The Respiratory Tract and the Environment
by Joseph D. Brain*

The primary determinants of pulmonary disease are environmental. The same thinness and delicacy of the air-blood barrier which allows rapid exchange of oxygen and carbon dioxide also reduce its effectiveness as a barrier to inhaled allergens, carcinogens, toxic particles, and noxious gases, and microorganisms. Adults breathe 10,000 to 20,000 liters of air daily. This volume of air contains potentially hazardous contaminating particles and gases. Future research should explore the diverse physiological mechanisms which prevent the accumulation and deleterious action of inhaled particles and gases. Since most pulmonary diseases are either initiated by or at least aggravated by the inhalation of particles and gases, the role of environmental factors in the development of respiratory disease is an area worthy of continued support.

Functions of the Respiratory System

Human beings cannot live without producing adequate amounts of energy, which is produced by oxidizing the organic molecules in food. Thus the consumption of oxygen and the production of carbon dioxide, a waste product of ATP synthesis, are indispensable to life. It follows that the human body must have an organ designed to exchange carbon dioxide and oxygen between the circulating blood and the atmosphere in sufficient volumes to sustain life. Within the lungs are hundreds of millions of alveoli which form a surface of approximately 70 m². Blood flowing through the lungs comes within 1 μm of gas contained in the alveoli. However, the respiratory tract is faced with conflicting demands. It must be an efficient organ of gas exchange, but it must also create a protective barrier which effectively excludes unwanted, harmful agents.

Three processes are essential for the transfer of oxygen from outside air to the blood flowing through the lung: (1) ventilation, (2) perfusion, and (3) diffusion within the air spaces and across the air-blood barrier. Ventilation is the tidal process in which air is moved in bulk between the atmosphere and the lung. Perfusion is the action of the cardiovascular system in pumping blood throughout the lungs. Diffusion is the passive movement of gases down a concentration gradient between the alveolar gas and pulmonary capillary blood.

In order to deliver the inhaled gas to the alveolar surface uniformly, the lung has an elaborate distribution system. After moving through the nasal cavity or through the mouth, air reaches the throat or pharynx. Unless swallowing occurs, the epiglottis does not cover the larynx, and the gas passes through the larynx into the trachea. The trachea branches into left and right bronchi, which branch repeatedly until the alveoli are reached.

Movement of air into the lungs is caused by the action of the respiratory muscles which expand the chest wall so that the lungs fill passively. At the end of a normal expiration, a large amount of gas remains in the lungs. Both the rate at which breathing occurs (respiratory frequency) and the amount of air coming in with each breath (tidal volume) are variable and can be altered to match metabolic demands. The ventilation rate is adjusted by the brain to keep the concentration of oxygen and carbon dioxide in the alveoli relatively constant.

Although ventilation is essential, gas exchange does not occur unless blood is uniformly distributed throughout the lungs. The right side of the heart pumps venous blood which is high in carbon dioxide and low in oxygen. The pulmonary blood vessels are highly branched, and a thin film of blood is

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spread just beneath the alveolar surface.

The third requirement for gas exchange in the lungs is diffusion. Diffusion is very effective over small distances. In the alveoli, the blood is separated from the air by two specialized layers of cells, which together are less than 1 μm thick. The gas-exchange capability of the lungs must be linked to the needs of the whole body by means of the circulatory system and by the unique properties of the blood.

During the last decade, it has become obvious that the lung also has many nonrespiratory functions; for example, the endocrine function of the pulmonary circulation has been emphasized. The lungs metabolize vasoactive substances and may be involved in such complex humoral processes as blood pressure homeostasis. The endothelial cells in the lung alter bradykinin, angiotensin-I, and prostaglandins of the E and F series. Preliminary evidence also suggests that the lung may synthesize prostaglandins E₂, F₂α, G₂, and H₂. Too few studies involving the uptake and release of pharmacologic substances in the lung have been conducted on human beings, although there is substantial information from animal experiments.

The Lung as a Portal of Entry: Pathways of Toxic Materials from the Outer to the Inner Environment

The respiratory tract is the most common route of entry for toxic particles present in the work environment and the inhalation of particles in polluted air by the general population is also of great concern. Furthermore, the major portal of entry for infectious particles is the respiratory tract; inhaled particles from tobacco smoke now represent the most important cause of emphysema, bronchitis, and lung cancer. This section briefly summarizes the nature of particle deposition and clearance in the respiratory tract. These principles have broad application to both toxic and therapeutic aerosols which are inhaled. Excellent reviews of this area are readily available (1–3).

Deposition is the process that determines what fraction of the inspired particulates will be caught in the respiratory tract and thus fail to exit with the expired air. It is likely that all particles deposit after touching a surface; thus the site of initial deposition is the site of contact. Distinct physical mechanisms operate on particles suspended in the inspired air, causing them to move towards the surface of the respiratory tract: inertial forces, sedimentation, Brownian diffusion, interception, and electrostatic forces.

Inertia is the tendency of moving particles to resist changes in direction and speed. Repeated breathing in the airways causes sudden changes in the direction of air flow. However, because of inertia, particles tend to continue in their original direction, crossing air-flow streamlines and eventually making contact with the airway walls. Gravity accelerates falling bodies downward, and terminal settling velocity is reached when viscous resistive forces are equal and opposite in direction to gravitational forces. Respirable particles reach this constant terminal or sedimentation velocity in less than 0.1 msec. Thus, particles are also removed as their terminal velocity causes them to strike the airway walls or alveolar surfaces. Aerosol particles also undergo Brownian diffusion, a motion caused by collisions of gas molecules with particles suspended in the air. This motion causes the particles to cross streamlines and thus increases the probability that they will deposit.

The effectiveness of these deposition mechanisms depends on: (1) the anatomy of the respiratory tract, (2) the effective aerodynamic diameters of the particles, and (3) the pattern of breathing. These factors determine the fraction of the inhaled particles that are deposited as well as the site of deposition.

The anatomy of the respiratory tract is important since it is necessary to know the diameters of the airways, the frequency and angles of branching, and the average distances to the alveolar walls. Furthermore, when considered with the volumetric flow rate, airway anatomy specifies the local linear velocity of the air stream and the character of the flow as a significant change in the effective anatomy of the respiratory tract occurs when there is a switch between nose and mouth breathing. There are inter- and intraspecies differences in lung morphometry; even within the same individual, the dimensions of the respiratory tract vary with changing lung volume, aging and pathological processes.

The effective aerodynamic diameters of the particles affect the magnitude of forces acting on them. For example, while inertial and gravitational effects increase with increasing particle size, diffusion produces larger displacements as particle size decreases. This effective aerodynamic diameter is a function of particle size, shape, and density. Hygroscopic properties may also alter particle size both within and without the respiratory tract. In order to predict deposition patterns, it is essential to describe both the distribution and the mean of aerodynamic diameters.

The remaining factor affecting deposition is the breathing pattern. Minute volume defines the aver-
age flow velocity of the aerosol-containing air in the lung and the total number of particulates to which the lung will be exposed. Respiratory frequency will affect the residence time of aerosols in the lungs and hence the probability of deposition by gravitational and diffusional forces. Changing lung volume will alter the dimensions of the airways and parenchyma.

Despite continuous exposure of the lung to particulates characteristic of occupational and urban environments, the surfaces of the respiratory tract are relatively free of foreign matter. Even the diseased and blackened lungs of miners who succumb to coal workers' pneumoconiosis, contain less than 10% of the dust originally deposited there. Even in lungs seriously compromised by disease, the respiratory system is still surprisingly efficient at cleaning itself. The healthy lung exhibits even greater potency in the pursuit of cleanliness. This is the result of a complex system of respiratory defense mechanisms which both prevents particle entry and removes particles that have been deposited. Clearance refers to the dynamic processes that remove particulates from the respiratory tract; it is the output of particulates previously deposited. Highly soluble particles and gases dissolve rapidly and are absorbed into the blood from the respiratory tract. Their metabolism and excretion resemble that of an intravenously injected dose of the same material.

Less soluble particles that deposited on the mucus blanket covering pulmonary airways are moved toward the pharynx by the cilia. Also present in this moving carpet of mucus are cells and particles which have been transported from the nonciliated alveoli to the ciliated airways. Similarly, particles deposited on the ciliated mucus membranes of the nose are propelled toward the pharynx. There, mucus, cells, and debris coming from the nasal cavities and the lungs meet, mix with salivary secretions, and enter the gastrointestinal tract after being swallowed. Since the particles are removed with half-times of minutes to hours, there is little time for solubilization of slowly dissolving materials. In contrast, particles deposited in the nonciliated compartments have much longer residence times; there, small differences in in vivo solubility can have great significance.

A number of factors can affect the speed of mucus flow. They may be divided into two categories: those affecting the cilia themselves and those affecting the properties of the mucus. The following aspects of ciliary action may be affected by environmental insult: the number of strokes per minute, the amplitude of each stroke, the time course and form of each stroke, the length of the cilia, the ratio of ciliated to nonciliated area, and the susceptibility of the cilia to intrinsic and extrinsic agents that modify their rate and quality of motion. The characteristics of the mucus are frequently even more critical. The thickness of the mucus layer and its rheological properties may undergo wide variations.

Many studies reported in the literature do not characterize ciliary motility and the quantity of the mucus separately. In most cases the interaction of the two processes, mucus transport, is the variable that is measured. Most evidence suggests that mucus secretion is a more sensitive process than is ciliary activity. In many instances the quantity and rheological characteristics of the polymeric gel which constitutes mucus may be affected independently of any change in the cilia.

Macrophages are usually credited with keeping the alveolar surfaces clean and sterile. Alveolar macrophages are large, mononuclear, phagocytic cells found on the alveolar surface. They do not form part of the continuous epithelial layer, which is made up of pulmonary surface epithelial cells (type I pneumocytes) and great alveolar cells (type II pneumocytes). Rather, the alveolar macrophages rest on this lining. These cells are largely responsible for the normal sterility of the lung. It is the phagocytic and lytic potential of the alveolar macrophages that provides most of the known bactericidal properties of the lungs. Like other phagocytes, alveolar macrophages are rich in lysosomes. The lysosomes attach themselves to the phagosomal membrane surrounding the ingested pathogen. Then the lysosomal membranes become continuous with the phagosomal membrane and the lytic enzymes kill and digest the bacteria. Among the hydrolases they are known to contain are proteases, deoxyribonuclease, ribonuclease, β-glucuronidase, and acid phosphatase. Although these enzymes constitute an important aspect of the lung's defensive posture, when kept in a chronically activated state, this digestive capacity may serve to damage pulmonary tissues. Release of lysosomal enzymes, particularly proteases, from activated macrophages and leukocytes may be involved in the development of emphysema. Release may occur as a consequence of cell death, cell injury, or exocytosis. Increased deposition of inert or infectious particles acts to recruit additional macrophages and thus the effect may be reinforced.

Hence, our concern is also directed toward their ability to ingest nonliving, insoluble dust and debris. Rapid endocytosis of insoluble particles prevents particle penetration through the alveolar epithelia and facilitates alveolar-bronchiolar transport. Schiller (5) and later Sorokin and Brain (6)
found little evidence that macrophages laden with
dusts can re-enter the alveolar wall; only free parti-
cles appear to penetrate. Thus phagocytosis plays
an important role in the prevention of the entry of
particles into the fixed tissues of the lung. Once
particles leave the alveolar surface and penetrate
the tissues subjacent to the air–liquid interface (type
I and II cells, interstitial and lymphatic tissues),
their removal is slowed. Particles remaining on the
surface are cleared with a biological half-time esti-
mated to be twenty-four hours in humans, while
particles that have penetrated into “fixed” tissues
are cleared with half-times ranging from a few days
to thousands of days. Therefore, the probability of
particle penetration is critical in determining the
clearance of particles from the non-ciliated regions
of the lungs.

Pathological processes, such as fibrosis, may also
impair particle clearance from these compartments.
The probability of particle entry into a fixed tissue
in which it would have a long biological half-time is
reduced if the particle is phagocytized by a free cell.
Therefore endocytosis emerges as a central theme
of macrophage activity and the ability to quantitate
endocytosis and to study variations in the rate of
endocytosis is essential to our understanding of
respiratory defense mechanisms. Only then can the
role of differing environmental conditions on en-
docytosis be understood. Endocytosis has been
much studied, but most techniques which are avail-
able measure phagocytosis in vitro. Attempts to
quantitate phagocytosis in vivo are considerably
less frequent. Hepatic macrophages have been
studied as the major component of the reticuloen-
dothelial system and the rate of disappearance of
various particulate materials from the circulation is
well described. In contrast, the rate at which
phagocytosis occurs in the living lung is not well
established. Serial sacrifice and visual observation
can give some clues, but other techniques to esti-
mate endocytosis in vivo must be created.

During the last few years, it has become increas-
ingly obvious that not all pulmonary macrophages
are alveolar macrophages. Another important subdi-
vision of pulmonary macrophages is the airway
macrophage. These mononuclear cells are present
in the conducting airways, both of large and small
caliber. They may be present as passengers on the
mucus escalator, or they may be found beneath the
mucus lining, apparently adhering to the bronchial
epithelium. These airway macrophages probably
represent the result of alveolar-bronchiolar trans-
port of alveolar macrophages, although it has been
suggested that they are the product of direct migra-
tion of cells through the bronchial epithelium.

The third subdivision of pulmonary macrophages
is interstitial macrophages found in the various
connective tissue compartments in the lung. These
include alveolar walls, sinuses of the lymph nodes
and nodules, and peribronchial and perivascular
spaces. Connective tissue macrophages have been
considered in some detail by Sorokin and Brain (6).
All pulmonary macrophages are usually considered
to be relatives of monocytes and macrophages
throughout the body. This extended family includes
the Kupffer cells of the liver, the free and fixed ma-
crophages in the spleen, lymph nodes, bone marrow,
the peritoneal macrophage of the serous cavity, and
the osteoclasts of bone.

A major aspect of macrophage function of great
importance to environmental health is the fate of
the pulmonary macrophage. Differences exist
among the varying classes of pulmonary macro-
phages and their fate must be considered sepa-
rately. Although little is known about the latter
stages of the life of the alveolar macrophage, the
possibilities are finite and easily enumerated.
They may be subject to alveolar-bronchiolar trans-
port mechanisms; they may enter the lymphatics or
connective tissue; or they may enter the circulation.
Finally, it is also possible that some never leave the
alveolar surface but persist for long periods of time,
die there, and are then ingested and digested by
younger, more vigorous siblings.

There is speculation about the mechanisms res-
ponsible for alveolar-bronchiolar transport but lit-
tle supporting evidence exists. We do know that
most particles deposited in alveoli are ingested by
alveolar macrophages. Some of these cells find their
way to the bronchioles, and are then carried to the
pharynx by ciliary action. Migration through alveo-
lar pores, or other collateral pathways between ad-
jacent bronchial paths cannot be excluded. How-
ever, almost all of the macrophages are located on
the surfaces of alveoli or bronchi. Thus, it seems
unlikely that macrophages migrate to the bron-
chioles by penetrating between alveolar cells or by
emerging from lymphatic pathways.

Since some macrophages find their way to air-
ways, we must ask how these cells move to the
mucus escalator. It is possible that macrophages
exhibit directed locomotion because of a concen-
tration gradient of a chemotactic factor. The
phenomenon of chemotaxis is well studied in vitro.
This is particularly true for neutrophils, though
much less so for macrophages. Little is known
about the chemotactic behavior of alveolar ma-
crophages and no observations of alveolar ma-
crophage movement in situ have been made. There
is also no evidence to suggest that other tropisms,
such as geotropism, account for a purposeful mi-
gration of macrophages. Little direct experimental

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evidence suggests that the fluid lining of the alveolar region moves mouthward, but many investigators assume that it does.

The direct entry of alveolar macrophages into lymphatic pathways and connective tissue has often been suggested but rarely proved. The presence of particle-containing macrophages in these compartments is compelling evidence for many investigators. However, the entry of alveolar macrophages on the one hand and the entry of bare particles which are subsequently ingested by connective tissue macrophages already present on the other cannot be readily distinguished. During alveolar clearance, some noningested particles may follow lymphatic or vascular channels from alveoli into the peribronchial, perivascular, or subpleural adventitia and thus penetrate into the connective tissue of the lung. They are then stored by resident macrophages already present. This pathway may be more common when conditions favor increased lymphatic permeability (pulmonary edema). Then a greater number of particles might pass into these vessels through clefts between endothelial cells, to be carried along lymphatic drainage paths until filtered out by macrophages located farther along in lymphoid foci. Although inhaled particles can be found in connective tissue macrophages, we are aware of little evidence that implicates movement of surface macrophages into connective tissue compartments. We cannot, however, totally exclude this possibility, however uncommon it may be. Yet such penetration may be of consequence to environmental immunologic lung disease since it provides a pathway for antigens in or on alveolar macrophages to meet reactive lymphocytes in the connective tissue.

When we consider the fate of airway macrophages, we should not assume that the particle-containing mononuclear cells in the airways are necessarily the product of alveolar-bronchiolar transport. Some may be, but others may derive from blood monocytes which have migrated from the bronchial circulation directly to the airways. Alternatively, they may derive from local monocytic cell renewal systems (albeit undescribed) subjacent to the bronchial epithelium.

Although the pulmonary macrophages are essential to host defense, the normal activity and movement of pulmonary macrophages may also cause harm. Because the macrophages are actively phagocytic, inhaled toxic, radioactive, or carcinogenic particles become concentrated within pulmonary macrophages. What begins as a diffuse and relatively even exposure becomes highly localized and nonuniform. “Hot spots” are formed, and if thresholds for certain effects exist, these “hot spots” of high dose may be of significance.

Similarly, adherence of some airway macrophages to the airway epithelium may increase airway exposure to inhaled toxic materials. More importantly, perhaps, this close association with the bronchial epithelium can lead to transbronchial transport of inhaled particles and subsequent reinigestion by subepithelial connective tissue macrophages. These cells, like their relatives in the alveolar and airway compartments, also segregate, retain, and perhaps metabolize carcinogenic and other toxic particles.

Additional research needs to be carried out on the structure and function of the pulmonary lymphatics. Classically, removal of particulates through these channels was often considered to be the major route for the clearance of material from the lower respiratory tract. The importance of lymphatic clearance was probably exaggerated for several reasons. Most of the early judgments were based on morphological studies. Histological procedures, however, usually washed away the particles and cells being cleared via the airways, and so the importance of this route was underestimated. It is also difficult to draw quantitative conclusions about dynamic processes when only static observations are available. The importance of a clearance pathway is a function not only of the number of particles in the pathway, but also of the rate at which the particles are moving. Yet, although our estimate of the percentage of particles cleared via the lymphatics has dropped, our appreciation of the importance of those particles entering the lymphatics has greatly increased. Because particles in lymphatics are cleared slowly, they attain great significance in the pathogenesis of many lung diseases. When months and years have passed after exposure to particles, these connective tissue burdens may constitute the major reservoir of retained particles.

Too little is also known regarding the nature and properties of the air-blood barrier and the potential for particles to penetrate it. The extent to which the pulmonary capillary bed serves as a protective filter eliminating debris, pathogens, and cells from the circulating blood is not well described.

Little is known about the relative importance of these clearance pathways in health and disease. This lack is not without good reason. Difficult experimental protocols would be necessary to accurately quantitate fractions carried by the different clearance pathways. Theoretically, clearance by the various routes could be measured by monitoring the final common pathways for the airways, the blood vessels and the lymphatics, using various ingenious approaches. In the airways, a balance must be made for material deposited more proximally if
alveolar clearance is to be measured. In the lymphatic route, a single final pathway would have to be rationalized surgically for many collateral openings into the bloodstream. Examination of lung washings can be helpful in determining what part of the uncleared particle residues are lodged in alveolar macrophages and what part remain in the connective tissue. Further allowance must be made for the possibility of crossover from one clearance path to another, and it is important to separate the amount of material cleared by extracellular from that cleared by cellular mechanisms. As yet few studies have provided quantitative details sufficient to describe and evaluate the complexities of alveolar clearance. It is also likely that the extent to which each pathway is used depends on the sizes, solubilities, and chemical properties of the deposited particles, as well as the time elapsed after the exposure and the degree of disease present. Finally, it should also be remembered that deposition and clearance must be considered together. Of prime importance is the mass of the toxic substance in the lung. The actual amount of a substance in the respiratory tract at any time is called the retention. When the exposure is continuous, the equilibrium concentration (achieved when the clearance rate matches the deposition rate) is also the retention. This, the relative rate constants of deposition and clearance determine the equilibrium level; it is this level, or retention integrated over time, and the properties of the particle that are presumably related to the probability of a pathological response.

The Lung As a Target Organ

The lung is more than an important portal of entry for environmental contaminants. It may also be the critical organ—that is, the organ most likely to be injured or compromised by inhaled particles or gases. The abundance of specific diagnostic labels for pulmonary diseases does not alter the reality that universal mechanisms are operating. The lung does respond to environmental insults in a variety of ways, but its repertoire of responses is not unique. The same catalog of responses and mechanisms that characterizes the injured respiratory tract is also common to other organ systems. Thus, general pathology (7, 8) provides an essential backdrop for our understanding of the lung and environmental insults. Further progress in basic pathology is essential. Table 1 lists some of the changes that may take place in the lung. These changes are direct or indirect consequences of the physical and physiologic changes in cells brought about by their contact with inhaled agents. The cell responses, in turn, are responsible for loss and distortion of alveoli, deposition of new alveolar tissue, alterations in capillary and airway diameter, and other anatomic alterations. These changes can occur in combination, and their severity is related to the duration and concentration of exposure.

<table>
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<tr>
<th>Table 1. Mechanisms of pulmonary injury.</th>
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<td>Proteolysis: Elastin and collagen destruction leading to emphysema</td>
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<td>Fibrosis: Increased connective tissue and scarring</td>
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<td>Pulmonary edema and altered alveolar stability</td>
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<td>Immunologic responses: Asthma, hypersensitivity lung diseases, extrinsic allergic alveolitis</td>
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<td>Inflammation: Irritation leading to mucosal edema, increased mucus production and bronchitis, enhanced cell renewal</td>
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<td>Altered susceptibility to infection: Cytotoxic and competitive effects on macrophage function, altered mucociliary transport due to changes in cilia or the quantity of rheological character of mucus</td>
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<td>Degenerative changes: Necrosis, calcification, and autolysis</td>
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<td>Bacterial, viral, and fungal infection</td>
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<td>Pulmonary carcinogenesis</td>
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Although these pathological mechanisms operative in the lung are not exclusive to it, many arise from unusual interactions with environmental factors. Certain mechanisms seem especially worthy of further study. Connective tissue proteins are an integral part of the lung. Collagen and elastin help maintain alveolar, airway and vascular stability, limit lung expansion, and contribute to lung recoil at all lung volumes. Because of their fundamental role in lung structure and function, collagen and elastin balance (synthesis and degradation) deserve additional attention.

Two groups of environmental lung disease are associated with aberrations of normal collagen and elastin balance: emphysematous and fibrotic disorders. To what extent this association involves changes in the rate and nature of synthesis, or in the role or nature of degradation of these proteins remains speculative. Analysis of collagen composition, synthesis and degradation rates would be extremely useful both for diagnosis and for assessment of therapy of these connective tissue lung diseases.

Unfortunately, the measurement of such parameters in the lung is difficult. Quantitation of rates and composition analysis have been described (9), and can be performed on lung biopsy tissue. However, the large number of different cell types, and the heterogeneity, interactions and insolubility of lung connective tissue have made accurate quantitation difficult. Improved approaches are needed
and in vitro models of lung biochemical function may be very useful. Appropriate animal models of emphysema and fibrosis could be exploited to quantitate the rates of elastin and collagen synthesis and degradation and to compare them to normal rates. In emphysema, further studies are needed to examine the release of proteolytic enzymes from leukocytes and macrophages, their effect on lung cells, and the protection afforded by serum and lung antiproteases. In fibrosis, investigations need to be pursued studying the role of lymphocytes, macrophages, fibroblasts and the effect of fibrogenic agents on the collagen balance and on the replication and differentiation of each parenchymal cell type (10).

Attention to this kind of injury has increased because of recent new knowledge about pulmonary emphysema associated with inborn α1-antitrypsin inhibitor deficiency in humans. Inbalances between proteolytic activity and its control or inhibition have important implications as a generalized mechanism of lung injury in other pulmonary pathological states caused by air pollution, or the inhalation of occupational dusts.

Much more needs to be known regarding the nature of the particles which trigger these connective tissue diseases. Dust particles of appropriate size and shape may deposit on alveolar surfaces and stimulate production of excess collagen in the alveolar membrane. Particle size, shape, and durability may be more important in fibrogenicity than chemical characteristics. Asbestos, glass, and other fibrous dusts all have been shown to stimulate collagen synthesis (11). Fibers over 5 μm in length are sometimes incompletely ingested by macrophages (12, 13), and this may result in macrophage death or release of mediators. Growth of fibroblasts in vitro has been shown to require a solid supporting particle of critical minimum dimensions (14).

In addition, there is some evidence that fibrogenesis may also occur as a two-step process (15). This would especially apply to highly fibrogenic particles such as silica which are highly symmetrical in shape (5 μm in diameter) and which are engulfed by macrophages following deposition. Silica has not been shown to exert a direct stimulatory effect on fibroblasts. Rather, the interaction of a particle with a macrophage is thought to release factors which stimulate local production of collagen by fibroblasts. It is unlikely that macrophages differentiate into collagen synthesizing fibroblasts (16).

Silica and asbestos have the added disadvantage of being cytotoxic to alveolar macrophages. Within a few minutes they can cause lysis of cells by direct interaction with the plasma membrane, or, if successfully ingested, in several hours cause rupture of secondary lysosomes, releasing lysosomal hydrolases into the cytoplasm (17). The resulting dead macrophages can become focal points for further fibrogenesis. In addition, the particles are released anew on the alveolar surface to cause more irritation.

The responses of the lungs to inhaled antigens and allergens and the general area of environmental allergic respiratory disease is emerging as an important area of basic investigation. Advances in this area may help to identify hyperreactors to a number of industrially important materials, such as toluene disocyanate (TDI), cotton dust, molds (Farmer's Lung and Bagassosis) and proteins causing a variety of forms of extrinsic allergic alveolitis. Immunological research addressed to the interaction of these inhaled materials, connective tissue, pulmonary cells and the lymphatic and vascular systems in the lung holds promise. Little is known about the degradation of proteins deposited on the respiratory tract surfaces. No doubt, in many instances macrophages and pulmonary clearance defend the body against excessive antigenic stimulation. However, there may also be circumstances when clearance pathways may cooperate with the immune system and preserve and present immunogenic molecules to the immune system. Thus the issue of how and when pulmonary macrophages either suppress or enhance the immunogenicity of antigens must be confronted.

It is known that several organic dusts can induce an immune response in bronchial or alveolar tissues. The type I and type III allergic reactions are most commonly seen (18, 19). The type I response, often termed immediate hypersensitivity, occurs when an inhaled antigen reacts with cell-sensitizing reaginic antibody (IgE). Cells of the bronchial wall may be sensitized by this antibody, and react to produce histamine, slow reacting substance of anaphylaxis, and bradykinin. In persons sensitized to a specific inhaled antigen, bronchoconstriction, mucosal edema, excessive secretions, and eosinophil infiltration occur.

The type III, or Arthus reaction, is characterized by formation of a complex between an antigen and a precipitating antibody in the presence of complement. Tissue damage occurs from inflammatory responses caused by deposition of these complexes along capillary basement membranes. The complex is phagocytized by leukocytes and causes them to release their lysosomes. In addition, histamine may be released from mast cells producing local edema. It is unknown whether antigens cross the alveolar and capillary membranes to react with circulating antibodies, or if lymphocytes and other reactive cells on the alveolar and bronchiolar surfaces are responsible for the initial antibody-antigen reaction.
The site of action of TDI and the active components of cotton dust need to be better described.

Particles inducing hypersensitivity pneumonitis tend to be derived from fungal, bacterial, or serum protein sources. Examples are the fungus *Thermoactinomyces vulgaris* in Farmer’s Lung, *Bacillus subtilis* in detergent workers’ lung and bird fanciers’ disease (20–22).

Neoplastic responses to inhaled particles is another area of major and growing concern. Although smoking has been determined to be the major cause of respiratory carcinogenesis, other factors, especially industrially produced agents, are gaining strong epidemiologic support. Asbestososis has been correlated with bronchogenic carcinoma, as well as mesothelioma of the pleura and peritoneum (22). In addition, a synergistic effect between asbestos and cigarette smoking may cause a substantially increased risk (23). Other agents imposing an increased risk are the polycyclic aromatic hydrocarbons, radioisotopes, chromates, and compounds involved in nickel refining (24–27).

Further study on the role of modifying influences is of particular importance. The pathogenetic mechanisms just discussed are in no way isolated from important host variables. Dose–response curves for interactions with environmental agents are altered by many characteristics of the exposed organism and its immediate environment. Some factors known to be important include: developmental aspects, aging; genetic factors (AHH inducibility and lung cancer, α₁-antitrypsin deficiency, nonspecific airway reactivity); sexual differences; species differences; interactions; circadian rhythms; effects of pre-existing disease (“the sensitive individual”); nutritional status; drugs; exercise and altered ventilation; tolerance and adaptation; and metabolic transformations.

Additional research concerning the effect of these variables on the respiratory tract and other organ systems is desirable.

Detection and Quantitation of Lung Injury

An essential aspect of research on environmental lung diseases relates to improved determination of dose–response curves. We need to know with precision to what the respiratory tract is being exposed. We must be able to measure lung damage with similar precision and sensitivity.

It is now more widely appreciated that it is essential to specify the mass, particle size, and solubility of inhaled particles. The ICRP lung model (2, 28) emphasized the importance of these parameters in determining burdens of particulates in the respiratory tract. It also provided a common framework which can be used in summarizing experimental data. But there are still major problems in quantitating the pulmonary dose. The relative merits of intrathoracic and inhalation exposures have recently been discussed (29). Switching from nose to mouth breathing and the effects of exercise are largely unexplored. Systematic descriptions of species differences are also essential. Although many species have been used for studies in aerosol deposition, we are unaware of any published systematic description of the differences between commonly used laboratory animals. It is difficult to abstract such a description from the literature because so many different kinds of animals and aerosols have been used in various combinations.

The lung responds to environmental injury in many ways. This diversity in response is matched by a dazzling array of possible tests designed to detect and quantitate lung damage. Table 2 lists some of the approaches which commonly have been used.

Almost all these approaches demand further refinement and many have serious limitations. For example, one of the major problems with pulmonary function tests is that they are rarely specific. Measurements of airway resistance reflect not only direct alterations in the airway but may also reflect parenchymal changes. For example, if the parenchyma becomes weakened by emphysema, support for the airways is diminished. They become more collapsible and airway resistance may increase. Similarly, compliance measurements do not exclusively reflect changes in the pulmonary parenchyma. If airway obstruction occurs, then the parenchyma served by the airway will largely be excluded and the lung will appear to be stiffer.

Further refinements are also needed for better histologic, histochemical and morphometric analysis of lung damage. Various fixation procedures have been used for the fixation of lung tissue. The main criteria are to preserve optimally the pulmonary parenchyma in accordance with the desired goal (examination of ultrastructure, investigation of acellular alveolar lining layer, application of histochemistry). Techniques exist which permit cutting sections of whole lungs for pathologic examination. These sections were originally intended for studying the pathology of coal workers’ pneumoconiosis, and for comparison with radiological examinations during the individuals’ lives. In addition to evaluating the lung sections qualitatively, quantitative morphometric methods (30) have been developed to assay the degree of tissue damage.

It would be desirable to identify enzymes or other factors released from damaged lung into serum.
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<th>Parameters and methods</th>
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<tr>
<td>Mechanical properties (pulmonary function)</td>
<td>Compliance, Resistance, Lung volumes, VC, TLC, RV, FRC, He dilution, Boyle's law, radiologic, FEV₁, Flow volumes, partial flow volume curves, Maximum breathing capacity, Closing volumes, Frequency dependence of compliance</td>
</tr>
<tr>
<td>Gas exchange</td>
<td>Adequacy of ventilation, Alveolar gas tensions</td>
</tr>
<tr>
<td>Arterial $pCO_2$, $pO_2$.</td>
<td>$N_2$ washout, $Xe^{133}$ scans, $a-A$ gradients, Perfusion scans—particles, or infused $Xe^{133}$, Diffusing capacity</td>
</tr>
<tr>
<td>Distribution of ventilation</td>
<td></td>
</tr>
<tr>
<td>Radiologic techniques</td>
<td>Atelectasis, Fibrosis, Degree of aeration, Bronchography (Tantalum), Lung volumes, Focal lesions</td>
</tr>
<tr>
<td>Mucociliary transport</td>
<td>$In$ vivo, $In$ vitro, Nasal, Airway, Mucus studies, Cilia studies</td>
</tr>
<tr>
<td>Lung lavage</td>
<td>Surfactant levels, Cell numbers, Cell differential counts, Viability, Biochemistry, Histochemistry, $In$ vivo functional assays of macrophage activity, Migration, Phagocytosis, Bactericidal activity</td>
</tr>
<tr>
<td>Histology on whole lung</td>
<td>Fixation techniques. Problems: lung volumes, cellular heterogeneity, Gough sections, Reid index, Airway and alveolar dimensions: morphometric approaches, Cell types: light and electron microscopy, Histochemistry, Vascular changes</td>
</tr>
<tr>
<td>Renewal of lung constituents</td>
<td>Metaphase counts—colchicine, Uptake of tritiated thymidine, Problems in identifying cell types, Collagen and elastin breakdown and synthesis</td>
</tr>
<tr>
<td>Identifying pulmonary carcinogens</td>
<td>Experimental pulmonary carcinogenesis (Saffiotti model), Ames assay</td>
</tr>
<tr>
<td>Microbicidal activity</td>
<td>Recognizable experimental pulmonary infections (morbidity and mortality studies), Bacterial aerosol models, $in$ vivo models, $In$ vitro killing, Phagocytosis, $In$ vitro, $In$ vivo</td>
</tr>
</tbody>
</table>
which could be sampled. Unfortunately, currently no biochemical parameters are known which clearly indicate the presence of toxic lung damage. It thus becomes important to obtain more complete knowledge about the biochemical events leading to cell death or to repair and pulmonary cell proliferation. Only then will we understand the mechanisms of response and perhaps obtain useful indicators of lung damage.

Techniques for sampling directly from the lung are also desirable. Improved biopsy techniques which can be used for repeated sampling from live animals need to be developed. Lung lavage both in sacrificed animals and in isolated segments of living animals is also a valuable approach (see Table 2). For example, research on pulmonary macrophages often depends on our ability to isolate pure populations of macrophages from the lungs of animals and man. Alveolar macrophages represent a relatively homogeneous cell population accessible by lung lavage. However, success in isolating pulmonary macrophages depends on the particular subclasses involved, and, unfortunately, not all categories of pulmonary macrophages can be recovered with the same ease and purity. To our knowledge, no investigator has been able to prepare a pure suspension of interstitial pulmonary macrophages. Techniques for dissolving the lung and liberating individual cells are being developed and intensive efforts are underway with regard to separation of isolated lung cells. However, interstitial macrophages are less numerous and little is known about the existence of unique physical or chemical properties which could be exploited in separating these cells. Conceivably, their tendency to attach themselves to glass or some specific surface receptor could be exploited. If a lung which has been washed to eliminate airway and alveolar macrophages, was dispersed, presumably those cells attaching to glass represent a relatively enriched population of interstitial macrophages.

Improved assays for identifying pulmonary carcinogens also need to be developed. One approach is to administer the suspected material to a laboratory animal by intratracheal instillation. Saffioti (31) has developed such an approach, and it has been frequently imitated. An alternative to experimental carcinogenesis has been implemented by Ames et al. (32). This test operates under the working hypothesis that carcinogens are also mutagens. Using an in vitro bacterial test system (Salmonella typhimurium) Ames et al. have been able to detect known classes of mutagens at very low concentrations. The Ames assay is based on the use of controlled mutation of the bacterial strain for detecting mutagens by a highly sensitive and convenient back mutation test. The relatively small genome (approx. 4 x 10^6 base pairs), the large number of organisms exposed (approx. 10^9 per plate), and positive selection of mutants are some of the advantages of this system. In addition, many chemical carcinogens become powerful mutagens through in vivo metabolic activation, so addition of various cell suspensions to the test culture is another important feature.

Conclusions

Pollutants characteristic of occupational and urban environments, bacteria, viruses, and other infectious particles, allergens, and antigens all may cause or aggravate pulmonary disease (Table 3). Furthermore, the magnitude of these effects is strongly influenced by the number of particles deposited, their site of deposition, and especially their ultimate fate. Thus, environmental factors represent a major area of concern. Death rates for lung cancer and pulmonary emphysema have been steadily increasing over the last half century. Chronic nonspecific pulmonary disease has become a leading cause of death and disability in adult males. Cigarette smoking is the major cause of this epidemic, but community air pollution and industrial exposures are also a major significance.

Recommendations

It is important to realize that factors within the laboratory environment may influence the response of animals to inhaled particles and vapors. Such diverse influences as dietary components and contaminants, caging conditions, (bedding, cleanliness, overcrowding, lighting, temperature), nutritional deficiency, disease, as well as such factors as insecticides and pesticides which are used to keep animal facilities insect- or pest-free must be better understood and quantified.

The properties of the bronchial epithelium may be critical in relation to the development of lung disease. The epithelium is both a surface that can respond, and a barrier which is occasionally crossed. A great deal is known about the epithelial lining of the gastrointestinal tract and its relationship to the movement of particles, molecules, electrolytes, and water. Similar information ought to be gathered about the bronchial epithelium.

Support for studying the effects of air pollution should be continued. Such studies are essential in determining mechanisms of biological response and as part of systematic studies of dose-response relationships. They also may be used as animal models
for the effects of new products. Systematic exposure of animals to materials produced by technological advance would permit some assessment of the effects of these materials.

The effects of parameters which may modulate the pulmonary response to atmospheric and industrial contaminants should be measured. The effects of medications, altered physiological states, and concurrent illness should be systematically explored. Meteorologic variables (temperature, fog) and factors in the organism such as exercise, disease, or allergic states, adaptation, and presence of other foreign chemicals should also be considered.

Support for lung research is supplied by a number of Federal agencies, such as NHLBI, EPA, NIOSH, ERDA, as well as NIEHS. Coordination and integration among these different organizations is frequently inadequate. Mechanisms should be developed for finding out about each other's grant programs, contract programs, and in-house research. Also, a published inventory of major facilities which are essential in health effects re-

<table>
<thead>
<tr>
<th>Type</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic dusts</td>
<td></td>
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<tr>
<td>Free silica</td>
<td>Quartz, tridymite cristobalite</td>
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<tr>
<td>Crystalline</td>
<td>Diatomaceous earth, silica gel</td>
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<tr>
<td>Amorphous</td>
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<tr>
<td>Silicates</td>
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<tr>
<td>Fibrous</td>
<td>Asbestos, sillimanite, talc, sericite</td>
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<tr>
<td>'Inert' metals</td>
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<tr>
<td>Biological 'dusts'</td>
<td></td>
</tr>
<tr>
<td>Vegetable</td>
<td>Moldy hay (''farmer's lung''), mushroom compost, bagasse (sugar cane), maple bark, B. subtilis enzyme (detergents), malt, grain-weevil, cork, roof-thatch, lycopoderm, cotton, flax, hemp, jute, sisal, thermophilic actinomycetes</td>
</tr>
<tr>
<td>Animal</td>
<td></td>
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<tr>
<td>Toxic chemicals</td>
<td></td>
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<tr>
<td>Irritant gases</td>
<td></td>
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<tr>
<td>Metallic fumes, vapors, oxides and salts</td>
<td>Beryllium, silicide, mercury, cadmium, platinum, magnanese, zinc, vanadium pentoxide, nickel, osmium</td>
</tr>
<tr>
<td>Plastics</td>
<td>Polytetrafluoroethylene, toluene diisocyanate (TDI)</td>
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<tr>
<td>Aerosols</td>
<td>Mineral oil, ''cutting'' oils, copper sulfate</td>
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<tr>
<td>Respiratory infections</td>
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<tr>
<td>Bacteria</td>
<td>Tuberculosis (miners, nurses, pathologists), Anthrax (wool, mohair, and alpaca workers)</td>
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<td></td>
<td>Glanders (grooming of horses, mules)</td>
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<tr>
<td>Virus/rickettsiae</td>
<td>Psittacosis (pet shops, turkey dressings, pigeons)</td>
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<tr>
<td></td>
<td>Variola (smallpox handlers)</td>
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<tr>
<td></td>
<td>Q fever (lab workers, abattoirs)</td>
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<tr>
<td>Fungi</td>
<td>Coccidiomycosis (irrigation, farm work, archeology)</td>
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<tr>
<td></td>
<td>Histoplasmosis (poultry, pigeons, starlings)</td>
</tr>
<tr>
<td></td>
<td>Cryptococcosis (pigeons)</td>
</tr>
<tr>
<td>Respiratory carcinogens</td>
<td>Arsenic, cobalt, nickel, hematite, uranium (pitch blends, ''yellow cake'') isopropyl oil, gas retort fumes, chromates, asbestos, other silicates (talc)</td>
</tr>
<tr>
<td>Consumer products</td>
<td>Hair sprays</td>
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<td></td>
<td>Deodorants</td>
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<td></td>
<td>Paints</td>
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<td></td>
<td>Pesticides</td>
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<tr>
<td>Tobacco products</td>
<td>Cigarettes</td>
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<tr>
<td></td>
<td>Cigars and pipes</td>
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<tr>
<td></td>
<td>Marijuana</td>
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</table>

*From Trieff and Corrigan (33).*
search would encourage more efficient use of scarce and expensive resources. Sophisticated environmental exposure chambers, for example, now exist and might be made available to visiting scientists.

A frequent response to environmental insult is the deposition of fibrous connective tissue (a scar) usually known as pulmonary fibrosis or proteolysis and alveolar wall destruction leading to emphysema. These responses are common to a number of inhaled particles and gases. Our understanding of these diseases and appropriate strategies for their prevention and control would be helped by a better understanding of the synthesis and degradation of collagen in the lung, since it is the primary connective tissue protein. Similar studies should be carried out with regard to pulmonary elastin.

Diseases such as bronchial asthma, Farmer's Lung, diffuse hypersensitivity pneumonitis, and sarcoidosis are all examples of pulmonary diseases characterized by immunologic abnormalities. Many of the symptoms of asthma appear to be mediated by various chemicals released from lung cells. Further research is needed to define other mediators, to determine where and how they act, and to learn what environmental materials govern their release.

Frequently important contributions to our understanding of environmental disease have depended upon the existence of appropriate animal models. These are most helpful when the animals react to disease as humans do. Additional research which will develop and exploit animal analogs of environmental disease processes common to man should be supported.

New indices of exposure need to be developed. Breath analysis may be exploited as an indicator of exposure. Lung analysis at autopsy may also yield useful information about retention levels of pollutants. Noninvasive approaches to intact animals and people, such as whole body counting of radioactive materials or the use of magnetic measurements as a possible index of asbestos exposure, should be exploited.

More sensitive and specific indices of injury should be developed. Early detection should be a major goal especially when reversibility is possible.

For large segments of the population, industrial and atmospheric contaminants aggravate existing lung injury. More research is needed concerning the influence of disease states on the response to environmental agents. Animal models of asthma, emphysema, lung cancer, and newborn and adult respiratory distress syndrome all need to be better developed and defined and then used in these kinds of experiments. Similarly, such important agents as tobacco and alcohol need to be studied in regard to possible synergistic action.

Pulmonary metabolism needs to be better described. We need to understand how environmental contaminants are either degraded or made more toxic by the lung. The use of isolated cells, the isolated perfused lung, whole animals, and other test systems can lead to improved understanding.

Species differences in lung structure, function, endemic disease, and susceptibility to environmental agents need to be better determined. Comparative studies of the physiology, biochemistry, and morphology of the lung should be encouraged in order to provide a logical basis for understanding the mechanisms of environmental influence and comparing results in animals and man.

Pyrolysis products, either as a result of industrial processes or as a result of unwanted fires, cause frequent exposure. Better analysis of the chemical composition of pyrolysis products and of their impact on the lung and other organs is needed.

Since so many diseases of the lung are poorly diagnosed and/or treated, more emphasis needs to be given to preventive strategies. The design of adequate legislation, testing of new materials and especially compliance with existing laws, all need to be studied. Frequently, we are dealing with factors influencing human behavior (i.e. use of protective equipment and safety strategies). Factors affecting individual compliance need to be better understood.

All organisms undergo a normal developmental sequence beginning with conception and leading to death. The conditions of each developmental stage may influence the response of the lung and of the organism to environmental insult and should be better studied. Very young and very old animals should be exposed to both air pollutants and particles and gases characteristic of industrial environments. Age-related responses must be more thoroughly described.

Chamber concentrations of pollutants do not adequately define dose since deposition is influenced by exercise, circadian rhythms, level of activity, and other factors. The effective dose is further modified by clearance pathways and by metabolic changes which make the compounds more or less toxic. Whenever possible, the effective tissue dose at the site of action in the lung should be measured.

The time course of damage, recovery, repair, and adaptation to inhaled particles and vapors must be described. This description is essential to an understanding of mechanisms involved in damage. This history also influences the design of experiments by predicting optimal times for sampling.

Our understanding of almost all environmental
lung disease would be greatly enhanced by a more thorough knowledge of the cell biology and physiology of the lungs. Important clues to the cause and prevention of environmental lung disease have frequently come from scientists working in unrelated areas. Thus, future progress in controlling environmental lung disease will depend in part on strong support for basic research in a variety of fields of science, as well as those fields directly concerned with the environment and the lung.

Too little is known about what influences an individual's decision to help or hinder his or her pulmonary health. Addictive cigarette smoking or compliance with preventive strategies such as respirators are two examples. Research should focus on the mechanisms of motivation for self-help or self-destruction. Similarly, research should be supported on the effectiveness of various education campaigns designed to influence human behavior.

Bronchial mucus is a major factor in the defense mechanisms of the lung as well as a component capable of response. The structure, synthesis, physical, chemical and rheological characteristics of mucus and periciliary fluid need to be better elucidated. Studies aimed at identifying specific causative agents in cigarette smoke should be continued. The presence of certain carcinogens, such as polycyclic hydrocarbons and 210Po, have been suggested as having major importance. Animal experiments should be carried out with animals exposed to cigarettes with these materials selectively removed. Steps should be taken to determine whether low tar and nicotine cigarettes are less hazardous than others. The possibility that combinations of agents rather than single components are responsible must be kept in mind. Research should not focus exclusively on carcinogenesis, but also on the many other deleterious effects of cigarette smoke on the respiratory and cardiovascular systems. Aggressive steps toward developing a safe cigarette should be undertaken.

Additional studies should be carried out to determine whether cigarette smoking has a synergistic action with other materials in the production of emphysema, cancer, asthma, and other pulmonary diseases. Such synergism seems to exist between cigarette smoking and asbestos exposure as well as exposure to radon-daughters, which are found in uranium mines. Experiments of this sort should be carried out in animals as well as in human epidemiological studies.

Our knowledge of the effects of indoor climate is limited. Widespread use of artificial ventilation, heating, and cooling of indoor air influence the lungs to a degree which is now uncertain. Building materials may emit noxious substances into indoor air, or conversely may serve to reduce indoor air pollution through absorption on indoor surfaces. Additional studies are needed to ascertain the influence of indoor climate on the lungs.

The lung is composed of many different cell types. Loss and replacement of these play an important role in the response to environmental pollutants. Measurements of turnover rates of lung cells and the changes in these rates relative to environmental insult require further study.

Because environmentally induced emphysema, chronic bronchitis, and lung cancer are usually irreversible by the time they are diagnosed, improved techniques and devices are needed to detect early biochemical, immunologic or physiologic abnormalities associated with development of these diseases.

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REFERENCES