Effects of Inhaled Acids on Lung Biochemistry

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Effects of respirable aerosols of sulfuric acid, ammonium sulfate, sodium sulfite, and ammonium persulfate on lungs of rats are reviewed. The literature regarding interactions between ozone or nitrogen dioxide and acidic aerosols (ammonium sulfate, sulfuric acid) is discussed. An unexpected interaction between nitrogen dioxide and sodium chloride aerosol is also discussed. An attempt is made to identify bases for prediction of how and when acid aerosols might potentiate effects of inhaled gases.

Effects of Sulfuric Acid and Ammonium Sulfate Aerosols

By most of the conventional biochemical and histopathological criteria applied to the determination of lung injury, sulfuric acid (H2SO4) aerosol is a remarkably benign substance when inhaled by experimental animals, especially rats. Last and Cross (1) reported on the effects of exposure of rats to 1 mg/m3 and higher concentrations of H2SO4 aerosol (nuclei mode, 0.02 μm CMD) on various parameters evaluated in lungs of rats exposed continuously for 3, 4, 7, or 14 days to up to 180 mg/m3 of the aerosol. In the same study, a significant potentiation of the effects of exposure of rats to 0.4 ppm of ozone (O3) (expressed as concentration determined by UV photometric analysis) by 1 mg/m3 of H2SO4 aerosol, as evaluated by several parameters, was also reported. These observations have led us into various studies in the last 10 years that have mainly focused on the following questions: (a) What are the apparent no-effect levels for O3 and H2SO4 in the positive interaction we have observed between them by various biochemical and morphological assays? (b) Can we develop a systematic data base by which we might generalize what specific properties of O3 and of H2SO4 give rise to their synergistic interaction? (c) Can we begin to understand the mechanisms underlying interaction between pollutants in the centriacinar region of the lung, thereby allowing predictions of potential interactions between compounds prior to their testing in animal exposures? and (d) Can we develop sensitive quantitative assays that can be performed on acutely exposed animals that are predictive of (sub)chronic effects upon the lung?

To approach the first question, we have done extensive dose-response (more correctly, exposure-concentration response) experiments to characterize minimal response levels for the oxidant gases O3 and nitrogen dioxide (NO2) and for the acid aerosols ammonium sulfate [(NH4)2SO4] and H2SO4, alone and in binary combinations of oxidant gases and acid aerosols. We have examined several biological end points, including lung collagen synthesis rate determined in explant cultures, protein content of lung lavage fluid, and total protein and DNA content of lung tissue. An important aspect of our work has been to examine various assays for quantifying the response of rat lungs to exposure to O3. We then selected for further use those methods that were sensitive indicators of response at low concentrations of O3 and that also showed proportional exposure (dose)-response behavior over a range of O3 concentrations. This approach requires a systematic optimization of each assay for dose/time response before challenging animals at different concentrations of pollutants. For example, protein content of lung lavage fluid shows peak elevations above control values after 1 day of exposure of rats to 0.63 ppm O3, but only after 2 days of exposure of rats to 0.20 ppm O3 (2).

Guth et al. (3) showed significant exposure concentration-related increases in the total protein content of whole lung lavage fluid from rats exposed for 2 days to either 0.12 or 0.19 ppm O3. Several other assays performed on lung lavage fluid were less sensitive indicators of response to O3. Activities of lactate dehydrogenase, acid phosphatase, and N-acetyl-β-D-glucosaminidase were not affected by O3 at these concentrations. Minimal effective concentrations of O3 with

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respect to increased enzyme activity in lung lavage fluid by these assays were 0.66, 0.40, and 0.40 ppm O₃, respectively. Transport of a tracer molecule, [³H]albumin, from blood to lavage fluid was also increased by exposure of rats to concentrations of O₃ of 0.40 ppm and above. Warren and Last (4) examined concentration-response relationships between O₃ and H₂SO₄ aerosols; Warren et al. (2) studied similar concentration-response relationships between O₃ and (NH₄)₂SO₄ aerosols. These papers extended earlier studies with O₃ and (NH₄)₂SO₄ or H₂SO₄ aerosols (5) performed at relatively high concentrations of O₃. These last three papers used the assays of lung lavage fluid described above, plus lung collagen synthesis rate and selected morphometric indices of lung inflammation or structural change, as quantifiable responses of the lungs to exposure. The collagen synthesis rate assay and the protein content of whole lung tissue and of lung lavage fluid are the three most sensitive indicators of lung response to exposure to oxidants that we have, so these indicators were taken as the standard for attempts to define apparent no-effect levels in this series of studies.

Since well-controlled dose-response exposures to binary mixtures and their constituents are expensive and tedious experiments to perform, it was clear at the outset that it would be impossible to examine every possible combination and permutation of binary mixtures of oxidant gas and acid aerosol potentially of interest. We therefore initially focused on concentration-response relationships between O₃ and (NH₄)₂SO₄ aerosol.

We found in this study (2) that there was a significant increase in lung collagen synthesis rate and in protein content of lung lavage fluid in rats exposed for 7 or 2 days, respectively, to 0.2 ppm of O₃. A significant increase in both parameters over the values observed in rats exposed to 0.2 ppm of O₃ alone was found for rats exposed to 0.2 ppm of O₃ plus 5 mg/m³ of (NH₄)₂SO₄ aerosol. We could, therefore, conclude that O₃-(NH₄)₂SO₄ synergy could be demonstrated at concentrations of O₃ as low as 0.2 ppm [with 5 mg/m³ of (NH₄)₂SO₄]. We found in subsequent studies an apparent no-effect level at 1 mg/m³ of (NH₄)₂SO₄ (0.20 and 0.64 ppm O₃ were used in these combined exposures) by the criterion of potentiating the response to O₃ in the panel of assays performed above (2). Thus, all of our subsequent experiments have been performed with H₂SO₄ aerosols, alone and in combination with O₃.

We also examined whether acidity of the aerosol or its sulfate (SO₄) content was the important determinant of interaction with oxidant gases (6). We quantitated lung collagen synthesis rate and whole lung protein content after 7 days of exposure of rats to 0.96 ppm O₃ ± 5 mg/m³ (NH₄)₂SO₄, Na₂SO₄, or NaCl aerosols. By both of the criteria tested, exposure of rats to O₃ increased values significantly as compared with control rats breathing filtered air. The animals exposed to O₃ plus (NH₄)₂SO₄ aerosol showed significant increases above the values seen with rats exposed to O₃ in both assays [for example, collagen synthesis rate was 150% of the values observed with rats exposed to 0.96 ppm O₃ alone for the animals exposed to the mixture of O₃ and (NH₄)₂SO₄. These results may be contrasted with the findings for rats exposed to O₃ plus Na₂SO₄ or NaCl aerosols, where there was no difference as compared to values observed with rats exposed to O₃ alone. Morphometric analysis of volume of lung lesion and of fibroblast accumulation in lung lesions gave results consistent with the biochemical analyses. We concluded that acidity, not SO₄ content, of an aerosol is the determinant of its potential to interact synergistically with O₃.

### Interaction of Ozone and Sulfuric Acid Aerosol

The response of rat lungs to exposure of animals to O₃ for 1 or 2 days is proportional to concentration of O₃ between 0.12 and 0.96 ppm (3). Elevations of lung collagen synthesis rates are proportional to exposure concentrations between 0.12 and 1.2 ppm of O₃ for 7 days (2,5). We thus titrated the response of rats to H₂SO₄ aerosols using low (0.12 or 0.20 ppm) and high (0.64 ppm) concentrations of O₃ as standard regimens. We found synergistic interaction between O₃ and H₂SO₄ aerosols upon exposure of rats to 0.20 ppm O₃ plus 100, 500, or 1000 µg/m³ of acid after 3 days of exposure by the criterion of significant increases in total lavageable protein (4). A small (not significant) increase in this parameter was observed in rats exposed to 0.20 ppm O₃ plus 40 µg/m³ H₂SO₄ aerosol. H₂SO₄ aerosol alone provoked no significant response by this assay (95 or 107% of control values for 100 or 1000 µg/m³ of acid aerosol alone, respectively). Synergistic interaction was also observed by the criterion of increased lung protein content (7- and 9-day exposures to ozone plus acid aerosol) in rats exposed to 0.2 ppm O₃ plus 40, 100, or 500 µg/m³ H₂SO₄ aerosol (4). Recent unpublished experiments have also shown significant increases in lung protein content in rats exposed for 9 days to 0.2 ppm O₃ plus 20 µg/m³ H₂SO₄ aerosol. An apparent no-effect level as defined by this assay (whole lung protein content) was observed (with a trend to slightly elevated values above those with O₃ alone) in preliminary experiments with rats exposed for 9 days to 0.2 ppm O₃ plus 5 µg/m³ H₂SO₄ aerosols.

Lung collagen synthesis rates were significantly elevated in rats exposed to 0.64 ppm O₃ in this study (4), with synergistic interaction observed between 0.64 ppm O₃ and 200, 500, and 1000 µg/m³ H₂SO₄ aerosol. A synergistic interaction was also observed between 0.20 ppm O₃ and 40, 100, 500, and 1000 µg/m³ H₂SO₄ in this study. Also of interest was the apparent interaction between 0.12 ppm O₃ and 500 µg/m³ H₂SO₄ aerosol observed with this assay (4). O₃-exposed rats had lung collagen synthesis rates that were 115% of control values (not significantly elevated); rats exposed to 0.12 ppm O₃ plus 500 µg/m³ H₂SO₄ had rates that
were 132% of control values, a significant increase.

Two aspects of this study deserve special mention. First, interaction of O₃ and H₂SO₄ aerosols seems to be an all-or-nothing response. That is, there is no apparent dose-response relationship between concentration of acid aerosol in the exposure chamber and extent of increase over values observed with O₃ alone in any of the assays we have performed. Second, we have examined the relationship between H₂SO₄ aerosol mass concentration added to the chambers and pH of eluates of sample filters (4). There is no evidence in our experiments for neutralization of H₂SO₄ by chamber NH₃ (putatively arising from microbial action on animal excreta or from the rats themselves), at least down to values of 100 µg/m³ of H₂SO₄ aerosol, by this criterion. That is, plots of aerosol concentration versus pH of filter eluates have a linear correlation of 0.98 between 100 and 1000 µg/m³ H₂SO₄ aerosol. The biological response of rat lungs to exposure to 0.20 ppm O₂ plus 40 or 20 µg/m³ H₂SO₄ aerosol suggests that any putative neutralization of H₂SO₄ by NH₃ is of little or no consequence at concentrations of acid at or above 20 µg/m³.

Finally, it should be emphasized that the interaction between O₃ and H₂SO₄ or (NH₄)₂SO₄ is clearly one in which the damaging effects of O₃ on the centriacinar region of the lung are enhanced; that is, the acid aerosol potentiates the effects of O₃ and not vice-versa (7). We have suggested a biochemical mechanism involving extension of the lifetime of active oxygen species (free radicals) at sites of O₃ interaction with the deep lung in more acidic environments as one potential mechanism to explain this effect (7). Clearly, other mechanisms are also possible, including altered patterns of breathing affecting sites of deposition of O₃, or altered mucociliary clearance affecting removal of mediators or products of cell damage. It remains to be proven what the actual mechanism underlying the synergistic interaction between oxidant gases and acidic aerosols may be.

Interaction of Nitrogen Dioxide and Sulfuric Acid Aerosol

Rats were exposed for 7 days to 10, 5, or 2 ppm of NO₂. Elevations of lung collagen synthesis rate as compared with control animals exposed to filtered air were 210, 120, and 99%, respectively. The values at 5 and 10 ppm NO₂ were significantly increased as compared with the controls. Thus, there was a reasonable concentration-response relationship by this assay, with an apparent no-effect level at 2 ppm NO₂. This is not an unexpected finding: Using the relationship that the ratio of toxicity of NO₂:O₃ = 18 (5), we would estimate that 2 ppm NO₂ is equivalent in biologic effect to 0.11 ppm O₃, while 5 ppm NO₂ is equivalent to 0.28 ppm O₃.

Protein content of lung tissue after 7 days of exposure was significantly increased to 122% of control values at 10 ppm NO₂. Values observed at 5 and 2 ppm were, respectively, 98 and 109% of controls; neither value was significantly different from the controls. Thus, an apparent no-effect level, as defined by this assay, was observed at 5 ppm NO₂.

Protein content of lung lavage fluid was quantified after 3 days of exposure. Values observed were 225, 175, and 106% of control for 10, 5, and 2 ppm, respectively; the first two values (at 10 and 5 ppm) were significantly greater than the controls. Enzyme activities quantified in the lavage fluid from the rats exposed to 5 ppm NO₂ included lactate dehydrogenase, acid phosphatase, and N-acetyl-β-D-glucosaminidase, none of which were significantly different than control values.

Thus, good concentration-response behavior was observed by the criteria of the lung collagen synthesis rate and the total lavagable protein content assays for rats exposed to 2 to 10 ppm NO₂, with an apparent no-effect level at 2 ppm. With this background information, we examined the response of rats by these assays to exposure to mixtures of NO₂ and H₂SO₄ aerosol.

Rats were exposed for 1, 3, or 7 days to 5 ppm NO₂ ± 1 mg/m³ H₂SO₄ aerosol. As discussed above, response of rats to 5 ppm NO₂ alone as quantified by the collagen synthesis rate assay (7 days) and by the protein content of lung lavage fluid (3 days) was significant, whereas protein content of lung lavage fluid after 1 day of exposure was indistinguishable from control values. The protein content of lung lavage fluid from rats exposed to NO₂ plus H₂SO₄ aerosol was significantly elevated to 215 and 180% of control values, respectively, for assays after 1 and 3 days exposure. The elevation at 1 day was significantly greater than that observed with NO₂ alone; the value at 3 days was comparable to that observed with NO₂ alone (175% of controls). These results illustrate the importance of considering the optimal time-response behavior of rats as a function of pollutant concentration in this assay when designing the experiment. Protein content of lavage fluid for rats exposed for 3 days to the acid aerosol alone was not significantly different from control values (113% of controls). For rats exposed for 3 days to 2 ppm NO₂ plus H₂SO₄ aerosol, lavagable protein content was increased to 132% of control values.

The rats exposed for 7 days to 5 ppm NO₂ plus acid aerosol had lung collagen synthesis rates of 145% of control values, significantly greater than the controls or values from rats exposed to 5 ppm NO₂ alone (120% of control values). The rate of collagen synthesis for lungs of rats exposed to H₂SO₄ aerosol alone was indistinguishable from control values (98% of controls). Rats exposed to 2 ppm NO₂ plus H₂SO₄ aerosol had lung collagen synthesis rates (129% of controls) significantly higher than rats exposed to either 2 ppm NO₂ (99% of controls) or to filtered air.

We conclude that there is a synergistic interaction between 1 mg/m³ of H₂SO₄ aerosol and 2 or 5 ppm NO₂ by two independent assays of response of rat lungs to exposure to these agents.
Effects of Other Aerosols Containing Sulfur Oxide Anions

We exposed rats to respirable aerosols of sodium sulfate (Na₂SO₄) over a concentration range of 0.1 to 15 mg/m³, equivalent to concentrations of sulfur dioxide (SO₂) of 0.02 to 2.7 ppm, for 3 days (24 hr per day). Additional groups of rats were exposed to 10 mg/m³ Na₂SO₄ and to the addition product of Na₂SO₃ and formaldehyde (sodium hydroxymethane sulfonate) at 6 mg/m³ (8). Aerosols had MMADs of 0.8 to 1.2 μm and were essentially SO₄⁻ and SO₂⁻ free. We measured various parameters of lung response to exposure, of which the most sensitive (in this study) was an increase in the wet to dry weight ratio of whole lung tissue. We interpret this assay as indicative of changes related to pulmonary edema and/or inflammation. Significant dose-related increases in lung wet to dry weight ratio were observed in rats exposed to 1, 6, and 14 mg/m³ of Na₂SO₃ aerosol, with nonsignificant trends toward increased wet to dry weight observed in the groups exposed to 0.1 mg/m³ Na₂SO₃ aerosol and to 6 mg/m³ sodium hydroxymethane sulfonate. No response was observed by this criterion in lungs of rats exposed to 10 mg/m³ sodium sulfate (Na₂SO₄) aerosol.

We have also exposed rats to 1 to 20 mg/m³ of respirable (0.8–1.3 μm MMAD) aerosols of ammonium persulfate [(NH₄)₂S₂O₈] for 7 days (9). Rats inhaling 4 to 20 mg/m³ of the persulfate aerosol lost body weight and had significantly increased lung wet weight, total lung protein content, and lung DNA content. We interpret these changes as indicative of changes related to pulmonary edema and/or inflammation. Small, not significant, increases in all of these parameters were observed in the lungs of rats exposed to 1 mg/m³ of persulfate aerosol in the same study. We also exposed a group of rats to 5 mg/m³ aerosol (NH₄)₂SO₄ (0.8 μm MMAD) containing 0.7 mg/m³ of gaseous hydrogen peroxide (H₂O₂), equivalent in oxidizing capacity to 5 mg/m³ (NH₄)₂S₂O₈ aerosol, to examine whether a highly soluble gaseous oxidant (H₂O₂) plus a sulfate aerosol gave comparable effects to the persulfate aerosol. No response was observed to the H₂O₂-sulfate combination by any of the assays used in this study.

Even though the concentrations of sulfate or persulfate aerosols required to elicit responses in these studies were very high as compared to realistic exposures likely to be encountered by humans, these results are suggestive that acid aerosols other than H₂SO₄ and/or HSO₄ could be potential toxicants in occupational or environmental atmospheres.

Effects of Sodium Chloride Aerosols

There is no observable interaction between O₃ (0.96 ppm) and respirable aerosols of 5 mg/m³ NaCl under conditions identical to those that we demonstrated had elicited a synergistic interaction in our experiments with O₃ and (NH₄)₂SO₄ aerosols (6).

In an earlier study (5), we exposed rats to aerosols of 5 mg/m³ (NH₄)₂SO₄, alone or in combination with 5, 10, 15, 20, or 25 ppm of NO₂. There was an approximate doubling of the lung collagen synthesis rate in rats exposed to the binary mixtures as compared with NO₂ alone at all concentrations tested; (NH₄)₂SO₄ aerosol alone had no effect on the rats in these studies. In later work (Last and Warren, unpublished data), we have examined responses of rats to 2, 5, or 10 ppm of NO₂ by most of the assays described above, observing an apparent no-effect level at 2 ppm NO₂ (discussed above).

Based upon these experiments we exposed rats to 5 ppm NO₂ with and without 1 mg/m³ H₂SO₄ or of NaCl aerosol (10).

Rats exposed to 5 ppm NO₂ for 7 days showed a significant increase in lung collagen synthesis rate (120% of control values). Groups of rats exposed to 5 ppm NO₂ and 0.9 mg/m³ H₂SO₄ aerosol had lung collagen synthesis rates of 145% of the control values, i.e., rats exposed to filtered air, values significantly greater than those observed with the rats exposed to NO₂ alone. Of interest here, however, is the response of rats exposed for 7 days to 5 ppm NO₂ plus 1.1 mg/m³ NaCl aerosol. These animals showed a lung collagen synthesis rate of 165% of the values observed with controls exposed to filtered air. In control experiments, we found values of 98 and 95% of control for rats exposed to H₂SO₄ aerosols and NaCl aerosols alone, respectively, by this assay.

We also quantified the protein content of the lung lavage fluid from rats exposed for 3 days to 5 ppm NO₂, with and without either 1 mg/m³ H₂SO₄ or NaCl aerosol. As compared with filtered air controls, we found values of 175% of controls in the rats exposed to NO₂ alone, 180% of controls in rats exposed to NO₂ + H₂SO₄ aerosol, and 210% of controls in rats exposed to NO₂ + NaCl aerosol. The increase in values for the NO₂ + NaCl group was significant as compared to those observed in rats exposed to NO₂ alone.

We interpret these results as suggesting that a reaction product of NO₂ and NaCl is responsible for the interaction observed in these experiments, since no interaction was observed between NaCl and O₃, which do not react chemically. We hypothesize that the interaction between NO₂ and NaCl is due to their reaction to form nitrosyl chloride (NOCl), the mixed anhydride of hydrochloric, nitrous, and nitric acids, which could give rise to strong acids in the centriacinar region of the lung upon hydrolysis. The possibility of acid aerosols (or acidogenic aerosols) arising in the atmosphere from sources other than SO₂ or direct dissolution of NO₂ to give nitric and nitrous acids opens some very interesting vistas toxicologically.

Summary and Conclusions

To return to the four questions asked at the beginning of this paper, we can attempt to answer them as

J. A. Last
follows: (a) Apparent no-effect levels for O\textsubscript{3} and H\textsubscript{2}SO\textsubscript{4} aerosol interactions: As of our present assays and data base, the apparent no-effect concentration value for O\textsubscript{3} is at or below 0.12 ppm, while that for H\textsubscript{2}SO\textsubscript{4} aerosol (at 0.20 ppm of O\textsubscript{3}) is below 20 \mu g/m\textsuperscript{3}. (b) Properties of O\textsubscript{3} and H\textsubscript{2}SO\textsubscript{4} responsible for their interaction: The combination of a relatively insoluble oxidant gas and a respirable-sized acidic (or acidogenic) aerosol, such that significant deposition and interaction can occur in the centriacinar region of the lung, seem to be the critical properties that are predictive for interactions to occur. (c) Interactions between pollutants: On a phenomenological level, synergistic interaction seems to occur between oxidant gases and acids. We do not yet understand on a mechanistic level what are the controlling factors underlying such interactions (i.e., what are the rules?). (d) Development of sensitive, quantitative assays predictive of chronic effects: Until now, essentially all of our work on O\textsubscript{3} (or NO\textsubscript{2})-acid aerosol interaction has been done with acute experiments (1–9 days of exposure), so we know little or nothing about possible chronic effects upon the lung of inhalation of such mixtures. We have one hint that the synergistic interaction between 0.4 ppm O\textsubscript{3} and 1 mg/m\textsuperscript{3} H\textsubscript{2}SO\textsubscript{4} persists for at least 50 days of continuous exposure (5), but far more work remains to be done in this area to define the nature and extent of any chronic interactions. A panel of sensitive assays for studying effects on acutely exposed animals discussed in this paper remain to be validated for their ability to predict chronic effects upon the lung.

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