The Lung Mucosa: A Critical Environmental Battleground

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The entirety of the lung mucous membrane and epithelial surface are exposed to the environment; react to noxious environmental gases, vapors, and particles; and are under physiologic and humoral mediator control. In recent years much information has been gained regarding the mucous membrane of the tracheobronchial tree, its physiology, and its reaction to environmental hazards. The pharmacologic control of secretion, ciliary beat rate, and net mucus flow governs both the clearance of mucus and the clearance of particles. The physiologic factors that govern this clearance mechanism can be influenced by pharmacologic agents in patients with lung disease and presumably also in patients with purely environmental injury.

The effects of ozone on lung function, lung compliance, and airway resistance have been well documented in adults and children. Environmental ozone also alters mucous membrane function, increasing mucociliary secretion rate and peripheral lung clearance. The speed-up in clearance implies an increase in mucous gland secretion, which may act unfavorably when ciliary beat is damaged, glandular hypertrophy is present, or flow-limiting segments exist, as is usually the case in bronchial asthma and chronic obstructive pulmonary disease. Thus, whereas the consequences of ozone may be modest for a normal, healthy individual, they presumably increase hazards for the individual with lung disease or damage. For this reason, efforts should be made to control or limit damage by ozone or other environmental inhalants in such individuals. This goal may be facilitated by a wider knowledge of the pharmacologic control of the mucous membrane.

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In view of what transpires when noxious environmental gases, vapors, and particles invade the mucous membrane, it is not unreasonable to call the lung mucosa a battleground. The mucous membrane is the landscape on which individual battles between invaders, that is, the tracheobronchial mucus escalator and alveolar macrophages, are won and lost. If the defense is inadequate, breakdown occurs in the form of structural changes in the lung, and this may be succeeded by revolutionary activity when influx of cells and cytokines to the battlefield does damage to their native host. One of the important agents of potential injury to the normal lung is atmospheric ozone, particularly in the freeway cities of the Sunbelt, where sunlight interacts with aerosols of hydrocarbons to raise ozone levels in our atmosphere to values three times greater than allowable federal standards. This article deals with the mucosal battlegrounds in asthma and chronic obstructive pulmonary disease (COPD), with particular reference to the effects of ozone on mucus and particle retention in the tracheobronchial tree and the potential for aggravation of pulmonary dysfunction in these entities.

THE CONTENDING FORCES: FACTORS AFFECTING LUNG MUCOSAL FUNCTION

As in any fight for supremacy, several players crowd the battlefield. In this analogy, the outside invaders that attack the lung mucosa include tobacco smoke, environmental gases, particles, vapors, bacteria, viruses, and allergens [1–3]. The defending armies call up the troops, chiefly macrophages and neutrophils. Societal breakdown is represented by structural lung disease, for example, asthma, COPD, and asbestosis. This saps energy from the war effort, preventing firm resistance to the invaders. Then, as often happens in protracted struggles, revolutionary activity breaks out, with immune and auto immune reactions in the battleground. Gross and Detreville [4] also conceived of the lung as a battlefield, but in World War II terms, not the Star Wars idiom.

APPROACHING THE BATTLEGROUND: MEASUREMENTS IN HUMANS

Information from the battleground can be obtained from several directions (**Table 1**). Direct in

vivo microscopic or macroscopic analyses of particulate movement or ciliary beat rate are difficult and cannot be done in humans but are useful in addressing specific questions in animal experiments. Microsampling of mucosal glands has been recently emphasized, with the discovery of electro-ionic pumping as a factor in the volume and viscosity of mucus. Classic electron microscopy (EM and scanning EM) is also an important tool in this area. However, this review will focus on the technique of clearance of inhaled and deposited particles visualized by external gamma camera imaging, because this lends itself very well to extensive studies and characterization of tracheobronchial function in humans with and without disease [1,5].

TABLE I

Approaches to Studies in Tracheobronchial Mucosal Function

- Animal
  - Direct in vivo macroscopy of
    - Ciliary beat rate
    - Artificial particulate movement
  - Mucosal explants for ciliary "spin" rate
  - Microsampling of mucosal gland electrolyte concentration
  - Microscopy, light and electron microscopy
  - Electrophysiology of whole mucosal surface (Ussing)
- Human
  - Gamma-camera imaging of inhaled, clearing radiolabeled particles
  - Gamma-camera imaging of diffusible solutes for "permeability"
  - Mucus analysis for mechanical performance and chemical constituents
  - Pulmonary function testing

OCCUPYING TROOPS: LUNG RETENTION OF INSOLUBLE PARTICLES

Particle Size and Site of Deposit

The effects of an inhaled agent on the lung depend on several factors, chiefly the physical and chemical state of the inhaled agent and its site of deposit [3]. The two factors are of course linked. Much has already been learned about factors that affect the deposition of a given particle. In a person who breathes through the nose, particles that range in size between 10 and 100 μm are generally filtered out and deposited on the nasal and pharyngeal mucus membrane. Particles of 5 μm or smaller are deposited in the tracheobronchial tree and pulmonary parenchyma. As the particles become smaller (3 to 1 μm in diameter), they are deposited in greater proportions in the alveolar parenchyma. Other factors affecting the site and extent of particle deposition for a given particle size are the inspiratory and expiratory flow rates, the anatomy of the lung (especially the presence of expiratory flow limitation in COPD), breath-holding time, and excess mucus. These all provide variations to the three physical modes that have long been needed to govern inhaled particle deposition in the lung: impaction, gravitational settlement, and diffusion. The lung mucosa is designed to defend against all invading particles, noxious or otherwise, and prevent their retention. But the different parts of the mucosal surface have different anatomic characteristics, and, no doubt as a result, different mechanisms whereby deposited particles are cleared.

Figure 1 illustrates three separate battlegrounds within the lung epithelial surface, showing the separate strategic considerations applicable to each area. The clearance of particles from alveoli may be the most complex. As represented in the figure, no mucociliary escalator exists at the level of the alveolus, and a particle deposited there may exit the alveolus through penetration to the epithelium into the interstitium of the lung (a bad outcome fraught with the possibility of interstitial inflammation and fibrosis) or by engulfment by macrophages, subsequent migration to its mucociliary escalator of larger bronchi, and ultimate clearance through cough or swallowing. An actual scanning electron microscopic view of the mucociliary escalator with cilia is shown in **Figure 2**. The size, number, and composition of the particles determine, no doubt, the direction particles take in the course of alveolar clearance, but observations on this relationship in humans or large animals is meager, although rodent data are available. All such data suggest a long clearance time from alveoli with a half-life of $\geq$20 days and a 90% clearance time >100 days (depending on particle characteristics). This timing is particularly obvious in large animals resembling humans, such as the sheep [8].

The overall appearance of the clearance curve, when a bland particle of polystyrene is inhaled and all clearance proceeds via egress from the alveoli onto the mucociliary surface, is shown in **Figure 3**. Here, alveolar clearance predominates from the third day after deposition, and even when a direct route out of the alveolus is utilized, a long process involving macrophage engulfment and subsequent migration is required for alveolar clearance.

By way of contrast, clearance of particles from the bronchial tree is much more rapid, largely independent of the macrophage carrier, and entirely mediated by the mucociliary escalator. But even this process has a slow and fast phase, well illustrated by the curve of tracheobronchial clearance of Figure 3. Here, the initial rapid response in the first 4 hours represents clearance of particles deposited in large bronchi >1 mm diameter. But clearance from bronchi <1 mm diameter is much slower and has an average 90% clearance time of 44 hours [8,9]. This difference is best attributable to the two different surface epithelial layers in these areas (Figure 1): when compared with tagged bron-

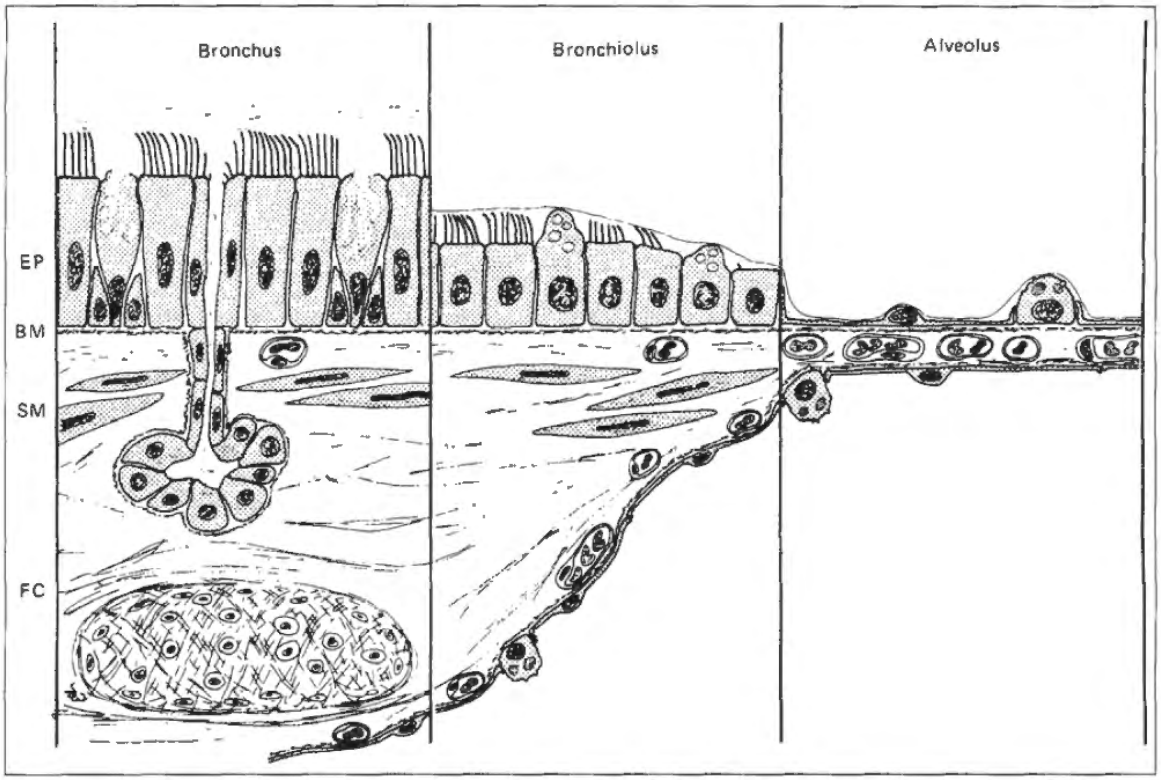

Figure 1. Characteristics of the lung mucosa affecting mucus secretion and particle clearance. The bronchus (>1 mm diameter) is outfitted with a full set of cilia in most columnar cells, goblet cells, and glands so that an extensive, though incomplete, blanket of moving mucus catches and clears deposited particles. The 90% clearance time of particle deposited in this region is 4 hours. The bronchiolus (<1 mm diameter) has fewer ciliated cells, no mucus glands, a less complete mucus blanket, and a 90% clearance time of 40 hours. The alveolus is outfitted with only macrophages for particle clearance, and the 90% clearance time from this region for a supramicron particle, though still via the tracheobronchial tree, is two orders of magnitude longer. Reprinted with permission from [5].

chi, the smaller bronchi have no mucous glands, depend on goblet cells alone for their sparse and incomplete mucus layer, and have only patches of cilia rather than a completely ciliated surface. Even with these differences, both large and small bronchi are under neural and mediator control, and the role of pharmacologic agents in regulating tracheobronchial clearance is easily demonstrated (**Figure 4**). Two β-adrenergic agents, epinephrine and isoproterenol by inhalation, greatly augment clearance [10]. Other studies have demonstrated that clearance from both the lung periphery (mostly small

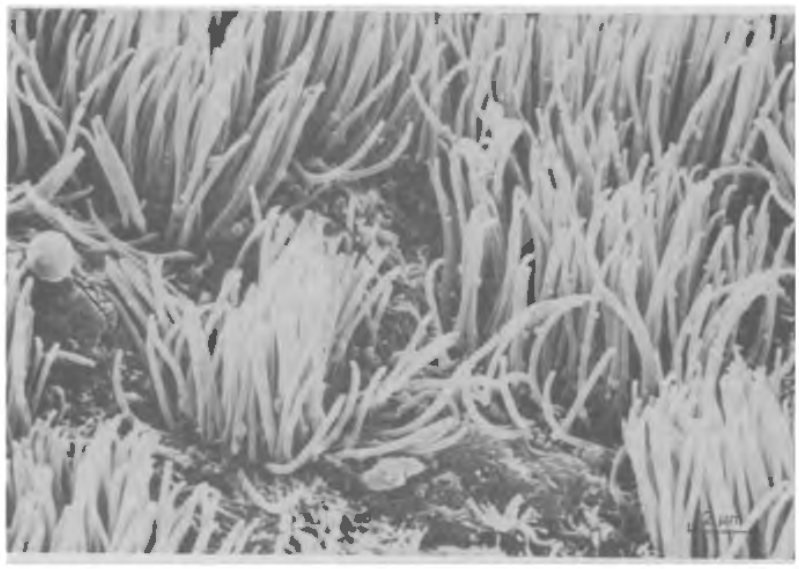

Figure 2. The ciliary surface of the human tracheobronchial tree by scanning electron microscopy, showing the relatively complete ciliary propelling mechanism for the overlying mucus blanket. Reprinted with permission from [6].

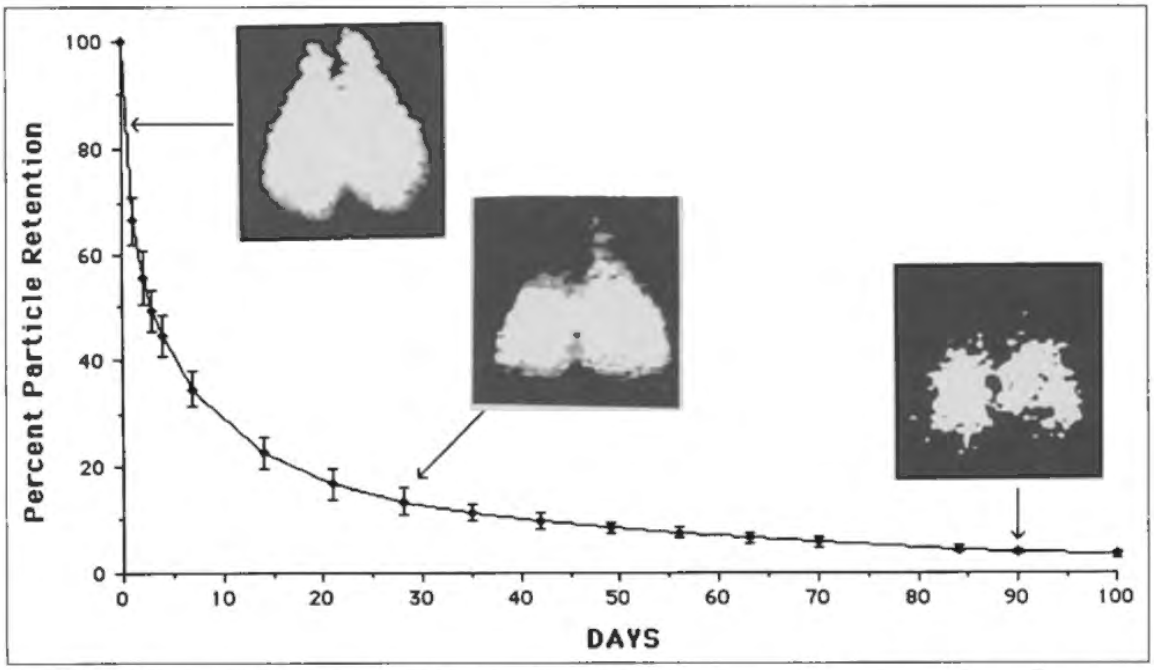

Figure 3. Clearance kinetics of particles deposited in the lung of the sheep, a large animal with lungs resembling in size and structure those of humans. The three gamma camera images demonstrate the appearance of the deposited particle load in the lung at various intervals during 100 days of clearance. The first 2 days of rapid clearance are tracheobronchial clearance. The remaining portion of the clearance curve shows a fast alveolar phase (about 2 weeks) and a slow phase lasting 50 weeks during which clearance averages about 10% of the remaining total lung particle per day. Reprinted with permission from [8].

bronchi) and the central lung (mostly large bronchi) are equally enhanced [11].

FEDERAL STANDARDS

Levels of ozone in the stratosphere have dropped considerably, which causes us to fear a greenhouse effect; yet levels of ozone in our immediate atmosphere are too high. As an example, in the last decade Southern California has achieved conformity with respect to federal air cleanliness standards for nitrogen dioxide, sulfates, lead, and sulfur dioxide, but not for carbon monoxide or ozone, for the latter of which federal standards are exceeded by almost 300% at times. In the case of ozone the federal standard is no more than 0.12 parts per million (ppm), averaged over 1-hour periods, throughout the year. In these Southern California counties the ozone levels exceeded federal standards by at least two 1-hour periods on 44% of the days of the year in 1986. Similar excesses are illustrated in **Table II**, where representative U.S. cities are listed with respect to the number of days on which the federal

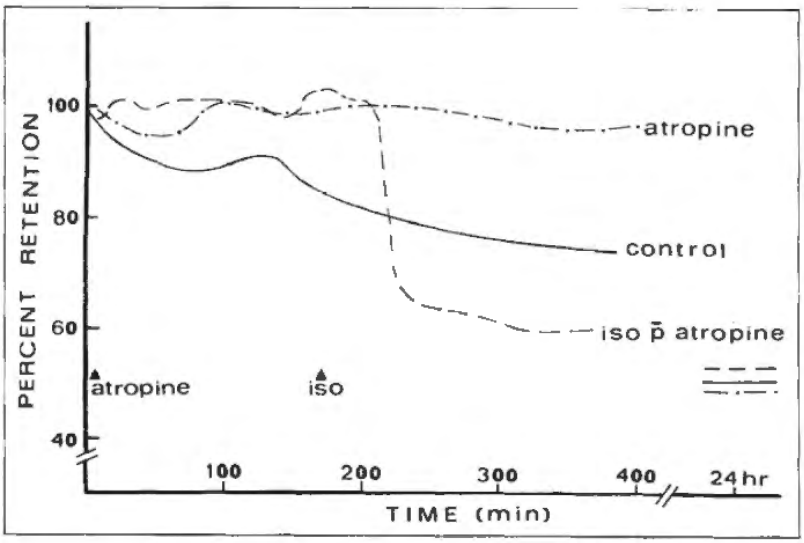

Figure 4. The pharmacologic control of tracheobronchial clearance in humans. Control clearance curve of particles is compared in the small individual with clearance curves modified after deposition of particle on subsequent days by the administration of (1) atropine (virtual cessation of all clearance over a 4-hour period), and (2) β-adrenergic agonist (virtual complete clearance of all tracheobronchially deposited particles within 1 hour). Reprinted with permission from [10].

TABLE II

Environmental Ozone Levels, 1986–1987, U.S. Cities Exceeding Federal Standard*

City	Number of Days Exceeded	Ozone ppm (peak hour)
Los Angeles	137.5	0.29
Bakersfield	44.2	—
Philadelphia	8.8	0.20
New York	17.4	0.17
Chicago	13.0	0.15
Baltimore	10.7	0.15

*Federal standard = 0.12 ppm ozone.

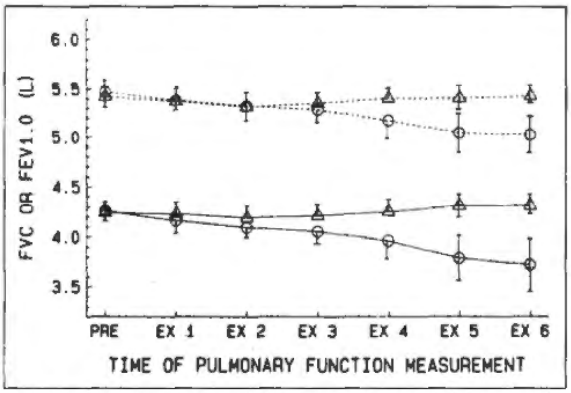

Figure 5. Effect of successive periods of exposure to ozone during successive periods of exercise on the forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) in a group of normal subjects. Control periods of breathing ambient air (△) showed no changes in lung function; ozone (○) caused significant decreases in FVC and FEV_1 beginning during the fourth 1-hour period of exercise. Reprinted with permission from [15].

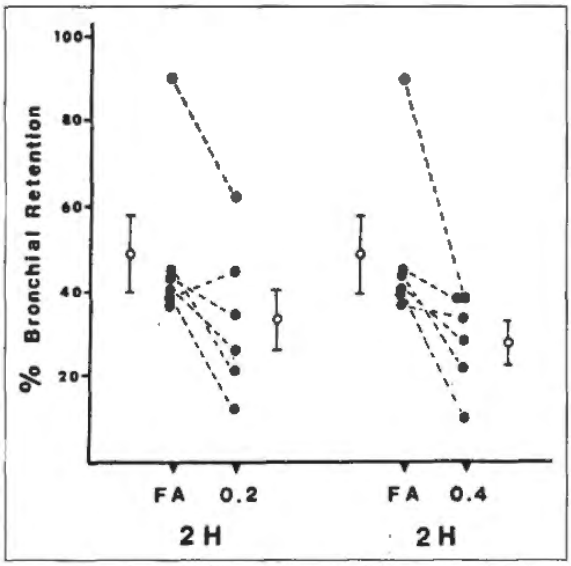

Figure 6. Effect of ozone inhalation on the rapidity of clearance of particles deposited on the peripheral tracheobronchial tree of normal human. **Left panel:** Ozone 0.2 ppm inhaled for 2 hours decreases bronchial retention of deposited particles (speeds clearance) compared with a control period breathing filtered room air (FA). **Right panel:** Ozone 0.4 ppm inhaled for 2 hours had a similar, but not necessarily greater, effect. Reprinted with permission from [16].

standard of 0.12 ppm is exceeded and the peak concentration reached on the day with the second highest ozone concentration [12]. Although Southern California cities have the worst records, eastern and midwestern cities also demonstrate significant excesses.

EFFECTS IN NORMAL INDIVIDUALS

Some rodents exposed to relatively high levels of ozone over a period of time will develop inflammatory disease of both airways and lung parenchyma, with some fibrosis [13,14]. Little is known about the effects of ozone in normal humans. Four major long-term effects of ozone on the lung are theoretically possible. These include a tracheobronchial effect (changes in mucus volume and viscosity, mucus–ciliary interaction, surface membrane integrity, and water secretion), a smooth muscle effect producing airway changes, a decrease in lung compliance, and cellular migration with neutrophilia in bronchoalveolar lavage [15–17]. The airway smooth muscle and compliance effects have been studied by measurements of lung function when volunteers exercised under filtered air conditions and under conditions of air containing either 0.12 or 0.2 ppm of ozone [15,16]. These results (**Figure 5**) showed a significant change as mean forced vital capacity (FVC) and forced expiratory volume in 1 minute (FEV_1) decreased over time. It is unclear whether the fall in FVC is connected with the fall in FEV_1 or whether there are two processes: one, an airway obstruction process and, two, a decrease in lung compliance. It is possible that both occur as independent functions.

As Figure 5 indicates, the fall in FEV_1 is dose and time dependent [15]. With respect to particle clearance, there is no significant change in the deposition pattern of an experimental inhaled aerosol of radioactive particles when volunteers breathe filtered air or air containing ozone at 0.2 ppm for a 2-hour period or a 4-hour period [16]. However, the rate of elimination of particles is significantly increased when ozone is applied at 0.4 ppm compared with filtered air; that is, clearance of particles is actually speeded up by ozone, not slowed down (**Figure 6**). It seems most probable that ozone increases mucus volume and clearance in the short term in these subjects. On the other hand, adaptation to ozone on a longer-term basis is apparent with respect to changes in mucociliary function [17]. This phenomenon must be distinguished from the persistence of the acute effects of ozone with repeated exposures, at least for the first 2 or 3 days of daily exposures [18].

Ozone exposure has been studied extensively in

normal exercising humans [19,20]. Although humans are relatively resistant to low-level ozone effects during exercise [21], exposure during exercise to ozone levels near the ambient air quality standard (0.12 ppm) does appear to affect lung function and exercise performance in elite cyclists in hot environments [20]. Older men and women experience significant parallel reductions in FVC and FEV_1, without apparent effect on mid-expiratory flow rates when exposed to 0.45 ppm ozone during exercise, and little difference is seen between the responses of men and women [21]. Children have been observed with reductions of 3.5% in FVC and 7% in peak flow during ambient ozone levels of 0.143 ppm [22]. Exercising young men and women showed reductions in specific airway resistance to 0.3 ppm of ozone while showing no response to nitric oxide, 0.6 ppm [23].

Several studies have dealt with ozone exposure in patients with asthma and COPD. Individuals with asthma who exercised while breathing 0.4 ppm of ozone showed significant reductions in FVC and FEV_1, which exceeded the reductions of FVC and FEV_1 in a control group exercising under the same conditions [24]. However, when Tennessee school children were serially tested for lung function, higher ambient ozone levels (.074 ppm) reduced FVC and FEV_1 uniformly in both normal children and those with asthma. These were short-term effects related to ozone level [25]. Total oxidants elicited greater symptoms in patients with COPD with long-term exposure compared with those with little exposure [26].

The role of inflammation, migration of neutrophils, and humoral mediators in humans with ozone exposure awaits to be defined. Access to such information is potentially available through the accepted procedure of bronchoalveolar lavage. Some preliminary valuable data already exist. Indomethacin pretreatment reduces ozone-related decreases in pulmonary function in normal individuals, which suggest a prostanoid role in the mediation of ozone effects [27]. In vitro, ozone exposure inhibits nitrogen-induced lymphocytosis and interleukin-2 production [28]. Although difficulties have been encountered with the radiolabeled diethylenetriamine pentaacetic acid (DTPA) colloid and its use in permeability studies, the available data suggest that exposure to 0.4 ppm ozone during exercise increases airway permeability in humans [29]. Finally, in young nonsmoking men, 0.4 ppm ozone for 2 hours produced an eight-fold increase in neutrophils and a 3.8-fold increase in neutrophil-derived elastase in the fluid of bronchoalveolar lavages performed 18 hours later [30].

CONSEQUENCES FOR INDIVIDUALS WHO HAVE LUNG DISEASE

Since humans in their normal habitat are probably exposed sporadically rather than repeatedly to ozone, each time humans breathe ozone they increase mucus volume and velocity. In healthy individuals an increase in mucus volume and velocity would probably have no effect. However, for patients with asthma, COPD, and other chronic lung diseases, these changes may increase hazards considerably. Asthmatic children in summer camps where ozone levels are relatively high have increased responses to methacholine challenge and decreases in lung volume and airflow [14]. Although specific data do not yet exist for patients who have COPD, one would expect that the increase in mucus flow would change upstream airway resistance, which would increase and further limit airflow. More important, if ozone increases parenchymal neutrophilia, one could expect that a greater extent of lung parenchyma would be destroyed by the release of neutrophil elastases.

V-DAY: PROSPECTS FOR FUTURE RESEARCH

Both clinical and basic investigation is needed to define the relationship between atmospheric ozone levels and lung disease. Clinical studies could include (1) the effects of ozone alerts on serial lung function in patients with established COPD and asthma; (2) the effects of aerosolized protectants to the irritant effects of ozone in these patients, that is, pharmacologic agents, steroids, and others; and (3) determination of precise atmospheric levels of ozone at which lung dysfunction and its aggravation can be averted. Although clinical clearance studies under ozone exposure are easily feasible, basic studies of the relationships between cellular-humoral events and the tracheobronchial surface are not yet available. In volunteers or in animal studies, bronchoalveolar lavage may be a potent tool to obtain critical dispatches from this all-important battleground.

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