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TRACHEAL AND BRONCHOALVEOLAR PERMEABILITY CHANGES IN RATS INHALING OXIDANT ATMOSPHERES DURING REST OR EXERCISE

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Permeability of tracheal and bronchoalveolar airways of rats was measured and used to examine the effects of inhaled oxidant-containing atmospheres. The atmospheres studied were (a) ozone (O_3) at 0.6 ppm (1.2 mg/m^3) or 0.8 ppm (1.6 mg/m^3); (b) nitrogen dioxide (NO_2)at 6 ppm (11.3 mg/m^3) or 12 ppm (22.6 mg/m^3); (c) O_3 + NO_2 at 0.6 ppm (1.2 mg/m^3) and 2.5 ppm (4.7 mg/m^3), respectively; and (d) a 7-component particle and gas mixture (complex atmosphere) representing urban air pollution in a photochemical environment. The rats were exposed for 2 h. The effects of exercise during exposure were evaluated by exposing additional groups in an enclosed treadmill. Exposure of resting rats to 0.8 ppm O_3 increased tracheal permeability to DTPA and bronchoalveolar permeability to diethylenetriamine pentaacetate (DTPA) and bovine serum albumin (BSA) at 1 h after the exposure. Bronchoalveolar, but not tracheal, permeability remained elevated at 24 h after the exposure. Exercise during exposure to O_3 increased permeability to both tracers in the tracheal and the brochoalveolar zones, and prolonged the duration of increased permeability in the tracheal zone from 1 h to 24 h, and in the bronchoalveolar zone from 24 h to 48 h. Permeability in the tracheal and brochoalveolar zones of rats exposed at rest to 6 or 12 ppm NO_2 did not differ from controls. However, rats exposed during exercise to 12 ppm NO_2 for 2 h developed a significant increase in tracheal and bronchoalveolar permeability to DTPA and BSA at 1 h, but not at 24 or 48 h, after exposure. Exposure at rest to 0.6 ppm O_3 plus 2.5 ppm NO_2 significantly increased bronchoalveolar permeability at 1 and 24 h after exposure, although exposure at rest to 0.6 ppm O_3 alone increased bronchoalveolar permeability only at 1 h after exposure. Exposure to O_3 + NO_2 during exercise led to significantly greater permeability to DTPA than did exercising exposure to O_3 alone. Resting rats exposed to a complex gas/aerosol atmosphere composed of the above O_3 and NO_2 concentrations, plus 5 ppm (13.1 mg/m^3) sulfur dioxide (SO_2) and an aerosol of insoluble colloidal Fe_2O_3 with an aerosol of manganese, ferric, and ammonium salts, demonstrated increased permeability at 1 and 24 h after exposure. Nitric acid vapor was formed in both the O_3 + NO_2 atmosphere and the complex gas/aerosol atmosphere. The particles in the latter also contained hydrogen ions equivalent in concentration to about 100 μg/m^3 of NH_4 HSO_4 suggesting that acidic components in the atmospheres produced effects that were additive upon the effect of O_3 in producing both increase and prolongation of permeability in tracheal and bronchoalveolar zones of the respiratory tract.

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INTRODUCTION

Exposure of resting animals to single oxidant pollutants is known to produce pulmonary injury detectable by morphologic, biochemical, physiologic, or immunologic criteria. The nature and extent of injury depend on the pollutant concentration and the duration of the exposure. Altered epithelial permeability has been utilized to analyze pulmonary injury after exposure of laboratory animals or humans to gaseous pollutants. Increased mucosal permeability was observed in humans smoking cigarettes (Jones et al., 1980; Mason et al., 1983) and animals exposed at rest to O_3 (Bhalla et al., 1986), NO_2 (Selgrade et al., 1981; Ranga et al., 1980), and cigarette smoke (Boucher et al., 1980; Hulbert et al., 1981).

Mustafa et al. (1984), noting the coexistence of NO_2 and O_3 in the atmosphere, compared the biochemical effects of these gases when administered alone or in combination. Their studies indicated that concentrations of either NO_2 or O_3 that produced nonsignificant biochemical changes in mouse lungs produced changes that were more than additive when the gases were combined. Watanabe et al. (1980) found that when NO_2 and O_3 were administered in sequence, the biochemical changes produced in mouse lungs were additive. Such additive or synergistic effects of exposure to NO_2 + O_3 are, however, not always observed. Ehrlich and Findlay (1979) reported additive effects of O_3 + NO_2 on pulmonary host resistance to inhaled bacteria, but Goldstein et al. (1974) suggested that response to O_3 and NO_2 is equivalent to that expected from each gas. Histologic changes produced by O_3 + NO_2 in rats were also similar to the changes produced by O_3 alone (Freeman et al., 1974).

Studies of the toxicologic effects of atmospheres that result from adding sulfur emissions from coal and oil burning to an atmosphere already dominated by photochemical air pollutants have been reported from this laboratory. Phalen et al. (1979, 1980) described the effects of O_3, sulfuric acid, and ammonium sulfate aerosols on clearance of labeled insoluble particles from rat lungs following single 4-h exposures to single or combined gases or aerosols at high or low relative humidity. Ozone slowed the early particle clearance and stimulated clearance during the later phase. High humidity usually amplified these effects of ozone, as well as many of the other atmospheres studied. Combinations of ozone and sulfate aerosols resulted in effects that were similar to those of ozone alone, sulfuric acid being a probable exception.

One consequence of studying the health effects of atmospheric mixtures is that the components will often interact to form new, possibly toxic, reaction products. In the case of mixtures containing NO_2, O_3, and nonacidic aerosols, it is possible to form acidic particles and acidic vapors that were not in the original mixture but that might significantly alter the biological response upon exposure. For example, in recent stud-

ies of atmospheric products formed by O_3 plus NO_2 and a combination of O_3, NO_2, SO_2, $(NH_4)_2SO_4$, $Fe_2(SO_4)_3$, and $MnSO_4$, Kleinman et al. (1985a, b) found the formation of HNO_3 vapor and of acid aerosol. Histologic sections of rat lungs were examined quantitatively at 18 and 48 h after exposure to score the percent of the alveolar zone of the section that was occupied by focal inflammatory lesions (Mautz et al., 1985a). The pathogenic effect of O_3 was stable over time, but the effect of the two atmospheres that formed HNO_3 was greater than for O_3 alone and was progressive with time, indicating potentiation of the O_3 effect by an acidic atmosphere. Last and Cross (1978) and Last et al. (1983, 1984) reported that $(NH_4)_2SO_4$ and H_2SO_4 aerosols potentiated the effect of O_3 on the inflammatory lesions of rat lung and induced increased collagen synthesis, with the conclusion that the more acidic aerosol was the major contributor. The present study is therefore relevant to the evolving description of the effects of O_3, and O_3 plus acidic atmospheres, on the rat respiratory tract.

In humans, exercise has been shown to alter pulmonary function (Silverman et al., 1976; DeLucia and Adams, 1977). Exercise increased minute ventilation and led to deeper penetration and a different distribution of inhaled toxic agents. In a recent study aimed at analyzing histopathologic changes in rats exposed to O_3 during exercise, Mautz et al. (1985a) found enhancement of lung lesions by exercise. O_3 concentrations that did not produce detectable histologic changes when the rats were exposed at rest induced lung lesions in exercising rats. In a series of similar studies, Last et al. (1985) reported that acid aerosols potentiated the effect of O_3 upon blood-to-lung transfer of serum proteins, and of radiolabeled serum albumin in rats exposed at rest.

Although the adverse effects of exposure to individual pollutant gases under resting conditions have been widely investigated (Plopper et al., 1973; Bils and Christie, 1980; Hu et al., 1982; Bhalla et al., 1986), the literature on the effects of combined gases inhaled during exercise is rather limited. From evidence available at the time this work was undertaken, exercise was known to modify the response produced by exposure to individual gases at rest. It is now clear that the response to inhaled pollutants depends on the level of ventilation as well as pollutant composition and concentration (Silverman et al., 1976; Mautz et al., 1985a; Last et al., 1985). Reaction products, including acids, can also be expected to modify responses measured in the laboratory.

This report represents an effort to study permeability changes in animals exposed under conditions that approach the complex atmospheres to which humans are exposed at rest or during periods of physical activity. The study analyzes permeability changes following exposure of rats to O_3, NO_2, O_3 + NO_2 or O_3 + NO_2 + SO_2 + ammonium sulfate + ferric and manganese salts that catalyze oxidation of S(IV) to S(VI) in the presence of an insoluble aerosol of colloidal Fe_2O_3.

METHODS

Animals

Male barrier-reared Sprague Dawley rats (Hilltop Laboratory, Scottdale, Pa.) that weighed 225–274 g (about 47–52 d of age) were delivered in filtered containers, maintained on a standard diet, and held in a laminar air barrier caging system for at least 1 wk prior to pollutant exposure. Ten percent of each batch was autopsied upon arrival to assure their freedom from lung disease.

Generation and Characterization of Atmospheres

Exposure Chambers and Air Purification Exposures were in stainless-steel chambers (Mannix et al., 1982) or an enclosed treadmill (Mautz et al., 1985b). Each system was supplied with purified air from a high-pressure air-purification system. Outside air was first filtered and then compressed to about 100 psig using a liquid ring compressor (Nash Engineering Company, Norwalk, Conn.). The high-pressure air subsequently passed through a fixed Purafil bed ($KMnO_4$ on alumina; H. E. Burroughs, Inc.) in order to remove gaseous contaminants such as O_3, SO_2, NO_2, and hydrocarbons. Carbon monoxide was removed as it passed through a catalyst bed; a heatless dryer located just upstream of the catalyst bed prevented catalyst inactivation by moisture. Following filtration and thermal equilibration to laboratory temperature, the high-pressure purified air was throttled down to ambient pressure and humidified to 85 ± 2% relative humidity (RH). Delivery of purified air to the control chamber followed a final HEPA filtration. In the pollutant exposure chamber, test gases and/or aerosols were injected into the airstream following the HEPA filtration, and these diluted atmospheric pollutants then entered an expanding cone-shaped diffuser-mixer (Walters et al., 1982) before reaching the exposure chamber (Figs. 1 and 2). Atmospheric "aging" time was about 2.5 min from initial pollutant mixing to the time of inhalation.

Generation Techniques Ozone was generated by passing medical-grade oxygen through an electrical ozone generator (Sander ozonizer, type III, Osterberg, West Germany). NO_2 and SO_2 were obtained from cylinders of research-quality compressed gas, metered into purified air using calibrated rotameters with needle valves and then injected into the chamber air-supply stream through separate ports located downstream of the O_3 injection port. Ammonium sulfate aerosols (about 0.25 μm mass median aerodynamic diameter) containing catalytic metal ions were generated by nebulizing an aqueous solution of ammonium sulfate, ferric sulfate, and manganese(II) sulfate using a Collison nebulizer (Ho et al., 1980). Aerosols were passed through an ^{85}Kr discharger to reduce static charges on particles to the Boltzmann equilibrium level. Ferric oxide particles were generated by nebulization of dilute colloidal suspensions of Fe_2O_3, yielding spherical aggregate particles. These particles had a mass

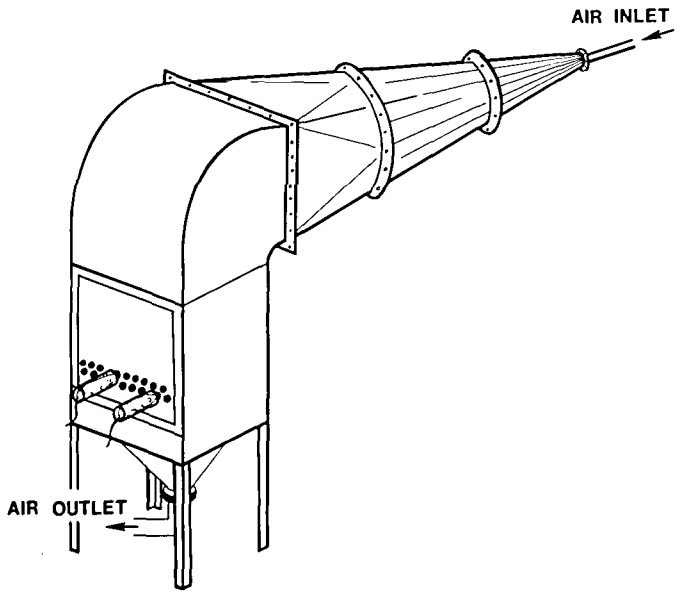

FIGURE 1. Nose-only exposure system. Air pollutants are introduced into the Walters' chamber air flow before the expanding cone-shaped diffuser-mixer. Rats in plastic tubes are exposed nose-only to chamber atmosphere by way of ports in the chamber wall. Arrows show direction of air flow.

median aerodynamic diameter of 0.3 μm and geometric standard deviation of about 2. The aerosols were diluted with warm, dry air, discharged by passage through an ^{85}Kr discharger and introduced into the chamber air.

Characterization Techniques The following methods have been described in greater detail by Kleinman et al. (1984). All atmospheric gas measurements were made at the rat breathing zone using Teflon tubing sampling lines. Nitrogen dioxide and nitric oxide were monitored using a chemiluminescent detector (Monitor Labs 8840, San Diego, Calif.). Sulfur dioxide was monitored using a Teco series 43 pulsed fluorescence detector (Thermal Electron Corp., Hopkinton, Mass.). Ozone was measured by ultraviolet (UV) absorption (Dasibi model 1003-AH, Dasibi Environmental Corp., Glendale, Calif.). During exposure, the aerosol stability was monitored using "real-time" detectors: a Climet 208 optical particle detector (Redlands, Calif.) for aerosols with diameters from 0.3 to 10 μm and a TSI model 3030 (St. Paul, Minn.) electrical aerosol size analyzer for particles with diameters ranging from 0.05 to 0.5 μm. Cascade impactor samples (Sierra eight-stage impactor, Anderson Samplers, Atlanta, Ga.) were collected to provide size-classified particles for gravimetric and chemical analyses. Aerosols were collected on precleaned quartz fiber or Teflon

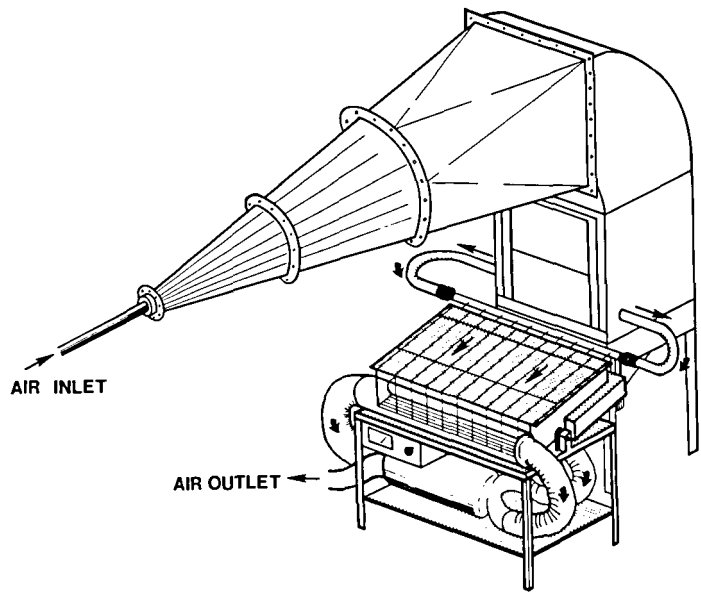

FIGURE 2. Exercise exposure system in which air pollutants are drawn from ports in the chamber walls and through a modified ten-channel Quinton small-animal treadmill shown in the foreground. Each channel is separated from the others by stainless-steel-lined partitions. Arrows show direction of air flow.

filters for gravimetric determinations and for chemical analyses of atmospheric components and reaction products. Nylon backup filters were used to collect nitric acid vapor from the reaction of NO_2 and O_3. Ion-exchange chromatography was used to analyze sulfate, sulfite, nitrate, and nitrite ions in extracts from both particulate and vapor-phase filters. Sample aliquots of extracts were analyzed for manganese, "soluble iron" (ferric sulfate), and "insoluble iron" (ferric oxide) using a differential extraction technique. Iron and manganese were determined by atomic absorption spectrophotometry and H^+ by potentiometry.

Exposure of Resting or Exercising Rats

Rats were exposed at rest or during exercise for 2 h to (a) air, (b) O_3 (0.6 or 0.8 ppm), (c) NO_2 (6 or 12 ppm), (d) O_3 (0.6 ppm) + NO_2 (2.5 ppm), (e) O_3 (0.6 ppm) + NO_2 (2.5 ppm) + SO_2 (5 ppm) + Fe_2O_3 (241 $\mu g/m^3$) + $(NH_4)_2SO_4$ (308–364 $\mu g/m^3$), $Fe_2(SO_4)_3$ (411–571 $\mu g/m^3$), and $MnSO_4$ (7–9 $\mu g/m^3$). In each experiment, at least 10 rats were exposed, either during rest or while exercising to each atmosphere. Only 9 of the 10 exposed rats were used for permeability measurements. The extra rats served as a backup in case of technical problems with any of the scheduled rats. Three of the exposed rats were tested for tracheal or bronchoalveolar permeability immediately after the exposure. Three of the remaining rats

were tested at 24 h and 3 at 48 h after the exposure to determine the duration of effect of exposure on permeability. Each experiment was repeated at least once for testing permeability immediately after the exposure and 24 h later. Since permeability at 48 h after the exposure was not different from controls in representative experiments, the repeats were not performed at this time point. The data for 48 h after the exposure are stated in the results but not included in the tables.

In experiments in which all exposures were at rest, rats were placed in cylindrical plastic tubes fitted with aluminum end pieces at the head end. The end piece bears a cone-shaped interior cavity tapered toward the forward end so that only the tip of the rat's nose protrudes into the atmosphere. The exterior of the end piece bears a cylindrical fitting by which it is inserted and sealed into one of a set of 26 exposure ports placed in the wall of the chamber (Fig. 1) as described by Mannix et al. (1982). Each port was supplied by a channel on the interior wall of the chamber; the channel directed the atmosphere downward past the nose of each rat, to prevent rebreathing of that atmosphere by the exposed rat and to prevent sharing of that sample of atmosphere by another rat.

In experiments designed to test the effects of exercise during exposure, all rats were trained on a 10-channel treadmill (Fig. 2) (Quinton model 42-15, Quinton Instruments, Seattle, Wash.) for 2 d prior to the experimental exposure; they ran at 15 m/min at 20% grade for 1 h on the first day and for 2 h on the second day. All rats underwent identical training and were then randomly assigned to exposure during rest or exercise. Rats exposed at rest were held in the restraining plastic tubes for nose-only exposure and placed in the exposure treadmill in order to maintain the exposure positions as close as possible to those for exercising rats to assure that the breathing zone of each resting rat was located at the same point in relation to entry of O_3 in the treadmill as the breathing zone of rats exposed while running. Concentrations of test gases were measured continuously during a 2-h exposure. The exposure treadmill (Mautz et al., 1985b) has been used to determine the mean metabolic rate of 10 rats at rest or during exercise; the exposure regimen produced about a twofold increase in oxygen consumption in exercising rats as compared to resting rats.

Permeability Measurement and Data Analysis

Methods for measuring transport of tracers across the tracheal and bronchoalveolar mucosa have been reported (Bhalla et al., 1986). The methods are reviewed briefly here. Animals were anesthetized intraperitoneally with sodium pentobarbital (Nembutal sodium, Abbott Laboratories, North Chicago, Ill.), 5 mg per 100 g body weight. A polyethylene tube (PE-90) was placed in the trachea, and a polyethylene catheter (PE-10, Clay Adams, Parsippany, N.J.) was placed in the femoral artery. The radiolabeled tracer inoculum contained [^{99m}Tc]diethylenetriamine pen-

taacetate (^{99m}Tc-DTPA, molecular weight 492) and ^{125}I-labeled bovine serum albumin (^{125}I-BSA, molecular weight 69,000).

For tracheal instillations, 0.1 ml of the inoculum was manually delivered into the trachea using a PE-10 tube attached to 1-ml syringe; inoculum was introduced through the PE-90 tracheostomy tube while the rat was held supine in a nearly horizontal position. The tracer solution was delivered into the middle zone of the trachea about 0.5 cm beyond the tracheostomy tube over a 5-min period.

For bronchoalveolar instillation, rats were held on an incline. Inocula were delivered directly to a main stem bronchus using a longer PE-10 tube inserted through the tracheostomy tube. When India ink was used as a tracer inoculum, the dye distributed in the major airways following instillation into the tracheas of supine rats, but the dye could be localized in the alveolar region upon instillation into a mainstem bronchus of rats held on an incline (Bhalla et al., 1986). After delivery of radiolabeled tracers but prior to blood sampling, heparin solution (20 units in 0.2 ml saline) was injected into rats through femoral-artery catheters prior to blood sampling. Blood samples of 0.10 ml were drawn 6, 7, 8, 9, and 10 min after the start of instillation. Blood samples were transferred to glass microtubes for immediate counting of ^{99m}Tc in a gamma counter (1275 Minigamma, LKB Instruments, Gaithersburg, Md.). Same blood samples were then counted for ^{125}I radioactivity.

Isotope counts in 0.10-ml blood samples at the 6, 7, 8, 9, and 10-min time points for the two tracers were expressed as the percent of the inoculum transferred from the site of application to the entire blood volume of each rat. Blood volume was calculated as 6.4% of the body weight (Altman and Dittmer, 1974). The label in blood samples was analyzed by obtaining the regression curve extrapolated to the intercept at the mid-time point, T_i, for the five samples. Differences in the fractions of each labeled molecule transferred to the blood at time T_i in both the control and the O_3-exposed groups were analyzed by analysis of variance, using a significance level of 0.05. Data from different experiments were pooled and permeability values in rats exposed to pollutant gases or their combinations were compared between the groups as well as with clean-air controls. To delineate the effect of exercise on permeability, comparisons were made between groups exposed to same pollutant while at rest or during exercise.

RESULTS

Atmospheres

Analytic results for 6 atmospheres of O_3 + NO_2 and for two 7-component atmospheres that include O_3 and NO_2 (Table 1) reveal that the dominant acidic product in these atmospheres was HNO_3 at concen-

trations of from 1179 to 2558 $\mu g/m^3$ (0.46–1.02 ppm). The amount of HNO_3 formed was consistent with calculated products of the reaction of O_3 and NO_2 at the concentrations employed in this study (M. T. Kleinman and B. Finlayson-Pitts, personal communications). Some formation of acid sulfates (most likely ammonium bisulfate, NH_4HSO_4) was also observed. Extracts from aerosols collected on the quartz-fiber or Teflon filters were analyzed for hydrogen ion content, shown in Table 1 in terms of microequivalents of hydrogen ion per cubic meter of air. The particles contained very little NO_3 ion; thus particle acidity values were regarded as arising from SO_2 conversion to SO_4. Some hydrogen ion is also released by hydrolysis of water by Fe^{3+} during storage of the extract prior to analysis; the values in Table 1 were empirically corrected by subtracting the amount of hydrogen ion formed by hydrolysis due to iron in the sample. The residual acidity, regarded as arising from NH_4HSO_4, is shown as acidic aerosol in Table 1.

Trachea-to-Blood Transfer

Rats Exposed to O_3 at Rest or during Exercise Exercise during sham-exposure to clean air did not alter tracheal mucosal permeability to DTPA or BSA as compared to permeability in rats exposed to clean air while at rest (Table 2). In control rats exposed to clean air, either at rest or during exercise, the fraction of the instilled quantity of DTPA transferred from the tracheal lumen to the blood was greater than the fraction of BSA (Table 2). In exposed rats, 1 h after a 2-h exposure to O_3 at rest, both DTPA and BSA fractions appearing in blood were higher than in control rats (Table 2). In a previous series of experiments (Bhalla et al., 1986), both DTPA and BSA were transferred in significantly greater fractions in O_3-exposed, as compared to control, resting rats, but in Table 2 the increase in transfer of DTPA only is significantly greater in rats exposed at rest to 0.8 ppm O_3 for 2 h.

Tracheal mucosal permeability to DTPA was significantly greater in animals examined 1 h after a 2-h exposure to ozone at rest and was not significantly elevated at 24 and 48 h after the exposure, while in rats exposed during exercise, tracheal permeability at 1 h was increased to both DTPA and BSA and remained elevated at 24 h after the exposure (Table 2). Although transfer of DTPA and BSA at 1 h and 24 h postexposure in rats exposed during exercise was greater than at the corresponding time points in rats exposed at rest, only DTPA transfer at 24 h postexposure was significantly different between the two groups ($p < 0.05$).

Rats Exposed to NO_2 at Rest or during Exercise Tracheal permeability in rats exposed to 6 or 12 ppm NO_2 while at rest, or to 12 ppm during exercise, has been studied twice. In rats exposed at rest to 12 ppm NO_2, the fractions of DTPA and BSA in blood were not consistently increased either immediately after, or at 24 h after, the exposure (Table 3). In rats exposed during exercise, there was a significant increase in transfer of

TABLE 1. Characteristics of Combined Gas–Aerosol Atmospheres: Data for Permeability Experiments of Table 6

	0.6 ppm O_3 + 2.5 ppm NO_2[a]	Multicomponent complex atmosphere[b]
	Gases	
O_3, ppm	0.61 ± 0.05[e]	0.61 ± 0.04
(mg/m^3)	(1.2 ± 0.1)	(1.2 ± 0.1)
NO_2, ppm	2.5 ± 0.2	2.6 ± 0.2
(mg/m^3)	(4.7 ± 0.4)	(4.9 ± 0.4)
SO_2, ppm		5.0 ± 0.1
(mg/m^3)		(13.1 ± 0.3)
RH, %	85.2 ± 1.4	84.5 ± 1.6
HNO_3, ppm	0.70 ± 0.23	0.87 ± 0.21
(mg/m^3)	(1.8 ± 0.6)	(2.2 ± 0.5)
	Aerosols[c] ($\mu g/m^3$)	
$(NH_4)_2SO_4$		236 ± 40
$Fe_2(SO_4)_3$		491 ± 113
$MnSO_4$		8 ± 1
$(NH_4)HSO_4$[d] calc.		100 ± 0
Fe_2O_3		241 ± 8
Total dry mass, calculated by mass balance		1076 ± 83
Total wet weight measured at 85% RH		1402 ± 68

[a]Data for O_3 + NO_2 at rest were compiled by pooling results from five exposures.
[b]Data for seven-component atmosphere were compiled by pooling results from two exposures.
[c]Mass median aerodynamic diameter = 0.35 μm.
[d]Acid aerosol computed as ammonium bisulfate ($[H^+]$ = 0.88 $\mu eq/m^3$).
[e]Mean ±SD.

DTPA and BSA 1 h after the exposure, but only DTPA transfer was significantly elevated at 24 h. Only DTPA transfer at 1 h after exposure to NO_2 was significantly greater ($p < 0.05$) in rats exposed during exercise than at the same time point in rats exposed at rest.

Bronchoalveolar Zone-to-Blood Transfer

Rats Exposed to O_3 at Rest or during Exercise Ozone increased the transfer of DTPA and BSA to blood at 1 h after exposure. The percent transferred was greater for DTPA than for BSA, and the permeability induced by exposure to O_3 persisted for DTPA at 24 h after the exposure (Table 4).

As in the case of trachea-to-blood transfer of molecules, exercise during exposure to clean air did not produce any consistent, significant change in permeability from the bronchoalveolar zone to the blood. Exercise during exposure to O_3 resulted in increased transmural transfer of both DTPA and BSA (Table 4). Bronchoalveolar permeability in rats ex-

TABLE 2. Tracheal Mucosal Permeability to DTPA and BSA at 1 and 24 h after 2-h Exposure of Resting or Exercising Rats to 0.8 ppm (1.6 mg/m^3) O_3[a]

	Percent of inoculum transferred to entire blood volume at time T_i after tracheal instillation of labeled molecules						
		Clean-air exercise		O_3 rest		O_3 exercise	
Labeled molecule	Clean-air rest	1 h	24 h	1 h	24 h	1h	24 h
DTPA	1.35 ± 0.45	0.96 ± 0.23	0.96 ± 0.13	3.31 ± 2.10[b]	1.91 ± 0.80	4.25 ± 1.73[b]	4.05 ± 2.38[b,c]
BSA	0.70 ± 0.30	0.95 ± 0.60	0.54 ± 0.20	1.08 ± 0.73	1.00 ± 0.64	1.80 ± 1.11[b]	1.50 ± 1.31[b]
	(*n* = 16)	(*n* = 6)	(*n* = 6)	(*n* = 6)	(*n* = 6)	(*n* = 7)	(*n* = 7)

[a]DTPA, [^{99m}Tc]diethylenetriaminepentaacetate; BSA, [^{125}I]labeled bovine serum albumin; data given as mean ± standard deviation.
[b]Significant at $p < 0.05$ when compared with clean-air rest.
[c]Significant at $p < 0.05$ when compared with corresponding time points after exposure to O_3 at rest.

TABLE 3. Tracheal Permeability to DTPA and BSA at 1, and 24 h after a 2-h Exposure of Resting or Exercising Rats to 12 ppm (22.6 mg/m^3) NO_2[a]

	Percent of inoculum transferred to entire blood volume at time T_i after tracheal instillation of labeled molecules				
		12 ppm NO_2 rest		12 ppm NO_2 exercise	
Labeled molecule	Clean air exercise	1 h	24 h	1 h	24 h
DTPA	1.25 ± 0.57 (n = 14)	1.20 ± 0.18 (n = 7)	2.09 ± 0.80[b] (n = 7)	2.53 ± 0.72[b,c] (n = 7)	2.56 ± 0.89[b] (n = 8)
BSA	0.45 ± 0.05 (n = 6)	0.64 ± 0.07 (n = 3)	0.59 ± 0.30 (n = 3)	0.88 ± 0.35[b] (n = 3)	0.50 ± 0.17 (n = 4)

[a]Data as mean ± standard deviation.
[b]Significant at $p < 0.05$ when compared with clean-air exercise.
[c]Significant at $p < 0.05$ when compared with corresponding time points after exposure to NO_2 at rest.

TABLE 4. Bronchoalveolar Mucosal Permeability to DTPA and BSA at 1, 24, and 48 h after a 2-h Exposure of Resting or Exercising Rats to 0.8 ppm (1.6 mg/m^3) O_3[a]

		Percent of inoculum transferred to entire blood volume at time T_i after tracheal instillation of labeled molecules								
		Clean air exercise			O_3 rest			O_3 exercise		
Labeled molecule	Clean air rest	1 h	24 h	48 h	1 h	24 h	48 h	1 h	24 h	48 h
DTPA	0.72 ± 0.23	0.69 ± 0.11	0.86 ± 0.24	0.82 ± 0.15	2.04 ± 0.67[b]	1.14 ± 0.31[b]	0.60 ± 0.12	3.27 ± 0.78[b,c]	2.59 ± 0.37[b,c]	1.08 ± 0.28[b]
BSA	0.70 ± 0.19	0.70 ± 0.14	0.71 ± 0.09	0.79 ± 0.14	1.46 ± 0.45[b]	0.84 ± 0.15	0.81 ± 0.21	2.15 ± 0.62[b,c]	1.50 ± 0.17[b,c]	1.19 ± 0.20[b,c]
	(n = 16)	(n = 6)	(n = 6)	(n = 6)	(n = 7)	(n = 8)	(n = 5)	(n = 7)	(n = 6)	(n = 5)

[a]Data as mean ± standard deviation.
[b]Significant at $p < 0.05$ when compared with clean-air rest.
[c]Significant at $p < 0.05$ when compared with corresponding time points after exposure to O_3 at rest.

posed during exercise to O_3 was greatest at 1 h after the exposure. Permeability to DTPA remained high at 24 h, while permeability to BSA was still significantly elevated at 48 h after the exposure. Permeability to DTPA and BSA in rats exposed during exercise was significantly greater than in rats exposed at rest ($p < 0.05$).

Rats Exposed to NO_2 at Rest or during Exercise Rats exposed while at rest or during exercise to 6 ppm or at rest to 12 ppm NO_2 did not differ from controls, but in rats exposed at exercise to 12 ppm, the bronchoalveolar zone showed significantly increased permeability to DTPA and BSA at 1 h after a 2-h exposure; the increased permeability was absent at 24 and 48 h (Table 5). Permeability to DTPA and BSA at 1 h after exposure of exercising rats to 12 ppm NO_2 was significantly greater than 1 h after exposure of resting rats ($p < 0.05$).

Rats Exposed to O_3 + NO_2 at Rest or during Exercise Bronchoalveolar permeability to DTPA in rats exposed at rest to 0.6 ppm O_3 alone was elevated only at 1 h after exposure, while in rats exposed at rest to 0.6 ppm O_3 + 2.5 ppm NO_2 for 2 h, permeability for DTPA was significantly greater both at 1 h and 24 h after the exposure (Table 6). The increase, however, was not significantly greater than in rats exposed to O_3 ($p < 0.05$).

In rats exposed during exercise to 0.6 ppm O_3 alone, the significant increase in permeability to DTPA that persisted at 24 h after the exposure was similar to that already described for 0.8 ppm O_3 (Table 4). The increase in permeability in rats exposed during exercise to 0.6 ppm O_3 + 2.5 ppm NO_2 was significantly greater than the increase induced by these gases in rats exposed at rest both at 1 h and at 24 h after the exposure ($p < 0.05$).

In rats exposed to O_3 + NO_2 during exercise, permeability was significantly greater at 1 h and 24 h after the exposure than at the corresponding time points in rats exposed during exercise to O_3 alone ($p < 0.05$).

Rats Exposed to Complex Atmosphere at Rest Bronchoalveolar permeability to DTPA in rats exposed for 2 h to a complex atmosphere containing O_3 (0.6 ppm) + NO_2 (2.5 ppm) + SO_2 (5 ppm) + Fe_2O_3 (0.3 mg/m^3) + ammonium, ferric, and manganese sulfates (1.0 mg/m^3) increased by over threefold immediately after the exposure when compared with clean-air-exposed controls (Table 6). As after exposure to O_3 + NO_2, increased bronchoalveolar permeability after exposure to the complex atmosphere persisted at 24 h after the exposure. The increase at 1 h after the exposure was greater than that after exposure of resting rats to O_3, or O_3 + NO_2. The difference between the effects of complex atmosphere and O_3 was, however, not significant ($p = 0.07$), but the difference between the effects of complex atmosphere and O_3 + NO_2 was significant ($p < 0.05$). The increase was significantly greater at 24 h after the exposure to complex atmosphere than at the corresponding time point in rats exposed to O_3 alone at rest ($p < 0.05$).

TABLE 5. Bronchoalveolar Mucosal Permeability to DTPA and BSA at 1 and 24 h after a 2-h Exposure of Resting or Exercising Rats to 6 ppm (11.3 mg/m^3) and 12 ppm (22.6 mg/m^3) NO_2[a]

		Percent of inoculum transferred to entire blood volume at time T_i after bronchoalveolar instillation of labeled molecules							
		6 ppm NO_2 rest		6 ppm NO_2 exercise		12 ppm NO_2 rest		12 ppm NO_2 exercise	
Labeled molecule	Clean-air exercise	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
DTPA	0.64 ± 0.28	0.51 ± 0.05	0.67 ± 0.07	0.66 ± 0.29	0.75 ± 0.31	0.82 ± 0.18	0.88 ± 0.40	1.58 ± 0.43[b, c]	0.82 ± 0.41
BSA	0.43 ± 0.08	0.46 ± 0.11	0.51 ± 0.09	0.43 ± 0.11	0.53 ± 0.19	0.37 ± 0.04	0.41 ± 0.03	0.73 ± 0.21[b, c]	0.46 ± 0.11
	(n = 16)	(n = 3)	(n = 3)	(n = 6)	(n = 6)	(n = 3)	(n = 3)	(n = 6)	(n = 6)

[a]Data as mean ± standard deviation.
[b]Significant at $p < 0.05$ when compared with clean-air exercise.
[c]Significant at $p < 0.05$ when compared with corresponding time points after exposure to 12 ppm NO_2 at rest.

TABLE 6. Bronchoalveolar Mucosal Permeability to DTPA at 1 and 24 h after a 2-h Exposure of Resting or Exercising Rats to 0.6 ppm (1.2 mg/m^3) O_3 + 2.5 ppm (4.7 mg/m^3) NO_2; 0.6 ppm (1.2 mg/m^3) O_3 + 2.5 ppm (4.7 mg/m^3) NO_2 + 5 ppm (13.1 mg/m^3) SO_2 + Fe_2O_3 (0.24 mg/m^3) + Ammonium Sulfate (0.24 mg/m^3), Ferric Sulfate (0.49 mg/m^3), and Manganese Sulfate (0.01 mg/m^3); and 0.6 ppm (1.2 mg/m^3) O_3.

		Percent of inoculum transferred to entire blood volume at time T_i after bronchoalveolar instillation of labeled molecules									
		0.6 ppm O_3 rest		0.6 ppm O_3 exercise		2.5 ppm NO_2 + 0.6 ppm O_3 rest		2.5 ppm NO_2 + 0.6 ppm O_3 exercise		7-Component-atmosphere rest	
Labeled molecule	Clean air rest	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
DTPA	0.75 ± 0.43 (n = 34)	1.89 ± 0.60[b] (n = 6)	1.80 ± 0.08 (n = 6)	1.97 ± 0.28[b] (n = 6)	1.57 ± 0.56[b] (n = 6)	1.72 ± 0.54[b] (n = 15)	1.54 ± 0.76[b] (n = 15)	3.40 ± 1.23[b,c,d] (n = 6)	2.30 ± 0.88[b,c,d] (n = 6)	2.53 ± 0.70[b,c] (n = 6)	1.97 ± 0.67[b,e] (n = 6)

[a]Data as mean ± standard deviation.
[b]Significant at $p < 0.05$ when compared with clean-air rest.
[c]Significant at $p < 0.05$ when compared with corresponding time points after exposure to NO_2 + O_3 at rest.
[d]Significant at $p < 0.05$ when compared with corresponding time points after exposure to O_3 during exercise.
[e]Significant at $p < 0.05$ when compared with corresponding time points after exposure to O_3 at rest.

DISCUSSION

We have previously reported (Bhalla et al., 1986) that the simultaneous application of DTPA and BSA for detecting airway permeability provides a reliable means for studying epithelial injuries localized to various airway regions, i.e., nose, trachea, and bronchoalveoli. No interaction between DTPA and BSA was evident, and the two tracers appeared to be transferred independently across the airway mucosa. A 2-h exposure of resting rats to 0.8 ppm O_3 resulted in an approximately twofold increase in tracheal and bronchoalveolar permeability to DTPA; permeability to BSA also increased, but to a lesser extent. The rates of transfer from lumen to blood and from blood to either a storage or excretion route were fit to a three-component model:

$$\text{lung} \xrightarrow{k_1} \text{blood} \xrightarrow{k_2} \text{output or storage}$$

The transfer coefficients k_1 and k_2 were estimated by an iterative fitting technique. In experiments where concentration in blood was monitored during the first 6 min, concentration rose rapidly in the first 2 min, reached a plateau when the rate of transfer into blood equaled the rate of transfer out of blood, and in some cases began to decline when the rate of transfer out of blood exceeded the rate of transfer in. The increased tracheal permeability was observed for at least 4.5 h after the exposure but not for 24 h after the exposure. The increased bronchoalveolar permeability persisted at 24 h after the exposure, suggesting a more persistent effect of O_3 in this region than in the trachea.

We have now extended these studies to evaluate permeability changes in the tracheal and bronchoalveolar regions of rats exposed under conditions that approach more realistic environmental exposures, i.e., exposure during periods of moderate exercise activity and to atmospheres composed of multiple pollutants. Due to the lack of information on the permeability changes produced by exercise or combined gases and aerosols, we compare our results with reported studies evaluating pulmonary-function changes following exposure during exercise, and with morphologic injuries or biochemical alterations produced by gas or gas-aerosol combinations.

In humans, pulmonary-function changes have been studied after exposure of exercising healthy subjects to O_3 concentrations that do not produce effects during resting exposures. Exercise during exposure to O_3 caused substantial decrements in pulmonary-function measures (Silverman et al., 1976; DeLucia and Adams, 1977; Adams and Schelegle, 1983). Exercise appeared to be a major enhancer of toxicity. Greater total dosages of O_3 inhaled during exercise and the delivery of higher concentra-

tions of the pollutant to distal alveolar surfaces are likely to be the causes of increased toxicity during exercise.

The exercise protocol used in our experiments has been shown to cause a twofold increase in oxygen consumption and was postulated to lead to a twofold increase in minute ventilation (Mautz et al., 1985b). Exposure to O_3 under these conditions increased the proportion of the alveolar zone of lung involved in focal lesions (as measured histopathologically) by about fourfold as compared to the percent of the lung parenchyma bearing lesions after exposure of rats at rest. This degree of alveolitis was greater than predicted on the basis of twofold increase in minute ventilation. A more widespread distribution of inhaled toxic gases in the lung was expected to increase alveolar lesion formation in proportion to increased minute ventilation upon exposure during exercise; hence, the reason for the disproportionately greater lesion formation is unknown (Mautz et al., 1985a).

Increased bronchoalveolar permeability in our studies is consistent with increased histologic changes following O_3 exposure in the alveolar zone (Mautz et al., 1985a; Bils and Christie, 1980; Evans, 1984). Delivery of greater cumulative O_3 doses to the bronchoalveolar region during exercise would explain both increased permeability and percent of alveolar tissue bearing lesions. Although the greater flow rate reduces the fractional absorption in the upper respiratory tract (DeLucia and Adams, 1977), there is an increase in total inhaled O_3. Exposures to higher pollutant concentrations for short periods may produce a greater effect than exposure to lower concentrations for extended periods (Silverman et al., 1976; Hazucha et al., 1973). It is likely that higher total dosage of O_3 per minute were delivered to the trachea and upper airways during exercise and compensated for shorter contact periods in the upper respiratory tract resulting from increased flow rates.

Following exposure of resting rats to O_3, we have noted a more prolonged permeability response in the bronchoalveolar region than in the trachea (Bhalla et al., 1986). A similar response after exposure to O_3 + NO_2 suggests that the gas combination tends to enhance the O_3 effect but does not necessarily alter the target sites for O_3. Since NO_2 by itself does not alter permeability or produce histologically detectable lesions even at much higher concentrations, i.e., 12 ppm exposure at rest, an NO_2 effect as such is not likely to be an influencing factor in the response elicited by the gas combination. Freeman et al. (1974) studied the alveolar lesions produced by a combination of O_3 and NO_2 and concluded that the injury following exposure to this gas combination reflected largely the effect of O_3. In our study, the O_3 injury was probably potentiated by nitric acid vapor formed at 85% relative humidity, rather than by NO_2.

The rationale for and the experimental system for producing experimental atmospheres of O_3 combined with gases that form acid or with

acid aerosols have been described by Kleinman et al. (1984). Kleinman et al. (1985a,b) have reported significant levels of nitric acid vapor in atmospheres containing a complex gas–aerosol mixture in which O_3 and NO_2 were present. Atmospheres of 0.6 ppm O_3, 2.5 ppm NO_2, 5 ppm SO_2, 1.2 mg/m^3 of an aerosol containing $(NH_4)_2SO_4$, $Fe_2(SO_4)_3$, and $MnSO_4$ produced, without aging, 1.4–2.4 mg/m^3 HNO_3 vapor and small amounts of acid aerosol, while O_3 + NO_2 without aerosol produced 2.1–2.8 mg/m^3 of HNO_3. In the alveolar zone of lung sections from rats exposed to ozone alone, 1.7–2.1% of the alveolar area was occupied by focal inflammatory lesions at 18 and 48 h after a single 4-h exposure. The percent of the alveolar area occupied by localized lesions after exposure to O_3 and NO_2 was 2.2% at 18 h and 5.1% at 48 h after exposure, while after exposure to the complex atmosphere, these values were 0.6 and 4.0% (Kleinman et al., 1985a,b). Last et al. (1983) have demonstrated potentiation of the O_3 effect on lung collagen synthesis by either ammonium sulfate aerosol or H_2SO_4. Last et al. (1985) have also reported that H_2SO_4 aerosol is more active than $(NH_4)_2SO_4$ in potentiating the effect of 0.2–1.2 ppm O_3. Mustafa et al. (1984) reported synergism between O_3 and NO_2 when a variety of biochemical parameters was studied in mouse lung. Our results indicate increase in permeability immediately after exposure to O_3, O_3 + NO_2 or the complex atmosphere consisting of these gases plus SO_2, Fe_2O_3, $(NH_4)_2SO_4$, $Fe_2(SO_4)_3$, and $MnSO_4$. The magnitude of increase was approximately the same after exposure to O_3 alone or its combination with other gases. However, significant increase in the persistence of enhanced permeability occurred after exposure to O_3 + NO_2 or to the complex atmosphere but not after exposure to O_3 alone, suggesting extension of the duration of biologic effects by gas combinations. If cellular alterations are more severe after exposure to O_3 combined with NO_2, HNO_3, and acid aerosols than after exposure to O_3 alone, the recovery period for resumption of normal transepithelial transport may be prolonged. Greater pulmonary lesions after exposure to the acid-containing complex atmosphere than after exposure to O_3 alone (Kleinman et al., 1985a,b) could then result from the cumulative effect of alterations that progress over a prolonged period following exposure. When an end point such as permeability is used, the effect of prolongation of injury is seen as the persistence of elevated permeability for a longer duration rather than as a greater elevation of permeability after exposure to the acidic atmospheres as compared to O_3 alone.

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