CHAPTER 5

RESPONSE OF THE RESPIRATORY TRACT TO INHALED POLLUTANTS

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INTRODUCTION

The respiratory system is a major area in which mammals contact their external environment. During a typical breath about 600 cm^3 of air enters the nose. A fraction of this fills the "deadspace"—about 150 cm^3 including the nose, trachea and bronchial tubes that have relatively thick walls and do not participate in oxygen and carbon dioxide exchange. The remainder, about 450 cm^3 in the moderately active adult enters the respiratory portion of the lung where it is diluted by the 2400 cm^3 of air that remained unexhaled from the previous breath. Each day approximately 10,000 liters of air is inspired by the average adult. Since the respiratory system is designed to efficiently deliver this air to 70 m^2 of delicate surfaces, the potential for disease from

air pollutants is understandably great. The respiratory tract has varied and elaborate systems of defense, including rapid healing of epithelial injury. Although the list of agents that cause or promote lung disease is a long and growing one, these defense systems may explain why many of the changes seen in laboratory investigations after short-term inhalation exposure are transient. Such brief changes cannot be appropriately classified as disease but do show that the material is toxic enough to cause some injury and might lead to progressive damage and disease on repeated exposure.

Broadly, the lung can respond to pollutants in as many ways as its total number of properties and functions. Thus, if one can define and measure a property or function, this measurable factor can be made the biologic endpoint in an inhalation study. Furthermore, it appears that no matter what is selected for measurement, there will be a great number of inhalable materials that, in sufficient concentration and over a suitably long exposure time, will produce a statistically significant change. But until the role of the measured property is understood in terms of its relationship to respiratory tract disease, we do not know just how to treat the result. From a toxicologic point of view we have either identified a toxin or generated basic scientific information, but we may not have the ability to distinguish between the two. Although new scientific findings are of great, even fundamental, importance to all scientific disciplines, they generally require further development before they can be readily justified in toxicologic investigations. Although many of us—the authors of this chapter included—engage in basic studies on the respiratory system, we will direct our attention here to toxicologically meaningful perturbations of basic processes in the lung with emphasis on materials inhaled in polluted air.

Although the science of inhalation toxicology is still in an early stage of development, a great number of meaningful measures of injury to the respiratory tract have been developed. For simplicity these measures can be categorized into three broad types: anatomic, physiologic and biochemical. These categories represent the specific scientific disciplines that have primarily been applied to assessing toxic injury in the respiratory tract. Each discipline has its own way of viewing the respiratory system, its own tools and animal models and its own state of sophistication. For this reason, each of the three main areas of investigation will be presented separately, though in reality the lines of separation are often blurred.

TESTING STRATEGIES

The increase in public awareness of toxicological phenomena in environmental, industrial and occupational settings appears to have led to a desire to test all materials in every possible testing system. Such activity has helped to produce increased costs for these tests and has helped to point out the

need for regulations and standards of performance of tests. One problem that results from this greater demand for toxicological testing involves attempts to select inexpensive or short-term tests over longer-term or traditional testing procedures. Another attempt to reduce the costs of testing involves the utilization of a single species for testing and making judgments as to the hazards of the test material in relation to the response of this single species. While it is true that certain tests, using a given species, can provide information concerning the relative hazard of two or more test materials, a single test using a single species is inadequate for toxicological characterization of any test material.

The respiratory tract will respond in one way or another to any airborne pollutant that is inhaled. A variety of toxicity tests, or specific endpoints, have been applied to detect and quantitate these responses. The eventual goal is to interpret for mankind the hazard of the inhaled pollutant, in view of the responses seen in one or more of these tests. Two items that plague an attempt to make precision measurements for statistical interpretation are biological variability and defense mechanisms. If measurements are so fine-tuned as to detect miniscule physiologic or biochemical excursions from control values, we are often confronted with the dilemma that such excursions might be considered normal in the next batch of animals tested. Even when we obtain a statistically significant result for a given material, lifetime exposures to this material may not indicate any toxicity or potential hazard. This may be due to our initial measurement of a pulmonary defense mechanism or other transient physiological or biochemical response that is not seen in longer-term exposures. Often "adaptation" or some other phenomenon is initiated to counter the response.

Alarie [1] has developed a simple rapid method for evaluating the degree of upper airway irritation in rodents resulting from exposures to gases and aerosols. The mechanism underlying the method is that irritant chemicals stimulate the free nerve endings in the nasal mucosa; and through a reflex pathway, mediated by the trigeminal nerve, the respiratory rate is depressed as a function of irritant concentration [2]. The test involves four female mice per exposure group and has been of considerable value in evaluating the irritancy of personal and household aerosol products. Perhaps the greatest value of the test comes in discriminating between two or more formulations and when used in this way, i.e., the ranking of upper airway irritants, it is a very useful tool. For test materials that are not upper airway irritants, including many toxic dusts, this test alone is not adequate.

The irritant response in guiena pigs has been characterized by Amdur and associates [3, 4]. The respiratory system of the guinea pig is capable of dramatic bronchoconstriction in response to inhaled pollutants. Bronchoconstriction results in increased pulmonary resistance and in decreased pulmonary compliance. Slight changes in pulmonary resistance and compliance

can be measured by placing the guinea pig in a plethysmograph. Results from this testing system are highly significant if one uses each guinea pig as his own control. However, it is quite difficult using this test to get statistically significant results by randomly choosing a group of control animals to be tested against an identical exposure group. Also, it is possible to obtain statistically significant results from otherwise nontoxic aerosols. Finally, long-term exposures to pollutants that give positive results in the plethysmograph system have, at times, failed to show any toxicity in either rats or guinea pigs, even in lifetime studies at levels 10- to 100-fold higher than those detected in the plethysmograph system [5, 6]. Thus, neither this test system alone nor the use of a single species is appropriate as the basis for regulation of the test material.

The changing of the breathing rate and/or bronchoconstriction are physiological responses that serve to reduce exposure to inhaled pollutants by limiting their penetration into the lungs. Other defense mechanisms can be activated once pollutants deposit in the deep lung. Particulates that deposit in the gas exchange region of the lung are taken up by macrophages in the lung which have been recruited by these deposition stimuli. Particle-laden macrophages are then swept up the mucociliary escalator or are transported via the lymphatics to bronchus-associated lymphoid tissue. When the particulate burden is greater than the recruitment process can handle, granulomas form in the lung and lymphoid tissue [7, 8]. The transient recruitment of neutrophils and macrophages to the lung may not indicate a toxic response per se. It is only when recruitment cannot handle the magnitude of the insult or repeated insults or when the macrophages cannot ingest the entire particle, e.g., asbestos fibers, that toxic effects are seen, and only then after a long latent period or after years of repeated insult.

Interestingly, recruitment stimulus and the ability to clear the recruited cells from the lung varies with each species. In granuloma formation in the rat, more neutrophils (3:1) than macrophages are present in the fluid that can be lavaged, or rinsed from the lungs, while, in guinea pigs, more macrophages (4:1) than neutrophils are obtained. This may relate to the inflammatory response seen in oxygen toxicity in these species. When activated in the lung neutrophils produce superoxide, O_2^-, perhaps as an antimicrobial agent. The presence of increased levels of superoxide induces the enzyme, superoxide dismutase in lung tissue [9]. A similar response occurs when adult rats are placed in 85% oxygen for five days. Once the induction of superoxide dismutase occurs, these rats can survive in 100% oxygen. On the other hand, guinea pigs seem unable to induce the enzyme superoxide dismutase in lung tissue and are unable to survive in 85% or 100% oxygen. Perhaps, the recruitment of much larger numbers of macrophages over neutrophils is a teleological explanation of why guinea pigs are sensitive to high oxygen concentrations.

They do not have the need to induce superoxide dismutase since relatively few neutrophils are recruited to the lung. Instead, they rely on macrophages to clear the lung of deposited materials.

The traditional method of histopathological examination of target organs does not always tell us which pollutants are toxic. For instance, it is possible to see histopathological evidence of toxicity that is, in actuality, the result of "super" stimulation of bronchoconstriction. The guinea pig bronchi are capable of constriction to the point of asphyxiation. Laryngeal or bronchial spasm can completely eliminate the supply of oxygen resulting in histopathological manifestations in lung tissue. Thus, using the guinea pig we may identify the potential hazards of a given pollutant but we cannot determine if these hazards are due to direct toxic effects or to "superstimulated" defense mechanisms.

The conclusion is that multiple tests with multiple species are necessary for hazard evaluation of airborne pollutants. It is not likely we will ever eliminate the hazards of airborne pollutants, but through proper evaluation, we can reduce or minimize the health hazards in the environment and the occupational workplace.

ANATOMICAL CONSIDERATIONS

Because the respiratory system is not one organ, but rather an organ system, it is useful to simplify by identifying major compartments, i.e., regions that exhibit some internal anatomic similarity. The compartmental scheme proposed by the Task Group on Lung Dynamics of the International Commission on Radiologic Protection (ICRP) that was devised in order to describe the deposition and retention of inhaled particles is a very useful one in inhalation toxicology [10].

The model divides the respiratory tract into three regions based on anatomical features and deposition and clearance phenomena. The regions, called the nasopharynx (N), the tracheobronchial region (TB) and the pulmonary or parenchymal region (P) also are to some extent characterized by their unique responses to air pollutants.

The nasopharynx begins at the anterior nares and includes the respiratory airway down to the level of the larynx, i.e., "voice box." The nasal cavity, which is roughly triangular in cross section, contains a central septum and turbinates that form narrow channels through which air flows. The forward portion of the nose is lined by cells not unlike those of the body skin. The remainder being mostly lined with ciliated epithelium. The cilia, i.e., small numerous motile hairlike projections, produce movement of overlying mucus rearward to a point where it is swallowed. The most common disease state, rhinitis, is characterized by inflammation; nasal membranes are swollen and

excessive secretion or excessive dryness are both possible states. Rhinitis, a term which includes the common cold, is commonly associated with infection by inhaled viruses, bacteria or fungi. Rhinitis can also be produced by allergens and by irritating gases such as chlorine or formalin vapor or by prolonged exposure to dusts like those which occur in milling and stone cutting [11]. Rhinitis often precedes the formation of benign growths or polyps. Other responses of the nasopharynx include ulceration, which can be produced by very irritating gases or particles. Cancer is seen in nickel refinery workers who chronically inhale nickel carbonyl [12]. Although inhalation toxicologic studies often neglect tissue changes in the NP region, the potential for disease in this compartment is real and should not be overlooked.

The tracheobronchial region begins at the larynx and includes the trachea and the 16 to 20 generations of cilia-covered bronchial airways down to and including the terminal bronchioles. Interspersed in the ciliated cells are goblet cells that, along with mucous glands deep in the bronchial walls, secrete the mucus. Various mammalian species differ in the number and location of mucous glands. Muscle bundles that control airway caliber are present throughout the bronchial tree [13].

The responses of the tracheobronchial region to inhaled materials are varied and include excessive constriction of muscles leading to increased resistance to airflow, hypersecretion of mucus, swelling or edema of bronchial walls, bronchial infection and cancer.

Bronchoconstriction can be induced in sensitive asthmatics and in normal human or animal subjects by a great variety of inhaled substances. The most potent are usually organic materials that first establish a state of allergic sensitization and later induce an allergic response; typical agents include gases or vapors and dusts. Representative causative agents are pollens, mold spores, animal danders, feathers, insecticides, fabric lint and grain dust. Direct stimulation of bronchoconstriction in nonsensitized subjects occurs with sulfur dioxide, ammonia, cigarette smoke, sulfuric acid mists, a number of sulfate salts and so-called "inert dusts" [14-17]. The severity of bronchospasm can range from mild—detectable only be sensitive pulmonary function tests—to life-threatening bronchospasm where death due to asphyxiation may occur as in status asthmaticus.

Bronchitis with inflammation and excessive secretion of thick tenacious mucus that may or may not be effectively removed by cough can be produced by many agents. Most commonly viruses or mycoplasma, and less commonly bacteria are causative factors with common air-pollutants probably playing a secondary, potentiating role [14]. In sufficient concentrations, sulfur dioxide, chlorine and nitrogen dioxide can lead to this condition [14]. In the previously healthy person bronchitis is not commonly fatal; cough and treatment with antibiotics usually results in complete resolution.

A serious, often fatal form, *bronchiolitis obliterans* (silo-fillers' disease). progresses to a condition where nodules of scar tissue completely occlude the bronchial tubes. Most commonly, inhalation of nitrogen dioxide from nitric acid spills, burning of nitrocellulose or working in silos is the precipitating factor. This progressive fatal disease is characterized by shortness of breath, unremitting cough and cyanosis, i.e., poor oxygenation of tissues [14].

The most common tumor of the respiratory tract of man, bronchiogenic (usually squamous) carcinoma, arises from the cells lining the tracheobronchial tree [18]. Such growths usually occur in the central bronchi involving the mainstem (lobar) bronchus or its largest branches and upon enlargement completely obstruct bronchi and lead to collapse and infection of tissues supplied by the affected airway. Although as long as 20 years may lapse between exposure to a causative agent and the development of disease, bronchial carcinoma, once established, invades extensively and can produce metastases, i.e., daughter growths, throughout the body [18]. The prime causative agent is tobacco smoke, but asbestos, nickel, chromium, arsenic, beryllium, coal tar, radioactive particles, vinyl chloride and polyurethane are among the airborne agents that appear to produce the disease in man and experimental animals [14, 18, 19]. Sulfur dioxide appears to act as an irritant cofactor in the production of experimental squamous carcinoma by benzo[a]pyrene in rat lung [19].

A less common class of bronchial tumors of man, the bronchial adenomas and adenocarcinomas, appear to derive from glands of the bronchi rather than epithelia of central bronchi per se [18]. The causative agents of this form are less certain than those of bronchial carcinoma [19]. There are also a number of rare forms of bronchial tumors, including lipomas, papillomas, cystadenomas and melanomas [19].

The third compartment, the pulmonary or parenchymal region represents the functional gas-exchange sites of the lung. It includes respiratory bronchioles–small bronchial tubes with some air-sacs, i.e., alveoli, opening into their lumens, alveolar ducts–ducts whose walls are composed totally of alveoli and alveolar sacs, i.e., terminating alveolar ducts. The basic unit of this compartment, the alveolus is a thin-walled polyhedral sac with one face open to the air. The interior walls are covered by flat,thin pulmonary epithelial cells. A dense network of small capillary blood vessels intimately surrounds the alveoli where exchange of oxygen and carbon dioxide between the air and blood occurs. Alveoli in man are about 300 micrometers in diameter, being much larger than the inhaled particles that penetrate to the deep lung. In addition to the alveolar epithelial (Type I) cells, alveolar macrophages (mobile cells that engulf and digest foreign bodies) and alveolar septal (Type II) cells (which secrete surfactant) are also present. Alveoli are lined by a thin layer of surfactant that prevents their collapse [20].

Responses of the parenchymal region that are commonly induced by inhaled substances include inflammation, edema, fibrosis and cancer. The term "pneumoconioses" refers to parenchymal diseases caused by dusts. It is perhaps best characterized by a failure of the self-cleansing mechanisms of the lung that results in the development of specific responses depending on the type of material that is deposited and retained [21]. As the variety and number of materials that produce parenchymal disease are large, no attempt will be made to completely explore this topic.

Gases that are relatively insoluble in water are not well absorbed in the NP and TB regions and penetrate into the deep lung. Ozone, or triatomic oxygen, a component of photochemical air pollution, is a good example of a gas that is sufficiently insoluble to pass beyond the NP and TB compartments to produce damage in the lung parenchyma. In concentrations of about 1 part per million (ppm), ozone has many effects, including killing of pulmonary alveolar epithelial cells and damaging alveolar capillary endothelium which produces hemmorhage and edema, i.e., filling of airspaces and interstitial tissues with plasma ultrafiltrate [22]. Even in concentrations below 1 ppm, ozone destroys cilia, interferes with the ability of phagocytic cells to inactivate microorganisms, produces edema with thickening of alveolar walls and causes various biochemical and breathing-pattern changes [22]. A major feature of this injury pattern is that it points to the vulnerability of the thin alveolar epithelial cell and the closely neighboring capillary endothelial cells.

Tissue destruction followed by inflammatory changes in the pulmonary region is produced by the very irritant gases ammonia, chlorine and phosgene. Inhalation of these types of gases can lead to acute pulmonary edema, a condition that may resolve or in severe cases may progress to fibrosis of the deep lung. Similar responses have been caused by the inhalation of a variety of dusts including cadmium, osmium tetroxide, bauxite fumes, beryllium and a variety of organic materials including molds from hay, sugar cane, sawdust, bark and cheese [18]. In many of the organic dust cases a hypersensitivity reaction apparently plays a role in the lung response which is described as extrinsic allergic alveolitis. In such reactions an allergic response involving an antibody against the toxin, that appears after repeated exposure, participates in the formation of products that injure cells of the lung [18].

Several dusts lead, on chronic inhalation, to fibrosis without an apparent inflammatory phase. Silicon dioxide, asbestos, aluminum and possibly talc are notable examples of important fibrogenic dusts [14, 18].

The epithelial cells of the bronchiolar-alveolar zone also give rise to neoplasms in man and animals. Alveolar adenomas occur spontaneously in certain mouse strains and can be induced by many agents of which some but not all are chemical carcinogens. Bronchoalveolar adenomas are relatively rare in

humans, probably equally prevalent in both sexes and not apparently related to smoking or occupation [18].

The foregoing has served to illustrate, by example, the variety of anatomical responses of the lung; it is by no means a complete treatment in any sense of the term.

Because the respiratory system is composed of a large and varied set of tissues and organs, one must devise careful morphologic sampling techniques in inhalation studies. The problem of obtaining an adequate number of representative samples from tissues that have been properly preserved is discussed in a review by Dungworth et al. [23].

PHYSIOLOGIC CONSIDERATIONS

Physiologic measures of lung injury are important because they assess life-sustaining functions. The measures are largely nondestructive and many are therefore applicable to both human and laboratory animals. A major challenge in assessing physiological lung injury results from the substantial functional reserve that is present in the respiratory system. This functional reserve permits the slightly injured lung to often appear near normal in clinical tests.

Gas exchange of the lung is one example which shows a large functional reserve. This prime function of the lung is to allow oxygen to move from the ambient air into the venous blood and carbon dioxide to move out. During rest, gas exchange across the lung is at a rate only 1/10 of that possible during exercise. Should any toxic agent impede gas exchange, then the lung reserve capacity is often more than adequate to supply the needed oxygen and remove the metabolic CO_2 during rest. Only after the reserve is exhausted does one see dramatic changes of arterial O_2 and CO_2.

Surely, one must seek measurements which show clinical degradation before such reserve capacity is exhausted. One way is to expend the reserve capacity through exercise while making measurements that detect a decrement in the lung function. Such an approach is used in several laboratories [22, 24], but is complicated in that measurements must be made in a moving experimental preparation.

A somewhat different approach is to look at the respiratory mechanical function of the lung, which reflects the cellular and tissue properties of the airway. If these properties are modified by experimental intervention, then the mechanical function of the lung may be altered.

The mechanical function of the lung is commonly described by pulmonary compliance and resistance. Compliance is the index of the functional stiffness of the lung; compliance will decrease if the lung becomes stiffer through such mechanisms as constriction of alveolar duct smooth muscle, cellular infiltration

or alveolar edema [25]. Airway closure, which is thought to occur in small airways, will also make the lung functionally stiffer by reducing the amount of parenchyma available to accept inspired gas.

Pulmonary compliance is the ratio of the pressure differences between the mouth and the pleural space and lung volume. Pulmonary compliance may be determined from the shape of a pressure-volume curve near the lung volume of interest at zero airflow.

Lung resistance is more difficult to measure than lung compliance. It is the relationship between pulmonary airflow and transpulmonary pressure and is complicated in that this transpulmonary pressure not only must overcome the resistance to airflow but compliance of the lung as well. To compute resistance, transpulmonary pressure and airflow are measured once during inspiration and again at the same lung volume during expiration.

Resistance to airflow is influenced by airway size, flow direction and lung tissue resistance. Although all patent airways have an influence on pulmonary resistance, the large airways account for the majority of the resistance to airflow because the aggregate cross section of small airways is much greater. The caliber of the upper and central airways can be altered by changing bronchomotor tone, by bronchial edema, or by the presence of increased amounts of mucus. Low doses of 0.1-1 ppm ozone have been shown to increase airway resistance in humans exposed for one hour over 75 days [26]. Resistance has been used in toxicological studies as an index of pulmonary response.

The frequency of respiration is under central nervous system control but may be influenced by mechanical factors such as resistance and compliance, or respiratory reflexes. Depending on the nature of the interaction between a given irritant and the central nervous system, a given stimulus may increase or decrease respiratory frequency [27].

Tests which measure the distribution of inspired gas to the alveoli require the use of gases such as N_2, H_2 or helium, which are so poorly soluble that they do not pass in great quantity from the alveolar gas into the blood or pulmonary tissue. The simplest of these tests, the "pulmonary N_2 emptying rate," requires only that the subject breathe O_2, usually for about 7 minutes. If the inspired O_2 is distributed evenly to all alveoli during the 7-min period, the N_2 will be washed out evenly and the final alveolar gas sample will contain less than 2.5% N_2. More detailed analysis may involve use of the entire washout curve, as by exponential analysis or moment analysis [28]. However, if some areas are markedly hypoventilated during normal breathing, they will still have a high N_2 concentration. When there is uneven ventilation in the lung, there may be different washout rates in different parts of the lung.

The cause of uneven ventilation may be a regional reduction of the number of elastic fibers in the lung such as occurs in advanced pulmonary

emphysema; regional obstruction such as occurs in asthma; or regional changes in expansion caused by fluid or exudate in the alveoli, pulmonary congestion or restrictive disease.

BIOCHEMICAL CONSIDERATIONS

Homeostatic mechanisms within an organism responsible for maintaining its integrity and well-being rely upon complex networks of biochemical reactions for rapid response to pollutant exposures. Thus, biochemical response to different classes of pollutants might be expected to be general and nonspecific. Recent reviews have dealt at length with this subject [29, 30]. We will consider here only general highlights of biochemical response to sulfur oxide and oxidant pollutants.

Sulfur Oxides

Sulfur dioxide is a highly soluble gas capable of rapid equilibration in an aqueous environment, producing sulfite:

$$SO_2 + H_2O \rightleftharpoons SO_3^{2-} + 2H^+ \quad (1)$$

Sulfite, in turn can react with free protein (R-SS-R) or amino acid disulfide groups, Reaction 2, and by exchange lytic processes produce S-sulfonate compounds capable of circulating in blood [31-33]:

$$R\text{-}S\text{-}S\text{-}R' + SO_3^{2-} \rightleftharpoons R\text{-}S\text{-}SO_3^- + R'\text{-}S \quad (2)$$

These circulating S-sulfonates have been measured in rabbit and dog plasma after exposure to high (10-25 ppm) levels of sulfur dioxide [31-33]. Sulfite can also be oxidized enzymatically to sulfate. This process, catalyzed by the enzyme sulfite oxidase can occur in many tissues, including lung, liver and kidney. It has been calculated [34] that even 100-500 times the maximum estimated average daily sulfite intake of humans could not saturate the capacity for oxidation to sulfate and excretion of that end product. More recent studies [35] point out the lack of correlation in different species between endogenous plasma S-sulfonate levels and sulfite oxidase activity, leaving the conclusion that other factors may be important in the partitioning between the competing reactions to form S-sulfonates and sulfates. However, within a single species, S-sulfonate formation correlates with sulfite dose [36] and may still serve as a useful indicator of exposure. Similar detailed studies need to be performed with inhalation exposures to sulfur dioxide, since much of the previous work has relied upon sulfate supplied in the diet, or by injection.

In vitro guinea pig lung slice exposure to various sulfate salts [37] has indicated that associated ammonium cations can cause histamine release. The physiological significance of the observation for humans has been questioned [38] in light of the high in vivo ammonia levels measured in exhaled breath [39].

Ozone and Nitrogen Dioxide

Ozone and nitrogen dioxide, strong oxidizing agents, are both believed to be harmful to biological tissue because of that particular property. And because of the physiological similarity of lung lesions produced by exposure to these gases and those caused by ionizing radiation, radiomimetic properties are ascribed to them [30].

Ozone has been studied both in vitro and in vivo. When exposure of organs, tissue homogenates or pieces occurs, lipid peroxidation, sulfhydryl group oxidation and loss of enzymatic activity can all occur [29]. However, these same changes, although postulated to occur in many models of ozone action, have not always been observed in the whole animal exposures to the gas. Evidence for malonaldehyde production (a product of lipid peroxidation) in lung tissue has been reported by some groups, but not found by others [29].

Nitrogen dioxide, more soluble than ozone and less reactive, can penetrate more deeply into the lung and react with endothelial tissue. On the basis of deep lung injury and a hypothesis expecting "neurohumoral factor" release [40], experiments using continuous low level exposure of guinea pigs to nitrogen dioxide were conducted [41]. Increases in urinary protein excretion were noted in exposed animals when compared to controls. Quantitating the changes or ascribing them to kidney damage response to released chemical factors from lung was not possible.

Since the lung is not only the central portal by which oxygen enters and carbon dioxide leaves animals, but also an important biosynthetic organ providing a wide range of essential "chemical services," it has been logical to study the impact of acute and chronic oxidant gas exposure on enzyme activities and metabolite levels. Details of these studies have been recently reviewed [29] and will be generally summarized here. Glucose metabolism, lipid metabolism, nucleic acid and protein synthesis, all essential for highly metabolically active tissue, all show significant decreases when lung homogenates or lung slices from animals are exposed to high levels of either nitrogen dioxide (greater than 5-10 ppm) or ozone (greater than 1-2 ppm) for short periods of time. However, in animals exposed to lower levels of oxidant gas for longer periods of time, an augmentation in enzyme activities and metabolite levels was observed. This suggests some type of repair and/or adaptive

processes occurring in the chronically exposed animals where massive initial tissue destruction had not occurred. Other in vitro testing in acutely and chronically exposed rats has shown similar results for hexose monophosphate shunt enzymes (key in producing NADPH, an essential intracellular reducing agent), monamine oxidase and glutathione.

Also, it has been shown that the nutritional status of an animal is important in reducing susceptibility to these oxidant-induced biochemical changes. Both vitamin C and vitamin E can protect against lethality from ozone and nitrogen dioxide. The exact extent and mechanism of this protection is not yet known.

Finally, although early indication of oxidant change can be observed in biochemical changes, specific initial responses need still be observed and understood.

ACKNOWLEDGMENTS

Lisa Lieberman provided valuable editorial and typing services in the preparation of this chapter.

REFERENCES

1. Alarie, Y. "Irritating Properties of Airborne Materials to the Upper Respiratory Tract," *Arch. Environ. Health* 13:433-449 (1966).
2. Ulrich, C. E., M. P. Haddock and Y. Alarie. "Airborne Chemical Irritants, Role of the Trigeminal Nerve," *Arch. Environ. Health* 24:37-42 (1972).
3. Amdur, M. O., R. Z. Schulz and P. Drinker. "Toxicity of Sulfuric Acid Mist to Guinea Pigs," *Arch. Ind. Hyg.* 5:318-326 (1952).
4. Amdur, M. O. "The Respiratory Response of Guinea Pigs to Sulfuric Acid Mist," *Arch. Ind. Health* 18:407-414 (1958).
5. Cavender, F. L., H. W. Sugg, B. Y. Cockrell and W. M. Busey. "Health Effects of Sulfuric Acid Mist," presented at the 71st Annual Meeting of the Air Pollution Control Association, Houston, TX, June, 1978.
6. Cavender, F. L. "Respiratory Defense Mechanisms and Inhalation Toxicology," presented at the Eighth Conference on Environmental Toxicology, Dayton, OH, October, 1977.
7. Steinhagen, W. H., F. L. Cavender and B. Y. Cockrell. "Respiratory Tract Lesions in Guinea Pigs Exposed to Sulfuric Acid Mist," *J. Environ. Path. and Tox.* 1:267-277 (1978).
8. Cavender, F. L., J. Campbell and B. Y. Cockrell. "Functional Changes in the Free-Cell Population Lavaged from Lungs of Rats and Guinea Pigs During Chronic Inhalation Exposures," presented at EPA Symposium, Williamsburg, VA, February, 1978.
9. McCord, J. and J. Crapo. Personal communication.

10. Task Group on Lung Dynamics. "Deposition and Retention Models for Internal Dosimetry of the Human Respiratory Tract," *Health Physics* 12:173-207 (1966).
11. Bell, E. T. In: *A Textbook of Pathology*, (Philadelphia, PA: Lea and Febiger, 1956), pp. 551-554.
12. Nelson, N. "Inhalation Carcinogenesis in Man: Environmental and Occupational Hazards," in *Inhalation Carcinogenesis*, M. G. Hanna, Jr., P. Nettesheim and J. R. Gilbert, Eds. (Oak Ridge, TN: U.S. Atomic Energy Commission, 1970), pp. 3-12.
13. Bloom, W., and D. W. Fawcett. *A Textbook of Histology* (Philadelphia: W. B. Saunders Company, 1968), pp. 629-651.
14. Beeson, P. B., and W. McDermott. *Textbook of Medicine* (Philadelphia: W. B. Saunders Company, 1967), pp. 492-551.
15. Altman, P. L., and D. S. Dittmer, Eds. *Environmental Biology* (Bethesda, MD: Federation of American Societies for Experimental Biology, 1966), pp. 269-310.
16. Alarie, Y. "Sensory Irritation by Airborne Chemicals," *Crit. Rev. Toxicol.* 2:299-363 (1973).
17. Amdur, M. O., J. Bayles, V. Ugro and D. W. Underhill. "Comparative Irritant Potency of Sulfate Salts," *Environ. Res.* 16:1-8 (1978).
18. Crofton, J. W., and A. Douglas. *Respiratory Diseases* (Oxford: Blackwell Scientific Publications, 1975), pp. 562-611.
19. Laskin, S., M. Kuschner, A. Sellakumar and G. V. Katz. "Combined Carcinogen-Irritant Animal Inhalation Studies," in *Air Pollution and the Lung*, E. F. Aharonson, A. Ben-David and M. A. Klingberg, Eds. (New York: John Wiley and Sons, Inc., 1976), pp. 190-218.
20. Macklin, C. C. "The Pulmonary Alveolar Mucoid Film and the Pneumocytes," *Lancet* 266:1099 (1954).
21. Committee on Pneumoconioses. "The Pneumoconioses," *Arch. Environ. Health* 7:130-171 (1963).
22. Committee on Medical and Biologic Effects of Environmental Pollutants. *Ozone and Other Photochemical Oxidants* (Washington, DC: National Academy of Sciences, 1977).
23. Dungworth, D. L., L. W. Schwartz, W. S. Tyler and R. F. Phalen. "Morphological Methods for Evaluation of Pulmonary Toxicity in Animals," *Ann. Rev. Pharm. Tox.* 16:381-399 (1976).
24. Folinsbee, L. J., F. Silverman and R. J. Shephard. "Exercise Response Following Ozone Exposure," *J. Appl. Physiol.* 38:996-1001 (1975).
25. Drazen, J. M. "Physiologic Basis and Interpretation of Common Indices of Respiratory Mechanical Function," *Environ. Health Pers.* 16:11-16 (1976).
26. Goldsmith, J. R., and J. A. Nadel. "Experimental Exposure of Human Subjects to Ozone," *J. Air Poll. Control Assoc.* 19:329-330 (1969).
27. Alarie, Y. "Sensory Irritation by Airborne Chemicals," *Crit. Rev. Tox.* 2:299-263 (1973).
28. Saidel, G. M., R. B. Salmon and E. H. Chester. "Moment Analysis of Multibreath Lung Washout," *J. Appl. Physiol.* 38:328-334 (1975).
29. Mustafa, M. G., and D. F. Tierney. "Biochemical and Metabolic Changes in the Lung with Oxygen, Ozone and Nitrogen Dioxide Toxicity," *Amer. Rev. Respir. Dis.* 118:1061 (1978).

30. Coffin, D. L. and H. E. Stokinger. "Biochemical Effects of Air Pollutnats," in *Air Pollution*, A. C. Stern, Ed. (New York: Academic Press, Inc., 1977), Vol. 2, pp. 232-360.
31. Gunnison, A. F., and A. W. Benton. "Sulfur Dioxide: Sulfite, Interaction with Mammalian Serum and Plasma," *Arch. Environ. Health* 22:381 (1971).
32. Gunnison, A. F., and E. D. Palmes. "A Model for Metabolism of Sulfite in Mammals," *Toxicol. Appl. Pharmacol.* 38:111 (1976).
33. Gunnison, A. F., and E. D. Palmes. "Persistence of Plasma S-Sulfonates Following Exposure of Rabbits to Sulfite and Sulfur Dioxide, Biological Origin and Metabolism of SO_2," *Toxicol. Appl. Pharamacol.* 24:206 (1973).
34. Rajagopalan, K. V., and J. L. Johnson. In: *Biochemical Effects of Environmental Pollutants*, S. D. Lee, Ed. (Ann Arbor, MI: Ann Arbor Science Publishers, Inc., 1977), pp. 307-314.
35. Gunnison, A. F., and E. D. Palmes. "Species Variability in Plasma S-Sulfonate Levels During and After Sulfite Administration," *Chem.-Biol. Interact.* 21:315 (1978).
36. Gunnison, A. F., C. A. Bresnahan and E. D. Palmes. "Comparative Sulfite Metabolism in the Rat, Rabbit and Rhesus Monkey," *Toxicol. Appl. Pharmacol.* 42:99 (1977).
37. Charles, J. M., and D. B. Menzel. "Ammonium and Sulfate Ion Release of Histamine from Lung Fragments," *Arch. Environ. Health* 30:314 (1975).
38. Faeder, E. J., and A. V. Colucci. "The Effects of Sulfate Aerosols on Human Health," Annual Meeting American Society of Civil Engineers, Chicago, Illinois, 1978.
39. Larson, T. V., D. S. Covert, R. Frank and R. J. Charlsen. "Ammonia in the Human Airways: Neutralization of Inspired Acid Sulfate Aerosols," *Science* 197:161 (1977).
40. Fairchild, E. J. "Nuralhumoral Factors in Injury from Inhaled Irritants," *Arch. Environ. Health* 6:79 (1963).
41. Sherwin, R. P., and L. J. Layfield. "Proteinuria in Guinea Pigs Exposed to 0.5 ppm Nitrogen Dioxide," *Arch. Environ. Health* 26:336 (1974).