

# Health Effects of Acid Aerosols Formed by Atmospheric Mixtures

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Under ambient conditions, sulfur and nitrogen oxides can react with photochemical products and airborne particles to form acidic vapors and aerosols. Inhalation toxicological studies were conducted, exposing laboratory animals, at rest and during exercise, to multicomponent atmospheric mixtures under conditions favorable to the formation of acidic reaction products. Effects of acid and ozone mixtures on early and late clearance of insoluble radioactive particles in the lungs of rats appeared to be dominated by the oxidant component (i.e., the mixture did cause effects that were significantly different from those of ozone alone). Histopathological evaluations showed that sulfuric acid particles alone did not cause inflammatory responses in centriacinar units of rat lung parenchyma (expressed in terms of percent lesion area) but did cause significant damage (cell killing followed by a wave of cell replication) in nasal respiratory epithelium, as measured by uptake of tritiated thymidine in the DNA of replicating cells. Mixtures of ozone and nitrogen dioxide, which form nitric acid, caused significant inflammatory responses in lung parenchyma (in excess of effects seen in rats exposed to ozone alone), but did not damage nasal epithelium. Mixtures containing acidic sulfate particles, ozone, and nitrogen dioxide damaged both lung parenchyma and nasal epithelia. In rats exposed at rest, the response of the lung appeared to be dominated by the oxidant gas-phase components, while responses in the nose were dominated by the acidic particles. In rats exposed at exercise, however, mixtures of ozone and sulfuric acid particles significantly (2.5-fold) elevated the degree of lung lesion formation over that seen in rats exposed to ozone alone under an identical exercise protocol.

## Introduction

Data obtained after severe air pollution episodes, such as those that occurred in London in the 1950s, suggest that acid particles, especially sulfuric acid ( $\text{H}_2\text{SO}_4$ ), were present in high concentrations and may have contributed to increased morbidity and mortality among London residents (1,2). In fact, many of the so-called killer smog episodes observed during the past 60 years have occurred under conditions that were favorable to the formation of airborne acids (3). The air during these episodes contained mixtures of gases, vapors and particles, including sulfur dioxide ( $\text{SO}_2$ ), nitrogen dioxide ( $\text{NO}_2$ ), nitric oxide (NO), substantial quantities of carbonaceous and noncarbonaceous particles, trace metals, and water in vapor and droplet forms. Under ambient conditions these

substances can react to yield sulfate- and nitrate-containing acidic particles and vapors and, when sunlight is present, ozone ( $\text{O}_3$ ) (4-8).

The amount of sulfate directly emitted to the global atmosphere by natural or human processes as sulfur trioxide of sulfuric acid is small. The major acid sulfate formation pathway is aqueous phase oxidation of  $\text{SO}_2$  in droplet aerosols at high relative humidity (9). The rate of conversion of  $\text{SO}_2$  to sulfate in coal-fired source plumes is variable but is generally estimated to be less than 15% per hour (and frequently is less than 5% per hour) (10,11). Oxides of nitrogen, emitted from anthropogenic stationary and mobile combustion sources, are converted to nitric acid ( $\text{HNO}_3$ ) vapor and particulate nitrates by photochemical processes involving hydrocarbons and  $\text{O}_3$  during daylight periods and via reactions with  $\text{O}_3$  at night (7,12) and indoors (13). In fogs, the rate of conversion of  $\text{SO}_2$  to sulfate is greatly accelerated (14) and, in addition, if aldehydes or ketones are present, strongly acidic hydroxymethanesulfonic acids can be formed (15). From

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this brief review, it is apparent that concentrations of acidic and oxidant compounds in ambient air could be strongly correlated. Such associations have, in fact, been reported in field epidemiological studies (16).

Animal toxicological studies have been performed to examine the expected range and mechanisms of health effects attributable to oxidant-acid mixtures. Some of these studies have indicated that synergistic interactions occur (17-19), but others have not (20,21). The aforementioned studies, however, were not designed to specifically examine the health effect potentials for reacting mixtures that lead to formation of airborne acidity. This paper will report on recent inhalation toxicologic studies using laboratory rats in which the atmospheres contained acid precursors and the exposure conditions permitted formation of acids by gas-phase and gas and particle reactions. The studies described involve complex atmospheric mixtures with up to seven intentionally added components, plus their reaction products. The end points that were selected as indices of relevant (to human populations) biological effects include: a) changes in clearance rates of inhaled insoluble radioactive tracer particles, both short term (presumably from nasal and tracheobronchial airways) and long term (presumably from alveolar regions of the lung); b) histopathologic focal lesions in the deep lung; and c) cell replacement (which is presumably stimulated by cell killing) in upper respiratory tract epithelium.

## Methods

### Atmosphere Generation and Characterization

The atmospheres for study contained acidic and nonacidic sulfates, SO<sub>2</sub>, O<sub>3</sub>, NO<sub>2</sub>, catalytic metal ions (Fe<sup>+3</sup>, Mn<sup>+2</sup>), and insoluble particles. Sulfate [H<sub>2</sub>SO<sub>4</sub> or ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] aerosols containing catalytic metal ions were generated by nebulizing a solution containing H<sub>2</sub>SO<sub>4</sub> or (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, ferric sulfate, and manganese (II) sulfate into highly purified dilution of air using a Collison nebulizer (22). Aerosols were passed through an <sup>85</sup>Kr discharger to reduce static charges to the Boltzmann equilibrium level. NO<sub>2</sub> and SO<sub>2</sub> were precisely metered into purified air from compressed gas cylinders. O<sub>3</sub> was generated from medical-grade oxygen using a commercial ozonizer (Sander Ozonizer, Type III). Insoluble particles of ferric oxide (Fe<sub>2</sub>O<sub>3</sub>) were generated by nebulization of dilute colloidal suspensions of Fe<sub>2</sub>O<sub>3</sub>, yielding spherical aggregate particles having an aerodynamic diameter of about 0.3 μm. The aerosols were dried at 50°C in a flow-through furnace, diluted with dry air, passed through an <sup>85</sup>Kr discharger, and introduced into the multicomponent atmosphere mixture.

NO<sub>2</sub> and NO were monitored using a chemiluminescent detector (Monitor Labs 8080). SO<sub>2</sub> was monitored using a TECO Series 43 pulsed fluorescence detector. O<sub>3</sub> was measured using a UV absorption mon-

itor (Dasibi Model 1003-AH). During exposure the atmosphere was monitored using real-time detectors: Climet 208 and GCA RAM-1 optical particle detectors for aerosols with diameters from 0.3 to 10 μm and a TSI Model 3030 electrical aerosol size analyzer for particles with diameters ranging from 0.05 to 0.5 μm. Cascade impactor samples (Sierra Model 212 8-stage impactor) were collected to provide size-classified particles for gravimetric and chemical analyses. Aerosols were collected on precleaned quartz fiber or fluorocarbon filters for gravimetric determinations and for chemical analyses of atmospheric components and reaction products. Nylon backup filters were used to collect HNO<sub>3</sub> vapor (from the reaction of NO<sub>2</sub> and O<sub>3</sub>). A point-to-plane electrostatic precipitator (23) was used to obtain aerosol samples for electron microscopic size characterization. Ion exchange chromatography was used to analyze for sulfate, sulfite, nitrate, and nitrite ions in filter extracts. Samples extracted in dilute acid were analyzed for manganese and iron by atomic absorption spectrometry.

### Animals and Exposures

Six-week-old, barrier-reared, male Sprague-Dawley rats (Hilltop Lab Animals, Inc.) were delivered to the laboratory in filter-equipped shipping containers. The rats were housed in a laminar-flow air barrier system (with chemical and particulate scrubbing) for 1 week prior to the start of each experiment. Microbiological assays supplied by the breeder and histopathological examinations performed at our laboratory were used to determine that the rats were free of major respiratory infections. Rats were randomly assigned to groups (6-12 per group); control groups were exposed to purified air, and matched groups were exposed to test atmospheres. In early experiments, rats were exposed whole-body in a chamber. This resulted in significant neutralization of acids by ammonia from excreta, hence the recent studies were performed using a nose-only exposure system, shown schematically in Figure 1A. In the earlier whole-body exposure studies with H<sub>2</sub>SO<sub>4</sub>, to attain a titratable acid concentration equivalent to 3.5 mg/m<sup>3</sup>, it was necessary to maintain the total aerosol sulfate mass at 5 mg/m<sup>3</sup> (24).

Exposures of exercising rats were performed using a modified Quinton 10-rodent treadmill. The treadmill (Fig. 1B) was enclosed and sealed against significant leaks (25), and a stainless-steel manifold equally distributed the flow to the 10 treadmill runways. The inner surfaces of the 10 treadmill runways were lined with a stainless-steel sheet to minimize reactions with corrosive gases. Ports in each runway permitted sampling of exposure atmospheres in the rat breathing zone. Exhaust lines at the rear of the treadmill conducted the atmosphere and expired respiratory gases to a mixing chamber for metabolic measurements. Measurements of O<sub>2</sub>, CO<sub>2</sub>, and flow rates of inspired and mixed expired gases permitted the determination of average metabolic rate of exercising animals. Rats

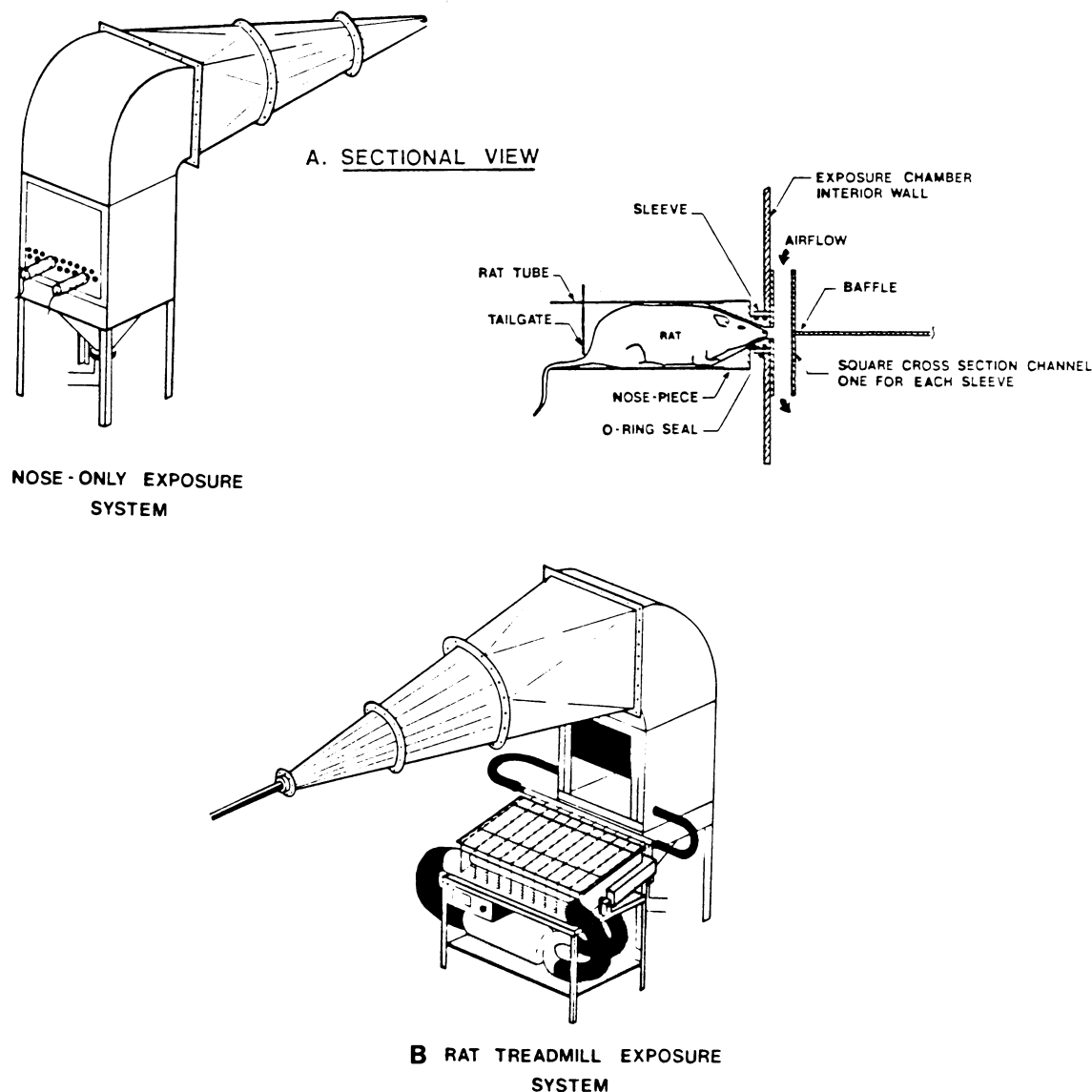


FIGURE 1. Systems used for exposing rats to acid-containing pollutant mixtures. (A) The nose-only system prevents neutralization of acids by ammonia liberated by excreta. (B) The treadmill systems allows for simultaneous exposures of up to 10 rats.

were exposed to pollutant atmospheres or to clean air (sham exposures) for 4 hr at rest, 3 hr at light (8 meters/min) continuous exercise, or 1.5 hr at intermittent moderate exercise (24 meters/min for 6 min, rest for 1 min).

### Particle Clearance

Clearance of inhaled radioactively labeled insoluble particles (26) by mucociliary and macrophage-mediated processes was quantitatively measured to determine the degree to which the ability of the respiratory tract to eliminate deposited particulate material (and by implication, toxic or infectious agents) was affected

by exposure to pollutant atmospheres. Tracer particles (1.5  $\mu\text{m}$  mass median aerodynamic diameter, MMAD) labeled with  $^{51}\text{Cr}$  (200 mCi/g particles) were suspended in distilled water, nebulized into dry dilution air, and the resulting dried particles passed through an  $^{85}\text{Kr}$  discharger and routed to a nose-only exposure chamber in which up to 20 rats could be simultaneously exposed (24,27). About 20 min was sufficient to deliver sufficient radioactivity to the rats' lungs. Initial chest counts were obtained on all of the rats, following which they were exposed for 4 hr to either purified air or to a pollutant atmosphere. The chest count data were used as baselines for the subsequent clearance measurements. Early clearance, presumably representing material removed from the upper

respiratory tract, was characterized by collecting and counting the rat's feces at 10 fixed times during the first 50 hr after the deposition of the tracer particles. Late, presumably deep lung, clearance was characterized by 5 chest counts during the subsequent 400 hr (27).

## Autoradiography and Histopathology

**Uptake of Radiolabeled DNA Precursors by Cells.** When inhaled pollutants kill respiratory tract cells, the damaged tissue is repaired by the replication of cells adjacent to the dead cells. In preparation for cell division, DNA precursors are taken up by the replicating cells and, by introducing tritiated thymidine ( $^3\text{HT}$ ), a radiolabeled nucleotide, it is possible to radioactively tag these cells and subsequently map their locations and numbers in the airways by autoradiography (28,29). These locations, then, represent epithelia in which cell death has been followed by a wave of new cell production. The number of cells that take up  $^3\text{HT}$  is an index of the magnitude of the tissue damage.

Seventeen hours after the end of a pollutant or clean air exposure, the animals were injected IP with  $^3\text{HT}$  (1  $\mu\text{Ci/g}$  of body weight), and, 1 hr later, were deeply anesthetized and killed. The lungs were excised and fixed by airway perfusion with 10% neutral-buffered formalin at 30 cm  $\text{H}_2\text{O}$  pressure for 72 hr (30). The trachea and larynx of each rat were dissected out and immersed in formalin. The heads were removed and the portions of the heads containing the intact nasal cavity fixed in formalin and decalcified with neutral TIS-buffered EDTA for 3 weeks. The trachea was split longitudinally, embedded in glycol methacrylate, and sectioned at 2  $\mu\text{m}$ . Specimens of the nasal cavity, 2 to 3 mm thick, were cut vertically through the hard palate and nasal septa at level II (31), embedded in glycol methacrylate, and sectioned at 2  $\mu\text{m}$ . Rates of cell turnover in tracheal and nasal sections were evaluated using autoradiography. Slides were dipped in Kodak NTB-2 nuclear track emulsion, and exposed for 30 days in a light-tight refrigerated box. After exposure, the slides were developed photographically, stained with toluidine blue and examined by light microscopy. Four distinct epithelial types were differentiated in the nasal sections (Fig. 2): squamous, olfactory, and two types of respiratory epithelia. Of the respiratory epithelia, one type, designated R, is a pseudostratified high columnar epithelium with ciliated and goblet cells and is located over the medial surfaces of the turbinates and the nasal septum. The second type, designated R1, is simple columnar epithelium with ciliated cells, but without goblet cells, and is found in the maxillary fossae lateral to the turbinates. Epithelial cells were quantitated by aligning a microscope eyepiece grid with the basement membrane and counting all cells, differentiating by cell type, within each of 10 grid spaces. The number of cells labeled with  $^3\text{HT}$  per unit grid space was expressed as the percent of labeled to total cells, by cell type.

**Lesions in Lung Parenchymal Tissue.** A single 2 to 4 hr inhalation exposure to  $\text{O}_3$  causes death of alveolar epithelial cells. Macrophages and some other leukocytes infiltrate the injured tissue to remove dead cells. The process of macrophage and leukocyte accumulation is called inflammation, and the locus of the inflammatory response is referred to as a lesion. Oxidant-related inflammatory responses occur in scattered centriacinar units of the alveolar parenchymal zone of the lung. Each centriacinar unit consists of the smallest airway, the terminal bronchiole, and the alveolar duct and alveolar sacs to which it leads. These units comprise most of the alveolar (parenchymal) zone of a mammalian lung, but only a fraction of these centriacinar units are injured after exposure and develop an infiltrate of inflammatory cells by which they are identified as lesions. Since most inflamed centriacinar units are surrounded by units of normal appearance, the lesions are isolated and are referred to as focal lesions. At exposure concentrations of about 0.6 to 0.8 ppm of  $\text{O}_3$ , focal lesions appear by 18 hr postexposure, reach maximum intensity at about 48 hr postexposure, and subsequently resolve. At higher concentrations,  $\text{O}_3$  exposure causes more widespread destruction of alveolar epithelial cells, greater numbers of lesions, and pulmonary edema, which can lead to death of the animal (29).

To assess parenchymal lung damage, groups of six or more rats were killed at 48 hr postexposure, and their lungs were fixed in the inflated state, as described previously. The volume of the right, middle lobe was measured by displacement. Longitudinal sections of this lobe, after paraffin embedding, were cut at 6  $\mu\text{m}$  thickness and stained with hematoxylin and eosin. In preliminary studies, we compared rat lung lobes for the distribution and volume fraction of inflammatory foci in the alveolar zone. There were no significant differences between lobes, hence the middle lobe of the right lung has been used routinely. Histologic sections were analyzed quantitatively by light microscopy using a morphometric process described by Elias and Hyde (32). Each lung lobe section was scanned systematically at low microscopic magnification to record the number of times the scanning point overlaid predominantly alveolar areas made up of centriacinar units as opposed to nonalveolar areas that contained structures including bronchi, blood vessels, and connective tissue. This process defines the alveolar and nonalveolar lung volume fractions. The alveolar volume density was recorded and the systematic scan was repeated at higher magnification to record the frequency with which the scanning point overlaid inflammation in a centriacinar unit in an alveolar zone. This frequency divided by the alveolar volume density was used to calculate the extent of lung injury expressed as the volume density of centriacinar units with inflammation within the total volume of the alveolar zone. This datum was multiplied by 100 and recorded as the percent lesion area.

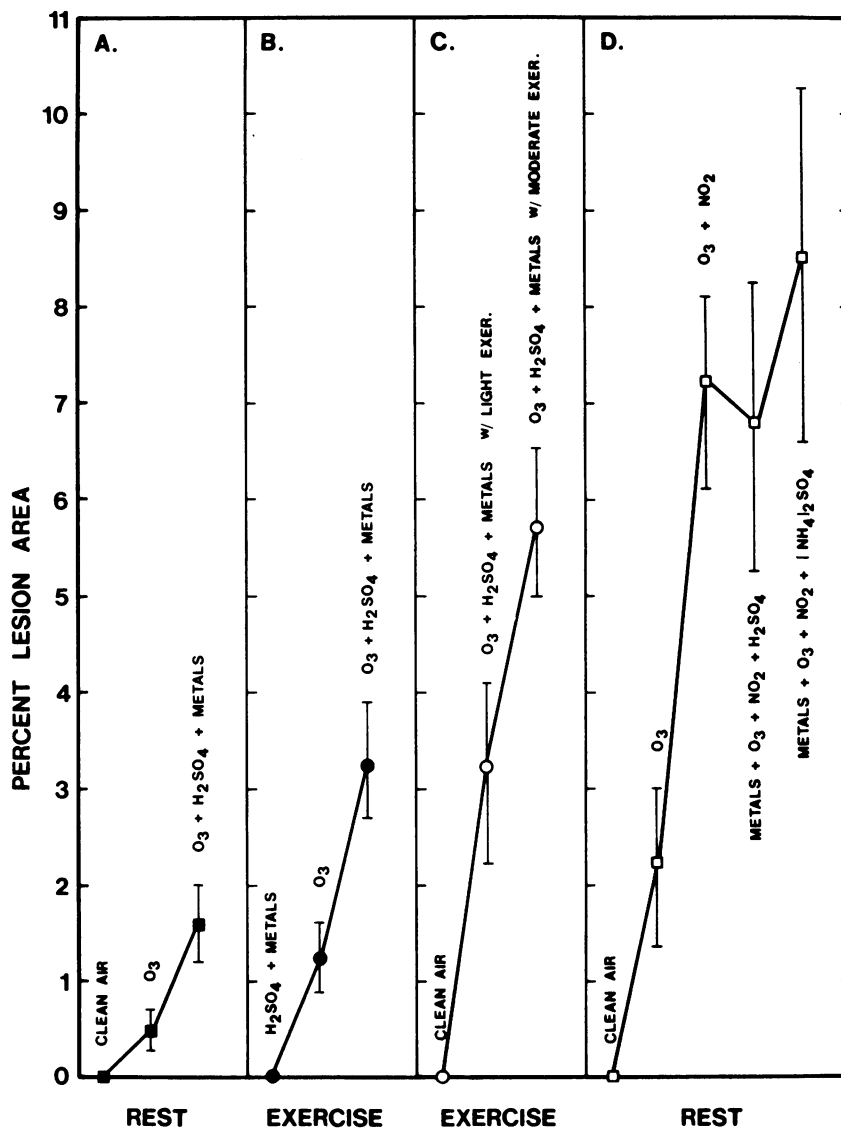


FIGURE 2. Inflammatory responses in centricinar units of lung parenchyma, expressed as percent lesion area, in rats exposed to acid-oxidant mixtures. The points on the graph represent the group mean, and the bars represent 1 SE of the mean. For details of exposure conditions and atmosphere composition, see text.

## Data Analysis

**Clearance.** Fecal clearance curves, which were representative of early clearance (0–50 hr), were fit to log-normal functions, and the time at which 50% of the total activity was excreted was determined for each rat ( $T_{50}$ ). For late clearance (2–17 days), a biological half-time ( $T_L$ ) for the radioactivity in the rat's chest (decay corrected to the time of deposition) was determined. Group mean values for control and pollutant atmosphere-exposed rats were compared using two-tailed  $t$ -tests, with  $p \leq 0.10$ .

**Histopathology and Autoradiography.** Group mean values of lung injury expressed as percent lesion area were contrasted among control and exposed groups using one-tailed analyses of variance. If there

were significant ( $p \leq 0.05$ ) mean differences between the atmosphere groups, pairwise comparisons were examined using the Tukey multiple comparison test.

## Results

### Effects of Acid Atmospheres on Clearance

Groups of rats were exposed to acidic and nonacidic sulfate aerosols alone and in combination with O<sub>3</sub>. Purified air-exposed rats were used as negative controls; rats exposed to O<sub>3</sub> (0.6–0.8 ppm) were used as positive controls.

H<sub>2</sub>SO<sub>4</sub> and non-acidic sulfate aerosols alone at 3.5

mg/m<sup>3</sup> produced relatively small effects on early and late clearance after whole body exposure (24). The greatest effects observed were by H<sub>2</sub>SO<sub>4</sub> and ferric sulfate at low (40%) relative humidity. The effects were of about the same order of magnitude, altering late clearance significantly when compared to the purified air-exposed group of rats.

Combinations of 0.8 ppm O<sub>3</sub> with 3.5 mg/m<sup>3</sup> of the acidic and nonacidic sulfate aerosols resulted in effects that were similar to those of O<sub>3</sub> alone. In general, the effects of the aerosols with O<sub>3</sub> were qualitatively similar to those of the O<sub>3</sub> alone, and in most cases the effects of the combination were less than those of O<sub>3</sub> alone at both high and low relative humidity did produce greater effects on early and late clearance than did the O<sub>3</sub> alone at high relative humidity. These effects were of greater magnitude than those produced by the nonacidic aerosols in combination with O<sub>3</sub>.

An atmosphere containing 0.8 ppm O<sub>3</sub>, 5 ppm SO<sub>2</sub>, and 1 mg/m<sup>3</sup> H<sub>2</sub>SO<sub>4</sub> at high relative humidity significantly delayed early clearance when compared with the purified air control group, but did not affect late clearance. However, the same atmosphere, but with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> substituted for H<sub>2</sub>SO<sub>4</sub>, affected early clearance to the same extent and produced a significant acceleration in late clearance; the effects of this mixture were slightly, but not statistically significantly, greater than those of 0.8 ppm O<sub>3</sub> alone.

## Histopathological Effects of Acid Atmospheres

Quantitations of inflammatory changes in centriacinar units in lung parenchyma and of rates of cell turnover in nasal and tracheal epithelia were undertaken in groups of rats exposed to a series of atmospheres ranging in complexity from clean air to a seven-component aerosol and gas mixture. Rats were exposed either at rest, nose-only, or while exercising on the rodent treadmill (Fig. 1B). The data shown in Figure 2 are a composite of independent experiments conducted over several years. Each experiment included its own control group and formed a self-consistent data set.

### Effects of Acid Atmospheres on Lung Parenchyma

The inflammatory changes in centriacinar units expressed as percent lesion area in the lung sections of each rat was averaged for groups of 6 to 10 rats. Data from rats exposed in four separate experiments are summarized in Figure 2. Within each experiment the group mean averages were contrasted between exposure conditions using analysis of variance. Tukey's multiple comparison test was used to test whether differences in effects on tissues between the experimental

conditions, on a pairwise basis, were significant at the  $\alpha \leq 0.05$  level.

Figure 2A examines a comparison of clean air, O<sub>3</sub> (0.6 ppm), and an acid-oxidant mixture containing H<sub>2</sub>SO<sub>4</sub> particles laced with Fe<sup>+3</sup> and Mn<sup>+2</sup> catalytic ions (1 mg/m<sup>3</sup>, 0.3  $\mu$ m MMAD), SO<sub>2</sub> (5 ppm), and O<sub>3</sub> (0.6 ppm) in rats exposed for 4 hr at rest. The acid-oxidant mixture was significantly more effective than O<sub>3</sub> alone in producing lung lesions. None of the clean air groups in this experiment, or in any of the other experiments, exhibited measurable inflammatory responses.

Figure 2B demonstrates the potentiating effect of 3 hr of low-level exercise. The control group was exposed to the acid mixture without O<sub>3</sub>, to O<sub>3</sub> alone, and to the acid-O<sub>3</sub> mixture, at the same concentrations as in the previous experiment. The acid mixture alone did not effect the parenchyma; the oxidant-acid mixture was about three times more potent than O<sub>3</sub> alone.

The potentiating effect of exercise is further explored in Figure 2C, where two different exercise levels are contrasted. The light exercise exposure was sufficient to approximately double the rat's resting oxygen uptake over a 3-hr period. The moderate, intermittent exercise rate increased oxygen uptake rate, but the animals were exposed for only 1.5 hr, and the quantities of pollutants inhaled under the two exercise regimes were approximately equivalent. While the light exercise group showed about the same level of lung damage as in the previous experiment, the moderate intermittent exercise protocol caused a significantly greater response.

Figure 2D examines the interaction of O<sub>3</sub>, NO<sub>2</sub> (2.5 ppm), and the SA aerosol mixture. In addition, one group of rats was exposed to an oxidant-aerosol mixture in which (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was substituted for H<sub>2</sub>SO<sub>4</sub> in the particle mixture. These exposures, which were conducted in resting rats exposed nose-only, demonstrated a striking synergism between O<sub>3</sub> and NO<sub>2</sub>, but the addition of either the acid or the nonacid sulfate mixture did not increase the lesion area compared to O<sub>3</sub> and NO<sub>2</sub> alone. One notes that significant concentrations of HNO<sub>3</sub> vapor (2 mg/m<sup>3</sup>; 0.8 ppm) were observed in atmospheric mixtures which contained NO<sub>2</sub> (2.5 ppm) and O<sub>3</sub> (0.6 ppm) together, both alone, or with other components.

### Cell Killing and Turnover in Nasal and Tracheal Tissue

Tissues from the same rats whose lung lesion data were summarized in Figures 2B and 2D were examined for uptake of <sup>3</sup>HT. Analyses of variance showed that there were no significant differences in cell labeling index values attributable to exposure atmospheres in the olfactory, respiratory, or tracheal epithelium. The group mean labeling index for squamous epithelium showed that there was a slight, but statistically significant ( $p \leq 0.05$ ), reduction in cell turnover in pollutant-exposed animals relative to the controls.

Analyses of variance demonstrated striking and significant ( $p \leq 0.01$ ) effects on cell killing and turnover in the R1 epithelium. As shown in Figure 3, the animals exposed while exercising to acid-containing atmospheres (SA and SA + O<sub>3</sub>) exhibited significantly greater rates of cell turnover than rats at rest exposed to either O<sub>3</sub> alone, clean air, or to the acid-oxidant mixture. As shown in Figure 4, rats exposed at rest to either O<sub>3</sub> or O<sub>3</sub> + NO<sub>2</sub> showed little or no increase in the cell labeling index compared to clean air. However, the addition of acidic or nonacidic sulfate aerosol to the oxidant gas mixture resulted in a 10-fold increase in the fraction of labeled cells. It should be noted that the nonacidic sulfate aerosol was in fact acidified by adsorption of HNO<sub>3</sub> from the vapor phase, which was generated by the reaction of O<sub>3</sub> and NO<sub>2</sub>. Reactions between the oxidant gases produced about 2 mg/m<sup>3</sup> (0.8 ppm) of HNO<sub>3</sub> vapor. The AS particles contained 370 µg/m<sup>3</sup> of nitrate ion (presumably from adsorption of HNO<sub>3</sub> vapor), which would provide acidity equivalent to about 290 µg/m<sup>3</sup> H<sub>2</sub>SO<sub>4</sub> on a mole-to-mole basis, which is about one-half of the acidity of the SA aerosol.

## Discussion and Conclusions

In general, our data do not indicate that the acidity of the aerosol is a major determinant of the effects upon particle clearance by pollutant atmospheres. In aerosol-only atmospheres, the acidic aerosol did not produce effects of greater magnitude than nonacid aerosols. In multicomponent atmospheres containing O<sub>3</sub>, the results support the hypothesis that H<sub>2</sub>SO<sub>4</sub> plays a minor role in altering respiratory tract clearance.

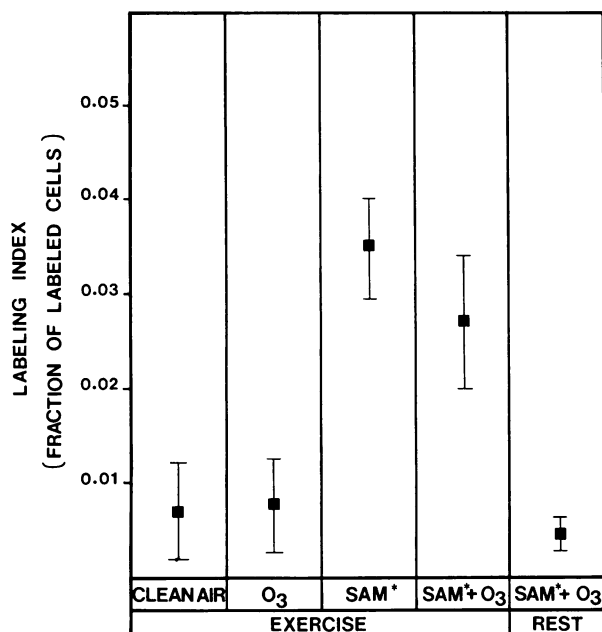


FIGURE 3. Damage to nasal respiratory epithelium in rats exposed to O<sub>3</sub>, acid aerosols (SAM), O<sub>3</sub>-acid aerosol mixtures.

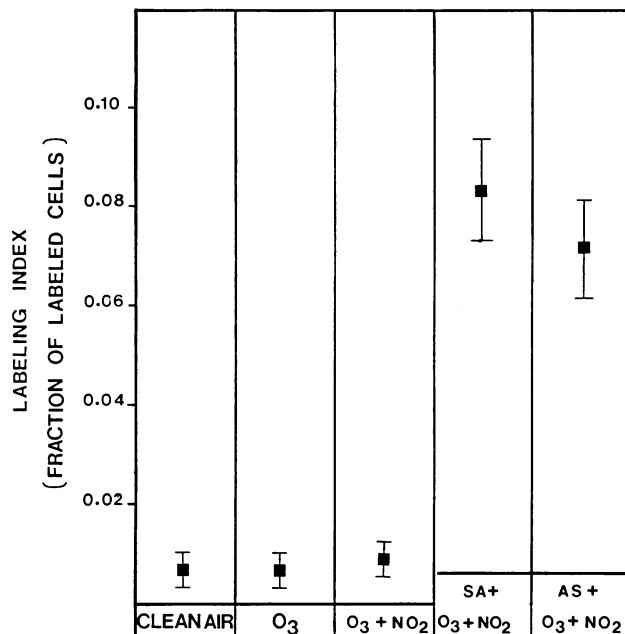


FIGURE 4. Damage to nasal respiratory epithelium in rats exposed to combined O<sub>3</sub> plus NO<sub>2</sub> with and without particulate sulfates (SA, AS). Note that in these studies all exposures were conducted with rats at rest.

O<sub>3</sub>, which has a relatively low solubility in water and which is not efficiently adsorbed in the upper respiratory tract, did not cause any significant increase in cell turnover rates, as measured by cell labeling with <sup>3</sup>HT. The type R1 respiratory epithelium in the noses of rats exposed while exercising was markedly affected by the atmospheres that contained the H<sub>2</sub>SO<sub>4</sub> mixture. When O<sub>3</sub> was added to the mixture, the labeling index was not significantly different. The addition, however, of NO<sub>2</sub> to the O<sub>3</sub>-particle mixtures produced a striking increase in the impact of the inhaled pollutants on the nasal epithelia. The component to which this effect is most likely attributable is the acid aerosol, either particles that were acidic intrinsically (H<sub>2</sub>SO<sub>4</sub>) or particles that were not originally acidic [e.g., (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] but which adsorbed HNO<sub>3</sub> formed by the reaction of O<sub>3</sub> and NO<sub>2</sub> and became acidic.

Some hypotheses suggested by these results are that the augmentation in centriacinar injury is due to (a) an increase in penetration and deposition of O<sub>3</sub> in the parenchyma when acidic pollutants are also present, because of changes in breathing pattern; (b) O<sub>3</sub> sensitization of tissues which were subsequently (or simultaneously) affected by acidic pollutants; or (c) the formation of intermediate chemical products which were toxic.

Alarie (30) reported that inhalation of sensory irritants caused reflex-driven changes in breathing patterns. Animals that inhaled substances such as H<sub>2</sub>SO<sub>4</sub> or SO<sub>2</sub> shifted to a pattern of slow, deep breathing. This type of pattern might result in enhanced deposition of gases such as SO<sub>2</sub> in the upper respiratory tract, but

could, in fact, allow greater numbers of small particles as well as gases of low water solubility such as O<sub>3</sub> to penetrate to the parenchyma more readily. The observations of damage to lung parenchyma in this study are consistent with these mechanisms.

With respect to formation of new toxic species in the oxidant/acid mixtures, we have observed significant levels of HNO<sub>3</sub> in oxidant mixtures containing O<sub>3</sub> and NO<sub>2</sub> and from chemical kinetics and stoichiometry we know that there must also be reactive intermediates such as nitrate radicals and nitrogen pentoxide present (the toxicology of these substances is not well understood). It is possible that more than one of the proposed mechanisms are active simultaneously. Current data are insufficient to identify or apportion the relative importance of each mechanism. The results discussed in this paper have only concerned short-term acute exposures. Data from current experiments of longer exposure duration are being evaluated and may shed additional light on the mechanisms by which acids and oxidants interact to cause damage to respiratory tract epithelium.

In summary, the effect of acid-oxidant mixtures on both early and late clearance appears to be dominated by the oxidant component, in short-term (4 hr) exposures at relatively high acid concentrations (3.5 mg/m<sup>3</sup>). Acid-O<sub>3</sub> mixtures are significantly more potent than O<sub>3</sub> alone, with respect to causing inflammatory changes in centriacinar units of the lung parenchyma in exercising animals, but the effects in animals exposed at rest again appear to be mainly attributable to O<sub>3</sub>. O<sub>3</sub>-NO<sub>2</sub> mixtures with associated HNO<sub>3</sub> vapor were much more potent than O<sub>3</sub> alone in terms of lung toxicity. The addition of acidic or nonacidic sulfate particles to the exposure atmospheres containing O<sub>3</sub> and NO<sub>2</sub> did not result in augmentation of the oxidant-related effects, suggesting that effects of vapor phase acidity were not augmented by the presence of particle acidity. Cell killing in the nasal respiratory epithelium is caused by H<sub>2</sub>SO<sub>4</sub> in exercising rats but neither O<sub>3</sub> nor O<sub>3</sub> plus NO<sub>2</sub> alone caused damage to nasal epithelia. Animals exposed at rest to mixtures of O<sub>3</sub>, NO<sub>2</sub>, and sulfate aerosols, under conditions favorable to the acidification of the particles by HNO<sub>3</sub> formed by reactions between the two oxidant gases, showed labeling in about 8% of the cells in the R1 nasal epithelium. Thus it appears that exposure atmospheres in which HNO<sub>3</sub>, and other reactive nitrogen compounds, are formed, i.e., those containing O<sub>3</sub> and NO<sub>2</sub>, are capable of causing health effects in excess of those attributable to either of the two oxidant gases alone.

An interesting interaction between O<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> was seen only in exercising rats. Exercising rats exposed to relatively high concentrations of an atmospheric mixture containing H<sub>2</sub>SO<sub>4</sub> aerosol (1 mg/m<sup>3</sup>) and SO<sub>2</sub> (5 ppm) did not exhibit parenchymal lesions. Exercising rats exposed to 0.6 ppm O<sub>3</sub> typically did show parenchymal lesions. When, however, both O<sub>3</sub> and the O<sub>3</sub> mixed with H<sub>2</sub>SO<sub>4</sub> were present, the degree of lesion formation was significantly elevated (2.5

times that observed in rats exposed to O<sub>3</sub> alone, at the same exercise level). Exercise was apparently an important factor in this process; rats exposed to an identical acid/O<sub>3</sub> mixture while at rest did not exhibit parenchymal lesions.

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