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Effects of Airborne Pollutants on Mucociliary Clearance

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The mucociliary clearance system is a first line of defense against inhaled agents, and so its compromise can adversely affect health. The purpose of this paper is to provide a review of data on the effect of *in vivo* air pollutant exposures on the clearance of test particles from airways. Data from both animals and humans are compared whenever possible, so that estimates of human health effects may be made. Mechanisms of action are also discussed, presenting the view that for low level exposures, changes in secretions are probably responsible for most observed changes in clearance. The pollutants pertinent to this review are those that are common in the environment and most likely to have impacts on large numbers of people: sulfur oxides, sulfuric acid mist, O_3, NO_2, particulates, diesel exhaust, and cigarette smoke.

Introduction

Mucociliary clearance is a lung defense mechanism by which inhaled and deposited particles, including toxic and infectious agents, are removed from the conducting airways of the lungs (*1,2*). Beating cilia propel the overlying mucus, containing deposited particles, up the airways to the trachea and larynx and then the mucus is swallowed. Impairment of this function could act to produce accumulations of secretions in airways or to give toxic materials a longer residence time in the airways. Abnormal elimination of tracheobronchial mucus is associated with, and is perhaps a factor in, the pathogenesis of chronic obstructive lung disease, especially chronic bronchitis (*1,3*). It is important to determine the response of the mucociliary system to challenges by airborne pollutants, especially at concentrations that are relevant to general community and occupational exposures.

There have been recent excellent reviews of the overall aspects of mucociliary clearance (*1,2,4*), secretions and their role in respiratory tract defense (*5,6*), and control of secretions in the airways (*7–9*). The primary intent of this review is to discuss the effect of *in vivo* air pollutant exposures on the clearance of test particles from airways as an indicator of the integrated system response.

Studies of *in vitro* exposures are not dealt with in any detail in this review. *In vitro* exposures of ciliated epithelium have been useful for showing the sensitivity of the mucociliary system to insult (*10,11*) and providing a means of ranking pollutants. However, *in vitro* tests are not necessarily good predictors of events in the intact respiratory tract. The concentration of pollutants reaching the trachea and conducting airways is often much lower (sometimes orders of magnitude) than the inhaled concentration, because of absorption and deposition processes. Also, many integrated processes such as reflex effects and influences from systemic circulation require the study of an intact animal or person.

Mucociliary clearance results from the interaction of epithelial cilia with overlying mucus secretions. Thus, when clearance of test particles is altered it could be as a result of changes in one or a number of factors: the number of morphology of cilia, ciliary activity or coordination, and secretion volume, composition, or viscoelasticity. It is important to determine the mechanisms responsible for observed changes in mucous clearance to evaluate their importance and their potential for long-term effects. If there have been changes in the depth or total surface area of mucus, in percentages of ciliated cells or in ciliary morphology after inhalation exposures, these observations can help predict the likelihood of long-term effects resulting from continued inhalation of irritant aerosols. Thus, in the intact animal or person there are many possible effects on the mucociliary system. The net effect on the clearance of test particles from the airways is one way to test the system response. A slowing in particle clearance would seem to be an obvious adverse effect. However, a speeding of clearance is not necessarily a beneficial effect, especially if it is associated with an increase in secretions. Excessive secretions produced for sustained periods following acute inhalation exposures may increase the possibility of airway plugging and possible initiation of lung disease.

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Mucociliary System

Rhodin (*12*) and, more recently, Breeze and Sheldon (*13*), have described the morphology of the airways. A ciliated pseudo-stratified respiratory tract mucosa extends from the nose down to the terminal bronchioles. Figure 1 shows a scanning electron micrograph of a cross section of a rat trachea showing the cilia and overlying mucus. The major sources of mucous glycoproteins are goblet cells and submucosal glands of both serous and mucous types (*7*). From the comparative volume of goblet cells and submucosal glands in normal airways, it has been suggested that mucosal glands contribute 40 times the volume of secretions secreted by goblet cells (*14,15*). As one moves to the periphery of the lung, the percentages of both ciliated and goblet cells decreases (*5,16*). Epithelial serous cells (*7*), with a distribution similar to that of goblet cells, are another source of mucus. Clara cells, found primarily in the bronchioles (*13*), are also possible sources of glycoprotein or lipid or both (*7*).

Submucosal glands are limited to the trachea and bronchi. There are considerable species differences. Submucosal glands are numerous in humans, cats, pigs, and ferrets, infrequent in rodents, and nonexistent in geese and chickens (*7,16,17*). Goblet cells are numerous in humans, cats, pigs, and geese and infrequent in rodents (*7*).

Inhalation of irritant gases and aerosols can cause hypertrophy of mucous glands and hyperplasia of goblet cells (*5,18*). It is not clear which of these responses is of greater importance; the nature of the response presumably depends at least somewhat on the type of irritant and the species studied. Most of the data available for pollutant responses are from rats that have fewer goblet cells and mucous glands than man.

Control mechanisms of mucus glycoprotein secretions are not entirely clear. There is strong evidence for efferent parasympathetic innervation of the mucous glands (*7*), and so they are at least partially under the influence of the nervous system. Goblet cells and serous cells are more likely to be influenced by local effects; however, goblet cell numbers are increased by parenteral administration of the sympathomimetic agent isoproterenol (*19*). Both parasympathetic and sympathetic stimulation have produced increased mucus glycoprotein production and volume of secretions (*19*). Parasympathetic agonists decreased secretions, whereas sympathetic agonists appeared to have no effect (*19*).

There is general agreement that the mucous blanket consists of two phases. An approximately 5μm thick layer of low viscosity periciliary fluid bathes the cilia in the trachea. Above this is a gel phase of higher viscosity material about 5 μm thick (*20*). Recent observations in our laboratory indicate that the mucus glycoprotein blanket preserved after fixation and drying is ca. 1 to 2 μm thick in normal rats (Fig. 1). The clawed tips of the cilia touch the upper layer at the top of their stroke (*5*). The existence of these two layers is supported by the work of Lucas and Douglas (*21*) and Bang and Bang (*22*) and Morgan et al. (*23*). The primary source of the upper layer is undoubtedly goblet cells and mucous glands; however, the source and control of the periciliary fluid has not been clearly demonstrated, although it appears to be related to water transport (*7*) across the epithelium.

Whether the upper mucous layer is a continuous blanket or consists of discrete "flakes" or "plaques" is a controversial issue. The work of Iravani and Van As (*24*), reviewed by Van As (*25*), supports the latter hypothesis. Many other workers have presented data indicating a continuous mucous blanket (*20–22,26,27*). From available data it appears most likely that the gel mucous layer is thinner and discontinuous as the airways become smaller (*5*), where there are fewer ciliated and mucous secreting cells, as noted earlier. Even in the trachea we have observed areas as shown in Figure 2*a* where there is no overlying mucous glycoprotein and in Figure 2*b* where it is relatively thin and strands of glycoprotein can be seen. In our observations, these regions represent 20 to 25% of the surface area of the trachea in normal rats. Certainly mucous transport velocities decrease from the trachea to peripheral airways as shown by Iravani and van As (*24*), Asmundsson and Kilburn (*28*), and Morrow et al. (*29*).

Tracheal mucous velocities have been observed to range from 2 to 20 mm/min (*1,2,5*) in a variety of species measured with a variety of techniques. When consistent methodology is used (*30*), smaller species have slower velocities than large species. Velocities in the smaller bronchioles have been found to be < 1 mm/min in rats (*24*).

It must be recognized that description of mucociliary clearance in terms of velocity has limitations. At best, it represents an average which has a wide standard deviation (*31,32*), and it represents the fastest movement of material when a leading edge of transported material is measured. The key point is that mucous clearance is not necessarily as relentless and uniform as it is sometimes portrayed. For instance, there are preferential routes of clearance, at least in the trachea (*32*), and presumably in the lower airways. Therefore, there is the chance for particles to remain in an area of slow clearance for some time. Clearance from the smaller airways is also probably slower than has been generally recognized. Lee (*33*) has calculated velocities as low as ~ 5 μm/min for terminal bronchioles by fitting a kinetic model to observed lung clearance curves.

Mucociliary Clearance Measurements

Techniques for measuring mucociliary clearance of animals or humans have been well reviewed (*34–38*) and will not be covered in detail here. All methods involve detection of the movement of test markers in the lung. The techniques divide into two basic types. (1) Inha-

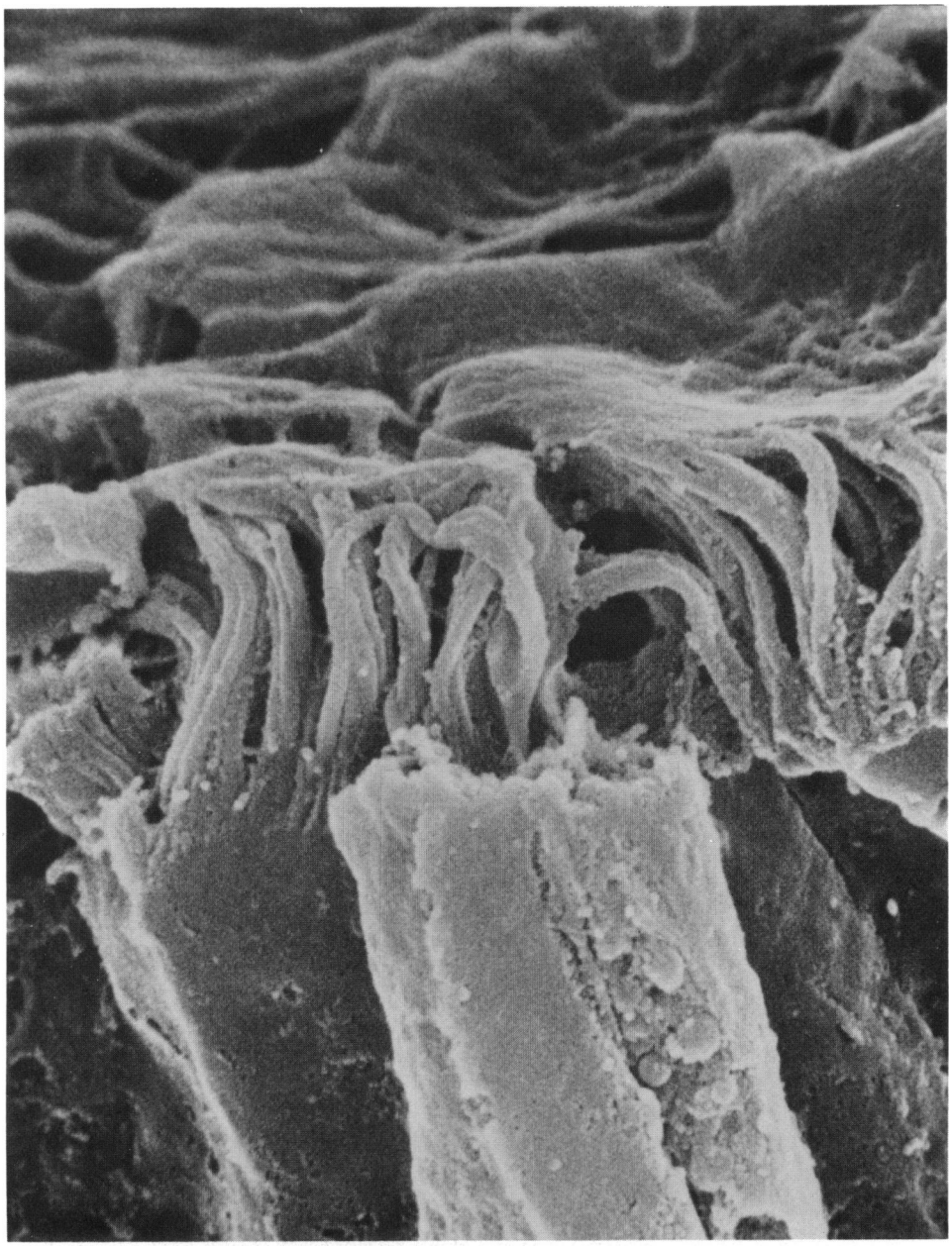
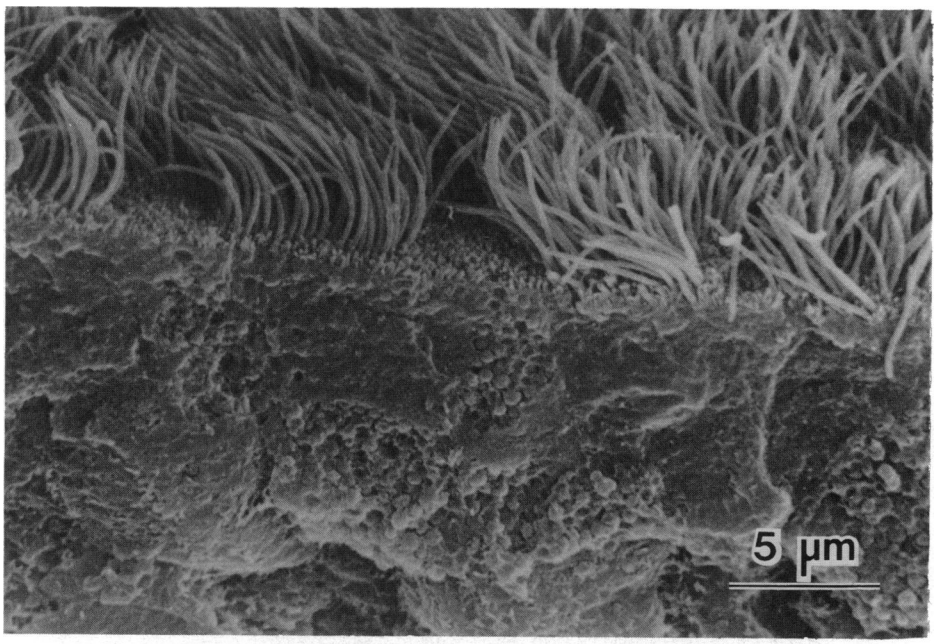


FIGURE 1. Scanning electron micrograph of a cross-section of a control rat trachea produced by cryofracturing. This is the normal appearance with the mucous glycoprotein blanket just at the tips of the cilia.

lation of radiolabeled particles of an appropriate size to result in significant tracheobronchial deposition is followed by external detection with NaI crystals or gamma cameras over a period of hours (up to 1 day) after exposure. Plots of retention of activity with time or lung clearance curves can then be obtained. (2) Instillation of either radiolabeled or radio-opaque materials into specific airways (usually the trachea) is followed by external detection of movement using NaI detectors or gamma cameras for radiolabeled materials and radiography or occasionally photography through a fiberoptic bronchoscope for nonradiolabeled materials. Photography of cell debris moving on mucus surfaces has also been used. Velocities of test markers are calculated and assumed to represent that of the mucus which is carrying them up the airways. The latter assumption appears valid, since the effects of particle size and type on observed velocities appear to be minimal except in extreme cases (*39–41*).

Both of these methods work well in practice. The inhalation method has some advantages in that it is a noninvasive procedure and clearance from the entire lung is measured. However, the instillation procedure has the advantage that a specific airway is studied.



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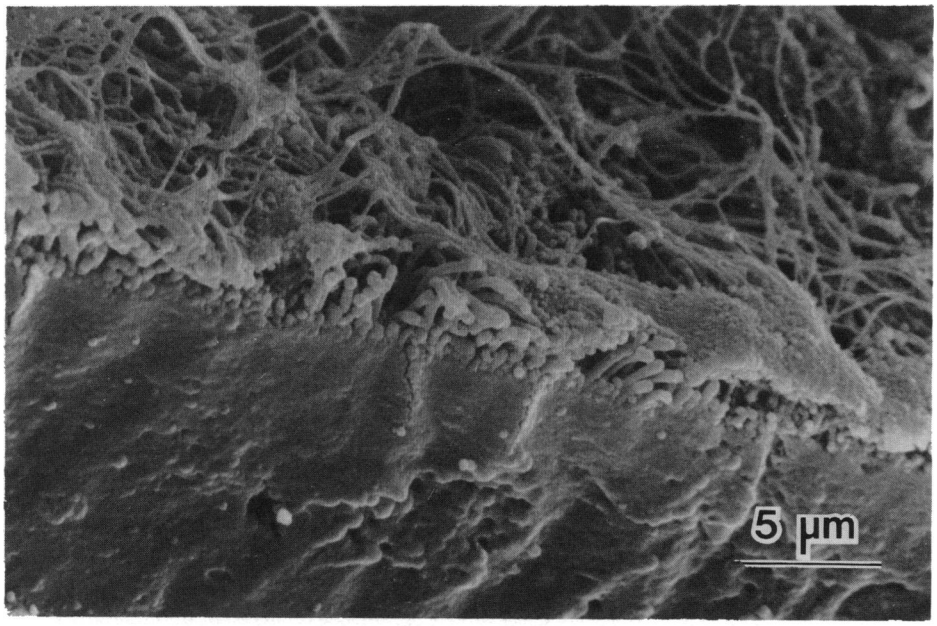

b

FIGURE 2. Scanning electron micrographs of areas of rat trachea (*a*) with no mucous blanket and (*b*) with a thin filamentous covering.

Yeates et al. (*42*) have shown that tracheal clearance rates are closely correlated with bronchial clearance rates. Also, the instillation technique allows determination of mucous clearance changes even in the presence of respiratory disease that might change the deposition pattern of inhaled particles and complicate the interpretation of results. If deposition patterns change, the observed clearance of inhaled markers will change as well even if there are no changes in mucous clearance, since larger airways clear faster than smaller airways.

Pollutant Effects

The main focus of this review is on pollutants commonly encountered in the environment and work place. Table 1 shows the exposure limits for some of the major regulated pollutants. These values will help put into perspective the various exposure concentrations used in the studies cited. A variety of different exposure lengths have also been used. If exposure length is not mentioned in the text, it can be assumed that it is an

acute exposure (defined as less than 8 hr, for the purposes of this review). When longer exposures have been used they have been noted.

Sulfur Dioxide (SO_2)

A number of studies have indicated alterations in mucociliary clearance with SO_2 exposure. Since SO_2 is highly water soluble, it is absorbed primarily in the nose and upper airways and concentrations drop off rapidly down the airways (*43,44*). Therefore, observed effects are related to the area of the respiratory tract being examined. Andersen et al. (*45*) showed significantly slower nasal mucous clearance with exposures of humans to 1 to 5 ppm SO_2. Wolff et al. (*46,47*) have shown alterations of bronchial clearance in both exercising (*47*) and sedentary humans (*46*) with exposures to 5 ppm SO_2. Speeding of clearance was observed in both cases with greater effects for the exercising subjects than sedentary. Mannix et al. (*48*) observed slower upper airway clearance following exposure to 20 ppm SO_2 but no changes in longer term clearance. Other studies have shown very little effects of SO_2 (*49,50*). On an equivalent sulfur concentration basis, the effects of SO_2 have been shown to be approximately 1/10 those of sulfuric acid mist with respect to altering mucociliary clearance (*47*) in healthy subjects. Hirsch et al. (*51*) have shown slight alterations in mucociliary clearance following 1 ppm SO_2 exposures of beagle dogs for 1 year. However, it takes prolonged exposure to SO_2 at very high levels (~ 500 ppm) to produce pronounced changes characteristic of chronic bronchitis (*52,53*). The predominant mechanism appears to be an increase of secretions as shown by Lamb and Reid (*54*) and Schultz et al. (*55*), since ciliary beat frequency has been shown not to be altered (*49*). Stimulation of secretions from the cholinergic innervated mucous glands is produced primarily through reflex effects originating from stimulation of irritant receptors (*7,55*).

Recently, concern for health effects from environmental exposures to SO_2 has centered on the heightened response of asthmatics. Increases of airway resistance in asthmatics have been demonstrated at concentrations down to 0.25 ppm (*56–58*). Limited data also indicate that mucociliary clearance may also be altered; one asthmatic showed cessation of bronchial clearance following exposure to 1 ppm SO_2 + 1 mg/m^3 carbon dust (*59*).

Sulfuric Acid Mist (H_2SO_4)

There is a more complete and comprehensive set of data for mucociliary clearance effects related to sulfuric acid mist exposures than for any other pollutant. The effects of sulfuric acid mist on mucociliary clearance have been studied in a variety of species. As shown in Figure 3, rats have a different pattern of response than man and larger animals. Rats had faster clearance at all exposure concentrations along with evidence of increased secretions on scanning electron micrographs (*60*). Studies by Phalen et al. (*61*) and Kenoyer et al. (*62*) showed very minor effects of sulfuric acid on mucociliary clearance in rats. Very few studies of H_2SO_4 have been carried out in other small rodents but the fact that very high concentrations (700–800 mg/m^3) are required to produce significant mortality in both mice and rats for H_2SO_4 exposure (*63*), suggests that mice are also not very sensitive to insult from H_2SO_4.

Other studies in rats and hamsters have also shown evidence of increased tracheal secretions (*64,65*) following exposures to sulfuric acid at levels down to 1 mg/m^3. There were also some indications of slight epithelial damage (*65*). Depressions of ciliary beat frequency at levels of 0.9 to 1.1 mg/m^3 (*66*) have also been observed in rats but not at levels of ~ 0.5 mg/m^3 (*67*). Although slower mucociliary clearance has not been observed directly in rats, the other changes outlined above indicate significant physiological responses.

Studies have shown that guinea pigs exhibit slowed mucous clearance at ~ 1 mg/m^3 H_2SO_4 levels (*68,69*). This fact indicates that guinea pigs can serve as useful small laboratory animals to study inhaled sulfate effects relevant to humans. It was interesting that, at very high concentrations (10 and 27 mg/m^3), clearance tended to be faster rather than slower. It appears quite likely that these effects are related to excessive production of secretions under highly irritant conditions (the "runny nose" and "tearing eyes" phenomena). Figure 4 shows an example of tracheal mucus increasing to a depth of over 20 μm in a guinea pig exposed to 27 mg/m^3 H_2SO_4. In these cases, overall clearance is probably increased to prevent "flooding" of the airways. Therefore, a faster

Table 1. Regulated air pollutants

	National ambient air quality primary standards	
	Concentration not to be exceeded for averaging (time)	Industrial threshold limit value (concentration allowable for 8 hr. exposure)
SO_2	0.14 ppm (24 hr)	2 ppm
H_2SO_4		1 mg/m^3
Particulates	260 μg/m^3 (24 hr)	10 mg/m^3[b]
O_3	0.12 ppm (1 hr)	0.1
Nitrogen oxides	0.05 ppm (1 year)	3
CO	35 ppm (1 hr)	50
Hydrocarbons	0.24 ppm (3 hr)	—[a]

[a] Not regulated.
[b] Nuisance dust, 5mg/m^3 respirable.

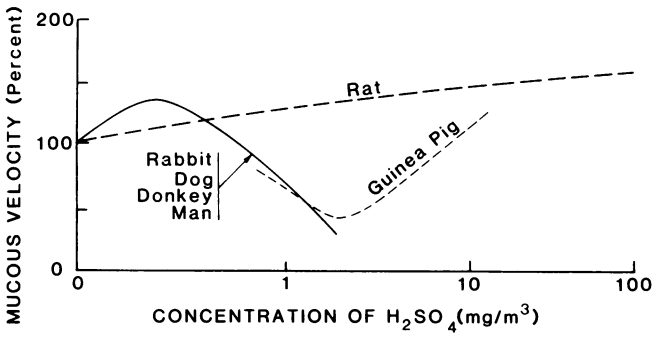

FIGURE 3. Conceptualization of the differences observed in mucous velocities in various species after acute exposures to sulfuric acid mist aerosols in the 0.5 to 0.9 μm size range.

clearance rate cannot necessarily be interpreted as reflecting a beneficial condition.

Data obtained by various investigators from large animals and humans agree relatively well and share the following common features. They indicate that exposure to low levels (0.1–0.5 mg/m^3) of sulfuric acid mist in the 0.6 to 0.9 μm mass median aerodynamic diameter (MMAD) size range can have an irritating effect that produces a speeding of tracheal clearance (Fig. 3). At the 1.0 mg/m^3 level, clearance is depressed after exposure of dogs (*70*), donkeys (*71*), humans (*72*), guinea pigs (*68*), and rabbits (*73*) (Fig. 3). A high degree of interindividual variability in response has been shown, particularly at levels below 1 mg/m^3. This was observed in studies of dogs at 0.5 mg/m^3 (*70*) and also in studies of humans at 0.11 and 0.33 mg/m^3 (*72*).

It seems most likely that the observed changes in mucous clearance following exposure to sulfuric acid mist were predominantly related to reflex-mediated changes in mucous properties similar to those described for SO_2 exposures. The depth, viscosity, and elasticity of mucus may be altered; this, in turn, alters mucous velocities (*5*). Changes in ciliary beat frequency are also possible. It appears that mild irritation produces an increase in mucus production which the clearance system can deal with by speeding mucociliary clearance. King's data (*74*) on the effect of cholinergic stimulation by methacholine showed a speeding of clearance at low concentrations correlated with an increase in mucous production and there was relatively little change in elasticity. These results suggest that the mucous clearance system can respond to changing "load" or volume of secretion, within limits. However, with higher concentrations of methacholine, mucous velocities fell dramatically. The irritant response to H_2SO_4 may be similar. Acute exposures to relatively low concentrations can produce some irritation and faster mucociliary clearance. At higher concentrations of H_2SO_4 or possibly with longer exposures, further changes in mucous properties may result in viscoelastic changes which impair clearance. Acute exposures to very high concentrations may produce airway "flooding" and faster clearance. It will be very important to determine if chronic exposures result in impaired clearance and eventual alteration in lung structure and deterioration of pulmonary function.

Sulfuric acid effects seem to be strongly particle size dependent. Exposures to 0.8 to 0.9 μm MMAD aerosols caused approximately five times more mortality in guinea pigs than exposures to 0.3 to 0.4 μm aerosols (*75*). No effect was observed on tracheal mucociliary clearance in dogs exposed to 5 mg/m^3 concentrations of 0.3 μm aerosols while decided effects were observed at 1.0 mg/m^3 concentrations of 0.9 μm aerosols (*70*). These results are consistent with those of Sackner et al. (*76,77*) who showed a lack of effect of 0.1 to 0.2 μm H_2SO_4 aerosols on mucociliary clearance in sheep at concentrations up to 14 mg/m^3. Mucociliary clearance effects have been noted in studies by others using H_2SO_4 particles greater than 0.5 μm MMAD in size (*70–72*). Thus, it appears that particles must be greater than 0.5 μm and perhaps larger to elicit significant physiological responses. The fact that the studies in beagle dogs (*70*) and guinea pigs (*75*) showed such dramatic differences with particle size is probably because much narrower size distributions were used than in other studies; geometric standard deviations (σ_g) of 1.2 to 1.3 were achieved as compared to the σ_g of 1.7 to 2.6 in other studies. For example, a considerable portion of the mass of 0.6 μm MMAD polydisperse aerosols is above 0.8 μm. In contrast, the mass of 0.4 μm MMAD aerosols (σ_g = 1.2) greater than 0.8 μm is negligible.

One explanation for the relationship of response to particle size may be regional differences in deposition. Data are accumulating which indicate that the major mechanism responsible for adverse effects of inhaled sulfuric acid mist is the stimulation of upper airway receptors. This can result in reflex-mediated bronchoconstriction (*78*) and also increases in secretions from the mucous glands (*5*). Therefore, if more material is deposited in upper airways, a greater effect may be elicited. Deposition studies have confirmed that there is greater upper airway deposition of 0.8 to 0.9 μm MMAD sulfuric acid aerosols than of 0.3 to 0.4 μm MMAD aerosols (*79*).

Another factor which may be important is the degree of neutralization of the acid droplets by endogenously produced ammonia in the upper airways (*80*). Experimental observations have shown a greater degree of neutralization of 0.5 μm MMAD aerosols than of 1.0 μm aerosols (*81*). This means that more of the smaller aerosols will be converted to the less reactive $(NH_4)_2SO_4$ and NH_4HSO_4 than the larger aerosols, probably causing a lesser physiological effect of smaller particles than larger particles.

The above results show impairment of an important lung defense mechanism following acute exposures to relatively low levels of sulfuric acid mist. Such an impairment might lead to greater toxicity of inhaled materials because of longer residence time in the lung and also increased susceptibility to infectious agents. These facts must be considered when setting industrial threshold limit values. These results are also important in determining mechanisms that might lead to chronic respiratory disease in people chronically exposed to urban atmospheres containing sulfate aerosols.

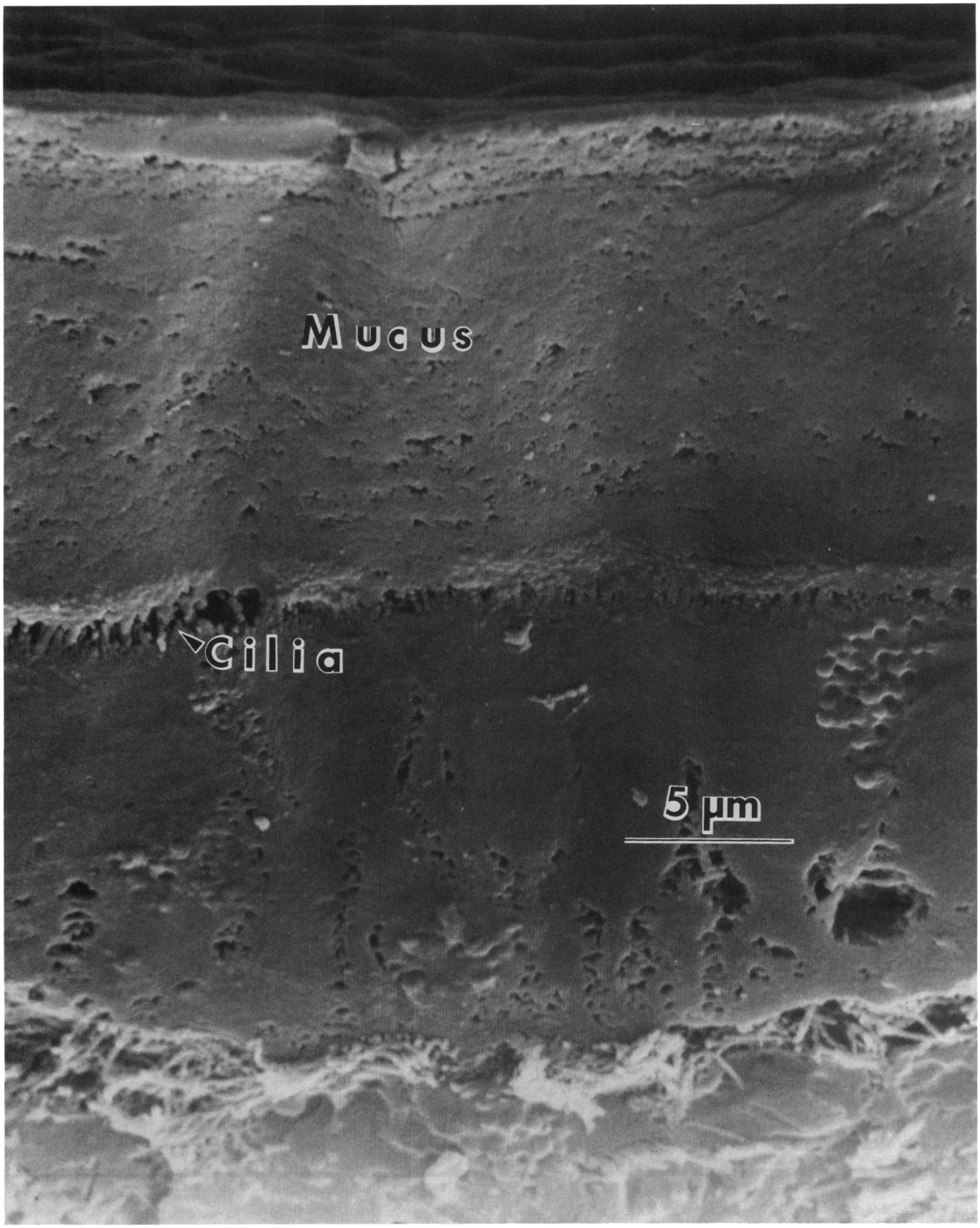

FIGURE 4. Scanning electron micrographs of a cryofractured cross section and overlying mucus from the trachea of a guinea pig exposed to 27 mg/m^3 H_2SO_4. Mucus depth is greater than 20 µm; this is many times thicker than observed in the normal state. (See Fig. 1 for an example in a rat.)

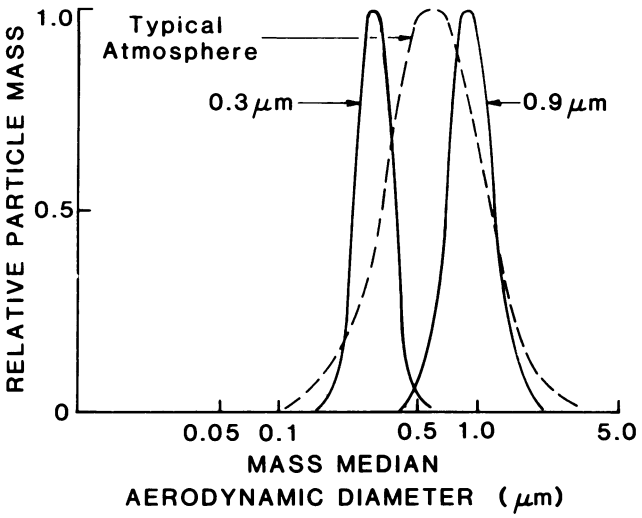

FIGURE 5. Aerosol mass distributions showing the distribution for 0.3 μm mass median aerodynamic diameter (MMAD) particles which did not show health effects compared to the 0.9 μm MMAD particles which did show effects (*58*). Also shown is the typical atmospheric distribution of sulfates for relatively polluted conditions (30 μg/m^3 sulfate).

These effects have particular significance in the light of atmospheric conditions during high pollution episodes and their relation to particle size. High humidity and high particle number concentration will tend to promote growth of aerosols, particularly hygroscopic materials like sulfuric acid mist. Data summarized by Whitby (*82*) showed that the mass median diameter of sulfate aerosols ranged from approximately 0.3 μm under lightly polluted conditions (~ 10 μg/m^3 sulfate) to approximately 0.7 μm under relatively heavily polluted conditions (~ 30 μg/m^3 sulfate). Again, the σ_g was relatively large (~ 2.0), so there would be considerable mass greater than 0.8 μm for 0.7 μm MMAD particles. Figure 5 compares ambient sulfate particle size with those used in the dog studies (*70*). The results suggest that larger particles, associated with more heavily polluted conditions, may be more harmful than smaller particles. Therefore, adverse health effects would be accentuated at these times. The data of Whitby (*82*) also show that even under heavily polluted environmental conditions total sulfate levels are a factor of approximately three times lower than those at which effects of sulfuric acid mist have been noted experimentally.

It is very important to relate changes in lung clearance to pulmonary function and structure. Studies with low concentrations of sulfuric acid mist have shown an effect on mucociliary clearance but no effect on pulmonary function in donkeys (*71*). Acute exposures of "normal" humans to H_2SO_4 have not shown changes in pulmonary function, or airway reactivity at levels of 1 mg/m^3 (*83,84*) and exposures of asthmatics have shown changes in airway reactivity only at 450 μg/m^3 (*84*). However, one of the major questions that remains unresolved is whether these changes contribute to the development of lung disease. It is crucial to determine if permanent structural damage occurs, eventually resulting in decrements in pulmonary function or if sulfates trigger only acute effects which are transitory. The most persuasive sets of data are the studies of chronic exposures of donkeys to 100 μg/m^3 sulfuric acid reported by Schlesinger et al. (*85*) and Lippmann (*86*). Decreases in mucociliary clearance were found in two of the four donkeys, and the other two showed variable responses. Acute exposures at the 100 μg/m^3 level had caused either no changes or speeding in clearance. Schlesinger et al. (*87*) have reported faster mucociliary clearance during and up to 2 weeks after 4 week exposures to 250 and 500 μg/m^3 sulfuric acid. These functional changes were also accompanied by observations of statistically significant increases in epithelial thickness and numbers of epithelial secretary cells in small airways. These results, coupled with the similarities of the human and large animal responses, suggest that high levels of sulfate sometimes found in urban environments may be a contributor to the development of lung disease. However, additional studies are needed to improve the certainty of this supposition. Mucociliary clearance effects appear to be one of the most sensitive indicators of potentially harmful health effects from inhaled sulfuric acid.

Other Sulfur Oxides

Sulfuric acid mist appears to be the most potent of the sulfur oxides in producing impairments in mucociliary clearance although there are limited data. Ammonium sulfate appears to produce minimal effects (*71*). Sulfur dioxide produces less effect (*46*), as does a ferric sulfite complex (*73*), sodium sulfite (*73*), or ammonium sulfite (*88*). Last (*89*) has shown ammonium persulfate to produce results similar to those of sulfuric acid mist. Studies by Sackner (*76,77*) have shown no effects on pulmonary function in dogs or tracheal mucociliary clearance in sheep exposed to submicron aerosols (0.05–0.2 μm) at concentrations ranging from 4 to 9 mg/m^3 of sodium sulfate, ammonium sulfate, zinc sulfate, zinc ammonium sulfate, ammonium bisulfate, aluminum sulfate, manganese sulfate, nickel sulfate, copper sulfate, ferrous sulfate, and ferric sulfate. The lack of response may be related to the small size of aerosols used. More recent studies in sheep with larger particles (0.5–1.5 μm) of aluminum sulfate, ammonium sulfate, zinc ammonium sulfate, zinc sulfate, and sulfuric acid have shown significant slowing of clearance (*90*).

From studies in which effects have been observed, it appears quite likely that the potency order of particulate sulfates for mucociliary clearance effects is similar to that developed by Amdur (*91*) using airway resistance effects in guinea pigs. She found that the irritant effect was generally related to the acidity in solution; sulfuric acid was the most potent and ammonium sulfate one of the least (*91*). Schlesinger (*92*) demonstrated that this appears to be true from the more limited number of compounds that have been studied. The potency order for the mucociliary clearance studies was H_2SO_4 > NH_4HSO_4 > $(NH_4)_2SO_4$, Na_2SO_4. The acidity of the

aerosols seems to be the predominant factor in influencing effects; however, chemical reactivity may play a modulating role given the fact that observable effects are found for some metal sulfates, sulfite, and SO_2 while they have near neutral pH values in aqueous solution. Reflex-mediated responses are probably primarily responsible for the observed effects in both cases; bronchoconstriction leading to airway resistance changes for Amdur's measurements and increases in secretions producing mucociliary clearance changes in other studies.

Ozone (O_3)

Only a few studies of O_3 effects on mucociliary clearance have been carried out, often in conjunction with sulfuric acid mist. Phalen et al. (*61*) showed impairment of early clearance in rats following 4-hr exposure to 0.8 ppm O_3. Co-exposure with 3.5 mg/m^3 H_2SO_4 did not appreciably alter the results; the depression in clearance persisted but was not augmented. Last et al. (*64,93*) have shown increases in secretions following 3 to 14 day exposures of rats to 0.4 to 0.5 ppm O_3. In this latter case, there were additive and even synergistic increases with co-exposures to H_2SO_4, with demonstrated effects down to 11 μg/m^3 (*64*). Grose et al. (*66*) have shown no changes in ciliary beat frequency following 2-hr exposures to 0.4 ppm O_3 and also no changes with co-exposures to 0.5 mg/m^3 H_2SO_4. Morphological studies indicate ciliary damage in the airways following O_3 exposure (*94*). However, studies by Abraham et al. (*95*) showed no changes in tracheal mucous velocities in sheep following exposures to 0.5 ppm O_3 while changes in bronchial reactivity were noted.

Nitrogen Dioxide (NO_2)

Studies of NO_2 effects are also very limited. Giordano and Morrow (*96*) demonstrated impairments of tracheal clearance of rats following a 6-week exposure to 6 ppm NO_2. Higher level exposures have been shown to inhibit clearance (*97,98*), but there are no reports of studies at lower levels. Two-hour exposures to 7.5 ppm NO_2 produced no changes in tracheal mucous velocity in sheep, while exposures to 15 ppm NO_2 produced slowing (*99*). Since both NO_2 and O_3 tend to produce effects predominantly in the region of the terminal bronchioles (*94,100*), it might be expected that effects on mucociliary clearance would not be as pronounced as for sulfuric acid mist. Other evidence for predominantly lower airway effects are the observations that acute and subchronic exposures to NO_2 (*101*), O_3, O_3 + sulfuric acid mist (*66,102,103*), and sulfuric acid + carbon dust (*104*), reduce resistance to respiratory bacterial infections. Such results have been ascribed primarily to effects on macrophages (*66,101*).

Other Irritant Gases

A variety of irritant gases have been shown to inhibit ciliary activity, particularly in *in vitro* studies (*10,11*). In addition to the effects of SO_2, NO_2, and O_3 mentioned above, Dalhamm (*105*) has shown that high concentrations of ammonia and formaldehyde depress mucus flow and ciliary activity following *in vivo* exposures. More recently, Mannix et al. (*48*) have reported slower upper airway clearance in rats following exposure to 20 ppm formaldehyde, and Morgan et al. (*106*) have reported ciliastasis in frog palates exposed to 4.4 ppm or more formaldehyde.

Particulates

Few studies of particulate effects have been conducted. Camner et al. (*107*) showed a speeding in bronchial clearance following brief exposure of human subjects to 50 mg/m^3 carbon dust. Schiff et al. (*65*) showed tracheal epithelial damage following combined exposures of hamsters to sulfuric acid and carbon dust. Inhalation of 37 mg/m^3 pulverized coal combustion fly ash and 36 mg/m^3 fluidized bed coal combustion fly ash for 4 weeks both produced no changes in early clearance of rats, but there were impairments of deep lung clearance (*108*). Similar results have also been obtained in rats at another laboratory (H. Muhle, personal communication). Accumulations of particles in the alveolar regions of the lung, characteristic of deep lung clearance impairments, have also been observed after exposures to high concentrations of coal dust (*109*), carbon black (*110*), diesel exhaust particles (*109,111–114*), and coal combustion fly ash (*108*). Abraham et al. (*115*) have shown that exposures of sheep to resuspended diesel exhaust particulate for 30 min at 0.4 to 0.5 mg/m^3 produced no changes in tracheal mucous clearance. There are no studies that show impairments of upper airway mucociliary clearance following particulate exposures to relative inert materials.

There have been some studies with trace metal aerosols which could indicate possible deleterious effects. Depressions in ciliary beat frequency have been seen after exposures to cadmium chloride (1.3 mg/m^3 for 2 days) (*116*) and nickel chloride (0.1 mg/m^3 for 2 hr) (*117*). These results suggest possible harmful effects if such high concentrations of soluble aerosols were encountered in an occupational situation.

Effects of particulate exposures appear to be relatively minor for mucociliary clearance but more pronounced for deep lung clearance when high lung burdens in the alveolar region are achieved. These results are not surprising since most of the materials studied are relatively innocuous and nonirritant.

Complex Mixtures

Diesel Exhaust. Diesel exhaust consists of a mixture of gases, vapors, and particles. The particles are composed of a carbonaceous core with adsorbed organics. Early studies (*118*) indicated impairments in mucociliary clearance following acute exposures of rats. More recent subchronic studies in rats indicated some transient changes over the first few weeks (*119*), which

subsequently resolved after 18 weeks of exposure (*120*). As noted in the previous section, acute exposures to resuspended diesel particles alone (*115*) also produced no changes. Despite the presence of irritant gases, diesel exhaust emissions appear to produce little change in mucociliary clearance. However, there are a number of studies which either show directly that deep lung clearance is impaired in rats following chronic diesel exhaust exposure (*120–125*) or strongly indicate such an effect because of increased lung burdens (*111–113*).

Cigarette Smoke. Like much of the scientific literature related to cigarette smoking, there have been some conflicting results. In general, most acute exposures using *in vitro* preparations have shown a depression of ciliary activity and mucus transport (*126–128*). Acute exposures of animals and humans to cigarette smoke have produced either a speeding, no change, or a slowing in mucociliary clearance. Some of these discrepancies may be methodological, but the studies of Yergin et al. (*129*), Isawa et al. (*130*), and Yeates et al. (*131*) support the view that acute smoking can have a variable effect on clearance.

Long-term smoking appears to depress mucociliary clearance. Again, methodological differences and interindividual variability have produced some conflicting data and so only the more definitive studies will be mentioned. By using radioaerosol inhalations and selecting healthy smokers and nonsmokers, Lourenco et al. (*132*) were able to show delayed overall clearance and an accumulation of material in central airways. Sanchis et al. (*133*) obtained similar results. Both Bohning et al. (*134*) and Camner and Philipson (*135*) were able to study smoking-discordant twins and showed depression of clearance in about half of the cases and no differences in others. Camner et al. (*136*) have seen that, in the absence of smoking, clearance in twins is strikingly similar. Goodman et al. (*137*) found dramatic reductions in tracheal mucus velocities in young healthy smokers. Some of the most informative studies are those in which a subject can be used as his own control. Camner et al. (*138*) found that cessation of smoking resulted in an improvement of mucociliary clearance after three months. Wanner et al. (*139*) found that chronic exposures of beagle dogs to cigarette smoke for 13.5 months depressed tracheal mucous velocities while lung mechanics did not change. These findings suggest that either mucociliary clearance defects may contribute to a chronic bronchitic state and/or they may provide an early warning of the possibility of development of the disease.

Mechanisms of Altered Clearance

As mentioned earlier, a number of factors may produce changes in mucociliary clearance. At threshold limit values and above, there is evidence for direct effects of pollutants on epithelium including such events as ciliary damage, cell sloughing, goblet cell discharge of secretions and probably serous cell discharge of secretions (*5*). *In vitro* studies have shown effects on mediators such as histamine (*140*) and prostaglandins (*141*) which might elicit local cellular responses including increased secretions. Lippmann et al. (*86,142*) have suggested that clearance is influenced primarily in the airways where H_2SO_4 is deposited. There is also evidence for adverse pH effects on the mucosa and ciliary activity (*143*).

There is considerable evidence for the alteration of the nature and volume of secretions by reflex events originating from stimulation of irritant receptors. The studies by Schultz et al. (*55*) clearly show that increases of tracheal mucous secretions can be observed in dogs even when SO_2 exposure is isolated to the larynx and no gas reaches the trachea. Keal (*18*) has stated that mucus secretory changes appear to precede effects on ciliary activity in rats, and Phipps (*19*) has shown that in the cat reflex secretory effects can be independent of bronchomotor effects, because increased secretions were produced from ammonia vapor inhalation when no bronchoconstriction was evident. Last et al. (*64*) has shown significant increases in secretion beyond those produced by O_3 exposure with the addition of only 14 $\mu g/m^3$ sulfuric acid to the atmosphere. All of these results indicate the degree of sensitivity of secretion response.

Effects on hydration of mucus, as evidenced by changes in ion transport (*7*), have not been clearly demonstrated as being produced by pollutants. However, drugs that show effects on ion transport indicating increases in watery secretion have also been shown to produce increases in mucus glycoproteins. It would not be too surprising if there were parallel effects on ion transport caused by pollutants, mediated by reflex pathways, since various pollutants have been shown to increase mucus glycoprotein production. Studies of airway permeability (*144*), which may be related to ion transport, have shown increases with exposures to cigarette smoke (*145*).

Evidence has been provided suggesting that the periciliary fluid level may be a very important determinant of particle and secretion movement. Stutts et al. (*147*) have suggested that increased periciliary fluid may uncouple the cilia from the overlying mucus. Proctor et al. (*148*) have observed cases where there was no movement of particles on the mucus layer while movement of the underlying periciliary fluid could be seen with dyes.

Most studies have shown relatively few effects of pollutants on ciliary activity at concentrations not causing a loss of ciliated cells. Cilia appear to be quite hardy and affected only when exposures are at or above threshold limit values.

The observed effects on mucociliary clearance are probably the result of some combination of all these factors. It is for this reason that mucociliary clearance studies may provide sensitive indicators of responses to irritant gases and aerosols since responses relate to a summation of effects.

The available evidence suggests that, for exposures near the current air quality limits, reflex mediated ef-

fects are most likely those that will predominate and be responsible for observed changes. Therefore, susceptible individuals would be those with the most pronounced reflex responses. Individuals such as asthmatics, with heightened bronchial reactivity, could be at greater risk to inhaled pollutants than the general population. However, clearance abnormalities may not be heightened in asthmatics because the secretory response is not directly tied to the bronchoconstrictive response (*19*). More studies on effects of pollutants on mucociliary clearance of asthmatics would be useful to clarify this issue.

It must be recognized that increases in secretions are probably a protective response. Nadel (*7*) and Phipps (*19*) have suggested that they could act to increase the barrier between the pollutant and the sensitive epithelium. However, it would appear that the key concern is whether a "biological backfire" phenomenon might be initiated. If there is continued stimulus, then increased secretions might well persist and eventually result in impaired clearance. Or, there may be interactions produced with other pollutants or respiratory infections which will tend to perpetuate an ongoing response and eventually lead to even greater problems. Lippmann (*142*) has suggested that pollutant exposures may affect clearance regulation resulting in erratic clearance behavior which is more characteristic of a bronchiticlike condition. It has to be recognized that ultimate adverse effects such as chronic bronchitis will probably occur only in a small fraction of people.

Summary

A variety of pollutants have been shown to impair mucociliary clearance following acute or subchronic exposures. The most convincing evidence for significant effects has been presented for sulfuric acid mist and sulfur dioxide and to a lesser extent for O_3 and NO_2 exposures. It appears that the more irritant the aerosol, the more pronounced the effects on mucociliary clearance.

The prime mechanism for observed alterations seems to be an increase in secretion produced predominantly by reflex effects following stimulation of upper airway receptors (*7*), although direct effects may also play a role (*7*). As such, mucociliary clearance studies provide a sensitive indication of response to irritant pollutants; they showed observable health effects at the lowest levels tested in the case of H_2SO_4 mist. Slowed clearance is a clear indication of adverse health effects but faster clearance, coupled with increases in secretion, must also be viewed with caution, since it probably indicates compensation in an adverse situation.

Whether mucociliary clearance data from acute or subchronic exposures can provide predictions of long-term lung damage which might result from chronic exposures is not clear. The main hypothesis has been that slowing in clearance and increases in secretion might be initiating factors in chronic obstructive respiratory disease (*1,3*). The observations to date indicate that mucociliary impairments found in acute exposures are good predictors of the development of lung disease following chronic exposure to H_2SO_4 mist. Most other pollutants have not been studied as extensively as sulfuric acid mist and so it is not clear if similar relationships will hold. It is also interesting that larger species show greater effects than rodents, in general, for both acute and chronic exposures. It should also be noted that the anatomic lesions observed for the chronic H_2SO_4 mist exposures were relatively subtle (*87,149*). Therefore, one would suspect that for chronic exposures of humans at high ambient levels of sulfate, lung disease would probably be initiated only in a small percentage of people and then probably only in concert with other factors such as respiratory infections and exposure to other agents. Challenges for future research are to further examine relationships between acute and chronic clearance effects, to determine sensitive populations at risk, and to investigate interactions of various environmental factors.

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REFERENCES

1. Wanner, A. Clinical aspects of mucociliary transport. Am. Rev. Respir. Dis. 116: 73–125 (1977).
2. Newhouse, M. T., Sanchis, J., and Bienenstock, J. Lung defense mechanisms. N. Engl. J. Med. 295: 990–998 (1976).
3. Hilding, A. C. Mucociliary insufficiency and its possible relation to chronic bronchitis and emphysema. Med. Thorac. 22: 329–345 (1965).
4. Proctor, D. F. The upper airway. I. Nasal physiology and defense of the lungs. Am. Rev. Respir. Dis. 115: 97–129 (1977).
5. Jeffery, P. K., and Reid, L. Respiratory mucous membrane. In: Respiratory Defense Mechanisms (J. D. Brain, D. F. Proctor, and L. M. Reid, Eds.), Marcel Dekker, New York, 1977, pp. 193–245.
6. CIBA Foundation Symposium. Respiratory Tract Mucus. Elsevier, Amsterdam, 1978.
7. Nadel, J. Control of mucus secretion and ion transport in airways. Ann. Rev. Physiol. 41: 361–381 (1979).
8. Cherniack, R. M. (Ed.), Lung Fluids and Secretions. Chest 81: May Supplement (1982).
9. King, M. Mucus and mucociliary clearance; basics of RD. Am. Mucociliary Thoracic Soc. News, 17–23 (1982).
10. Tremer, H., and Falk, H. L. The effect of air pollutants on ciliated mucus-secreting epithelium. J. Natl. Cancer Inst. 23: 979–999 (1959).
11. Falk, H. L. Responses of ciliated epithelium to irritants. Am. Rev. Respir. Dis. 93 (Suppl. 3): 86–92 (1966).
12. Rhodin, J. A. G. Ultrastructure and function of the human tracheal mucosa. Am. Rev. Respir. Dis. 93 (Suppl. 3): 15 (1966).
13. Breeze, R. G., and Sheldon, E. B. The cells of the pulmonary airways. Am. Rev. Respir. Dis. 116: 705–776 (1977).
14. Kilburn, K. H. A hypothesis for pulmonary clearance and its implications. Am. Rev. Respir. Dis. 98: 449–463 (1968).

15. Reid, L. Measurement of the bronchial mucous gland layer: a diagnostic yardstick in chronic bronchitis. Thorax 15: 132–141 (1960).
16. Miller, W. S. The epithelium of the lower respiratory tract. In: Special Cytology, 2nd ed. (E. V. Cowdry, Ed.), Hofner, New York, 1932, pp. 133–150.
17. Basbaum C., Ueki, I., and Nadel, J. Morphology of ferret tracheal glands stimulated *in vitro* with adrenergic and cholinergic agonists. Physiologist 23: 99 (1980).
18. Keal, E. E. Physiological and pharmacological control of airways secretion. In: Respiratory Defense Mechanisms, Marcel Dekker, New York, 1977, pp. 357–401.
19. Phipps, R. J., and Richardson, P. S. The effects of irritation at various levels of the airway upon tracheal mucus secretion in the cat. J. Physiol. 231: 563–581 (1976).
20. Hulbert, W. C., Forster, B. B., Laird, W., Pihl, C. E., and Walker, D. C. An improved method for fixation of the respiratory epithelial surface with the mucous and surfactant layers. Lab. Invest. 47: 354–363 (1982).
21. Lucas, A. M., and Douglas, L. C. Principles underlying ciliary activity in the respiratory tract. II. A comparison of nasal clearance in man, monkeys and other mammals. Arch. Oto-Laryngol. 20: 518–541 (1934).
22. Bang, B. G., and Bang, F. B. Effect of water deprivation on nasal mucous flow. Proc. Soc. Exptl. Biol. Med. 106: 516–521 (1961).
23. Morgan, K. T., Jiang, X., Patterson, D. L., and Gross, E. A. The nasal mucociliary apparatus: correlation of structure and function in the rat. Am. Rev. Respir. Dis. 130: 275–281 (1984).
24. Iravani, J., and Van As, A. Mucus transport in the tracheobronchial tree of normal and bronchitic rats. J. Pathol. 106: 81–93 (1972).
25. Van As, A. Pulmonary airway clearance mechanisms: a reappraisal. Am. Rev. Respir. Dis. 115: 721–726 (1977).
26. Sturgess, J. M. The mucous lining of major bronchi in the rabbit lung. Am. Rev. Respir. Dis. 115: 819–827 (1977).
27. Luchtel, D. L. Ultrastructural observations on the mucous layer in pulmonary airways. J. Cell Biol. 70: 350a (1976).
28. Asmundsson, T., and Kilburn, K. H. Mucociliary clearance rates at various levels in dog lungs. Am. Rev. Respir. Dis. 102: 388–397 (1970).
29. Morrow, P. E., Gibb, F. R., and Gazioglu, K. M. A study of particulate clearance from the human lungs. Am. Rev. Respir. Dis. 96: 1209–1221 (1967).
30. Felicetti, S. A., Wolff, R. K., and Muggenburg, B. A. Comparison of tracheal mucous clearance in rats, guinea pigs, rabbits and dogs. J. Appl. Physiol.: Respirat. Environ. Physiol. 51: 1612–1617 (1981).
31. Friedman, M., Stott, F. D., Poole, D. O., Dougherty, R., Chapman, G. A., Watson, H., and Sackner, M. A. A new roentgenographic method for estimating mucous velocity in airways. Am. Rev. Resp. Dis. 115: 67–72 (1977).
32. Wolff, R. K., and Muggenburg, B. A. Comparison of two methods of measuring tracheal mucous clearance in anesthetized beagle dogs. Am. Rev. Respir. Dis. 120: 137–142 (1979).
33. Lee, P. S., Gerrity, T. R., Hass, F. J., and Lourenco, R. V. A model for tracheobronchial clearance of inhaled particles in man and a comparison with data. IEEE Trans. Biomed. Eng. BME 26: 624–630 (1979).
34. Pavia, D., Bateman, J. R. M., and Clarke, S. W. Deposition and clearance of inhaled particles. Bull. Eur. Physiopathol. Resp. 16: 335–366 (1980).
35. Lippmann, M., Yeates, D. B., and Albert, R. E. Deposition, retention and clearance of inhaled particles. Brit. J. Ind. Med. 37: 337–362 (1980).
36. Yeates, D. B., Gerrity, T. R., and Garrard, C. S. Particle deposition and clearance in the bronchial tree. Ann. Biomed. Eng. 9: 577–592 (1981).
37. Clarke, S. W., and Pavia, D. Lung mucus production and mucociliary clearance: methods of assessment. Brit. J. Clin. Pharmacol. 9: 537–546 (1980).
38. Camner, P. Clearance of particles from the human tracheobronchial tree. Clin. Sci. 59: 79–84 (1980).
39. Stewart, W. C. Weight carrying capacity and excitability of excised ciliated epithelium. Am. J. Physiol. 152: 1–5 (1948).
40. Wolff, R. K., Hardy, S. C., and Muggenburg, B. A. Effect of radiolabeled materials on tracheal mucous clearance in beagle dogs. Am. Rev. Respir. Dis. 126: 505–508 (1982).
41. Ahmed, T., Januszkiewicz, A. J., Landa, J. F., Brown, A., Chapman, G. A., Kenny, P. J., Finn, R. D., Bondick, J., and Sackner, M. A. Effect of local radioactivity on tracheal mucous velocity of sheep. Am. Rev. Respir. Dis. 120: 567–575 (1979).
42. Yeates, D. B., Pitt, B. R., Spektor, D. M., Karron, G. A., and Albert, R. E. Coordination of mucociliary transport in human trachea and intrapulmonary airways. J. Appl. Physiol. Respir. Environ. Exercise Physiol. 51[5]: 1057–1064 (1981).
43. Speizer, F. E., and Frank, N. R. A comparison of changes in pulmonary flow resistance in healthy volunteers acutely exposed to SO_2 by mouth and by nose. Brit. J. Ind. Med. 23: 75–79 (1966).
44. Frank, N. R., Yoder, R. E., Brain, J. D., and Yokoyama, E. SO_2 [^{35}S labeled] absorption by the nose and mouth under conditions of varying concentration and flow. Arch. Environ. Health 18: 315–322 (1969).
45. Andersen, I., Lundqvist, G. R., Jensen, P. L., and Proctor, D. F. Human response to controlled levels of sulfur dioxide. Arch. Environ. Health 28: 31–39 (1974).
46. Wolff, R. K., Dolovich, M., Rossman, C. M., and Newhouse, M. T. Sulphur dioxide and tracheobronchial clearance in man. Arch. Environ. Health 30: 521–527 (1975).
47. Wolff, R. K., Newhouse, M. T., Dolovich, M., and Obminski, G. Effect of TLV levels of SO_2 and H_2SO_4 on bronchial clearance in exercising man. Arch. Environ. Health 33: 24–32 (1978).
48. Mannix, R. C., Phalen, R. F., Walters, R. B., and Kurosaki T. T. Effects of sulfur dioxide and formaldehyde on particle clearance in the rat. J. Toxicol. Environ. Health 12: 429–440 (1983).
49. Fraser, D. A., Battigelli, M. C., and Cole, H. M. Ciliary activity and pulmonary retention of inhaled dust in rats exposed to sulfur dioxide. J. Air Pollut. Control Assoc. 18: 821–823 (1968).
50. Spiegelman, J. R., Hanson, G. D., Lazarus, A., Bennet, R. J., Lippmann, M., and Alpert, R. D. Effect of acute sulfur dioxide exposure on bronchial clearance in the donkey. Arch. Environ. Health 17: 321–326 (1968).
51. Hirsch, J. A., Swenson, E. W., and Wanner, A. Tracheal mucous transport in beagles after long-term exposure to 1 ppm sulfur dioxide. Arch. Environ. Health 30: 249–253 (1975).
52. Chakrin, L. W., and Saunders, L. Z. Experimental chronic bronchitis. Pathology in the dog. Lab. Invest. 30: 145–154 (1974).
53. Greene, S. A., Wolff, R. K., Hahn, F. F., Henderson, R. F., Mauderly, J. L., and Lundgren, D. L. Sulfur dioxide-induced chronic bronchitis in beagle dogs. J. Toxicol. Environ. Health 13: 945–958 (1984).
54. Lamb, D., and Reid, L. Mitotic rates, goblet cell increase, and histochemical changes in mucus in rat bronchial epithelium during exposure to sulphur dioxide. J. Pathol. Bacteriol. 96: 97–111 (1968).
55. Schultz, H. D., Roberts, A. M., Hahn, H. L., Nadel, J. A., Coleridge, H. M., and Coleridge, J. C. G. Mechanism of airway constriction and secretion evoked by laryngeal administration of SO_2 in dogs. Physiologist 25: 226 (1982).
56. Sheppard, D., Wong, W. S., Uehara, C. F., Nadel, J. A., and Boushey, H. A. Lower threshold and greater bronchomotor responsiveness of asthmatic subjects to sulfur dioxide. Am. Rev. Respir. Dis. 122: 873–878 (1980).
57. Sheppard, D., Nadel, J. A., and Boushey, H. A. Inhibition of sulfur dioxide-induced bronchoconstriction by disodium cromoglycate in asthmatic subjects. Am. Rev. Respir. Dis. 124: 257–259 (1981).
58. Kirkpatrick, M. B., Sheppard, D., Nadel, J. A., and Boushey, H. A. Effect of the oronasal breathing route on sulfur dioxide-induced bronchoconstriction in exercising asthmatic subjects. Am. Rev. Respir. Dis. 125: 627–631 (1982).
59. Wolff, R. K., Obminski, G., and Newhouse, M. T. Exposure of symptomatic steelworkers to SO_2 and carbon dust: Effects on mucociliary transport, pulmonary function, and bronchial reactivity. Brit. J. Ind. Med. 41: 499–505 (1984).
60. Wolff, R. K., Henderson, R. F., Dahl, A. R., Felicetti, S. A.,

and Gray, R. H. Effects of H_2SO_4 on tracheal mucous clearance. In: Pulmonary Toxicology of Respirable Particles (C. L. Sanders, F. T. Cross, G. E. Dagle, and J. A. Mahaffey, Eds.), CONF-791002, National Technical Information Service, Springfield, VA, 1980.
61. Phalen, R. F., Kenoyer, J. L., Crocker, T. T., and McClure, T. R. Effects of sulfate aerosols in combination with ozone on elimination of tracer particles inhaled by rats. J. Toxicol. Environ. Health 6: 797–810 (1980).
62. Kenoyer, J. L., Phalen, R. F., and Davis, J. R. Particle clearance from the respiratory tract as a test of toxicity. Exp. Lung Res. 2: 111–120 (1981).
63. Runkle, B. K., and Hahn, F. F. The toxicity of H_2SO_4 aerosols to CD-1 mice and Fischer-344 rats. In: Inhalation Toxicology Research Institute Annual Report, LF - 58, National Technical Information Service, Springfield, VA, 1977, pp. 435–439.
64. Last, J. A., and Cross, C. E. A new model for health effects of air pollutants: evidence for synergistic effects of mixtures of ozone and sulfuric acid aerosols on rat lungs. J. Lab. Clin. Med. 91: 328–339 (1978).
65. Schiff, L. J., Bryne, M. M., Fenters, J. D., Graham, J. A., and Gardner, D. E. Cytotoxic effects of sulfuric acid mist, carbon particulates, and their mixtures on hamster tracheal epithelium. Environ. Res. 19: 339–354 (1979).
66. Grose, E. C., Gardner, D. E., and Miller, F. J. Response of ciliated epithelium to ozone and sulfuric acid. Environ. Res. 22: 377–385 (1980).
67. Grose, E. C., Richards, J. H., Illing, J. W., Mitler, F. J., Davies, D. W., Graham, J. A., and Gardner, D. E. Pulmonary host defenses responses to inhalation of sulfuric acid and ozone. J. Toxicol. Environ. Health 10: 351–362 (1982).
68. Wolff, R. K., Henderson, R. F., Gray, R. H., Carpenter, R. L., and Hahn, F. F. Effects of sulfuric acid mist inhalation on mucous clearance and on airway fluids of rats and guinea pigs. J. Toxicol. Environ. Health, in press.
69. Wolff, R. K., Muggenburg, B. A., Silbough, S. A., Carpenter, R. L., Rowatt, J. D., and Hill, J. O. Lung clearance mechanisms following inhalation of acid sulfates. Inhalation Toxicology Research Institute Report, LMF-99, National Technical Information Service, Springfield, VA, 1982.
70. Wolff, R. K., Muggenburg, B. A., and Silbaugh, S. A. Effect of 0.3 and 0.9 μm sulfuric acid aerosols on tracheal mucous clearance in beagle dogs. Am. Rev. Respir. Dis. 123: 291–294 (1981).
71. Schlesinger, R. B., Lippmann, M., and Albert, R. E. Effects of short-term exposures to sulfuric acid and ammonium sulfate aerosols upon bronchial airways function in donkeys. Am. Ind. Hyg. Assoc. J. 39: 275–286 (1978).
72. Liekauf, G., Yeates, D. B., Wales, K. A., Spektor, D., Albert, R. E., and Lippmann, M. Effect of inhaled sulfuric acid mist on respiratory mechanics and mucociliary particle clearance in healthy nonsmokers. Am. Ind. Hyg. Assoc. J. 42: 273–282 (1981).
73. Chen, L. C., and Schlesinger, R. B. Response of the bronchial mucociliary clearance system in rabbits to inhaled sulfite and sulfuric acid aerosols. Toxicol. Appl. Pharmacol. 71: 123–131 (1983).
74. King, J., Cohen, C., and Viires, N. Influence of vagal tone on rheology and transportability of canine tracheal mucus. Am. Rev. Respir. Dis. 120: 1215–1219 (1979).
75. Wolff, R. K., Silbaugh, S. A., Carpenter, R. L., and Mauderly, J. L. Toxicity of 0.4 and 0.8 μm sulfuric acid aerosols in guinea pigs. J. Toxicol. Environ. Health 5: 1037–1047 (1979).
76. Sackner, M. A., Ford, D., Fernandez, R., Cipley, J., Perez, D., Kwoka, M., Reinhart, M., Michaelson, E. D., Schreck, R., and Wanner, A. Effects of sulfuric acid aerosol on cardiopulmonary function of dogs, sheep and humans. Am. Rev. Respir. Dis. 118: 497–514 (1978).
77. Sackner, M. A., Dougherty, R. L., Chapman, G. A., Cipley, J., Perez, D., Kwoka, M., Reinhart, M., Brito, M., and Schreck, R. Effects of brief and intermediate exposures to sulfate submicron aerosols and sulfate injections on cardiopulmonary functions of dogs and tracheal mucous velocity of sheep. J. Toxicol. Environ. Health 7: 951–972 (1981).
78. Brownstein, D. G. Reflex mediated desquamation of bronchiolar epithelium in guinea pigs exposed acutely to sulfuric acid aerosol. J. Pathol. 98: 577–590 (1980).
79. Dahl, A. R., and Griffith, W. C. Deposition of sulfuric acid mist in the respiratory tracts of guinea pigs and rats. J. Toxicol. Environ. Health 12: 371–383 (1983).
80. Larson, T. V., Covert, D. S., Frank, R., and Charlson, R. J. Ammonia in the human airways: neutralization of inspired acid sulfate aerosols. Science 197: 161–163 (1977).
81. Larson, T. V., Frank, R., Covert, D. S., Holub, D., and Morgan, M. Measurement of respiratory ammonia and the chemical neutralization of inhaled sulfuric acid aerosol in anesthetized dogs. Am. Rev. Respir. Dis. 119: 226 (1979).
82. Whitby, K. T. The physical characteristics of sulfur aerosols. Atms. Environ. 12: 135–159 (1978).
83. Avol, E. L., Jones, M. P., Bailey, R. M., Chang, N. N., Kleinman, M. T., Linn, W. S., Bell, K. A., and Hackney, J. D. Controlled exposures of human volunteers to sulfate aerosols: health effects and aerosol characterization. Am. Rev. Respir. Dis. 120: 319–327 (1979).
84. Utell, M. J., Morrow, P. E., and Hyde, R. W. Airway reactivity to sulfate and sulfuric acid aerosols in normal and asthmatic subjects. J. Air Pollut. Control Assoc. 34: 931–935 (1984).
85. Schlesinger, R. B., Halpern, M., Albert, R. E., and Lippmann, M. Effect of chronic inhalation of sulfuric acid mist upon mucociliary clearance from the lungs of donkeys. J. Environ. Pathol. Toxicol. 2: 1351–1367 (1979).
86. Lippmann, M., Schlesinger, R. B., Leikauf, G., Spektor, D., and Albert, R. E. Effects of sulphuric acid aerosols on respiratory tract airways. Ann. Occup. Hyg. 26: 677–690 (1982).
87. Schlesinger, R. B., Naumann, B. D., and Chen, L. C. Physiological and histological alterations in the bronchial mucociliary clearance system of rabbits following intermittent oral or nasal inhalation of sulfuric acid mist. J. Toxicol. Environ. Health 12: 441–465 (1983).
88. Rothenberg, S. J., Dahl, A. R., Barr, E. B., and Wolff, R. K. Generation, behavior, and toxicity of ammonium sulfite aerosols. J. Air Pollut. Control Assoc. 36: 55–59 (1986).
89. Last, J. A., Dasgupta, P. K., De Cesari, K., and Tarrington, B. K. Inhalation toxicology of ammonium persulfate, an oxidant aerosol, in rats. Toxicol. Appl. Pharmacol. 63: 257–263 (1982).
90. Abraham, W. M., Chapman, G. A., and Kim, C. S. Airways effects of low level [0.5 μm and 1.5 μm] sulfate aerosols in conscious sheep. Am. Rev. Respir. Dis. 125 (Suppl.): 146 (1982).
91. Amdur, M. O., Bayles, J., Ugro, V., and Underhill, D. W. Comparative irritant potency of sulfate salts. Environ. Res. 16: 1–8 (1978).
92. Schlesinger, R. B. Comparative irritant potency of inhaled sulfate aerosols—effects on bronchial mucociliary clearance. Environ. Res. 34: 268–279 (1984).
93. Last, J. A., Jennings, M., Schwartz, L. W., and Cross, C. E. Glycoprotein secretion by tracheal explants cultured from rats exposed to ozone. Am. Res. Respir. Dis. 116: 695 (1977).
94. Schwartz, L. W., Moore, P. F., Chang, D. P., Tarkington, B. K., Dungworth, D. L., and Tyler, W. S. Short-term effects of sulfuric acid aerosols on the respiratory tract. A morphological study in guinea pigs, mice, rats and monkeys. In: Biochemical Effects of Environmental Pollutants (S. D. Lee, Ed.), Ann Arbor Science Publishers, Ann Arbor, MI, 1977, pp. 257–271.
95. Abraham, W. M., Januszkiewicz, A. J., Mingle, M., Welker, M., Wanner, A., and Sackner, M. A. Sensitivity of bronchoprovocation and tracheal mucous velocity in detecting airway responses to O_3. J. Appl. Physiol. Respir. Environ. Exercise Physiol. 48[5]: 789–793 (1980).
96. Giordano, A. M., and Morrow, P. E. Chronic low-level nitrogen dioxide exposure and mucociliary clearance. Arch. Environ. Health 25: 443–449 (1972).
97. Dalham, T., and Sjoholm, J. Studies on SO_2, NO_2, and HN_3: effect on ciliary activity in rabbit trachea of single *in vitro* exposure and resorption in rabbit nasal cavity. Acta Physiol. Scand. 58: 287–291 (1963).
98. Cralley, L. V. The effect of irritant gases upon the rate of ciliary activity. J. Ind. Hyg. Toxicol. 24: 193–198 (1942).

99. Abraham, W. M., Welker, M., Oliver, W., Mingle, M., Januszkiewicz, A. J., Wanner, A., and Sackner, M. A. Cardiopulmonary effects of short-term nitrogen dioxide exposure in conscious sheep. Environ. Res. 22: 61–72 (1980).
100. Freeman, G., Crane, S. C., Stephens, R. J., and Furiosi, N. J. The subacute nitrogen dioxide induced lesion of the rat lung. Arch. Environ. Health 18: 609–612 (1969).
101. Ehrlich, R. Interaction between environmental pollutants and respiratory infections. Environ. Health Perspect. 35: 89–100 (1980).
102. Gardner, D. E., Miller, J., Illing, J. W., and Kirtz, J. M. Increased infectivity with exposure to ozone and sulfuric acid. Toxicol. Letters 1: 59–64 (1977).
103. Aranyi, C., Vana, S. C., Thomas, P. T., Bradof, J. N., Fenters, J. D., Graham, J. A., and Miller, F. J. Effects of subchronic exposure to a mixture of O_3, SO_2; and $[NH_4]_2SO_4$ on host defenses of mice. J. Toxicol. Environ. Health 12: 55–71 (1983).
104. Fenters, J. D., Bradof, J. N., Aranyi, C., Ketels, K., Ehrlich, R., and Gardner, D. E. Health effects of long-term inhalation of sulfuric acid mist-carbon particle mixtures. Environ. Res. 19: 244–257 (1979).
105. Dalhamn, T. Mucus flow and ciliary activity in the trachea of healthy rats and rats exposed to respiratory irritant gases [SO_2, H_3N, HCHO]: a functional and morphologic [light microscopy and electron microscopy] study, with special reference to technique. Acta Physiol. Scand. 36 (Suppl.): 1–161 (1956).
106. Morgan, K. T., Patterson, D. L., and Gross, E. A. Frog palate mucociliary apparatus: structure, function, and response to formaldehyde gas. Fundam. Appl. Toxicol. 4: 58–68 (1984).
107. Camner, P., Helstrom, P. A., and Philipson, K. Carbon dust and mucociliary transport. Arch. Environ. Health 26: 294–296 (1973).
108. Hobbs, C. H., Carpenter, R. L., Bice, D. E., Mauderly, J. L., Pickrell, J. A., Silbaugh, S. A., Hahn, F. F., Henderson, R. F., and Wolff, R. K. Comparative inhalation toxicity of fly ash from pulverized and fluidized bed combustion of coal. Inhalation Toxicology Research Institute Annual Report, LMF-102, 1982, pp. 401–407.
109. Karagianes, M. T., Palmer, R. F., and Busch, R. H. Effects of inhaled diesel emissions and coal dust in rats. Am. Ind. Hyg. Assoc. J. 42: 382–391 (1981).
110. Nau, C. A., Neal, J., Stembridge, V. A., and Cooley, R. N. Physiological effects of carbon black. Arch. Environ. Health 4: 415–431 (1962).
111. Vostal, J. J., Schreck, R. M., Lee, P. S., Chan, T. L., and Soderholm, S. C. Deposition and clearance of diesel particles from the lung. In: Toxicological Effects of Emissions from Diesel Engines (J. Lewtas, Ed.), Elsevier Biomedical, NYU, New York, 1982, pp. 143–159.
112. McClellan, R. O., Brooks, A. L., Cuddihy, R. G., Jones, R. K., Mauderly, J. L., and Wolff, R. K. Inhalation toxicology of diesel exhaust particles. In: Toxicological Effects of Emissions from Diesel Engines (J. Lewtas, Ed.), Elsevier Biomedical, New York, 1982, pp. 99–120.
113. Henderson, R. F., Wolff, R. K., Mauderly, J. L., and McClellan, R. O. Accumulation of diesel soot in lungs of rodents exposed in life span studies to diluted diesel exhaust. In: Inhalation Toxicology Research Institute Annual Report, LMF-102, National Technical Information Service, Arlington, VA, 1982, pp. 224–228.
114. Vostal, J. J., White, H. J., Strom, K. A., Siak, J. S., Chen, K. C., and Dziedzic, D. Response of the pulmonary defence system to diesel particulate exposure. In: Toxicological Effects of Emissions from Diesel Engines (J. Lewtas, Ed.), Elsevier Biomedical, New York, 1982, pp. 201–221.
115. Abraham, W. M., Kim, C. S., Januszkiewicz, A. J., Welker, M., Mingle, M. A., and Schreck, R. Effects of a brief low-level exposure to the particulate fraction of diesel exhaust on pulmonary function of conscious sheep. Arch. Environ. Health 35: 77–80 (1980).
116. Adalis, A., Gardner, D. E., Miller, F. J., and Coffin, D. L. Toxic effects of cadmium on ciliary activity using a tracheal ring model system. Environ. Res. 13: 111–120 (1977).
117. Adalis, A., Gardner, D. E., and Miller, F. J. Cytotoxic effects of nickel on ciliated epithelium. Am. Rev. Respir. Dis. 118: 347–354 (1978).
118. Battigelli, M. C. Effects of diesel exhaust. Arch. Environ. Health 10: 165–167 (1965).
119. Wolff, R. K., Gray, R. H., and Hahn, F. F. Effect of subchronic diesel exposure on tracheal mucous clearance in rats. Toxicologist 1: 75 (1981).
120. Mauderly, J. L., Benson, J. M., Bice, D. E., Henderson, R. F., Jones, R. K., McClellan, R. O., Mokler, V. B., Pickrell, J. A., Redman, H. C., and Wolff, R. K. Life span study of rodents inhaling diesel exhaust: experiments design and results through 6 months. In: Inhalation Toxicology Research Institute Annual Report, LMF-91, National Technical Information Service, Springfield, VA, 1981, pp. 212–330.
121. Mohr, U., and Stober, W. Investigation of toxic and carcinogenic effects of diesel exhaust in long-term inhalation exposure of rodents. In: Toxicological Effects of Emissions from Diesel Engines, (J. Lewtas, Ed.), Elsevier Biomedical, New York, 1982, pp. 225–242.
122. Griffis, L. C., Wolff, R. K., Henderson, R. F., Griffith, W. C., Mokler, B. V., and McClellan, R. O. Clearance of diesel soot particles from rat lung after a subchronic diesel exhaust exposure. Fund. Appl. Toxicol. 3: 99–103 (1983).
123. Chan, T. L., Lee, P. S., and Hering, W. E. Deposition and clearance of inhaled diesel particles in the respiratory tract of Fischer rats. J. Appl. Toxicol. 1: 77–82 (1981).
124. Chan, T. L., Lee, P. S., and Hering, W. E. Pulmonary retention of inhaled diesel particles after prolonged exposures to diesel exhaust. Fundam. Appl. Toxicol. 4: 624–631 (1984).
125. Wolff, R. K., Henderson, R. F., Snipes, M. B., Mauderly, J. L., and McClellan, R. O. Diesel exhaust exposure: particle clearance and accumulation in rats and mice. Toxicol. Appl. Pharmacol., submitted.
126. Falk, H. L., Tremer, H. M., and Kotin, P. Effect of cigarette smoke and its constituents on ciliated mucus-secreting epithelium. J. Natl. Cancer Inst. 23: 999–1012 (1959).
127. Kensler, C. J., and Battista, S. P. Components of cigarette smoke with ciliary-depressant activity. N. Engl. J. Med. 269: 1161–1166 (1963).
128. Dalhamn, T. *In vivo* and *in vitro* ciliatoxic effects of tobacco smoke. Arch. Environ. Health 21: 633–634 (1970).
129. Yergin, B., Goodman, R. M., Landa, J. F., Golinvaux, M. H., and Sackner, M. A. Effects of cigarette smoking on tracheal mucous velocity in young smokers with and without small airway obstruction. Am. Res. Respir. Dis. 115 (Suppl.): 183 (1977).
130. Isawa, T., Teshima, T., Hirano, T., Kebina, A., and Konno, K. Mucociliary clearance mechanism in smoking and nonsmoking normal subjects. J. Nucl. Med. 25: 352–359 (1984).
131. Yeates, D. B., Aspin, N., Levison, H., Jones, M. T., and Bryan, A. C. Mucociliary tracheal transport rates in man. J. Appl. Physiol. 39: 487–495 (1975).
132. Lourenco, R. V., Klimer, M. F., and Borowski, C. J. Deposition and clearance of 2μ particles in the tracheobronchial tree of normal subjects: smokers and nonsmokers. J. Clin. Invest. 50: 1411–1420 (1971).
133. Sanchis, J., Dolovich, M., Chambers, R., and Newhouse, M. T. Regional distribution and lung clearance mechanisms in smokers and non-smokers. In: Inhaled Particles and Vapours, Vol. III (W. H. Walton, Ed.), Unwin Brothers, Old Working, Surrey, England, 1972, pp. 183–188.
134. Bohning, D. E., Albert, R. E., Lippmann, M., and Foster, W. M. Tracheobronchial particle deposition and clearance. Arch. Environ. Health 30: 457–472 (1975).
135. Camner, P., and Philipson, K. Tracheobronchial clearance in smoking: discordant twins. Arch. Environ. Health 25: 60–63 (1972).
136. Camner, P., Philipson, K., and Friberg, L. Tracheobronchial clearance in twins. Arch. Environ. Health 24: 82–87 (1972).
137. Goodman, R. M., Yergin, B. M., Landa, J. F., Golinvaux, M. H., and Sackner, M. A. Relationship of smoking history and pulmonary function tests to tracheal mucous velocity in nonsmokers, young smokers, ex-smokers and patients with chronic

bronchitis. Am. Res. Respir. Dis. 117: 205–214 (1978).
138. Camner, P., Philipson, K., and Arvidsson, T. Withdrawal of cigarette smoking. Arch. Environ. Health 26: 90–92 (1973).
139. Wanner, A., Hirsch, J. A., Greeneltch, D. E., Swenson, E. W., and Fore, T. Tracheal mucous velocity in beagles after chronic exposure to cigarette smoke. Arch. Environ. Health 27: 370–371 (1973).
140. Charles, J. M., and Menzel, D. B. Ammonium and sulfate ion release of histamine from lung fragments. Arch. Environ. Health 30: 314–316 (1975).
141. Iravani, J., and Melville, G. N. Mucociliary activity in the respiratory tract as influenced by prostaglandin. E. Respiration 32: 305–315 (1975).
142. Lippmann, M., Schlesinger, R. B., and Leikauf, G. Effects of sulfuric acid aerosol inhalations. Am. J. Ind. Med. 1: 375–381 (1980).
143. Holma, B., Lindegren, J., and Andersen, J. M. pH effects on cilia motility and morphology of respiratory mucosa. Arch. Environ. Health 32: 216–226 (1977).
144. Hogg, J. C., Park, P. D., and Boucher, R. C. Bronchial mucosal permeability. Fed. Proc. 38: 197–201 (1979).
145. Boucher, R. C., Johnson, J., Inoue, S., Hulbert, W., and Hogg, J. C. The effect of cigarette smoke on the permeability of guinea pig airways. Lab. Invest. 43: 94–100 (1980).
146. Leikauf, G. The effects of sulfuric acid aerosol on respiratory mechanics and mucociliary particle clearance in healthy and asthmatic adults. Ph.D. Dissertation, NYU, Institute of Environmental Medicine, 1982.
147. Stutts, M. J., Guyman, L. F., and Boucher, R. C. Interaction of Pseudomonas aeruginosa products with bronchial epithelium. Chest 81 (Suppl.): 135–145 (1982).
148. Proctor, D. F., Adams, G. K., Andersen, I., and Man, S. F. P. Nasal mucociliary clearance in man. In: Respiratory Tract Mucus. Elsevier, Amsterdam, 1978, pp. 219–234.
149. Alarie, Y. C., Krumm, A. A., Busey, W. M., Ulrich, C. E., and Kantz, R. J. Long-term exposure to sulfur dioxide, sulfuric acid mist, fly ash, and their mixtures. Results of studies in monkeys and guinea pigs. Arch. Environ. Health 30: 254–262 (1975).