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Effects of Inhaled Acids on Respiratory Tract Defense Mechanisms

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The respiratory tract is endowed with an interlocking array of nonspecific and specific defense mechanisms which protect it from the effects of inhaled microbes and toxicants, and reduce the risk of absorption of materials into the bloodstream, with subsequent systemic translocation. Ambient acids may compromise these defenses, perhaps providing a link between exposure and development of chronic and acute pulmonary disease. This paper reviews the effects of inhaled acids upon the nonspecific clearance system of the lungs.

Introduction

The respiratory tract presents a large area (ca. 150–180 m^2) in direct contact with the external environment, with most of its surface providing very little physical barrier between this environment and the circulatory system. But in addition to being a "vast" portal of entry into the blood for airborne materials, it contains potential target sites for numerous inhaled toxic agents and pathogens. The success an inhaled agent has in producing any local or systemic damage is the result of a number of different influences acting upon the host, one of which is the ability of the material to overload or subvert a sophisticated array of defenses.

The respiratory defense arsenal consists of a system of interlocking mechanisms, which may be conveniently divided into two broad categories: nonspecific and specific (immunologic) (Table 1). The former consists of nonselective mechanisms, which protect against a wide variety of materials; the latter requires antigenic stimulation for activation. Although one system may act without the other, they are linked, such that, for example, response to an immunologic insult may enhance subsequent response to nonantigenic materials as well. The overall efficiency of the defense system determines the residence times of deposited agents within the respiratory tract, and it is this which has a major influence upon the degree of local and/or systemic response; furthermore, dysfunction or overactivity of these systems may be involved in the pathogenesis of certain lung diseases.

Acids are irritants and, as such, inhaled acids may interfere with normal host defense, perhaps increasing the potential for acute or chronic disease. The study of altered defenses due to inhaled acids has concentrated on examination of bronchial reflex response in terms of ventilatory changes, and upon conducting and respiratory airway clearance function; there are essentially no direct studies of effects upon immunologic competence. Ventilatory effects have been described by others in this symposium; this paper discusses the role of inhaled acids in affecting clearance. In terms of the predepositional phase of acidic precipitation, the chemical species of interest are the acidic sulfur and nitrogen oxides.

Mechanisms of Respiratory Tract Clearance

Clearance is the physical removal of material that deposits on airway surfaces. The mechanisms involved are regionally distinct.

In conducting airways, clearance occurs via the mucociliary system. The nasal passages (except for the anterior nares and the posterior nasopharynx) and all airways of the tracheobronchial tree through the terminal bronchioles are lined with a ciliated epithelium overlaid by a fluid layer called mucus. Depending upon the species, this fluid lining is derived from various sources, which include specialized epithelial cells, and submucosal glands (*1*). The mucus is moved by the coordinated beating of cilia towards the naso- or oropharynx. The result is, in general, removal of deposited, insoluble material from the conducting airways within ~ 24 hr (*2,3*).

In the respiratory (alveolated) region of the lung, clearance may occur via a number of mechanisms and pathways, but the relative importance of each is not always certain and may depend to some extent upon the physicochemical properties and amount of material deposited, or the nature of any injury which occurs. Nevertheless, the first-line defense against microbes and nonviable particles is the alveolar macrophage, which isolates, transports and detoxifies deposited material. These large cells, which are part of the body's

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mononuclear phagocytic system, rest freely within the fluid lining of the alveolar epithelium and move via ameboid motion. They likely originate from precursors in bone marrow, and most reach the lungs, via the bloodstream, as monocytes, maturing in the pulmonary interstitium from which they traverse the epithelium to reach the alveolar surface; a certain percentage may arise from resident cells in the interstitium, which then migrate onto the alveolar surface (*4,5*). Macrophages comprise over 95% of the free cells obtainable by lavage from healthy lungs (*6*).

Via phagocytic ingestion, macrophages help prevent the penetration of deposited material through alveolar epithelium and subsequent translocation to other sites. They contain proteolytic enzymes that allow them to digest a wide variety of organic materials, and they also kill bacteria through peroxide-producing oxidative mechanisms (*4*). In addition, macrophages are involved in the induction and expression of cell-mediated immune reactions. Thus, the macrophage provides a link between the lung's nonspecific and specific defense systems.

Macrophages may be cleared from the respiratory region via a number of pathways, the primary one being via the mucociliary system (*5,7,8*). However, the mechanism(s) by which these cells reach the distal terminus of the mucus blanket is not definitely known. Nevertheless, the effective overall operation of clearance depends upon the integrated functioning of the macrophage and the mucociliary apparatus; dysfunction of the latter could affect the protective function of the former.

Clearance from respiratory airways is generally much slower than that from conducting airways (*9*). There are few useful data on alveolar clearance rates in man, while those for experimental animals vary widely due to different techniques used in the various studies (*10*). Nevertheless, alveolar clearance has been represented by three exponential phases which may relate to specific physiological processes: an initial fast phase, having a half-time of days to weeks, representing relatively rapid clearance via macrophages; an intermediate phase of slower macrophage clearance or movement of particles through alveolar epithelium, having a half-time on the order of months; and a phase of slow clearance, with a half-time of months to years, representing removal by dissolution (*8,9*).

Table 1. Defense mechanisms of the respiratory tract.

Nonspecific	Specific
Clearance	Antibody mediated (B-cell)
Via mucociliary transport (conducting airways)	
Via macrophages (with possible detoxification) (respiratory airways)	Cell-mediated (T-cell)
Local detoxification in airway fluids	
Buffers	
Antimicrobial agents	
Reflex responses	
Sneeze	
Cough	
Airway constriction	
Altered breathing pattern	

Effects of Acidic Sulfur Oxides

The major acidic sulfur oxide species found in ambient air are ammonium sulfate [$(NH_4)_2SO_4$], ammonium bisulfate [(NH_4HSO_4)], and sulfuric acid (H_2SO_4) (*11,12*). Both H_2SO_4 and NH_4HSO_4 are strong acids, with the former the stronger of the two; $(NH_4)_2SO_4$ is weakly acidic.

Mucociliary Clearance

The assessment of effects upon mucociliary clearance due to inhaled acid sulfates often involves examination solely of mucous transport rates in the trachea, since this is a readily accessible airway and tracheal mucociliary clearance measurements are more straightforward to perform than those aimed at assessing clearance from the entire tracheobronchial tree. Table 2 describes the available studies.

The most likely reason for the lack of effect in most studies, some of which involved high concentrations, is that the particular size of the H_2SO_4 aerosol used precluded significant tracheal deposition. This is supported by noting that Wolff et al. (*14*) found tracheal transport rates in dogs to be depressed only when using a 0.9 μm H_2SO_4 mist, while no effect was seen with a 0.3 μm aerosol at an equivalent mass concentration. Although the persistence of response after a single H_2SO_4 exposure seen by Wolff et al (*14,18*) in both dogs and rats is important, the use of tracheal clearance rates as the sole endpoint to assess the potential exposure-response relationship for acid sulfates, in terms of altering mucociliary clearance, may be misleading, inasmuch as studies in this laboratory (*15–17,20*) have demonstrated changes in bronchial clearance which were not associated with any change in tracheal transport.

The results of studies aimed at assessing the effects of acid sulfates upon bronchial clearance following acute exposures are outlined in Table 3; all of the effects are transient, unless noted otherwise. The lowest concentration of an acidic sulfate shown, to date, to produce any change at all after a single, brief (1 hr) exposure is ~ 0.1 mg/m^3 H_2SO_4, and this occurred in healthy human volunteers breathing via nasal mask (*16*). In addition, studies with H_2SO_4 indicate that the direction of clearance change, i.e., slowing or speeding, is exposure concentration dependent. Figures 1*A* and 2 show the concentration–response profiles for H_2SO_4 determined in humans (*16*) and rabbits (*21*), respectively, in this laboratory. The ability of an inhaled irritant to stimulate mucociliary clearance at low exposure concentrations while slowing it at higher levels was previously demonstrated in this laboratory on both animals and human volunteers using another irritant, whole fresh cigarette

Table 2. Effects of inhaled sulfates on tracheal clearance.

Aerosol	Species	Concentration, mg/m³	Particle size, μm	Exposure duration, hr	Effect[a]	Reference
H_2SO_4	Sheep	14	0.1	0.3	nc	(13)
		4	0.1	4	nc	(13)
	Dog	1	0.3	1	nc	(14)
		5	0.3	1	nc	(14)
	Donkey	0.2–1.4	0.4	1	nc	(15)
	Human	0.1–1.0	0.5	1	nc	(16,17)
	Dog	1	0.9	1	↓ (at 30 min, 1 day, 1 wk)	(14)
		0.5	0.9	1	↓ (at 1 wk)	(14)
	Rat	1	0.6	6	↑ (at 3 wk)	(18)
		10	0.7	6	↑ (at 1 day)	(18)
		100	0.8	6	↑ (at 1 and 3 wk)	(18)
	Rat	10	0.6	0.5	↑ (at 1 wk)	(18)
		50	0.4	0.5	↑ (at 1 wk)	(18)
		100	0.5	0.5	↑ (at 1 day, and 1 wk)	(18)
NH_4HSO_4	Sheep	1	0.1	4	nc	(19)
$(NH_4)_2SO_4$	Donkey	0.3–3.0	0.4	1	nc	(15)
	Sheep	1.1	0.1	4	nc	(19)

[a] Explanation of symbols: nc = no significant change from control; ↑ = significant acceleration of clearance; ↓ = significant slowing of clearance.

Table 3. Effects of inhaled sulfates on bronchial clearance of acute exposures.

Aerosol	Species	Concentration, mg/m³	Particle size, μm	Duration, hr	Effect[a]	Reference
H_2SO_4	Human	1	0.5	2.5	↑	(25)
	Human	0.1–1.0	0.5	1	↑, ↓ (depending on concentration)	(16,17)
	Rabbit	0.1–2.2	0.3	1	↑, ↓ (depending on concentration)	(21,26)
	Donkey	0.2–1.4	0.4	1	↓ (persistent effect in 2 of 4 animals	(15)
	Mouse	1.5	0.6	4	nc	(27)
		15	3.2	4	↓	(27)
	Rat	3.6	1.0	4	nc	(28)
NH_4HSO_4	Rabbit	0.6–1.7	0.4	1	↓ (only at highest conc.)	(29)
$(NH_4)_2SO_4$	Rabbit	2	0.4	1	nc	(29)
	Donkey	0.3–3.0	0.4	1	nc	(15)
	Rat	3.6	0.4	4	nc	(28)

[a] Explanation of symbols: nc = no significant change from control; ↑ = significant acceleration of clearance; ↓ = significant slowing of clearance.

smoke (22–24).

The actual H_2SO_4 exposure level needed to produce an observed acceleration may be dependent upon the region within the bronchial tree from which clearance is being measured, in relation to the region which is most affected by the inhaled acid mist. Figure 1*B* shows results of a study from this laboratory with human volunteers using a smaller tracer aerosol (4.2 μm) than that used in the study depicted in Figure 1*A* (7.5 μm); with the former, clearance was slower (rather than accelerated) at the 0.1 mg/m³ concentration level.

Deposition model calculations (17) indicate that the submicrometer H_2SO_4 aerosol used in both of these studies should be concentrated in distal conducting airways (generations 10–16). The 2.4 μm tracer should have substantial deposition fractions in both large (generations 0-9) and small airways, while the 7.5 μm particles should be concentrated primarily within the large airways. Based upon this model of tracer deposition and, therefore, the region from which observed clearance was occurring, it appears that the lowest H_2SO_4 exposure concentration, i.e., 0.1 mg/m³, accelerated clearance from the large proximal airways, where little deposited, while slowing clearance from the distal ciliated airways, where there was greater acid mist deposition. At the highest exposure concentrations, i.e., 1 mg/m³, both proximal and distal ciliated airway clearance was depressed.

The above scheme is supported by the results of single H_2SO_4 exposures in rabbits (21), shown in Figure 2. The tracer aerosols used in this study likely had a similar regional deposition pattern to the 7.5 μm aerosol used in the human tests. Thus, it seems that the low H_2SO_4 exposure concentrations act as small, stimulatory doses to mucociliary transport in the larger bronchial airways and, at these concentrations, the overall observed effects as measured in rabbits and humans (with the larger tracer) were dominated by those occurring in

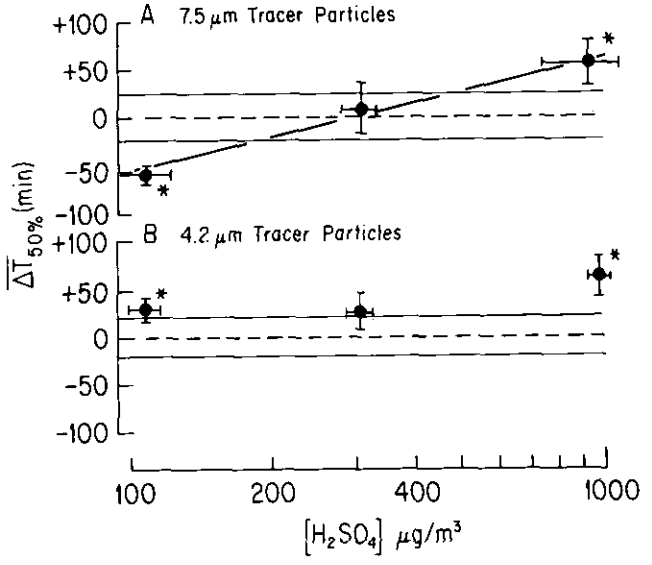

FIGURE 1. (*A*) *Effects of individual 1-hr exposures to* H_2SO_4 *upon* bronchial clearance of tracer particles (7.5 μm) from the lungs of healthy human volunteers, derived from data of Leikauf et al. (*16*). Clearance is quantitated in terms of half-time (T_{50}), i.e., the time required for completion of half of the bronchial clearance; an increase represents a slowing of clearance, while a decrease represents speeding. Each point represents the average change in bronchial clearance half-time ($\Delta\overline{T_{50}}$) of six subjects at each exposure concentration. Vertical error bars are ± 1 SE of $\Delta\overline{T_{50}} = 0$; horizontal error bars are ± 1 SE of mean $[H_2SO_4]$. 95% C.L. = 95% confidence limits of $\Delta\overline{T_{50}} = 0$. The linear regression line calculated from individual data for each subject is shown. The asterisk (*) denotes significant change ($p < 0.05$) at an individual concentration. From Schlesinger et al. (*21*). (*B*) Effects of individual 1-hr exposures to H_2SO_4 upon bronchial clearance of small tracer particles (4.2 μm) from the lungs of healthy humans, derived from data of Leikauf et al. (*17*). Each point represents the average change in bronchial clearance half time of 8 subjects at each exposure concentration. Error bars, 95% C.L., and asterisk as in Figure 1*A*.

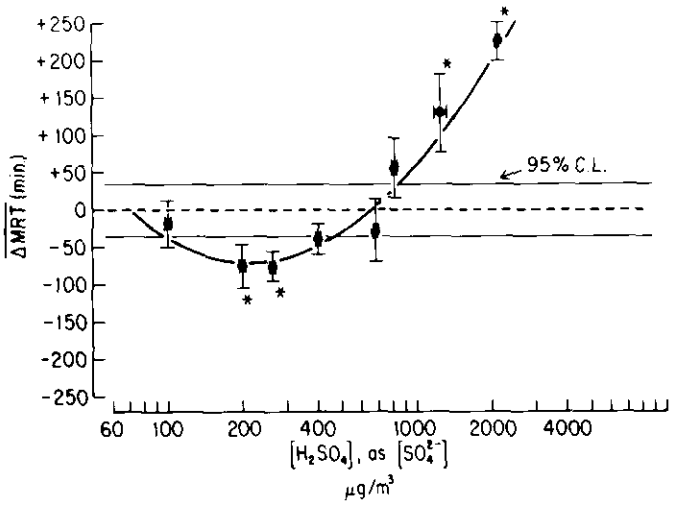

FIGURE 2. Effects of individual 1-hr exposures to H_2SO_4 upon bronchial clearance of tracer particles from the lungs of rabbits. Clearance is quantitated in terms of changes in mean residence time (MRT); an increase represents a slowing of clearance, while a decrease represents speeding. Each point represents the average change ($\Delta\overline{MRT}$) of a group of eight rabbits versus the mean H_2SO_4 concentration of all tests performed at each nominal concentration level. Vertical error bars are ± 1 SE of $\Delta\overline{MRT} = 0$; horizontal error bars are ± 1 SE of mean $[H_2SO_4]$. 95% C.L. = 95% confidence limits for $\Delta\overline{MRT} = 0$ based upon preexposure sham controls. The solid curved line is the polynomial regression calculated from the individual data for each animal. The asterisk (*) denotes significant change ($p < 0.05$) at an individual concentration. From Schlesinger et al. (*21*).

these airways, even though the H_2SO_4 dose delivered to the distal ciliated airways was sufficient to depress mucociliary transport there (Fig. 1*B*). On the other hand, at high concentrations, when the dose delivered to the entire bronchial tree was increased, there was likely slowing throughout the system.

The above scheme may be carried one step further. Examination of Figure 2 shows that from the apparent threshold level to ca. 0.25 mg/m³, there is an increase in the degree of acceleration. Thus, it is likely that, in fact, at low levels just beyond the threshold, overall bronchial clearance is stimulated by exposure to H_2SO_4, but a maximum acceleration is reached, and exposures at increasing concentrations result in a reduction in the degree of overall observed acceleration, as the dose delivered to distal bronchial regions is presumably sufficient to initiate a slowing of mucus transport. As this trend progresses, the regression curve begins to pass through the zero band at approximately 0.45 mg/m³ (Fig. 2), until the degree of retardation is sufficient to produce a net observable slowing of clearance. Within this crossover band, the H_2SO_4 exposures produce no "apparent" change in clearance. This observation should not be interpreted as indicating "no effect" exposure levels. Rather, at these concentrations, the differential responses to H_2SO_4 may have been equal (but opposite) in the upper and lower tracheobronchial tree, resulting in no apparent net change in tracer particle clearance from the lungs as a whole.

From Table 3, it is evident that in most studies where effects due to H_2SO_4 were found, levels > 1 mg/m³ resulted in bronchial clearance depression. However, in a study with exercising nonsmoking adults, Newhouse et al. (*25*) observed a speeding of bronchial clearance following exposures to H_2SO_4 at 1 mg/m³. This apparent discrepancy may be due to differences in the method of exposure and level of activity. In the Newhouse et al. study, the subjects had their nasal passages blocked, and were mouth breathing in an exposure chamber. There is greater neutralization of inhaled H_2SO_4 by endogeneous ammonia in oral than in nasal exposures, and perhaps some neutralization by exhaled ammonia in the chamber prior to H_2SO_4 inhalation. This methodology, thus, may have resulted in partial neutralization of the H_2SO_4 to the less irritating NH_4HSO_4 or $(NH_4)_2SO_4$ (see below). The remaining H_2SO_4 levels may have been low enough to result in accelerated clearance, as discussed above.

From the above discussion, the speeding of tracheal transport (Table 2) found by Wolff et al. (*18*) in the rat

at H_2SO_4 levels up to 100 mg/m^3 seem anomalous, since in other species, levels at $\geq$ 1 mg/m^3 produced mucociliary depression. The reasons for this observation are not known. It is known that the rat is less susceptible to the lethal effects of H_2SO_4 and that they do not have strong bronchoconstrictive reflex responses following H_2SO_4 exposures (*18*). These characteristics, together with the lack of effect of H_2SO_4 on bronchial clearance found by Phalen et al. (*28*) at 3.6 mg/m^3 for 4 hr, and in view of the similarity in bronchial clearance response in humans, donkeys, and rabbits to acute, 1-hr exposures, suggest that the rat mucociliary system may also differ in sensitivity from the other species studied.

In evaluating the potential public health significance of a pollutant, it is generally accepted that all individuals in any given population may not be equally susceptible to its actions. For example, recent studies have shown that asthmatics may be more sensitive to the bronchoconstrictive effects induced by inhaled irritants such as SO_2 (*30*), O_3 (*31*), and H_2SO_4 (*32*). In order to examine whether such individuals were more susceptible to H_2SO_4-induced alterations in mucociliary clearance, Spektor et al. (*33*) studied the effects of 1 hr nasal inhalations of submicrometer H_2SO_4 aerosols in a group of 10 asthmatic subjects. Of this group, six subjects were not on routine medication, and were found to have similar responses to those of healthy nonsmokers, i.e., 1 mg/m^3 H_2SO_4 produced a transient slowing of clearance. The four asthmatics who were on daily medication exhibited mucociliary clearance patterns that were too variable to permit detection of any H_2SO_4 effect. Although the magnitude of the clearance response in the nonmedicated asthmatics was not greater than for healthy nonsmoking volunteers, the asthmatics did have lower baseline rates for bronchial clearance which, combined with the observations of an effect on respiratory mechanics indices not seen in healthy people (*16,33*), suggests that asthmatic subjects may indeed be more sensitive to H_2SO_4 aerosols.

Sulfuric acid is the most potent of the acid sulfates in terms of altering mucociliary clearance. Schlesinger et al (*15*) showed altered bronchial clearance in donkeys exposed to H_2SO_4 for 1 hr at levels above ~ 0.2 mg/m^3, while exposures to $(NH_4)_2SO_4$ at up to 3 mg/m^3 produced no response.

The relative irritant potency of the major ambient sulfates was specifically examined in a study by Schlesinger (*29*) using rabbits. Figure 3 shows the results of 1 hr (oral) exposure to submicrometer aerosols of NH_4HSO_4, $(NH_4)_2SO_4$, and Na_2SO_4 (nonammonium control). NH_4HSO_4 at concentrations of ca. 0.6–1.7 mg/m^3 produced a significant depression of clearance rate only at the highest exposure level. No significant effects were observed with the other sulfates at levels up to ca. 2 mg/m^3. When these results were compared to those from a study using H_2SO_4 (*21*) (Fig. 2), the ranking of irritant potency was $H_2SO_4 > NH_4HSO_4 > (NH_4)_2SO_4$, Na_2SO_4; this strongly suggests a relation between the hydrogen ion (H^+) concentration and the extent of bronchial mucociliary clearance alteration. However, the mechanism(s) by which deposited acid aerosol alter(s) clearance after acute exposures is not certain.

The effective functioning of mucociliary transport depends upon optimal beating of the cilia and the presence of mucus having appropriate physicochemical properties. Both ciliary beating as well as mucus viscosity may be affected by the deposition of acid. Normally, tracheobronchial mucus has a pH of ca. 6.5 to 8.2 (*34–37*). *In vitro* studies have shown that, at alkaline pH, mucus is more fluid than at acid pH; the inflection point occurs at a pH between 7.5 to 7.6 (*38*). An increase in viscosity which may occur due to deposited acid could "stiffen" the mucus blanket, perhaps promoting the clearance mechanism and, thus, increasing its efficiency (*37*). This may occur at low H_2SO_4 exposure concentrations, where ciliary activity would not be affected, and is consistent with clearance acceleration observed at these concentrations.

At higher exposure concentrations, H_2SO_4 may affect ciliary beating. Schiff et al (*39*) and Grose et al (*40*) found that 2 to 3 hr *in vivo* exposures of hamsters to ca. 0.9 to 1 mg/m^3 H_2SO_4 resulted in a depression of ciliary beating frequency in tracheal explants prepared after exposure. *In vitro* studies clearly indicate that compelte ciliostasis will occur if the pH is low enough (*37*); however, ciliostasis in only some regions, presumably with a change in clearance function, may occur at pH values above this critical level. Thus, an H^+-induced depression of ciliary beating, with or without a change in mucus viscosity, could account for the observed retardation of clearance observed at high exposure concentrations of H_2SO_4 (or NH_4HSO_4).

There is, however, some evidence that the effects of H_2SO_4 may not be entirely due to H^+. Schiff et al. (*39*) exposed hamster tracheal rings *in vitro* to H_2SO_4 in media for 3 hr and examined ciliary beat frequency and cytology; the pH of the media was ca. 5. There was no effect upon beat frequency when the tissue was examined within 1 hr after exposure, although morphological damage was evident at this time; at 24 hr after exposure, beat frequency was depressed. They then exposed tracheal explants for 3 hr to the same media as above, but with the pH adjusted to 5 using hydrochloric acid (HCl). Exposure under this condition resulted in a 50 to 75% reduction in beating frequency, but no change in cell morphology. When these latter explants were transferred to fresh culture media, the cilia resumed beating at their normal frequency. According to the investigators, these results indicated that media at pH 5 itself produced no lasting morphological effects, and that acidity alone was not responsible for the observed morphological and functional effects produced by H_2SO_4.

The nature of any other factors is unknown; however, the lack of any significant effect upon mucociliary clearance due to $(NH_4)_2SO_4$ or Na_2SO_4 observed in other studies (Table 3), at concentrations which would provide an equivalent amount of sulfate ion (SO_4^{2-}) as the effective concentrations of the strong acid sulfates, demonstrates both that the sulfate component is not re-

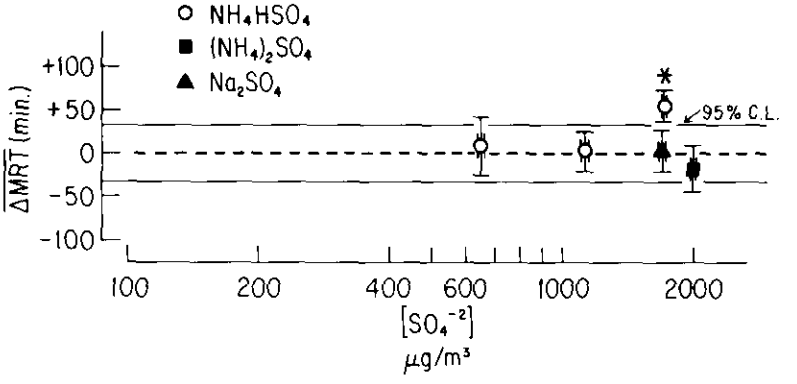

FIGURE 3. Effects of individual 1-hr exposures to various sulfate aerosols upon bronchial clearance of tracer particles from the lungs of rabbits. Each point represents the mean change in MRT of a cohort of five rabbits plotted versus the mean concentration of a pair of tests performed at each nominal concentration level. Error bars, 95% CL and asterisks as in Figure 2. From Schlesinger (*29*).

sponsible for the observed effects of NH_4HSO_4 or H_2SO_4, and that the ammonium ion does not mask or mitigate any potential response which may have been due to SO_4^{2-}.

Another (or an additional) mechanism by which an irritant may affect mucociliary clearance is via alteration in the rate and/or amount of mucus secreted. An increase in the quantity of mucus is consistent with the initial increase in bronchial clearance observed *in vivo* following exposures to low levels of H_2SO_4. In a study which directly examined secretory rate, Last and Cross (*41*) showed that *in vivo* exposure of rats for 23.5 hr/day for 3 day to submicrometer H_2SO_4 at ca. 1 mg/m^3 did not affect the secretion of mucus glycoprotein from tracheal explants prepared after exposure; however, the effect on mucus secretion from smaller conducting airways was not examined. An indirect examination of bronchial airway secretion was performed by Henderson et al. (*42*), using analysis of lung lavage fluid to detect responses in the lungs of rats exposed to 1, 10, or 100 mg/m^3 H_2SO_4 for 6 hr. They showed a dose-related increase in sialic acid content (a component of mucous glycoproteins), which they suggested was due to increased bronchial mucus secretion.

The pathological significance of transient alterations in bronchial clearance rates in healthy individuals is not certain, but such changes are an indication of a lung defense response. On the other hand, persistent impairment of clearance may render the host more susceptible to the inception or progression of acute or chronic respiratory disease and, as such, may be a plausible link between inhaled pollutants and respiratory pathology.

Short-term exposures to acids may lead to persistent clearance changes. Schlesinger et al. (*15*) demonstrated that weekly, 1 hr exposures of donkeys to submicrometer H_2SO_4 at 0.2 to 1 mg/m^3 produced transient slowing of bronchial clearance in three of four animals. However, two of the four (including one which did not respond after any individual test) developed persistently slowed clearance after about six of the exposures, and which persisted for about 2 months after all exposures had ceased.

The development of persistent alterations after a relatively small number of 1 hr weekly exposures emphasizes that in order to fully evaluate the impact ambient exposures to H_2SO_4 might have upon the inception and progression of respiratory disease, it is essential to consider the effects of intermittent exposures, especially at low levels. Thus, as a follow-up to the above study, Schlesinger et al. (*20*) exposed the two donkeys which had shown only transient responses, as well as two previously unexposed animals, to 0.1 mg/m^3 H_2SO_4 for 1 hr/ day, 5 days/week for 6 months (Fig. 4). Within the first few weeks of exposure, all four animals developed erratic clearance rates, i.e., rates that on specific test days were either significantly slower than, or significantly faster than, those in the preexposure period. The two previously unexposed animals developed persistently slowed bronchial clearance during the second 3 months of exposure, and during 4 months of follow-up clearance measurements, while the two previously exposed animals adapted to the exposures in the sense that their clearance times fell consistently within the normal range during the last few months of exposure. However, after the end of the exposure series, their clearance rates were significantly faster than in their preexposure control tests, and remained so for the 4-month follow-up period (*24*).

In a recent study, Schlesinger et al (*43*) examined the

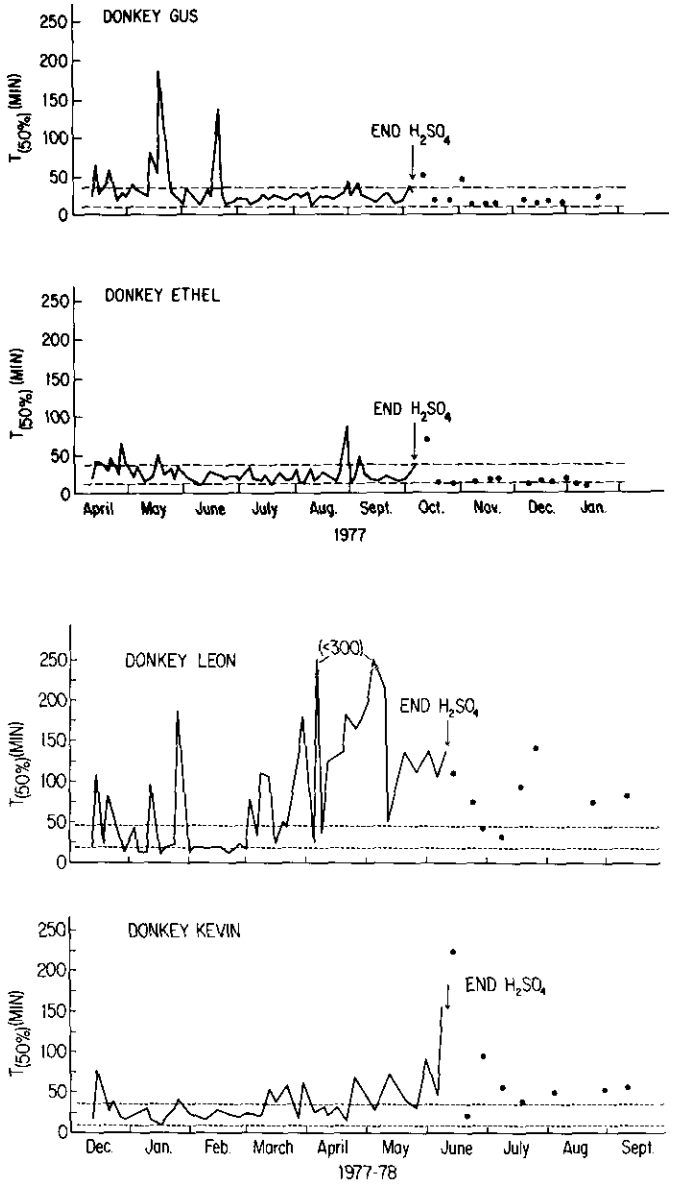

FIGURE 4. Effects of daily, 1-hr exposures to H_2SO_4 upon bronchial clearance from the lungs of four donkeys. The exposures were given over a period of 6 months, with a 4-month follow-up. The horizontal lines define the 95% tolerance interval for T_{50} (clearance half time) based upon a series of pre-exposure control tests which preceded the H_2SO_4 exposure series. From Schlesinger et al. (*20*).

response to repetitive H_2SO_4 exposures in rabbits. Groups of five rabbits were exposed to submicrometer H_2SO_4 for 1 hr/day, 5 days/week for 4 weeks, during which time bronchial mucociliary clearance was monitored. One group was exposed orally to 0.25 mg/m^3, another to the same concentration via the nose, and a third to 0.5 mg/m^3, also via nasal breathing. Clearance was accelerated on specific individual days during the course of the acid exposures, especially at 0.5 mg/m^3. In all exposure series, clearance was significantly faster, compared to preexposure controls, during a 2-week follow-up period after acid exposures had ceased. (This is a similar response to that observed in the donkey, as described above.) At the end of this period, the rabbits were sacrificed, and histological sections were obtained from the tracheobronchial tree. Significantly increased epithelial thickness of small conducting airways, compared to sham exposure controls, was noted in rabbits exposed orally at 0.25 mg/m^3, or nasally at 0.5 mg/m^3; additionally, the lumen of the smallest airways of the former group was narrower than in the controls. The number of airways containing epithelial secretory cells was also significantly greater in these acid exposure groups compared to sham controls. The only change in the rabbits exposed nasally at 0.25 mg/m^3 was a significant increase in the number of small airways containing epithelial secretory cells. Differences in site and degree of histological response and degree of physiological change between the two groups exposed to identical acid concentrations appear to have been due to differences in exposure mode, and resultant effects on breathing pattern, aerosol size distribution, and H_2SO_4 concentration penetrating beyond the upper respiratory tract.

The appearance of persistently increased secretory cell number in peripheral airways due to H_2SO_4 was a significant finding, since excess mucus production in small airways, which is a likely consequence of this increase, may be an early feature in the pathogenesis of chronic bronchitis (*44*). Furthermore, this demonstrates an underlying histological change consistent with the observed physiological effects of the H_2SO_4. For example, the exposure regime which produced the greatest degree of alteration in clearance rate was also associated with the most extensive change in secretory cell number in the small intrapulmonary airways.

An ongoing study in our laboratory is aimed at examining the temporal course of histological changes as they may relate to altered bronchial clearance. Groups of rabbits are being exposed (nasally) to H_2SO_4 at 0.25 mg/m^3 for 1 hr/day, 5 days/week for up to 52 weeks, during which time mucociliary clearance is monitored at 1 to 3 week intervals. Preliminary results from one group exposed, to date, for 30 weeks is shown in Figure 5. In this group, erratic clearance rates occur in all of the animals. In one (A3), there was a general tendency towards a slowing of clearance, while two others (A2 and A4) tended towards acceleration. The relationship between the direction of change and epithelial secretory histology is currently being examined.

Alveolar Defense

Antimicrobial Activity. The alveolar macrophage represents the initial defense against pathogenic organisms depositing in the alveolar region; under normal circumstances, there is rapid killing of microbes that do deposit. Since the development of an infectious disease requires both the presence of the appropriate pathogen as well as host vulnerability, the ability of pollutants to modify resistance to infection could, perhaps, result from an alteration in normal macrophage mi-

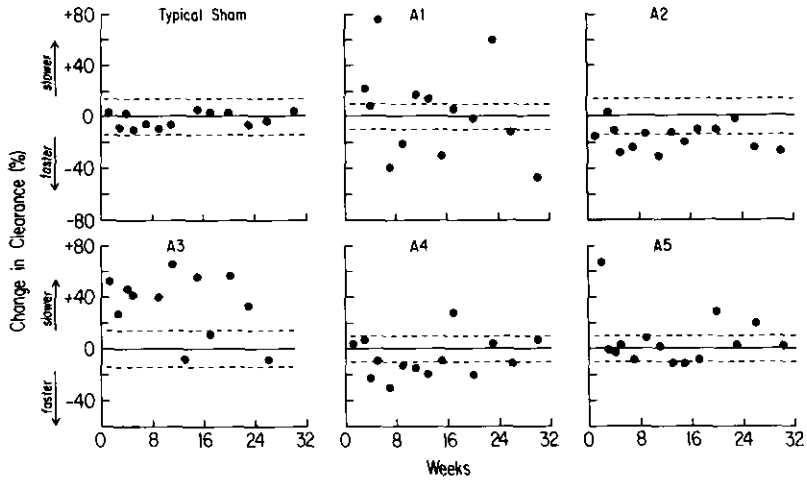

FIGURE 5. Effects of daily, 1-hr exposures to H_2SO_4 upon bronchial clearance from the lungs of five rabbits. Clearance is quantitated in terms of the amount of tracer particles cleared by 4 hr after exposure (C_4). The horizontal lines define the 95% tolerance interval for C_4 based upon a series of control tests prior to initiation of H_2SO_4 exposures.

crobicidal function. To test this possibility, the rodent infectivity model has been used (*45*). In this test system, animals (usually mice) are challenged with a bacterial aerosol after exposure to the pollutant of interest; mortality rate and survival time are then examined within a particular time period, usually 14 to 15 days post-exposure. Any decrease in the latter or increase in the former indicates impaired defense against respiratory infection, and suggests that the site of pollutant injury is likely the alveolar macrophage. Studies which have used the infectivity model to assess effects of acid sulfates are outlined in Table 4. It is evident that these aerosols are apparently not very effective in enhancing susceptibility to bacterial mediated respiratory disease in test animals. The only acid aerosol study which demonstrated any response was that of Coffin (*48*), and increased mortality occurred only at an extremely high exposure level.

In a related type of study, Fairchild et al (*27*) examined the clearance rate of viable bacteria from the lungs of mice, using a colony count assay involving culturing ground lung tissue. They found that exposures to 1.5 mg/m^3 of submicrometer H_2SO_4 for 1.5 hr for 4 days prior to, or for 4 hr after, exposure to a bacterial aerosol produced no alteration, compared to control, in the removal of this aerosol from the lungs. Since the bulk of microbial clearance was likely due to macrophage activity, these results are another, albeit indirect, indication that H_2SO_4 exposures at modest levels produce no measurable effects on bacterial infectivity.

Macrophages are also involved in antiviral defense. Cells harvested from mice exposed to submicrometer H_2SO_4 at high levels, i.e., 125 to 154 mg/m^3, for 10 to 14 days showed decreased interferon titers in their culture media (*53*).

Alveolar Clearance. Since acidic sulfates can alter the rate of mucociliary clearance from conducting airways, their potential for altering macrophage-mediated clearance from the respiratory region should be considered, so as to provide a more complete picture of

Table 4. Effects of inhaled sulfates on bacterial infectivity in mice.

Aerosol	Concentration, mg/m^3	Exposure duration	Effect	Reference
H_2SO_4	0.9	2 hr	nc[a]	(*47*)
	0.543	2 hr	nc	(*49*)
	80	3 hr	nc	(*48*)
	150	3 hr	nc	(*48*)
	300	3 hr	Increased mortality	(*48*)
	0.365	2 hr/day, 5 days	nc	(*49*)
NH_4HSO_4	6.7	3 hr	nc	(*50*)
$(NH_4)_2SO_4$	1.1–5.3	3 hr	nc[b]	(*51*)
	1	3 hr/day, 20 days	nc	(*52*)

[a] nc = no significant change from control.
[b] An increase in mortality was observed at the 2.1–3 mg/m^3 exposure level, but there was no relation between mortality and concentration in the study as a whole.

the role these pollutants may play in affecting lung defense systems. The only published study to date which examined this is that of Phalen et al. (*28*). Rats exposed to 3.6 mg/m^3 H_2SO_4 (1 μm) for 4 hr at low relative humidity exhibited a showing of alveolar region clearance as measured 2 to 17 days post-exposure.

In a study being completed in our laboratory (*55*), rabbits were exposed (orally) to submicrometer H_2SO_4 at 1 mg/m^3 for 1 hr to assess the effects upon alveolar clearance during the period 2 to 14 days post-exposure. Clearance of the tagged polystyrene latex tracer aerosol in the acid exposed group was found to be accelerated compared to that in the sham control group.

In order to adequately perform their role in clearance, macrophages must be competent in a number of functions, e.g., phagocytosis, mobility, and attachment to a surface (*54*). Alterations in any one, or combination, of these individual factors could perhaps result in altered clearance function. Thus, as part of the above study, and to examine the mechanism(s) underlying clearance alterations, rabbits were sacrificed at six selected times after H_2SO_4 exposure, bronchopulmonary lavage performed, and the functional characteristics of recovered free cells were examined *in vitro* (*55*). The acid exposure produced no change in the viability or numbers of macrophages recoverable by lavage. This is not surprising, since Coffin (*48*) found no change in the number of recoverable macrophages from mice exposed to 300 mg/m^3 H_2SO_4 for 3 hr. However, a marked increase in the number of neutrophils was observed in the rabbits sacrificed at 4 hr after either acid or sham exposure, compared to nonexposed colony controls. Although the number of neutrophils was back to normal by 12 hr in the sham group, an elevated level continued to be observed in the acid exposure group through the 1 day sacrifice time (Fig. 6).

The ability of macrophages to phagocytose latex particles *in vitro* was not affected by the H_2SO_4 exposure. However, by using a density-gradient technique to separate macrophages from free latex particles, it was found that the actual uptake of the tracer microspheres by macrophages *in vivo* was enhanced during the first 5 hr after acid exposure. In addition, *in vitro* phagocytosis by neutrophils was also increased through 48 hr post-exposure in the acid exposed group. Finally, reduced *in vitro* adherence (to a glass substrate) was observed for macrophages obtained from the H_2SO_4-exposed rabbits.

The only other study to examine the response of macrophages to acid assayed cells obtained by lavage from rabbits 4 hr after an intratracheal injection of hydrochloric acid (*56*). No change in the number of macrophages recovered was found; however, the number of neutrophils recovered was significantly increased, and microscopy indicated a mild inflammatory response. In addition, adherence of macrophages to a glass surface was decreased compared to controls, which the investigators attributed to the influx of neutrophils in the acid treated animals. On the other hand, *in vitro* macrophage phagocytosis and chemotaxis were not affected by acid exposure.

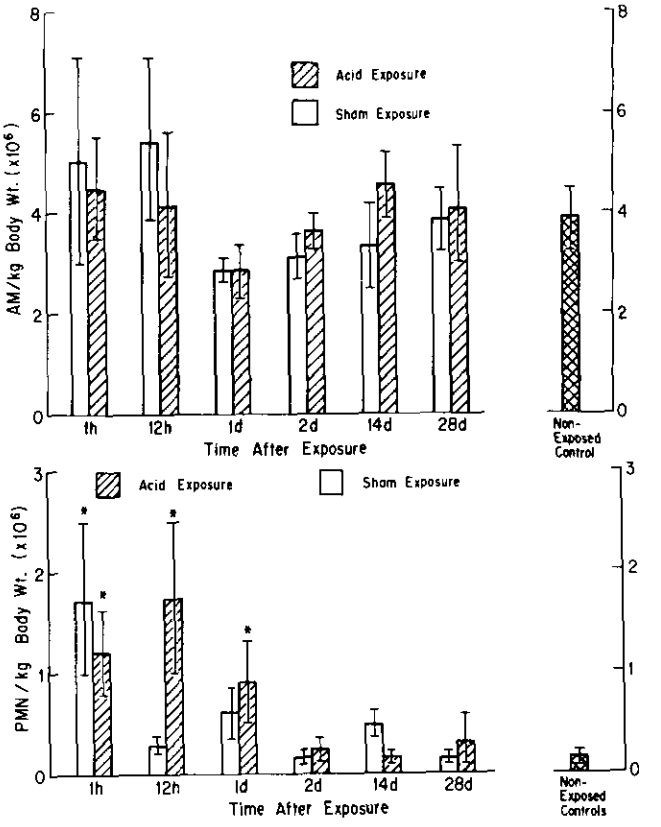

FIGURE 6. Numbers of macrophages (AM) and neutrophils (PMN) obtained by lavage from rabbits at selected time points after a single exposure to H_2SO_4 (1 mg/m^3) (Mean ± SE). From Naumann and Schlesinger (*55*).

Recruitment of neutrophils is evidence of an inflammatory response; thus, the exposure to 1 mg/m^3 H_2SO_4 performed in our laboratory apparently resulted in such a response, and one which was prolonged over that seen in the sham animals. In addition, mild inflammation appears to be associated with accelerated alveolar clearance, a cause-effect relationship described by other investigators (*57,58*).

The results from these alveolar macrophage studies are consistent with the lack of effects seen using the rodent infectivity model. Sulfuric acid does not reduce macrophage numbers nor phagocytic efficiency, but apparently enhances macrophage, as well as neutrophil, phagocytosis, and alveolar clearance. This inflammatory-induced clearance rate change should not, however, be deemed beneficial, since the cells involved are pivotal elements in the delicate balance between defense and disease, as will be discussed in a later section.

The relative potency of acid sulfates in terms of altering alveolar clearance has not been examined in any detail. However, Aranyi et al. (*52*) found no change in total or differential counts of free cells lavaged from mice exposed to $(NH_4)_2SO_4$ at 1 mg/m^3 for 3 hr/day for 20 days; this suggests a lack of inflammatory response to this sulfate aerosol.

A study in our laboratory (*59*) is examining early alveolar clearance in rabbits at various time intervals during the course of a series of intermittent (1 hr/day, 5 days/week) exposures to submicrometer H_2SO_4 at 0.25 mg/m^3. Three clearance tests, using tagged latex tracers, have been performed, one beginning on day 1 of H_2SO_4 exposure, with clearance measurements continuing until day 14, one on day 57, with measurements continuing until the 71st day, and one on day 240, with measurements until day 253. All tests show accelerated clearance, compared to sham controls, and the degree of acceleration was similar in all cases. Although lung cell assays were not performed in this study, it is interesting to speculate, based upon the results discussed previously, that the H_2SO_4 exposures, by producing and maintaining a mild inflammatory response, result in the observed persistent (to date) acceleration of alveolar clearance.

In the study involving a single exposure to 1 mg/m^3 H_2SO_4, it was noted that phagocytosis by neutrophils *in vitro* was enhanced in the acid treated animals. If this is also a reflection of *in vivo* effects, this functional change likely did not affect observed clearance rates for the tagged latex tracer, since the bulk of the neutrophil influx was observed to occur at ca. 4 to 5 hr post-exposure, while the latex tracer particles were found to be essentially completely ingested by macrophages prior to this time. However, if moderate neutrophil recruitment occurred in the intermittent exposure series, these cells may be playing a role in observed clearance since, in this case, they would be present at the time of the second tagged latex exposure test on day 57. Neutrophils generally contain less phagocytized particles than do macrophages, especially under high particle loading conditions (*58*). However, under other conditions, especially with low particle loading (such as is the situation in the described tests with latex), uptake by neutrophils may account for as much as half of all particles phagocytized by the free cells of the lungs (*58,60*).

Effects of Acidic Nitrogen Oxides

There are essentially no data concerning effects of inhaled acidic nitrogen oxides on lung clearance systems. Stutts et al. (*61*) found that ammonium nitrate (NH_4NO_3) could alter sodium and chloride transport across canine tracheal epithelium, which could affect the nature of the liquid lining of the airway lumen. However, the response was ascribed to NH_4^+ rather than to NO_3^-, since sodium nitrate ($NaNO_3$) had no effect.

Vassallo et al. (*62*) examined the effects of nitrite (NO_2^-) at concentrations of 5 to 20 mM upon the rate of *in vitro* bacterial ingestion and killing by rabbit macrophages. They found that incubation with the highest concentration used, i.e., 20 mM, reduced intracellular killing of the bacteria, as well as inhibited their phagocytic uptake. However, these effects were not due to acidity, since the culture system was maintained at a pH > 7.1. In addition, the relation between these results and *in vivo* exposures is not clear, since the concentration needed to produce an effect was very high.

Considerations Concerning Pollutant Mixtures

In any assessment of the toxicology of air pollutants, it is always necessary to bear in mind that the usual manner of inhalation involves mixtures of materials. The finding of "no effect" upon some endpoint using a single chemical does not necessarily mean that the presence of this material in the ambient air poses no potential health problem. Thus, the effects of ambient acids may be influenced by various copollutants. Although this topic is beyond the scope of this review, some examples from the limited data base will suffice.

Last and Cross (*41*) exposed rats for 3 and 14 days (23.5 hr/day) to a mixture of 1.1 mg/m^3 (0.5 μm) H_2SO_4 and 0.5 ppm O_3 and found a significant increase in the secretion of mucus glycoproteins into the trachea, while exposures to each material alone at these concentrations had no effect. When the H_2SO_4 concentration was lowered to 0.011 mg/m^3, a 3-day exposure to this plus 0.5 ppm O_3 still produced an increase in secretion.

Although H_2SO_4 does not appear to influence respiratory infection in the rodent model system, mixtures of H_2SO_4 (1.4 mg/m^3) and carbon (1.5 mg/m^3) resulted in a reduced degree of bacterial inactivation in mice after 4 weeks (3 hr/day, 5 days/week) of exposure (*63*). After 20 weeks, this same mixture produced a decreased mean survival time following challenge with airborne influenza virus after the last exposure. On the other hand (*52*), exposures of mice (5 hr/day, 5 days/week for 103 days) to mixtures of O_3 (0.2 mg/m^3) plus SO_2 (13.2 mg/m^3) plus $(NH_4)_2SO_4$ (1.04 mg/m^3) produced enhanced bacteriocidal activity of macrophages, compared to O^3 alone.

Alterations in Clearance and the Pathogenesis of Lung Disease

The relationship between clearance and lung disease is somewhat uncertain. Dysfunction of mucous transport may be involved in the pathogenesis of both acute and chronic respiratory disorders, but the clinical relevance of mucociliary changes is only recently being elucidated, and their role in pulmonary disease development is only beginning to be experimentally established.

To assess the actual importance of mucociliary transport and the effect of its dysfunction upon respiratory disease, as well as to provide information on the role of mucociliary clearance in maintaining the integrity of the lung, one may study individuals with a congenital disease syndrome, i.e., primary ciliary dyskinesia (PCD). The lack of mucociliary function in PCD is directly responsible for the early development of recurrent respiratory tract infections and, eventually, chronic bronchitis and bronchiectasis (*64,65*). It is, however, not

certain whether partial or transient impairment of the mucociliary system will increase the risk of disease.

Neither a slowing nor speeding of mucociliary clearance is beneficial; both are, most likely, indications of a pathophysiological response of the bronchial tree to insult. Furthermore, slowing of clearance may result in the prolonged retention of deposited materials, which would normally be rapidly removed, increasing the risk of local damage by and/or systemic uptake of toxic agents.

The rate of mucociliary clearance may affect the development of infectious disease. Pathogenic organisms depositing in the conducting airways are confronted with the mucociliary barrier, and the extent of penetration of vectors through the mucus to the underlying cells may be important in disease development. This rate, relative to that of mucociliary transport out of the respiratory tract, could determine the effectiveness of inhaled pathogens in initiating disease (*66,67*). In conditions characterized by retarded clearance from conducting airways, e.g., chronic bronchitis, there is a predisposition to respiratory infection (*68*). Destruction of the functional integrity of the ciliated epithelium can result in impaired defense against bacteria (*69*), and impaired transport has been observed associated with viral respiratory infections (*70,71*).

Retardation of mucociliary clearance may be a factor in the pathogenesis of bronchial cancer. Inhaled aerosols tend to selectively deposit at airway branching sites in the bronchial tree (*72*), and histological studies (*73,74*) have indicated that neoplastic and preneoplastic lesions tend to predominate within these regions. Macklin (*75*) and Hilding (*76*) have suggested that bifurcations are areas of normally slower mucus movements; this would add to the residence time of agents which deposit within these areas, or are being carried through them on the mucociliary escalator. Thus, any selective distribution of lesions at bifurcations within the upper bronchial tree may result from selective deposition and/or slower clearance, resulting in prolonged retention of high local concentrations of deposited carcinogens. Although there is no direct evidence that ineffectual clearance is a contributory factor to the development of bronchogenic carcinoma, a causal relation has been suggested between adenocarcinoma and sites of local particle retention and inadequate clearance in the nasal passages of furniture workers (*77,78*).

There is accumulating evidence that dysfunction of bronchial clearance plays a role in the pathogenesis of chronic bronchitis. Mucus transport is impaired in bronchitic individuals (*79*). In addition, although there is much interindividual variability in the rate at which healthy humans clear deposited particles from the lungs, cigarette smokers and persons with chronic obstructive pulmonary disease exhibit a somewhat wider variation (*80,81*). In such groups, the within-subject variation is also greatly increased, suggesting that a loss of control of mucociliary transport could cause and/or result from disease.

Mucociliary dysfunction may be an early indication of impending disease. For example, retarded clearance has been demonstrated in bronchitics who showed no sign of airway obstruction (*82*), while young smokers having various degrees of impairment of tracheal mucus transport rates had no overt bronchitic symptoms and had normal pulmonary function (*83*). Schlesinger et al. (*43*) showed, in an experimental animal, that small changes in bronchial mucociliary clearance rates may be associated with secretory epithelial changes in small bronchi and bronchioles. Whether these physiological and histological changes merely predispose to chronic bronchitis or are the actual initiating events in a pathogenic sequence remains a question, since the observed response of the mucociliary system may have merely been adaptive. However, pathological changes appear when adaptive capacity is overloaded. Thus, in a pathogenic scheme for chronic bronchitis proposed by Albert et al. (*80*), chronic irritant inhalation initially results in an acceleration of clearance as excess mucus is produced, but continued increases in the level of secretion result in an overloading of the mucus transport system, retarding clearance and leading to disease.

The hypothesis for a role of H_2SO_4 in the development of chronic bronchitis is supported by a comparison of results from studies of submicrometer H_2SO_4 mist and cigarette-smoke exposures conducted in this laboratory (*24*); the latter is an agent known to be involved in the etiology of human chronic bronchitis. The effects of both cigarette smoke and H_2SO_4 on bronchial mucociliary clearance patterns were found to be essentially the same following either single or intermittent exposures.

The pathogenic implications for lung disease of alterations in clearance from the alveolar region are even cloudier than those for mucociliary clearance. Alveolar clearance rates appear to be reduced in people with chronic obstructive lung disease (*84*) and in cigarette smokers (*85,86*), suggesting some relation between altered defense and disease development. Alveolar clearance dysfunction has also been demonstrated in experimental animals having viral infections (*86*).

A major role in rapid clearance from the alveolar region is played by the alveolar macrophage. The adequate performance of macrophages is critical in determining the effectiveness of pulmonary region defense in minimizing the residence time of deposited toxicants. For example, phagocytosis may prevent toxicant entry into the interstitial tissue of the lung, a region from which clearance is very slow; accumulations of several types of dust have been directly linked to the development of lung disease. Dysfunction of macrophages is associated with an increased risk of viral and bacterial infections (*4*). Furthermore, the viability and functional activity of macrophages is impaired in people with asthma (*87*).

Although the major function of the macrophage is protective, these cells are likely involved in the pathogenesis of two classes of chronic disease, namely, interstitial fibrosis and emphysema (*5,46,88–90*). Disease development may be related not only to actual cell damage or dysfunction, but may be the result of the mac-

rophage pursuing its protective role after exposure to noxious materials. Contact with an inhaled agent may result in macrophage activation, and it is during the period when these cells are activated that they may play a role in the pathogenesis of these lung diseases (*90*). For example, evidence suggests that cigarette smokers' lungs contain greater numbers of macrophages and inflammatory cells, i.e., neutrophils, than do those of nonsmokers, and that these macrophages are in an activated state (*5*). Activation involves the release of numerous biologically active materials, plus recruitment of inflammatory cells. These latter are themselves a source of potent mediators of tissue degradation, e.g., proteases. Thus, inflammation, while an essential response in defense of the lungs, is also likely involved in chronic disease development.

Epilogue

The respiratory tract has an array of intricate and interlocking specific and nonspecific defense mechanisms to detoxify and physically remove inhaled material via cellular and acellular processes. Inhaled acids may impair these defenses, leading to increased systemic absorption of inhaled materials, or increasing the susceptibility to acute and chronic respiratory disease.

The bronchial mucociliary clearance system is sensitive to inhaled acids; fairly low levels of strong acids such as H_2SO_4, not greatly above current ambient levels, may produce alterations in mucociliary transport, perhaps an early sign of pulmonary dysfunction, in previously healthy individuals. Although transient excursions from the norm may be adaptive, helping to maintain organ homeostasis, these changes are more likely a patho-physiological response of the airways, and such changes may foreshadow more permanent alterations or progressive changes following continued exposures.

Exposures to acids, in particular H_2SO_4, may alter the rate of clearance from the alveolar region, by affecting certain functions of the alveolar macrophage. But since inhalation of 1 mg/m^3 for 1 hr accelerated alveolar clearance and retarded mucociliary clearance, it is still not clear how these two defense systems are coupled. Although the implications of altered alveolar clearance are not certain, the production of a persistent inflammatory response could predispose the lung to chronic disease.

Almost all of the data concerning inhaled acid effects upon lung defense involve acid sulfates. A major gap exists concerning the effects of inhaled acidic nitrogen oxides, e.g., HNO_2 and HNO_3, which may be formed in the atmosphere from NO_2. In addition, these acids may be formed from inhaled NO_2 which deposits upon the liquid surfaces of the lungs.

Since a competent system of defenses is essential to host well-being, an understanding of how they maintain operation under environmental assault is necessary information in the attempt to clarify the pathogenesis of environmentally induced pulmonary (and, perhaps, systemic) disease.

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