filled with keratin. The mass may compress the adjacent lung parenchyma and occasionally invades it. These lesions are classified by some pathologists as bona fide tumors, whereas other pathologists characterize this type of lesion as a cyst filled with keratin. Classification of such a lesion as a tumor is important because these lesions often are found in long-term tests in animals that have been exposed to agents that are not considered carcinogenic, such as carbon black, titanium dioxide, and certain human-made fibers (ILSI, 2000).

**AGENTS KNOWN TO PRODUCE LUNG INJURY IN HUMANS**

The prevention and treatment of acute and chronic lung disease will eventually be based on a knowledge of the cellular and molecular events that determine lung injury and repair. During the past 20 years, a large body of evidence has accumulated. Table 15-1 lists common toxicants that are known to produce acute and chronic lung injury in humans. In the following sections, a few examples of our current understanding of lung injury at the mechanistic level are discussed, with emphasis on agents directly responsible for human lung disease.

**Airborne Agents That Produce Lung Injury in Humans**

**Asbestos** The term “asbestos” describes silicate minerals in fiber form. The most commonly mined and commercially used asbestos fibers include the serpentine chrysolite asbestos and the amphiboles crocidolite, anthophyllite, amosite, actinolite, and tremolite. Exposure to asbestos fibers occurs in mining operations and in the construction and shipbuilding industries, where asbestos was at one time widely used for its highly desirable insulating and fireproofing properties. During the last few years, concern about asbestos in older buildings has led to the removal of asbestos-based insulating material; abatement workers may now represent an additional population at risk.

Asbestos causes three forms of lung disease in humans: asbestosis, lung cancer, and malignant mesothelioma. Asbestosis is characterized by a diffuse increase of collagen in the alveolar walls (fibrosis) and the presence of asbestos fibers, either free or coated with a proteinaceous material (asbestos bodies). Malignant mesothelioma (a tumor of the cells covering the surface of the visceral and parietal pleura), a tumor that otherwise occurs only extremely rarely in the general population, is unequivocally associated with asbestos exposure. There is some discrepancy between human observations and animal data. In animal experiments, chrysotile produces mesothelioma much more readily than do the amphibole fibers. In humans, amphibole fibers are implicated more often even when the predominant exposure is to chrysotile asbestos. Chrysotile breaks down much more readily than do the amphiboles. It is possible that in small laboratory animals chrysotile fibers, even if broken down, are retained longer relative to the life span of the animal than they are in humans, thus explaining the higher rate of mesothelioma development.

The hazards associated with asbestos exposure depend on fiber length. Fibers 2 μm in length may produce asbestosis; mesothelioma is associated with fibers 5 μm long, and lung cancer with fibers larger than 10 μm. Fiber diameter is another critical feature: Fibers with diameters larger than approximately 3 μm do not readily penetrate into the peripheral lung. For the development of mesothelioma, fiber diameter must be less than 0.5 μm, since thinner fibers may be translocated from their site of deposition via the lymphatics to other organs, including the pleural surface. Once asbestos fibers have been deposited in the lung, they may become phagocytized by alveolar macrophages. Short fibers are completely ingested and subsequently removed via the mucociliary escalator. Longer fibers are incompletely ingested, and the macrophages become unable to leave the alveoli. Activated by the fibers, macrophages release mediators such as lymphokines and growth factors, which in turn attract immunocompetent cells or stimulate collagen production. Asbestos-related lung disease thus may be mediated through the triggering of an inflammatory sequence of events or the production of changes that eventually lead to the initiation (DNA damage caused by reactive molecular species) or promotion (increased rate of cell turnover in the lung) of the carcinogenic process.

The surface properties of asbestos fibers appear to be an important mechanistic element in toxicity. The protection afforded by superoxide dismutase or free radical scavengers in asbestos-related cell injury in vitro suggests that the generation of active oxygen species and concomitant lipid peroxidation are important mechanisms in asbestos toxicity. The interaction of iron on the surface of asbestos fibers with oxygen may lead to the production of hydrogen peroxide and the highly reactive hydroxyl radical, events that have been associated with asbestos toxicity (Timblin et al., 1999).

**Silica** Silicosis in humans may be acute or chronic; this distinction is important conceptually because the pathological consequences are manifested quite differently. Acute silicosis occurs only in subjects exposed to a very high level of aerosol containing particles small enough to be respirable (usually less than 5 μm) over a relatively short period, generally a few months to a few years.
# Table 15-1

## Industrial Toxicants That Produce Lung Disease

<table>
<thead>
<tr>
<th>TOXICANT</th>
<th>COMMON NAME</th>
<th>OCCUPATIONAL SOURCE</th>
<th>ACUTE EFFECT</th>
<th>CHRONIC EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asbestos</td>
<td>Asbestosis</td>
<td>Mining, construction, shipbuilding, manufacture of asbestos-containing material</td>
<td>Fibrosis, pleural calcification, lung cancer, pleural mesothelioma</td>
<td></td>
</tr>
<tr>
<td>Aluminum dust</td>
<td>Aluminosis</td>
<td>Manufacture of aluminum products, fireworks, ceramics, paints, electrical goods, abrasives</td>
<td>Cough, shortness of breath</td>
<td>Interstitial fibrosis</td>
</tr>
<tr>
<td>Aluminum abrasives</td>
<td>Shaver’s disease, corundum smelter’s lung, bauxite lung</td>
<td>Manufacture of abrasives, smelting</td>
<td>Alveolar edema</td>
<td>Interstitial fibrosis, emphysema</td>
</tr>
<tr>
<td>Ammonia</td>
<td></td>
<td>Ammonia production, manufacture of fertilizers, chemical production, explosives</td>
<td>Upper and lower respiratory tract irritation, edema</td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td></td>
<td>Manufacture of pesticides, pigments, glass, alloys</td>
<td>Bronchitis</td>
<td>Lung cancer, bronchitis, laryngitis</td>
</tr>
<tr>
<td>Beryllium</td>
<td>Berylliosis</td>
<td>Ore extraction, manufacture of alloys, ceramics</td>
<td>Severe pulmonary edema, pneumonia</td>
<td>Fibrosis, progressive dyspnea, interstitial granulomatosis, lung cancer, cor pulmonale</td>
</tr>
<tr>
<td>Cadmium oxide</td>
<td></td>
<td>Welding, manufacture of electrical equipment, alloys, pigments, smelting</td>
<td>Cough, pneumonia</td>
<td>Emphysema, cor pulmonale</td>
</tr>
<tr>
<td>Carbides of tungsten, titanium, tantalum</td>
<td>Hard metal disease</td>
<td>Manufacture of cutting edges on tools</td>
<td>Hyperplasia and metaplasia of bronchial epithelium</td>
<td></td>
</tr>
<tr>
<td>Chromium (VI)</td>
<td></td>
<td>Manufacture of pulp and paper, plastics, chlorinated chemicals</td>
<td>Cough, hemoptysis, dyspnea, tracheobronchitis, bronchopneumonia</td>
<td></td>
</tr>
<tr>
<td>Coal dust</td>
<td>Pneumoconiosis</td>
<td>Coal mining</td>
<td>Nasal irritation, bronchitis</td>
<td>Lung cancer, fibrosis</td>
</tr>
<tr>
<td>Cotton dust</td>
<td>Byssinosis</td>
<td>Manufacture of textiles</td>
<td>Chest tightness, wheezing, dyspnea</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>Hydrogen fluoride</td>
<td></td>
<td>Manufacture of chemicals, photographic film, solvents, plastics</td>
<td>Respiratory irritation, hemorrhagic pulmonary edema</td>
<td></td>
</tr>
<tr>
<td>Iron oxides</td>
<td>Siderotic lung disease; silver finisher’s lung, hematite miner’s lung, arc welder’s lung</td>
<td>Welding, foundry work, steel manufacture, hematite mining, jewelry making</td>
<td>Cough</td>
<td>Silver finisher’s lung: subpleural and perivascular aggregations of macrophages; hematite miner’s lung: diffuse fibrosislike pneumoconiosis; arc welder’s lung: bronchitis</td>
</tr>
<tr>
<td>Isocyanates</td>
<td></td>
<td>Manufacture of plastics, chemical industry</td>
<td>Airway irritation, cough, dyspnea</td>
<td>Asthma, reduced pulmonary function</td>
</tr>
</tbody>
</table>
These patients have worsening dyspnea, fever, cough, and weight loss. There is rapid progression of respiratory failure, usually ending in death within a year or two. No known treatment modality influences the relentless course of acute silicosis.

Chronic silicosis has a long latency period, usually more than 10 years. Uncomplicated silicosis is almost entirely asymptomatic; little alteration is shown on routine pulmonary function tests even after the disease is radiographically demonstrable. The x-ray picture presents fibrotic nodules, generally in the apical portion of lung. The hilar lymph nodes have peripheral calcifications known as eggshell calcifications. Simple silicosis may progress into complicated silicosis, which is defined as the presence of conglomerate nodules larger than 1 cm in diameter. These nodules usually occur in the upper and midlung zones. At an advanced stage they may be surrounded by emphysematous bullae. Chronic silicosis is associated with an increased incidence of tuberculosis.

Crystalline silica is a major component of the earth’s crust; after oxygen, silicon is the most common element. As a pure mineral, silicon exists primarily in the form of its dioxide, silica (SiO₂), which has a crystalline form in which a central silicon atom forms a tetrahedron with four shared oxygen atoms. The three principal crystalline isomeric forms are quartz, tridymite, and cristobalite. The tetrahedral structure is linked to fibrogenic potential.

Stishovite, a rare crystalline variant without the tetrahedral conformation, is biologically inert. Amorphous forms of silica such as kieselguhr and vitreous silica have very low fibrogenic potential. The ubiquitous presence of silica has made it an occupational hazard ever since humans began shaping tools from stone, and silicosis remains a significant industrial hazard throughout the world in occupations such as mining and quarrying, sandblasting, and foundry work. The main factors that affect the pathogenicity of silica both in vivo and in vitro, in addition to its structure, are particle size and concentration. Many studies have examined the relationship of silica particle size to fibrogenicity. In studies with humans, the most fibrogenic particle size appears to be about 1 μm (range 0.5 to 3 μm). In animal experiments (rats, hamsters), the comparable values appear to be 1 to 2 μm (range 0.5 to 5 μm). In animal models, there appears to be a direct relationship between the concentration of silica dust to which an animal is exposed and the intensity and rapidity of the histologic reaction in the lung.

The pathophysiological basis of pulmonary fibrosis in chronic silicosis is probably better understood than is the etiology of any other form of lung fibrosis. The role of pulmonary alveolar macrophages in the ingestion of silica as an initiating event has been established. Apparently, as part of the cytotoxic response of a macrophage to silica ingestion, the macrophage may release cy-

Table 15-1

<table>
<thead>
<tr>
<th>TOXICANT</th>
<th>COMMON NAME OF DISEASE</th>
<th>OCCUPATIONAL SOURCE</th>
<th>ACUTE EFFECT</th>
<th>CHRONIC EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolin</td>
<td>Kaolinosis</td>
<td>Pottery making</td>
<td>Acute pneumonia, often fatal</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>Manganese</td>
<td>Manganese pneumonia</td>
<td>Chemical and metal industries</td>
<td>Pulmonary edema, delayed by 2 days (NiCO)</td>
<td>Recurrent pneumonia</td>
</tr>
<tr>
<td>Nickel</td>
<td></td>
<td>Nickel ore extraction, melting, electronic electroplating, fossil fuels</td>
<td>Pulmonary congestion and edema</td>
<td>Squamous cell carcinoma of nasal cavity and lung</td>
</tr>
<tr>
<td>Oxides of nitrogen</td>
<td>Welding, silo filling, explosive manufacture</td>
<td>Pulmonary edema</td>
<td>Bronchiolitis obliterans</td>
<td></td>
</tr>
<tr>
<td>Ozone</td>
<td></td>
<td>Welding, bleaching flour, deodorizing</td>
<td>Pulmonary edema</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>Phosgene</td>
<td></td>
<td>Production of plastics, pesticides, chemicals</td>
<td>Edema</td>
<td>Bronchitis, fibrosis</td>
</tr>
<tr>
<td>Perchloroethylene</td>
<td>Dry cleaning, metal degreasing, grain fumigating</td>
<td>Edema</td>
<td>Cancer, liver and lung</td>
<td></td>
</tr>
<tr>
<td>Silica</td>
<td>Silicosis, pneumoconiosis</td>
<td>Mining, stone cutting, construction, farming, quarrying, sand blasting</td>
<td>Acute silicosis</td>
<td>Fibrosis, silicotuberculosis</td>
</tr>
<tr>
<td>Sulfur dioxide</td>
<td>Manufacture of chemicals, refrigeration, bleaching, fumigation</td>
<td>Bronchoconstriction, cough, chest tightness</td>
<td>Chronic bronchitis</td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>Talcosis</td>
<td>Rubber industry, cosmetics</td>
<td>Fibrosis</td>
<td>Widespread mottling of x-ray without clinical signs</td>
</tr>
<tr>
<td>Tin</td>
<td>Stanosis</td>
<td>Mining, processing of tin</td>
<td>Airway irritation and mucus production</td>
<td>Chronic bronchitis</td>
</tr>
<tr>
<td>Vanadium</td>
<td></td>
<td>Steel manufacture</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lung Overload Caused by Particles  Investigators studying the kinetics of the pulmonary clearance of particles have observed a slowing of the rate of alveolar clearance when deposited lung burdens are high. At about the same time, investigators evaluating inhaled particles in carcinogenesis bioassays observed excess tumors in animals that inhaled very high concentrations of apparently inert so-called nuisance dusts, which were included in such experiments as negative controls. From these observations came a unifying hypothesis (Morrow, 1992) that clearance mechanisms in the deep lung depend on species differences that were noted previously (Franklin et al., 1997). These pyrrole adducts may be particularly susceptible to removal from globin chains following either enzymatic degradation or upon the RBCs, mainly to the globin chains. Monocrotaline (MCT) is a pyrrolizidine alkaloid, one of many structurally related naturally occurring products of plants that have been identified in grains, honey and herbal teas. These compounds produce liver toxicity (hepatocellular necrosis and veno-occlusive disease). Although the hepatic metabolism of the pyrrolidine alkaloids is usually completed by 24 h, the administration of a single dose of monocrotaline is known to initiate a delayed lung injury. This pulmonary lesion is characterized by remodeling of the vascular bed with hyperplasia of capillary endothelial cells, thickening of the arterial media, formation of microthrombi, and eventually capillary occlusion with resulting hypertrophy of the pulmonary arterial system and hypertrophy of the right side of the heart.

Monocrotaline propagates changes in the contractile response of arterial smooth muscle, changes in smooth muscle Na/K-ATPase activity, release of platelet factors, and decreased serotonin transport by vascular endothelial cells (Wilson et al., 1992). Monocrotaline is metabolized in the liver by cytochrome P450 3A to a highly reactive pyrrole, a bifunctional alkylating agent, where some of the reactive pyrrole forms nontoxic conjugates with either glutathione or cysteine. A percentage of the remaining pyrrole is released from the liver and travels to other organs, such as the lung and possibly the kidney, via red blood cells (RBCs), where it initiates endothelial injury. It had been previously shown that RBCs circulated through an isolated perfused buffer containing [14C]MCT, subsequently washed and recirculated through an isolated lung preparation could transfer electrophiles to pulmonary granulocytes, mainly to the β-chains of hemoglobin (Lamée et al., 1997). These pyrrole adducts may be particularly susceptible to removal from globin chains following either enzymatic conjugation to the cells of the alveolar-capillary septum. Type I epithelial cells and capillary endothelial cells develop necrotic changes. Capillary damage leads to leakage of proteinaceous fluid and formed blood elements into the alveolar space. Hyaline membranes formed by cellular debris and proteinaceous exudate are a characteristic sign of pulmonary oxygen toxicity. In animals returned to air after the development of acute oxygen toxicity, there is active cell proliferation (Frank, 1997).

Blood-Borne Agents That Cause Pulmonary Toxicity in Humans

Paraquat  The bipyridylium compound paraquat, a widely used herbicide, produces extensive lung injury when ingested by humans. In patients who survive the first few days of acute paraquat poisoning, progressive and eventually fatal lung lesions can develop. Paraquat lung disease is characterized by diffuse interstitial and intraalveolar fibrosis. The initial damage consists of widespread necrosis of both type I and type II epithelial cells of the alveolar region. Extensive proliferation of fibroblasts in the alveolar interstitium and the largely collapsed alveoli follows. Paraquat accumulates in the cells of the lung through the polyamine uptake system. Once inside the cells, paraquat continuously cycles from its oxidized form to the reduced form, with the concomitant formation of active oxygen species. A mechanistic hypothesis to explain paraquat toxicity involves oxidation of cellular NADPH and eventual depletion of the NADPH content of pulmonary cells (Smith, 1997).

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Bleomycin

Bleomycin, a mixture of several structurally similar compounds, is a widely used cancer chemotherapeutic agent. Pulmonary fibrosis, often fatal, represents the most serious form of toxicity. The sequence of damage includes necrosis of capillary endothelial and type I alveolar cells, edema formation and hemorrhage, delayed (after 1 to 2 weeks) proliferation of type II epithelial cells, and eventually thickening of the alveolar walls by fibrotic changes.

In many tissues, the cytosolic enzyme bleomycin hydrolase inactivates bleomycin. In lung and skin, two target organs for bleomycin toxicity, the activity of this enzyme is low compared with that in other organs. Bleomycin stimulates the production of collagen in the lung. Before increased collagen biosynthesis, steady-state levels of mRNA coding for fibronectin and procollagens are increased, presumably subsequent to a bleomycin-mediated release of cytokines such as TGF-β and TNF. Bleomycin also combines with DNA, single- and double-strand breaks are produced by a free radical reaction (Hoyt and Lazo, 1997).

Cyclophosphamide and 1,3-Bis-(2-Chloroethyl)-1-Nitrosourea (BCNU)

Cyclophosphamide is widely used as an anticancer and immunosuppressive agent. The undesirable side effects include hemorrhagic cystitis and pulmonary fibrosis. Cyclophosphamide is metabolized by the cytochrome P450 system to two highly reactive metabolites: acrolein and phosphoramid mustard. In the lung, cooxidation with the prostaglandin-H synthase system, which has high activity in the lung, is a possibility. Although the exact mechanism of action for causing lung damage has not been established, studies with isolated lung microsomes have shown that cyclophosphamide and its metabolite acrolein initiate lipid peroxidation. Carmustine (BCNU) is an effective chemotherapeutic agent that exerts its antitumor properties by reacting with cellular macromolecules and forms inter- and intrastrand cross-links with DNA.

In humans, a dose-related pulmonary toxicity is often noticed first by a decrease in diffusion capacity. Pulmonary fibrosis caused by this drug can be fatal. The mechanism of action is not entirely clear. It is possible that BCNU inhibits pulmonary glutathione disulfide reductase, an event that may lead to a disturbed GSH/GSSG state in pulmonary cells. Eventually, this state leaves the cell unable to cope with oxidant stress. High concentrations of oxygen in the inspired air may enhance the pulmonary toxicity of BCNU and also that of the other anticancer drugs known to affect lung tissue: cyclophosphamide and bleomycin. Several other chemotherapeutic agents can produce lung damage and pulmonary toxicity in patients treated with these drugs can be a significant problem (Ramu and Keher, 1997).

Cationic Amphophilic Drugs

Several drugs with similar structural characteristics that are called cationic amphophilic drugs (CADs) produce pulmonary lipidosis. The antiarrhythmic amiodarone and the anorexic chlorthalidone elicit such changes in humans. Pulmonary lipidosis is characterized by the intracellular presence, particularly in macrophages, of large concentric membranous structures now known to be secondary lysosomes. CADs inhibit phospholipases A and B, presumably because these drugs combine with phospholipids and form indigestible complexes. Degradation of pulmonary surfactant is impaired, and the material accumulates in phagocytic cells. In humans, amiodarone may cause dyspnea and cough. In animals and humans, the condition is fully reversible on drug withdrawal (Reaor, 1997).

METHODS FOR STUDYING LUNG INJURY

Inhalation Exposure Systems

The generation of a gas available in high purity as a compressed “tank gas,” for example, SO₂, O₃, or NO₂, is relatively straightforward, and metering and dilution produce appropriate concentrations for exposure. Monitoring and quantifying gaseous pollutants require either expensive detectors that need frequent calibration (and usually a computer to process the tremendous amount of data generated) or very labor intensive wet chemical analysis procedures after sampled gases from the chambers are bubbled through traps. Particle generation is difficult, and specialized references must be consulted (Wong, 1999).

Exposure chambers must allow for the rapid attainment of the desired concentrations of toxicants, maintenance of desired levels homogeneously throughout the chamber, adequate capacity for experimental animals, and minimal accumulation of undesired products associated with animal occupancy (usually ammonia, dander, heat, and carbon dioxide). Modern chambers tend to be fabricated from inert materials (usually glass, stainless steel, and Teflon) and have flow patterns that are designed to promote mixing and homogeneity of the chamber atmosphere and prevent a buildup of undesirable contaminants. A major concern with regard to exposure to acid aerosols has been the putative buildup of ammonia in chambers because of microbial action on animal excreta. Thus, maximal loading factors and sanitation also must be considered in chamber usage. As a general rule, the total body volume of the animals should not exceed 5 percent of the chamber volume. Nose-only exposure chambers avoid some of these problems. Finally, concern for the environment and the safety of facility personnel suggest prudence in how chambers are exhausted.

In inhalation studies, selection of animals with a respiratory system similar to that of humans is particularly desirable. The respiratory system of monkeys most closely resembles that of humans. Guinea pigs and rabbits have been used to predict the response of humans to sulfuric acid (Amdur, 1989).

Pulmonary Function Studies

Numerous tests are available with which to study pulmonary function and gas exchange in humans and experimental animals. Commonly used tests include measurement of VC, TLC, functional residual volume, TV, airway resistance, and maximum flow (Fig. 15-6). Additional tests evaluate the distribution of ventilation,
invasive. The subject is asked first to inhale deeply and then to ex-
this is an easy test to perform in animals, requiring little specialized
apparatus. Cannulation of the excised lungs and attachment to a
syringe and manometer to measure volume and pressure are all that
is needed. Volume-pressure curves can be obtained from lungs
filled with air or physiological saline. The latter test is much more
sensitive to structural changes in lung parenchyma, as the effects
of surfactant are eliminated in a saline-filled lung.

To accomplish proper oxygenation of venous blood and elimin-
ation of CO₂, the gases have to diffuse across the air-blood bar-
rier. Gas exchange may be hindered by the accumulation of fluids
or cellular elements in the alveoli (edema, pneumonic infiltrates),
thickening of the alveolar wall (fibrosis), insufficient ventilation
of the alveolar region (emphysema), or insufficient presence of oxy-
gen transport elements (reduced alveolar blood volume or reduced
amount of hemoglobin in the blood). Gas exchange can be evalu-
ated by measuring the arterial partial pressure of both oxygen and
CO₂. In experimental animals, the collection of arterial blood may
require the presence of indwelling catheters.

In general, blood gas analysis is a comparatively insensitive
assay for disturbed ventilation because of the organism’s buffering
and reserve capacities. While it is a useful tool in clinical medici-
ne, only the most severe obstructive or restrictive pulmonary
alterations cause signs of impaired gas exchange in animals.
Measurement of diffusion capacity with CO, a gas that binds with
250 times higher affinity to hemoglobin than does oxygen, is more
sensitive. The test is comparatively easy to perform in both hu-
mans and laboratory animals and is widely used in toxicology
studies.

Morphologic Techniques

The pathology of acute and chronic injury may be described after
examination of the respiratory tract by gross inspection and under
the microscope. Morphologic evaluation should not be limited to
the peripheral lung; nasal passages, the larynx, and major airways
must be examined as carefully as is the lung parenchyma. For ex-
ample, formaldehyde produces nasal tumors but not deep lung tu-
mors in the rat. In hamsters exposed to cigarette smoke, cance-
ers changes are found in the larynx but not in the more distal airways.

Careful consideration must be given to tissue fixation and
preparation. Nasal passages must be flushed with fixative. After
decalcification, cross sections should be cut at multiple levels; the
regional distribution of lesions may vary from agent to agent.
Proper fixation of the lung is done by vascular perfusion with fix-
ative through the pulmonary artery or by instillation of fixative
through the trachea. Perfusion fixation does not dislodge material
(lining fluid, deposited particles) or cells in the lumen of the air-
ways or the alveoli from their original position. Fixation by instil-
lation does this, but it also keeps the alveoli open. It is done un-
der controlled pressure, usually 30 cm H₂O, and is required if
semi quantitative or quantitative measurements will be made. The
choice of fixative depends on how the lung will be further ana-
lyzed. Formalin-based fixatives are satisfactory for routine
histopathology, whereas the use of more sophisticated techniques
such as electron microscopy, immunohistochemistry, and in situ
hybridization require careful selection of the fixative.

Ordinary paraffin sections of respiratory tract tissue are suit-
able for routine histopathologic analysis; gross pathological
changes such as inflammation and the presence of cancerous tis-
sue can be detected easily. Plastic or epon sections about 1 μm
thick are required for proper identification of different cell types
lining the airways or alveoli and for recognition of cytoplasmic
changes in damaged Clara cells. Other structural alterations, such
as degenerative changes or necrosis of type I epithelial cells or cap-

Note that there is (1) a slowing of forced expiration in addition to gas trap-
ning (an increase in residual volume) in obstructive disease and (2) a gen-
eral decrease in lung volumes in restrictive disease. Note that the mea-
surements read from left to right.

Lung and chest wall compliance, diffusion capacity, and the oxy-
gen and carbon dioxide content of the arterial and venous blood
(Costa et al., 1991).

Many pulmonary function tests require active collaboration by
the subject examined, for example, the so-called FEV₁ (forced
expiratory volume) during the first second of an active exhalation.
This is an easy test to administer to humans, does not require so-
phisticated equipment or a hospital setting, and is completely non-
invasive. The subject is asked first to inhale deeply and then to ex-
hale the air as quickly as possible. The test is often used in
epidemiological studies or controlled clinical studies designed to
assess the potential adverse effects of air pollutants. A reduction
in FEV₁ is usually indicative of impaired ventilation such as that
found in restrictive (increased lung stiffness) or obstructive (ob-
structed airflow) lung disease. Experimental animals, by contrast,
cannot be made to maximally inhale or exhale at the investigator’s
will. In experimental animals, FEV₁ can be obtained, but the test
is done under anesthesia. Expiration is forced by applying exter-
nal pressure to the thorax or negative pressure to the airways.

Analysis of breathing patterns has been widely used to assess
the effects of irritants. This technique allows one to differentiate
between sensory or upper airway irritants and “pulmonary” irri-
tants. Highly water soluble irritants such as ammonia, chlorine, and
formaldehyde produce upper respiratory tract irritation, whereas
less soluble gases such as nitrogen dioxide and ozone generate pul-
monary irritation. The sensory, irritant pattern has been described
as slowing down respiratory frequency while increasing TV. Pul-
monary irritants usually increase respiratory frequency and de-
crease minute volume. The result is rapid, shallow breathing.

Analysis of volume-pressure curves of the lung provides some
indication of lung compliance. Compliance (volume/ pressure) is
measured as the slope of the volume-pressure curve; it gives some
indication of the intrinsic elastic properties of the lung parenchyma
and, when measured in vivo, the thoracic cage. This is a compar-
atively easy test to perform in animals, requiring little specialized
apparatus. Cannulation of the excised lungs and attachment to a
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illar endothelial cells, usually are detected by transmission electron microscopy (TEM). TEM is essential for an unequivocal identification of cells in the alveolar interstitium and is used mainly in morphometric analysis of the lung. Scanning electron microscopy allows visualization of the surface of interior lung structures, reveals alterations in the tissue surface, and detects rearrangement of the overall cell population. Confocal microscopy, consisting of a laser microscope coupled to a computer, allows examination of thick sections and discovery of specific cell types deep within the tissue; it is an ideal tool for three-dimensional reconstruction of normal and damaged lung.

Morphometry, the quantitative description of structure, refers to a quantitative analysis of tissue (Gehr et al., 1993). Measurements made in two dimensions on photographs taken under the microscope allow one to measure areas, the thickness of a structure, and numerical density. With the help of appropriate formulas, values such as the volume occupied by a specific cell population in the entire lung parenchyma can be calculated. The method is particularly useful for the detection of subtle toxic effects in the lung parenchyma (Witschi et al., 1999).

Additional tools for the study of toxic lung injury include immunohistochemistry, in situ hybridization, and analysis of cell kinetics. Antibodies to a variety of enzymes, mediators, and other proteins are available. It is possible to identify cell types that carry certain enzymes and their anatomic locations. This information is important for mechanistic studies. In situ hybridization allows one to visualize anatomic sites where a specific gene product is expressed, for example, collagen production in a fibrotic lung. This technique is especially important in an organ such as the lung where there are more than 40 morphologically distinct cell types present. Ascribing a given metabolic capability to a specific cell type requires evaluation of gene expression and/or protein production in specific cells in situ. Flow cytometry is valuable in the study of cell populations prepared from the lung. The technique requires dissociation of the lung parenchyma into its individual cell populations. Different lung cells then can be identified and isolated.

**Pulmonary Lavage**

Pulmonary edema and/or pulmonary inflammation appear to be obligatory early events in acute and chronic lung injury. Markers of these processes generally are chosen to reflect lung edema or cellular changes in the lung. The most popular of these types of assays have quantified various parameters in lung lavage fluid from animals exposed to pneumotoxic substances. Generally, the lungs of exposed and control animals are washed with multiple small volumes of isotonic saline. This technique has the further advantage of allowing direct comparisons with data accessible from normal human volunteers or patients undergoing bronchopulmonary lavage for therapeutic purposes. Current emphasis seems to be on the measurement of polymorphonuclear leukocytes, macrophages, and monocytes (and their phagocytic capabilities) in the cellular fraction and the measurement of lactate dehydrogenase (and its substrate isoenzymes), N-acetylglucosaminidase, acid or alkaline phosphatase, other lysosomal hydrolases, lavageable total protein and/or albumin, and sialic acid. Although such measurements have often formed the basis of mechanistic interpretations, we really do not have a rigorous theoretical understanding of the exact source of any of these parameters.

Measurement of apparent changes in the permeability of the air-blood barrier by quantification of intravenously injected tracer in lung lavage fluid is another useful index of lung damage. The movement of low-molecular-weight tracers such as $^{51}$Cr EDTA across the blood-air barrier occurs rapidly (within 10 min of IV injection). High-molecular-weight tracers such as radiolabeled albumin also have been used for this purpose.

**In Vitro Approaches**

In vitro systems are particularly suited for the study of mechanisms that cause lung injury. The following systems are widely used (Postlethwait and Bidani, 1997).

**Isolated Perfused Lung**  The isolated perfused lung method is applicable to lungs from many laboratory animal species (rabbit, rat, mouse, guinea pig). The lung, in situ or excised, is perfused with blood or a blood substitute through the pulmonary arterial bed. At the same time, the lung is actively (through rhythmic inflation-deflation cycles with positive pressure) or passively (by creating negative pressure with an "artificial thorax" in which the lung is suspended) ventilated. Toxic agents can be introduced into the perfusate or the inspired air. Repeated sampling of the perfusate allows one to determine the rate of metabolism of drugs and the metabolic activity of the lung.

**Lung Explants and Slices**  Slices and explants from the conducting airways or the lung parenchyma allow one to examine biochemical and morphologic changes in the lung parenchyma without intervening complications from cells migrating into the tissue (e.g., leukocytes). If the lung is first inflated with agar, the alveolar spaces remain open in the explant. Slices prepared in this way can be kept viable for several weeks, and the mechanisms of development of chronic lesions can be studied.

**Microdissection**  Many inhalants act in circumscribed regions of the respiratory tract, such as the terminal bronchioles, a region especially rich in metabolically highly competent Clara cells. Microdissection of the airways consists of the stripping of small bronchi and terminal bronchioli from the surrounding parenchyma and maintenance of the isolated airways in culture. Specific biochemical reactions predominantly located in the cells of the small airways can then be studied with biochemical or morphologic techniques.

**Organotypic Cell Culture Systems**  Tissue culture systems have been developed in which epithelial cells maintain their polarity, differentiation, and normal function similar to what is observed in vivo. Epithelial cell surfaces are exposed to air (or a gas phase containing an airborne toxic agent), while the basal portion is bathed by a tissue culture medium. Epithelial cells may be seeded on top of a suitable supporting material (e.g., collagen or nitrocellulose membranes) with mesenchymal cells seeded on the other side to observe epithelial cell–fibroblast interactions.

**Isolated Lung Cell Populations**  Many specific lung cell types have been isolated and maintained as primary cultures in vitro. Alveolar macrophages are easily obtained from human and animal lungs by lavage. Their function can be examined in vitro with or without exposure to appropriate toxic stimuli. Type II alveolar ep-
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Epithelial cells are isolated after digestion of the lung. Direct isolation of type I epithelial cells has also been successful. Systems for the isolation and culture of Clara cells and neuroepithelial cells are available. Lung fibroblasts are easily grown and have been studied in coculture with epithelial cells. Multiple primary cell cultures and cell lines have been established from lung tumors found in experimental animals and humans. Isolated cell techniques suffer from possible enzymatic digestion of critical cellular components. Caution should be exercised in the final interpretation of experiments utilizing this approach.

REFERENCES


