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CELLULAR AND IMMUNOLOGIC INJURY WITH PM-10 INHALATION

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Airborne particles less than 10 μm (PM-10) in mass median aerodynamic diameter (MMAD) are associated with adverse effects on human health including chronic lung diseases and mortality, but the mechanisms by which these particles might cause or aggravate diseases are not specifically known. PM-10 represents a complex mixture, both in terms of size and chemical composition, and it contains both aqueous-media soluble and insoluble particles. Furthermore, the ambient aerosol composition varies markedly in different locations and at different times in the same location. To test the effects of PM-10 on pulmonary defenses in relation to specific cell targets, barrier-reared Sprague-Dawley rats were exposed to purified air (control), to two important constituents of the fine-particle ($<1 \mu\text{m}$ MMAD) fraction of PM-10—ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4^{2-}]$ (20 or 70 $\mu\text{g SO}_4^{2-} \text{ m}^{-3}$, 0.2 μm MMAD) and ammonium nitrate $[\text{NH}_4\text{NO}_3]$ (90 or 350 $\text{NO}_3^- \mu\text{g m}^{-3}$, 0.6 μm MMAD). Rats were also exposed to resuspended road dust (300 and 900 $\mu\text{g m}^{-3}$, 4.0 μm MMAD), an important contributor to the coarse ($>2.5 \mu\text{m}$ MMAD) fraction of PM-10. Exposures were 4 h/day, 4 days/wk for 8 wk. Macrophage-dependent lung defense functions (antigen binding to Fc receptors and respiratory burst activity) were significantly depressed by NO_3^- , SO_4^{2-} , and the high-concentration road dust exposures, compared to purified air controls. Lung permeability, as determined from measurements of albumin concentrations in bronchoalveolar lavage fluid, was significantly greater in rats exposed to high concentrations of road dust and NO_3^- , but not to SO_4^{2-} , when compared to air-exposed controls. Quantitative histopathologic analyses, which included measurements of alveolar nuclear density, alveolar chord length, alveolar septal thickness, and alveolar cross sectional area, showed moderate to substantial changes. In general, the severity of the responses was in the order of $\text{SO}_4^{2-} > \text{NO}_3^- >$ road dust. The findings are consistent with those of epidemiological studies. This study also supports the hypothesis that the fine fraction of PM-10 is more toxic than the coarse fraction.

In southern California, the air quality standard for airborne particles less than 10 μm in diameter (PM-10) is one of the most frequently violated of the state's ambient air standards. Inhalation of PM-10 at levels near the state's (50 $\mu\text{g}/\text{m}^3$, 24-h average) and below the federal (150 $\mu\text{g}/\text{m}^3$, 24-h average) ambient air quality standards are associated with increased hospital admissions and emergency room visits for respiratory illnesses (Martin, 1964; Greenburg et al., 1967; Samet et al., 1981; Knight et al., 1989), increased incidences of asthma attacks (Whittemore & Korn, 1980; Schenker, 1993), increased asthma medication use (Pope et al., 1991), reduced pulmonary function

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(Pope et al., 1991; Stern et al., 1989; Pope & Kanner, 1993), and increased daily mortality (Schwartz, 1991, 1993; Schwartz & Dockery, 1992; Dockery et al., 1993). Exposure to respirable fine particles and sulfates is consistently and significantly associated with reported total annual mortality rates (Ozkaynak & Spengler, 1985; Ozkaynak & Thurston, 1987). People whose deaths have been associated most significantly with PM-10 exposures have been generally over 60 yr of age, often bedridden (at home or in a medical facility), and often having preexisting cardiovascular or chronic pulmonary disease (Utell, 1994).

The state, as well as the federal, standard is expressed in terms of sampled mass, and does not discriminate between particles of different chemical composition or different sizes within the overall particle diameter (d_p) ≤ 10 μm size classification. The health effects associated with PM-10 toxicity are likely to be dependent upon both chemical composition and particle size. However, laboratory studies have not demonstrated convincing mechanistic links between exposure to PM-10 components and pathology. The association between PM-10 exposure and adverse health effects has not been previously tested in a systematic manner to determine whether the effects were attributable to specific components of the ambient aerosol and whether specific aspects of the lung's defensive systems were impaired. Such studies could elucidate the mechanisms by which PM-10 might be associated with acute mortality. This study used the rat as a model to assess the potential of selected PM-10 components for modifying biological responses that could be related to the mechanisms by which PM-10 might affect human health. The primary hypothesis tested was that PM-10 toxicity would be dependent upon the chemical composition of the aerosol.

The relationship between PM-10 inhalation and acute mortality is an area of continuing concern. The mechanisms by which inhaled particles might precipitate fatalities are not clear; however, there are some likely possibilities. For example, inhaled particles might impair the integrity of the lung's epithelial barrier, causing increased leakage of serum and proteins into the lung, ultimately leading to pulmonary edema and death. Impairment of pulmonary host defenses by inhaled particles could permit development of acute respiratory infections, or could cause acute inflammatory reactions with the release of reactive oxygen species and cytotoxic chemicals in the lung. These could lead to the exacerbation of existing chronic pulmonary diseases. It is also possible that mediators and cytokines released by particle-injured pulmonary cells could enter the circulation and cause cardiac arrhythmia. These acute effects might be seen in humans with a long-lasting exposure to ambient particles. We therefore planned studies of an 8-wk duration.

This study analyzed the effects of three PM-10 components (ammonium sulfate, ammonium nitrate, and road dust), selected because they represented important fractions of ambient PM-10 aerosols (Chow et al., 1992). Two concentrations of each component were used: a low level representative of

estimated peak 4-h concentrations, based on extrapolations from ambient air data, and a high level based on estimated worst-case peak concentrations. The sizes of the particles used were chosen based upon reported sizes of fine- and coarse-mode inorganic aerosols in ambient air (John et al., 1990). The primary endpoints that were measured to detect adverse effects included assessing changes in airway permeability; histopathological alterations in the lung, including thickness and cellularity of alveolar walls, alveolar chord lengths and alveolar cross-sectional diameters; and assays related to host defenses (macrophage Fc receptor binding capacity and release of biocidal compounds such as superoxide anion during respiratory bursts). Exploratory endpoints that were measured but that did not show any significant responses included determinations of the numbers of goblet cells in the respiratory epithelium, releases of the inflammatory mediators prostaglandin E₂ (PGE₂) and leukotriene B₄ (LTB₄), and measurements of respiratory frequency and tidal volume during exposure.

METHODS

Atmosphere Generation and Characterization

Ammonium nitrate and ammonium sulfate aerosols were generated by nebulization of dilute aqueous solutions. Road dust, which was collected by vacuum sweeping of freeway surfaces by the State of California transportation authority, CALTRANS, was disaggregated, sieved, and sterilized. The freeway sampled was relatively new, and dust was collected from a region that had not been subject to any known spills of hazardous materials. Road dust aerosol was produced using a custom-built fluidized-bed generator. Particles thus generated were passed through a verticle elutriator at a linear rate of flow equivalent to the settling velocity in air of 10- μ m-diameter unit density particles. The nitrate, sulfate, and road dust particles were mixed with dry dilution air, brought to Boltzmann charge equilibrium by passage through an ⁸⁵Kr aerosol charge neutralizer, equilibrated to 60% relative humidity, and then introduced into the exposure system airstream.

Size-classified aerosol samples were collected biweekly using cascade impactors (Sierra model 226 and Andersen model 298; Graseby/Andersen, Atlanta, GA). A real-time aerosol monitor (RAM-1; MIE, Inc., Billerica, MA) provided real-time mass concentration data during each exposure. Integrated 4-h samples of aerosol particles were collected daily from the rat's breathing zone on preweighed and equilibrated (50% relative humidity) Pallflex T60A20 Teflon-coated glass fiber filters (Pallflex Co., Putnam, CT). The filters were equilibrated to 50% relative humidity and weighed to determine total mass. The filters were extracted with dilute aqueous 3 mM Na₂HCO₃, 2 mM Na₃CO₃, and the extracts were analyzed for SO₄²⁻, and NO₃⁻ by ion chromatography. Elemental carbon was determined on samples collected on quartz-fiber filters. These filters were combusted on pure oxygen, and the

resulting CO₂ was quantitated using a modified infrared absorption monitor (model 3003, Dasibi Environmental, Glendale, CA). Samples of road dust collected on membrane filters from the breathing zones of rats during exposures were analyzed for elemental components by x-ray fluorescence spectrometry. Ozone was monitored using an ultraviolet (UV) absorption monitor (model 1003 AH, Dasibi Environmental, Glendale, CA).

Animal Housing and Exposure

A total of 144 F344/N rats (Simonsen Laboratories, Inc., Gilroy, CA), barrier reared and maintained in laminar flow isolation units supplied with filtered air, was randomly assigned to treatment groups and exposed nose-only to prevent artifacts due to airborne dander, ammonia, and dried excreta in the inspired air. Groups were exposed to high or low concentrations of pollutants. Exposures were 4 h/day on 4 consecutive days per week for a total exposure time of 32 days over an 8-wk period. Rats were exposed to purified air, road dust, nitrate, or sulfate. Separate groups were assessed for histopathology endpoints and for macrophage and permeability-related endpoints. The high-concentration exposure groups were tested in one set of exposures and the low-concentration groups were tested in a separate set of exposures. Separate purified air control groups were used for high- and low-concentration exposures. Between exposures, rats were housed in a purified air-barrier environment and had access to clean water and dry laboratory feed ad libitum. The vivarium was AAALAC accredited, and all animal protocols were reviewed and approved by an Institutional Review Board.

Necropsy, Tissue Preparation, and Morphometric Analysis

Rats were killed 1–2 h after the end of exposure. Rats were anesthetized with ip pentobarbital (75 mg/kg body mass), and the tracheas were tied off just below the larynx. The lung and trachea were removed and fixed by inflation with neutral buffered formalin (pH 7.2) at a constant 30 cm H₂O pressure for 72 h. After fixation the left lung was embedded in paraffin and sectioned longitudinally along the left main airway. Sections (5 μ m thick) were cut from the paraffin-embedded blocks (one section per block per animal) and stained with alcian blue/periodic acid Schiff (ABPAS) reagent. Section area was measured from slabs before embedding and from slides after staining, and shrinking during embedding did not differ among groups. Lung sections were analyzed morphometrically using stratified random selection of 25 fields per section and avoiding bronchi and large blood vessels. Measurements included alveolar wall thickness, alveolar wall cellularity (nuclear density), alveolar chord length, and alveolar cross-sectional area, as described by Rasmussen and McClure (1992).

Macrophage Function Analysis

Bronchoalveolar lavage was performed on rats ($n = 12$ per atmosphere) to obtain macrophages for immunological testing and proteins for assessment of

epithelial permeability (Kleinman et al., 1993). The rats were anesthetized, the abdominal aortas were severed, and the tracheas were exposed. A catheter was inserted into the trachea and tied in place. The lungs were lavaged with HEPES-buffered (pH 7.2) Hanks balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+} (GIBCO, BRL, Gaithersburg, MD). The lavage volume was 7 ml, and it was instilled and aspirated 3 times at a rate of about 0.5 ml/second. The lavage was repeated three times per animal and the recovered fluid from each lavage was placed on ice. The lavage fluid from each animal was centrifuged $300 \times g$, 4°C , for 10 min. The fluid from the first lavage was reserved for protein and biochemical assays. The cell pellets from all three lavages were pooled and resuspended in 3 ml HBSS with Ca^{2+} and Mg^{2+} .

The cells were counted using a bright-line hemocytometer. Viability was assessed by trypan blue exclusion. The cell suspension was adjusted to 10^6 viable cells/ml. The yield by this lavage procedure was typically 3 million cells/rat, of which more than 95% were macrophages with an average viability of greater than 90%. A 0.1-ml aliquot of cells was plated onto a glass microscope slide using a cytocentrifuge (Shandon Inc., Pittsburgh, PA). The cells were stained with Wright-Giemsa stain, and a differential count was made using previously described procedures (Nadziejko et al., 1992; Kleinman et al., 1993).

Changes in functional characteristics of alveolar macrophages (12 rats/atmosphere) were quantified by a rosette assay (Prasad et al., 1988; Kleinman et al., 1993) for determining Fc receptor binding capacity, and by determination of the production of superoxide anion during respiratory burst activity. To measure the ability of macrophages to bind sheep red blood cells (SRBCs) to Fc receptors, adherent macrophages (10^5 cells) were incubated in eight-well microtiter chambers (Fisher Scientific, Tustin, CA) with rat anti-sheep red blood cell antibody (30 min at 37°C) to allow the antibody to bind to the macrophage Fc receptors. The excess, unbound antibody was removed by rinsing, and the macrophages were incubated with 10^6 SRBCs (30 min at 37°C). Excess red blood cells were removed by rinsing, and the number of macrophages forming rosettes with 3 or more SRBCs were counted out of a total sample of 300 cells per chamber.

Superoxide anion production was measured using a cytochrome c reduction method (Nadziejko et al., 1992; Kleinman et al., 1993), as follows. Aliquots (100 μl) of the cell suspension (10^6 macrophages/ml) from each exposed rat were added to the wells of a 96-well microtiter plate. These wells then received 50 μl of cytochrome c (200 μM in HBSS) and 50 μl of freshly opsonized zymosan (5 mg/ml) was added, and the cultures were incubated in the dark at 37°C for 60 min. Absorbance at 540 and 550 nm was read using a microtiter plate reader. The difference in absorbance ($A_{550} - A_{540}$) was multiplied by the molar extinction coefficient for reduced cytochrome c, and the results were expressed as nanomoles per milligram protein per 60 min.

Permeability Methods

Permeability was determined by measurement of albumin concentrations in the BAL. An enzyme-linked immunosorbent assay was used for determining albumin in BAL samples (Bhalla et al., 1992). Polystyrene nonflexible 96-well microtiter plates (Costar, Cambridge, MA) were coated with 100 μl of 2 ng/ μl goat anti-rat albumin antibody (GAR) (Organon Teknika; Durham, NC) in carbonate buffer, pH 9.6. The plates were covered and refrigerated overnight (18 h). The plates were washed two times with freshly made carbonate buffer. Nonspecific binding was blocked by adding 150 μl of a gelatin-carbonate buffer (4 mg gelatin/ml carbonate buffer) to each well, which was then removed by washing 2 times with a solution of PBS-Tween 20-gelatin (0.5 ml Tween 20/L PBS + 1.0 mg gelatin/ml) after 2 h of incubation at 20°C. Serial dilutions of a standard (15 mg/ml) rat albumin (Sigma Chemical, St. Louis, MO) from 1 : 128,000 to 1 : 4,096,000 and rat lavage fluid from 1 : 800 to 1 : 3200 were made using PBS-Tween 20-gelatin solution. Each well received 100 μl of the diluted standard or lavage fluid. The plates were covered and incubated in a humid chamber for 1–2 h at room temperature and then washed 3 times with PBS-Tween 20-gelatin. A 1 : 2000 dilution of rabbit anti-rat immunoglobulin G (IgG) peroxidase-conjugated albumin (5.0 mg/ml) was made and 100 μl was added to each well. The plates were incubated in a humid chamber at room temperature for 1 h, washed 2 times with PBS-Tween 20-gelatin, and once with PBS-Tween 20. The color was developed by the addition of 100 μl /well of citrate-phosphate buffer (pH 5.0), which contained 1 mg/ml *o*-phenylenediamine dihydrochloride (OPD) and 1 μl /2 ml of 30% H_2O_2 . The plates were covered and incubated in the dark at room temperature for 20 min. The reaction was stopped by the addition of 50 μl /well of 2 N H_2SO_4 . The plates were read at 492 nm with a Tekan Microtiter plate reader (Hillsboro, NC).

Statistics

Data were analyzed using one-way or two-way analyses of variance, as appropriate for a given endpoint. The Tukey multiple comparison test was used for a posteriori testing of differences between group means. The criterion for statistical significance was set at $p \leq .05$.

RESULTS

Particulate Characteristics

The sizes and concentrations of nitrate, sulfate, and road dust (used as a surrogate for crustal material) to which rats were exposed are shown in Table 1, along with the concentrations and aerodynamic diameters of PM-10 and the nitrate, sulfate, and crustal components measured during intensive sampling campaigns in three heavily polluted southern California communities.

TABLE 1. Maximum 24-h PM-10 Concentrations in Southern California Ambient Air and Studied Concentrations (mg/m³)

City (year)	Sulfate	Nitrate	Crustal	PM ₁₀
Azusa (1986)	0.020	0.060	—	0.183
Rubidoux (1988)	0.021	0.093	0.172	0.243
Riverside (1988)	0.017	0.092	0.151	0.202
Size range (μm)	0.2–0.5	0.4–0.8	4–5	≤10
Studied concentrations				
Low	0.020	0.090	0.300 ^a	
High	0.070	0.350	0.900	
MMAD (μm)	0.2	0.6	5	

^aRoad dust used as surrogate.

Histopathology

Detailed morphometric analyses were performed on lungs of rats exposed to purified air or to the high concentrations of PM-10 components. The results demonstrate significant ($p < .05$) PM-10-induced changes. Alveolar wall nuclear density (Figure 1a) was increased slightly, but not significantly, by road dust and sulfate, but was significantly increased by nitrate. Alveolar septal wall thickness was increased significantly by all three PM-10 components (Figure 1a). Although the nitrate-exposed rats tended to exhibit greater increases in

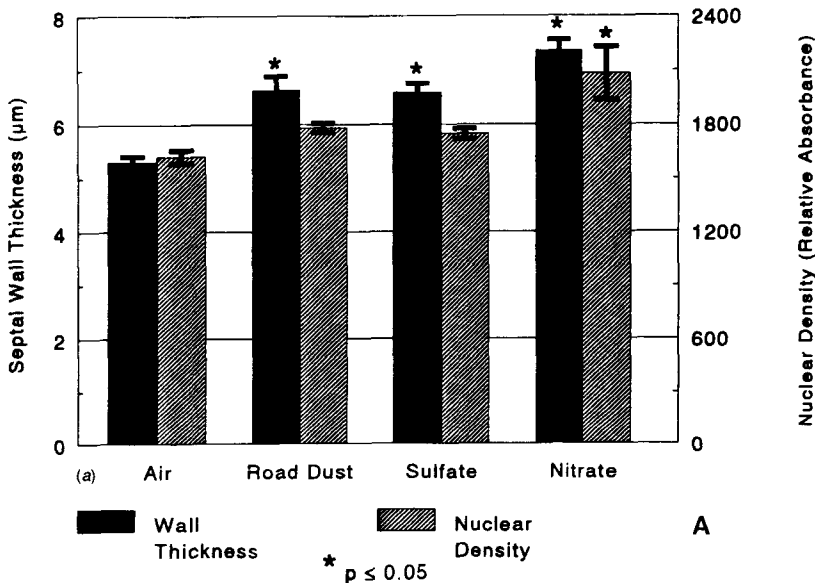


FIGURE 1. (a) Changes in alveolar wall thickness and alveolar wall cellularity, as measured by nuclear density, following exposure to road dust (900 μg/m³), ammonium sulfate (70 μg/m³), and ammonium nitrate (350 μg/m³). Means and SE are shown.

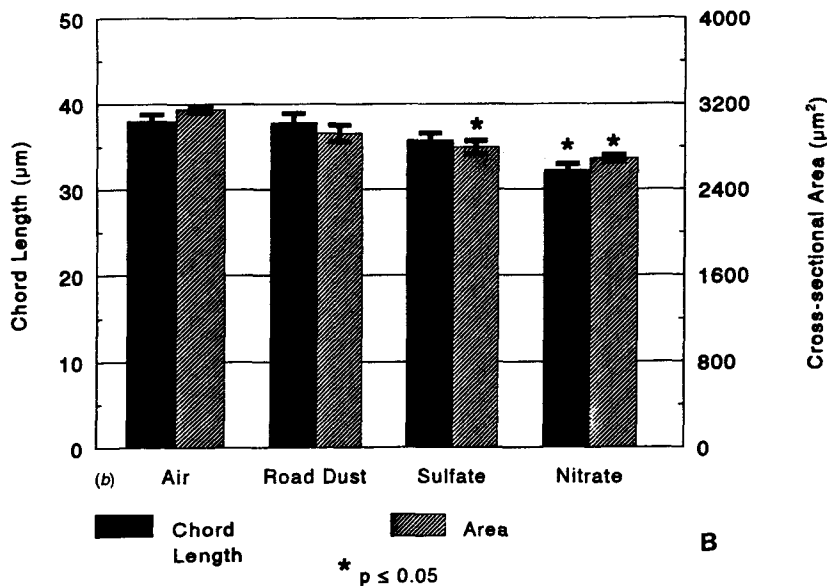


FIGURE 1. (Continued) (b) Changes in alveolar chord length and alveolar cross-sectional area following exposure to road dust ($900 \mu\text{g}/\text{m}^3$), ammonium sulfate ($70 \mu\text{g}/\text{m}^3$), and ammonium nitrate ($350 \mu\text{g}/\text{m}^3$).

both nuclear density and wall thickness than the sulfate- or road dust-exposed groups, the group mean values were not significantly different. Both alveolar chord length, a measure of average alveolar linear dimension, and alveolar cross-sectional area tended to decrease in the groups exposed to PM-10 components as compared to groups exposed to purified air; groups exposed to sulfate showed a significant decrease in area, and groups exposed to nitrate showed significant decreases in both chord length and area (Figure 1b).

Macrophage Function

Cells were recovered from rats by bronchoalveolar lavage. On the average, viability was greater than 90%, there were no exposure-related increases in numbers or percentages of lymphocytes or polymorphonuclear cells, and macrophages represented between 95 and 100% of the recovered cells. Macrophages from rats exposed to PM-10 components exhibited depressed ability to attack antigenic material (SRBC) via Fc receptor-mediated processes (Figure 2). A significant ($p = .018$) main effect of atmosphere was observed after the low-concentration exposures, but paradoxically, effects after high-concentration exposures were not statistically significant. The group mean effects were small, overall, and at the low concentration significant reductions were seen only after exposures to SO_4^{2-} . The production of superoxide during respiratory burst activity (Figure 3) was significantly depressed by high concentrations of SO_4^{2-} and road dust ($p < .05$). Exposures to all three pollutants at low concentrations produced a trend toward increased

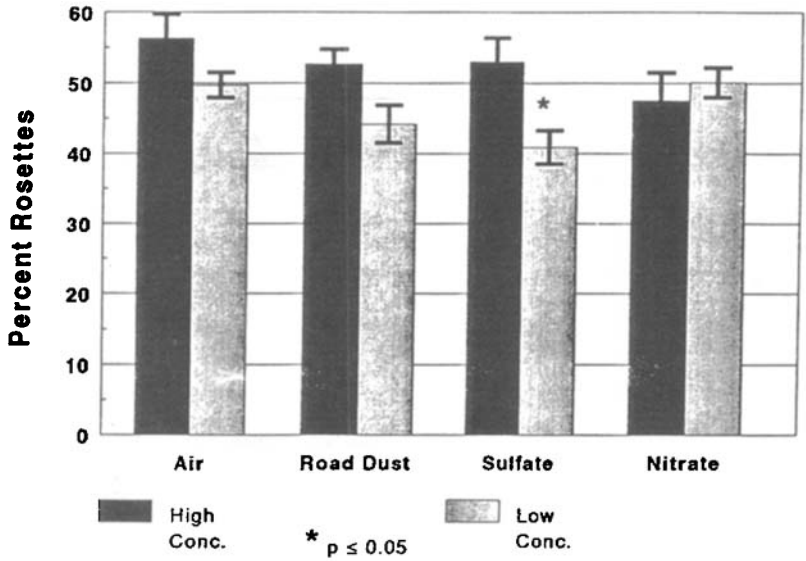


FIGURE 2. Changes in macrophage Fc receptor binding activity after exposure to PM-10 components at high and low concentrations: road dust, 900 or 300 $\mu\text{g}/\text{m}^3$; ammonium sulfate, 70 or 20 $\mu\text{g}/\text{m}^3$; and ammonium nitrate, 350 or 90 $\mu\text{g}/\text{m}^3$.

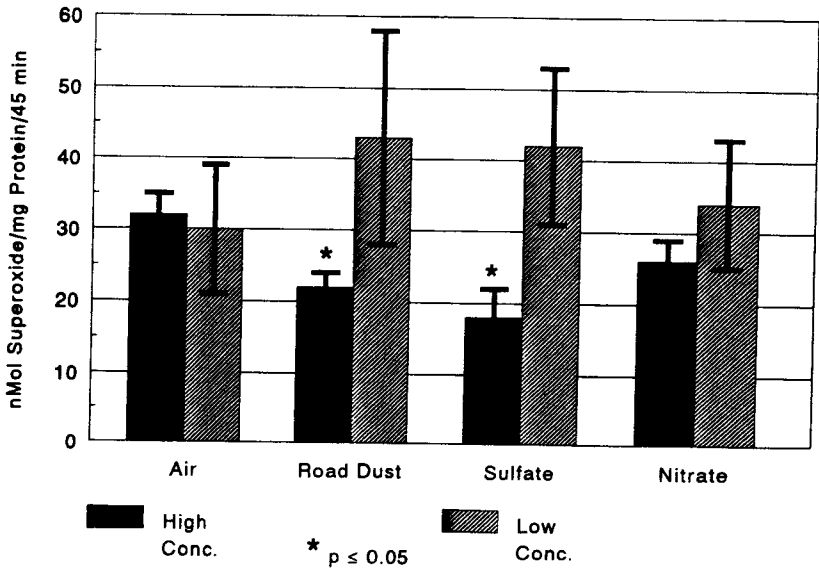


FIGURE 3. Production of superoxide anion during respiratory burst activity after exposure to PM-10 components at high and low concentrations: road dust, 900 or 300 $\mu\text{g}/\text{m}^3$; ammonium sulfate, 70 or 20 $\mu\text{g}/\text{m}^3$; and ammonium nitrate, 350 or 90 $\mu\text{g}/\text{m}^3$.

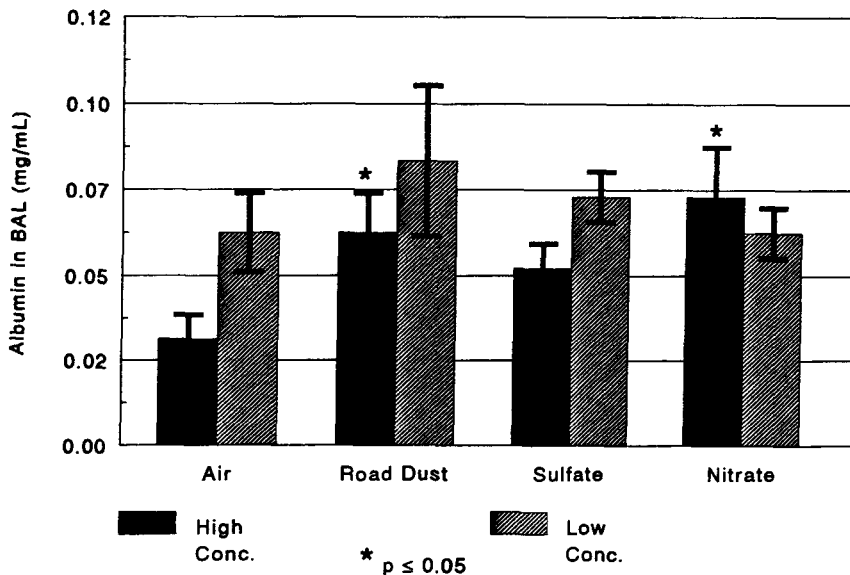


FIGURE 4. Changes in epithelial permeability as measured by the concentration of albumin in bronchoalveolar lavage fluid after exposure to PM-10 components at high and low concentrations: road dust, 900 or 300 $\mu\text{g}/\text{m}^3$; ammonium sulfate, 70 or 20 $\mu\text{g}/\text{m}^3$; and ammonium nitrate, 350 or 90 $\mu\text{g}/\text{m}^3$.

production of superoxide by macrophages, but the changes were not significantly different from the purified air group values.

Permeability

Bronchoalveolar lavage fluid (BAL) was analyzed for albumin, which served as an indicator of epithelial disruption and increased mucosal permeability. The results are shown in Figure 4. Increased ($p < .05$) permeability was observed in rats exposed to high concentrations of road dust and nitrate-containing atmospheres. After low-concentration exposures, albumin level in the lavage fluid from all of the exposure groups was slightly, but not significantly, elevated when compared to the controls.

DISCUSSION

This study demonstrated several biological effects in rats exposed to selected components of PM-10 and provided insights into possible mechanisms linking ambient PM-10 exposures with increased acute human morbidity and mortality. The histopathologic changes observed in this study included increases in alveolar wall thickness and decreases in alveolar lumen cross-sectional area. These findings could result from a decrease in compliance, or a "stiffening" of the lung, which, under constant fixative inflation pressure, could cause the lungs to become fixed with a smaller inflation volume. The possible loss of compliance could be due to early fibrogenic changes in the

lung, or it could be due to increased infiltration of fluid and cellular components associated with the increased permeability we observed. The latter mechanism is consistent with our observation of increased alveolar wall nuclear density. It is likely that both factors may contribute, since permeability changes may be early indications of inflammatory processes, which could eventually result in fibrogenesis and the development or aggravation of chronic lung disease. This study did not examine chronic effects, and it is an open question as to whether human populations with high ambient exposures to PM-10 might exhibit decreased lung compliance and possibly decreased pulmonary function as measured by forced expiratory maneuvers. While some epidemiologic study findings might support this contention, the specific role played by PM-10 as compared to that played by oxidant gases such as ozone is not clear, since acute ozone exposure can cause many of the same effects and the same populations might be exposed to both high PM-10 and high ozone concentrations (albeit not necessarily at the same time).

The permeability data shown in Figure 4 demonstrate a "batch" effect. These data represent two independent studies, one with high-concentration atmospheres and one with low-concentration atmospheres, which used batches of rats purchased at separate times. The protein concentrations measured in the high-concentration study were higher overall than those in the low-concentration study. In each study, the statistical comparisons are with a control group derived from the same batch of rats, treated in an identical fashion to the atmosphere groups and sacrificed on the same day. The increase in lung permeability observed in this study suggests that following exposures to PM-10 components, plasma or serum factors could enter the lung, infiltrating through disrupted tight junctions between epithelial cells (Bhalla et al., 1986). It is not established whether the tight junction disruption is due to changes in the characteristics or receptors for specific cell adhesion molecules, or if it is due to disarrangement of the cytoskeletal structure of these epithelial cells (Bhalla et al., 1990). In extreme cases, this increase in permeability could presumably produce pulmonary edema, which in individuals with compromised cardiopulmonary systems could be life threatening. Another aspect of this permeability barrier breakdown is that it offers an opportunity for toxic or allergenic materials that are deposited in the lung to infiltrate into the subepithelial layers (Bhalla et al., 1988), from which they may be removed much more slowly than they would be from the epithelial surface. This would result in increased contact time of potentially sensitive cells with compounds that could provoke asthmatic responses in some individuals and increase the risk of toxic, mutagenic, or carcinogenic effects. One aspect that could increase the possible importance of permeability changes as a key mechanism for linking PM-10 exposure to increased acute mortality would be evidence of age-related susceptibility. After reviewing the available literature, it is not clear that elderly individuals are more susceptible to permeability changes than are healthy younger adults. How-

ever, in subjects with interstitial lung disease, increased concentrations of albumin in epithelial lining fluid were related to increased age (Roberts et al., 1993). There is evidence from animal studies that neonates and infants show larger increases in permeability than animals with mature lungs when challenged with histamine (Arakawa et al., 1992) or barotrauma (Adkins et al., 1991), and baseline permeability of the newborn lung is greater than that in more mature lungs (Mills & Haworth, 1991; Adkins et al., 1991; Hutchison et al., 1985). This suggests that neonates and infants may represent a sensitive population subgroup whose response to PM-10 exposure should be evaluated.

Macrophages from rats exposed to PM-10 components exhibited decreased antibody-directed ability to bind antigenic material to Fc receptors on the macrophage cell membrane but only SO_4^{2-} produced a group mean value significantly different from that of the control group. We did not observe a significant effect of road dust at either high or low concentration. Ziegler et al. (1994), on the other hand, showed reduced expression of Fc receptors on macrophages from road dust-exposed rats, but not nitrate-exposed rats (SO_4^{2-} exposed rats were not studied). This discrepancy may be due to methodological differences (Ziegler studied fixed cells while this study examined live cells) or may relate to differences in specific Fc receptors. More research will be needed to resolve these differences. The present study also demonstrated that high-concentration road dust and sulfate exposures reduced the ability of macrophages to mount a respiratory burst to generate superoxide and presumably other biocidal and bacteriocidal compounds that are an important part of the lung's defense against inhaled pathogens. It is interesting to note, however, that at lower concentrations there was a tendency (which was not statistically significant) for macrophages from PM-10 component-exposed rats to generate excess superoxide. Ruiz et al. (1988) have reported that macrophages from children exposed to high environmental levels of airborne particles in Santiago, Chile, show increased production of reactive oxygen compounds and concomitantly that there was a reduction of circulating antioxidant compounds, indicative of oxidative stress. Oxidative stress has been linked with the development of cardiopulmonary and cardiovascular diseases. Our findings do not rule out a mechanistic link between PM-10 exposure and the development of heart and lung disease and suggest the need for additional studies.

This study demonstrated significant changes in lung morphometry, permeability, and alveolar macrophage functions following exposures to PM-10 components. The most consistent effects were observed in rats exposed to the two water-soluble PM-10 components, nitrate and sulfate. In terms of potency relative to the concentrations of each component administered, the effects could be ordered as $\text{SO}_4^{2-} > \text{NO}_3^- > \text{road dust}$. Both sulfate and nitrate are predominantly found as submicrometer particles in ambient PM-10, and the sizes generated for this study were 0.2 and 0.6 μm MMAD, respectively. Although, in ambient air, road dust is mostly found in the supermicrometer particle fraction of PM-10, there are small but significant contri-

butions of crustal elements from road dust to the submicrometer particle fraction of PM-10 (Ehrman & Pratsinis, 1992). In this study only 4- μm MMAD particles were tested. Further studies with other sizes of road dust particles will be needed before this component can be ruled out from among the possible contributors to the apparent effects of ambient PM-10 exposure on human health. The results of this study suggest that the mechanisms responsible for specific airway responses may be different for individual PM-10 components, although the nature of these mechanisms is not yet clear.

In summary, this study demonstrated the capability of soluble and insoluble PM-10 components to produce pulmonary effects following repeated exposures. Submicrometer PM-10 components altered morphometric measures of the lung parenchyma, depressed macrophage functions related to defenses against respiratory infections, and increased lung permeability. Such changes could increase susceptibility to respiratory infections and could exacerbate asthma in sensitive individuals. The findings, then, are consistent with those of epidemiological studies, and demonstrate some mechanisms by which PM-10 exposure may be related to morbidity or mortality.

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