THE EFFECTS OF INHALED SULFURIC ACID AEROSOLS
ON ALVEOLAR MACROPHAGE PHAGOCYTOSIS

by

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ABSTRACT

New Zealand white rabbits, 1-2 kg, were exposed, in vivo, to individual sulfuric acid aerosol concentrations of 46 to 323 mg/m$^3$ for 0.5 to 4 hours. Pulmonary alveolar macrophages were recovered and incubated with radiolabelled microspheres, in vitro, to determine percent phagocytosis.

No relationship between percent phagocytosis and a CT index (concentration x exposure length) was observed, although a steady increase in phagocytosis with an increase in incubation time was seen in test and control rabbits. The phagocytic index was depressed in all exposed rabbits at all incubation times employed. The maximum depression occurred at the 1.0 hour incubation and decreased at the longer incubation times. The phagocytic index approached the control index at 4 hours incubation, suggesting recovery. Viability and percent macrophages in recovered populations were not affected by exposure to H$_2$SO$_4$ aerosols regardless of concentration and exposure duration. Conversely, the concentration of red blood cells increased as the H$_2$SO$_4$ concentration increased. Morphological changes, observed by scanning electron microscopy, revealed shortened and matted cilia in the deeper segments of the superior rami. Signs of toxicity, i.e., sneezing and pawing at the nose, increased in frequency and duration as the H$_2$SO$_4$ concentration increased.
INTRODUCTION

Solid and liquid fossil fuels generally contain appreciable amounts of sulfur. Combustion of these fuels in power plants, ore smelters, petroleum refineries, and other industries forms sulfur oxides, primarily sulfur dioxide. The oxidation of atmospheric sulfur dioxide results in the formation of aerosols of sulfuric acid, bisulfates, and sulfates. These account for about 5% to 20% of the suspended particulates in the air.

Atmospheres polluted with sulfur oxides directly and indirectly attack and damage a wide range of materials and property. Various species, including man, respond to sulfur dioxide with constriction of the pulmonary bronchioles. This is manifested by an increase in airway resistance. Sulfuric acid has been shown to be a more potent irritant than $\text{SO}_2$. Insufficient data are available for the quantitative assessment of the health hazards associated with sulfuric acid.

Sulfuric Acid Formation

Sulfur dioxide emitted from any given source will be dissipated and diluted readily because of its mobility as a gas. Sulfuric acid aerosol is much less mobile and will persist for a longer time. Approximately 2-3% of the $\text{SO}_2$ formed in fuel combustion is oxidized to sulfur trioxide ($\text{SO}_3$) in boiler-flue systems.
Part of this $H_2SO_4$ may be deposited on stack walls and the rest emitted to the ambient air. When emitted to the ambient air, drops of concentrated $H_2SO_4$ react according to (a) in Figure 1 (Brosset, 1973). These drops pick up water and eventually form drops of approximately $5\ M\ H_2SO_4$.

The $SO_2$ that is not oxidized in the stack reacts by either photochemical or catalytic oxidation. The predominant mechanism, and extent, of oxidation for the conversion of $SO_2$ is determined by a number of factors. These include the $SO_2$ concentration, residence time in the atmosphere, temperature, humidity, and presence of other pollutants such as metal oxides, hydrocarbons, and nitrogen oxides. Catalytic oxidation is illustrated in (b) of Figure 1. In the atmosphere, $SO_2$ comes in contact with very small particles covered by an aqueous film or with water drops. The film on the drops may contain dissolved or suspended substances, e.g., iron compounds. Solutions of $SO_2$ in water form $H^+$, $HSO_3^-$, and $SO_3^{2-}$ ions. Under the influence of the iron compounds acting as catalysts, $HSO_3^-$ and $SO_3^{2-}$ are rapidly oxidized to $H_2SO_4$ (Johnstone and Moll, 1960). The process ceases when the solution reaches $pH = 2$ (Junge and Ryan, 1958).

Junge and Ryan (1958) also showed that: a) the maximum possible sulfate formation is a linear function of the partial pressure of $SO_2$ in the atmosphere; b) sulfate formation can account for observed sulfate concentrations in rain, fog, and smog only if some neutralizing cation such as $NH_4^+$ is present; c) no appreciable oxidation takes place in the
Figure 1. Formation of sulfuric acid mist by catalytic oxidation. -- From Brosset (1973).
absence of a catalyst; and d) initial oxidation is rapid and controlled by \( \text{SO}_2 \) concentration.

The photochemical reaction rate of \( \text{SO}_2 \) in clean air is very slow (Urone, Schroeder, and Miller, 1970). The oxidation rate is enhanced by oxidant(s) produced in the NO-olefin photochemical system (Cox and Penkett, 1971; Urone et al., 1968). According to Cox and Penkett (1971), the initiating reaction is the photolysis of \( \text{NO}_2 \) to give an oxygen atom. A complex series of reactions involving ozone and the hydrocarbons leads to the formation of photochemical smog and \( \text{H}_2\text{SO}_4 \).

Urone et al. (1968) exposed known amounts of radiochemically tagged \( \text{SO}_2 \) in air to controlled amounts of \( \text{NO}_2 \), hydrocarbons, water vapor, particulates, and sunlight or 3500 Å ultraviolet irradiations. High reaction rates (approximately 50% of the residual \( \text{SO}_2 \)) were observed for irradiated mixtures containing \( \text{NO}_2 \) and hexane. Low reaction rates occurred for mixtures having large excesses of \( \text{NO}_2 \), or \( \text{NO}_2 \) in the absence of hexane. Thus, the rate of disappearance of \( \text{SO}_2 \) and the formation of \( \text{H}_2\text{SO}_4 \) aerosol increase when \( \text{SO}_2 \) is photolyzed in the presence of olefinic hydrocarbons and \( \text{NO}_x \) (Bufalini, 1971; Urone et al., 1970; Urone et al., 1968).

Gartrell, Thomas, and Carpenter (1963) investigated, in situ, the atmospheric oxidation of \( \text{SO}_2 \) in the effluent from the coal-burning Colbert power plant. They found that \( \text{SO}_2 \) oxidation ranged from 0% at 1-1.5 miles to 55% at 8-10 miles from the plant. An oxidation rate of approximately 0.5%/minute was maintained in fog or mist. In the absence of fog or mist but with a high relative humidity (approximately 75%),
oxidation rates of 1-2%/minute were found. Oxidation rates in natural sunlight were 0.1-0.2% in the absence of a catalyst.

Physical Effects of Sulfuric Acid Aerosol

Sulfuric acid aerosol is oxidized in the atmosphere and plays a role in reduced visibility. Visibility is decreased by the scatter and absorption of visible radiation by molecules in the air and by aerosol particles. Sulfuric acid is a hygroscopic material which absorbs water to form light-scattering droplets of sulfuric acid mist. The density of the haze depends on the prevailing relative humidity, i.e., the higher the relative humidity, the more water is available for absorption; larger particles are produced and result in a more dense haze. Complete oxidation of 0.05 ppm SO$_2$ at 50% relative humidity results in a significant reduction of visibility (Gartrell et al., 1963).

Under normal conditions, damage to metal surfaces by the oxides of sulfur increases with increasing relative humidity and temperature. Sulfur oxides generally accelerate corrosion by first being converted to sulfuric acid, either in the atmosphere, or on the metal surfaces themselves. The corrosion products are primarily sulfate salts of the exposed metals. In addition, atmospheric sulfuric acid can react with some suspended particulates to form sulfate salts that can also accelerate corrosion ("Air Quality Criteria for Sulfur Oxides," 1969).

Atmospheres containing sulfur oxides also attack and damage a wide variety of building materials, e.g., limestone, marble and mortar, as well as statuary and other works of art, causing discoloration and physical deterioration ("Air Quality Criteria for Sulfur Oxides," 1969).
Toxicity of Sulfuric Acid Aerosol

The toxicity of airborne material is related to the particle size as well as to its atmospheric concentration. Determination of the size of sulfate particulates has shown that the major portion are smaller than 0.5 μm in diameter (Brosset, 1973). These are sufficient grounds for assuming that acid particles also fall within the respirable range, 5 μm. This, to a large extent, determines the amount of toxic substance that penetrates the upper respiratory tract and also the pattern of deposition of the aerosol in the lungs.

Sulfuric acid has been shown to create adverse biological effects, i.e., bronchoconstriction and irritation, at air concentrations less than those of SO₂. In addition, sulfuric acid has been found to be 20-60 times more potent in causing airflow resistance than is SO₂ (Air Quality and Stationary Source Emission Control, 1975; Alarie et al., 1975; Amdur, 1969).

Basic information on the nature of irritant action is important in assessing the irritant potential of an air pollutant. Submicron particles will most likely provoke an irritant response because a) they penetrate the deeper areas of the lung, and b) they have a large surface area for absorption and possible chemical interaction. The degree of irritation decreases with a decrease in aerosol concentration. The evaluation of irritant action of H₂SO₄ is further complicated in that the magnitude of response is also dependent upon the exposure duration because with small particles (0.8 μm) the onset of effect is rapid. With the larger particles (2.5 μm), the time to reach the maximum
response is slower (Amdur, 1958). In addition, exposure to combinations of irritant gases delays the return to control values of pulmonary functions, as compared to the recovery from exposure to individual gases (Amdur, 1969).

Species selectivity of sulfuric acid aerosol was investigated by Treon et al. (1950). Using concentrations ranging from 0.087 mg/m$^3$ to 1.61 mg/m$^3$, of which 93-99% contained particles less than 2 μm, they compared the toxicity among guinea pigs, mice, rats, and rabbits. Based on increased mortality, respiratory irrigation, and lung lesions, they determined that relative sensitivity to the H$_2$SO$_4$ aerosol was rabbit < rat < mouse < guinea pig.

Using the guinea pig, Amdur, Schultz, and Drinker (1952) determined that the age of a species may also influence sensitivity to sulfuric acid. They found the 8 hour LC$_{50}$ for 1-2 month old guinea pigs significantly lower, 18 mg/m$^3$, 1.0 μm (MMD), than the 8 hour LC$_{50}$ for 18 month old animals, 50 mg/m$^3$, 1.0 μm (MMD).

Further studies by Pattie, Burgess, and Cullumbine (1956) indicated that the particle size of H$_2$SO$_4$ aerosol also affected the lethal concentrations for the guinea pig. The 8 hour LC$_{50}$ for a particle size of 2.7 μm (MMD) was 27 mg/m$^3$, while 60 mg/m$^3$ was required for a particle size of 0.8 μm (MMD).

The effects of sulfuric acid aerosol on lung structure have been examined by several investigators.

Amdur et al. (1952) showed that H$_2$SO$_4$ aerosol produces deep-seated damage to 30% of the lung tissue of guinea pigs and that repair
of this damage is very slow. This damage was illustrated by an out-
pouring of leucocytes and erythrocytes into the alveolar spaces as well
as considerable congestion of bronchi, alveoli, and blood vessels.
Animals that survived the exposure were killed at various intervals up
to three weeks. Gross pathologic examination of the lungs disclosed
spotty areas of old hemorrhage. Closer examination revealed fibrosis.

Pattie et al. (1956) reported similar findings in the lungs of
exposed guinea pigs. Animals dying after short exposures of less than
two hours showed grossly distended lungs with trapping of air but no
other serious lesions. The cause of death appeared to be asphyxia
caused by bronchostenosis and laryngeal spasm. Animals dying after
longer exposures showed capillary engorgement and hemorrhage of lung
tissue (Pattle et al., 1956; Amdur et al., 1952).

These results led Amdur et al. (1952) to postulate that $H_2SO_4$
aerosol had two toxic actions: 1) laryngeal and bronchial spasms which
may result in death, and 2) parenchymal lung damage which is dependent
upon total dosage.

Bushtueva (1957) investigated the effect of concentration on
pathological changes in the lung. After 5 days of uninterrupted expo-
sure, edema and hemorrhage were observed in the lungs of animals exposed
to 8 mg/m$^3$. A marked accumulation of fluid and free epitheleal cells in
the alveolar spaces as well as thickening and swelling of alveolar
septae were also found.

Exposures to 4 and 2 mg/m$^3$ of the aerosol showed less clearly
expressed edemas and thickening of alveolar septae. Evidence of
developing focal pneumonia of a "catarrhaldesquamation type" was also observed.

Alarie et al. (1973) have presented work describing lesions resulting from H₂SO₄ aerosols of varying concentration and particle size. Groups of guinea pigs were exposed to 0.10 mg/m³, 2.8 μm MMD or 0.08 mg/m³, 0.8 μm MMD H₂SO₄ aerosols. After a continuous 52-week exposure, no deleterious effects were found, regardless of the particle size.

Experiments with the cynomolgus monkey (Alarie et al., 1973) disclosed pulmonary damage. Continuous exposures for 78 weeks, using concentrations of 2.43 and 4.79 mg/m³ with particle sizes of 3.60 μm and 0.73 μm (MMD), respectively, produced deleterious effects on pulmonary structures and a decrease in pulmonary function. At low concentrations, 0.38 and 0.48 mg/m³, small particles produced no alterations on pulmonary structures, while differences were observed with the large particles.

The physiological responses to mixtures of SO₂, H₂SO₄, and fly ash have been examined by Alarie et al. (1975) and Busey and Frankenberg (1973). Exposure to sulfuric acid mist at concentrations above 1.0 mg/m³, regardless of particle size (1 μm or 1 μm to 4 μm) induced pulmonary lesions and, at the highest concentrations, pulmonary function impairment in cynomolgus monkeys. At concentrations between 0.1 and 1.0 mg/m³, regardless of the particle size (1 μm or 1 μm to 4 μm), these effects were less pronounced or absent (Alarie et al., 1975; Busey and Frankenberg, 1973). Sulfuric acid, 1 mg/m³, 1 μm, in combination with fly ash or SO₂, 0.5 mg/m³ and 0.99 ppm, respectively, showed adverse
microscopic changes in the lungs of cynomolgus monkeys exposed continuously for 18 months. No adverse effects were detected at lower concentrations (Busey and Frankenberg, 1973).

Results of 18 month exposures in 6 groups of cynomolgus monkeys to ternary combinations of \( \text{SO}_2 \), \( \text{H}_2\text{SO}_4 \), and fly ash indicated morphological changes in the lungs of monkeys exposed to 1.01 ppm \( \text{SO}_2 \), 0.88 mg/m\(^3\) \( \text{H}_2\text{SO}_4 \) (1 \( \mu \)m) and 0.41 mg/m\(^3\) fly ash (Alarie et al., 1975; Busey and Frankenberg, 1973). The deleterious effects detected from the exposures to these mixtures were attributed to the presence of the acid mist alone.

A respiratory irritant may produce pathological changes in the lung which may be manifested by altered clearance rates of particles from the respiratory tract. This may render the host more susceptible to respiratory infection and disease.

Fairchild, Stultz, and Coffin (1975) exposed guinea pigs to 3.02 mg/m\(^3\), 2.6 \( \mu \)m (MMD), \( \text{H}_2\text{SO}_4 \) aerosol for 1 hour. Subsequent exposure to \( \text{H}_2\text{SO}_4 \) aerosol concentration was reduced to 0.32 mg/m\(^3\), there was no detectable effect.

Additional exposures (Fairchild et al., 1975) to concentrated \( \text{H}_2\text{SO}_4 \) mist (15 mg/m\(^3\), 3.2 \( \mu \)m CMD) for 4 days, 90 minutes/day, resulted in a decreased clearance rate of nonviable, radiolabelled streptococci from the noses and lungs of mice. No effect on the clearance of viable streptococci was observed. This may have been due to the relatively large particle size resulting in deposition of the \( \text{H}_2\text{SO}_4 \) on the ciliated
portion of the trachea. Therefore, the macrophages may not have received a substantial exposure.

Coffin (1972) exposed mice for 3 hours to $\text{H}_2\text{SO}_4$ aerosol with a mass median diameter less than 1 $\mu$m, at concentrations of 80, 150, and 300 mg/m$^3$. The mice were subsequently exposed to aerosolized, virulent streptococci. The results indicated that only the highest concentration enhanced mouse mortality. Exposure of rabbits to these concentrations did not alter the number of macrophages or polymorphonuclear cells in lavage fluid. Direct instillation of 0.1 N $\text{H}_2\text{SO}_4$ into the trachea produced a large influx of polymorphonuclear lymphocytes. It is conceivable that low penetration into the deeper portion of the lung may have accounted for the failure of the inhaled $\text{H}_2\text{SO}_4$ mist to elicit a response in the number of polymorphonuclear lymphocytes (Coffin, 1972).

**Effects of Sulfuric Acid Aerosol on Human Health**

Sim and Rattle (1957) exposed healthy males to dosages of 41 to 2384 mg-min/m$^3$ $\text{H}_2\text{SO}_4$ mist. Results can be seen in Table 1. Dosages from 41 to 1230 mg-min/m$^3$ failed to produce significant changes in physiological responses from the normal.

A subsequent series of exposures was performed with levels as high as 2384 mg-min/m$^3$. A dry mist (62% relative humidity) at a concentration of 39.4 mg/m$^3$ and mass median diameter of 0.99 $\mu$m produced a 35.5 to 100% increase in lung resistance to air flow in all 12 men. A wet mist (91% relative humidity) produced intense coughing, lacrimation, and rhinorrhea at a concentration of 20.8 mg/m$^3$, 1.54 $\mu$m MMD. This aerosol was intolerable from the onset and resulted in an increase in
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resistance to air flow of 43 to 150%. These authors suggested that the greater irritancy of the wet mist was probably due to a larger particle size and, as a consequence, an increased deposition in the throat and trachea.

French et al. (1973) examined the relationship between air pollution exposure and the frequency and severity of acute respiratory disease. They found that $\text{SO}_2$ and suspended sulfates, at an average annual concentration of $10-12 \mu g/m^3$, were linked to excess morbidity for upper and lower respiratory tract infections.

In a study of cardiopulmonary patients in metropolitan New York, the strongest and most consistent pollutant effects were associated with suspended sulfates for aggravation of shortness of breath, cough, and increased phlegm production. In addition, exposure to elevated levels of suspended sulfates, when accompanied by elevated temperatures, may contribute to excess risk of asthmatic attacks (French, 1975).

**The Alveolar Macrophage**

Because of the airborne nature of sulfur oxides, the primary route of exposure is via the respiratory tract. Alveolar macrophages are free cells located in the pulmonary alveoli. Their principal function is the maintenance of a sterile alveolar surface for gas exchange. Pulmonary alveolar macrophages (PAM) are characterized by their ability to phagocytize foreign particles which reach the alveoli. This material is then removed to ciliated areas of the lung where it moves up the mucociliary escalator and is eliminated.
The blood monocyte, derived from bone marrow precursors, is the cell of origin for macrophages. The blood monocyte may be considered as a transport element which, by the blood stream, reaches the pulmonary interstitial tissue. After adaptation to local circumstances, the cell enters the pulmonary alveoli (Klika et al., 1975; Sbarra et al., 1976).

The alveolar macrophage is a cell of varying, irregular shape, about 15-22 μm in size. The surface is often observed to have slender cytoplasmic projections or blunt, broad pseudopodia which aid in phagocytosis.

Phagocytosis is the ingestion of particles by single cells and is the fundamental defense mechanism of the host against foreign invaders. This process is comprised to a number of separate steps and activities: 1) the initial event which is the "signal" that there is a foreign particle to be phagocytized in a given location; followed by 2) the pursuit, i.e., locomotion of the phagocyte toward the foreign particle; 3) surface recognition of the particle as self or non-self; and 4) engulfment and destruction of the particle (Van Oss, Gillman, and Neumann, 1975).

It is likely that specific receptors on macrophage membranes may initiate phagocytosis (Carr, 1973; Al-Ibrahim et al., 1976). The presence or absence of opsonins (complement and immunoglobulins) on the surface of a particle is the most important factor governing the fate of the particle. Particles coated with ligands such as IgG and/or complement are avidly ingested by phagocytes, while ingestion does not occur in the absence of these opsonins (Griffin et al., 1975).
Griffin et al. (1975) studied the interaction of mouse peritoneal macrophages with sheep erythrocytes coated with immunoglobulins and with complement. Their results indicated that attachment of a particle to specific receptors on the macrophage plasma membrane is not sufficient to trigger ingestion of that particle. They found that ingestion requires the sequential, circumferential interaction of particle bound ligands with specific macrophage plasma membrane receptors not involved in the initial attachment process.

The biochemical activities of rabbit alveolar macrophages during phagocytosis were investigated by Ouchi, Selvaraj, and Sbarra (1965). They found that maximum phagocytosis occurs at 37°C with no measurable phagocytosis below 5°C or above 65°C. Any change greater than 20% in isotonicity of the cell medium produced a reduction in phagocytosis. The optimal pH for phagocytosis was between 6 and 7. Also, a minimum incubation of 45 minutes was required for optimal phagocytosis.

Alveolar macrophage function is impaired in areas of the lung in which oxygen tensions are greatly reduced (Ouchi et al., 1965; Cohen and Cline, 1971). The oxygen requirement for phagocytosis indicates that oxidative energy is required. Partial inhibition of phagocytosis has been demonstrated when glycolysis, cytochrome, and electron transport or total oxidative metabolism were inhibited (Cohen and Cline, 1971). Thus, they state that it is doubtful that the tricarboxylic acid cycle is involved in supplying the necessary energy for phagocytosis. This suggests that alveolar macrophages are highly dependent on oxidative metabolism for optimal phagocytic activity. Because alveolar macrophages
have an adequate and continuous supply of oxygen, they have adapted to
derive their energy by aerobic means (Vernon-Roberts, 1972). Conflicting results have been reported by Hahn et al. (1973-1974). They found that sodium fluoride, \(4 \times 10^{-2} \text{ M}\), completely inhibited particle uptake by rabbit alveolar macrophages. This indicates that glycolysis may be necessary for phagocytosis. These data indicate that the source of energy for phagocytosis by rabbit alveolar macrophages cannot be identified with certainty.

After the initial ingestion, the phagosome, or particle within the vacuole, fuses with one or several lysosomes containing acid phosphatase and other lysosomal enzymes. This allows for the delivery of highly active enzymes to the ingested substance without subjecting the cell's cytoplasm to potentially injurious effects (Goldstein et al., 1976).

Lockard and Kennedy (1976) observed alterations in lysosomal release in rabbit alveolar macrophages as a result of traumatic shock. An increased release of lysosomal enzymes in resting and phagocytizing macrophages, decreased total lysosomal enzymes alterations in cellular ultrastructure, and a decrease in bactericidal activity were seen 1 hour after shock. Electron microscopy showed many cells with greatly reduced or a complete absence of lysosomes. Abnormal release at lysosomal enzymes from macrophages could result in injury to lung tissue. The inability of shocked cells to destroy ingested microorganisms could be an important factor in the establishment of pulmonary infections.
The extent to which a noxious agent is dangerous to the host depends on how it is handled by that host. When aerosols or gases are inhaled, the concentrations in various parts of the respiratory tree will depend upon respiratory dynamics, particle size, and chemical and physical characteristics of the gases. Rapid and complete elimination of the agent(s) by competent macrophages will lead to rapid resolution. Incomplete elimination by vitiated macrophage function, either due to particular properties of the inhaled agent (e.g., cytotoxic properties of silica) or intrinsic defects within the host macrophage allow for continuing cycles of inflammation and infection (Turner-Worwich, 1976).

A number of in vitro tests have been devised to measure rates of phagocytic ingestion. Macrophage monolayers can be prepared and tested with microbial suspensions (Mackaness, 1960; Murphey et al., 1975; Reynolds and Thompson, 1976). Alternatively, bacteria can be injected intratracheally into animals, alveolar macrophages lavaged, and the number of intracellular bacteria counted (Coffin et al., 1968; Gardner, Holzman, and Coffin, 1969). Hahn et al. (1973-1974) assessed the rate of phagocytic uptake by measuring intracellular radioactivity of montmorillonite fused clay labelled with $^{169}$Yterbium.

Few studies of the effect of airborne toxicants on the phagocytic function of alveolar macrophages have been performed to date. Richmond (1974) incubated alveolar macrophages with $^{32}$Phosphorus labelled bacteria with subsequent exposure to ambient air and air containing 0.8 ppm ozone. He found little difference in the measurable uptake of the labelled bacteria. However, if air containing 5% CO$_2$ was introduced,
the macrophages ingested less than 50% of the control values. Richmond postulated that this may have been a result of a salt effect because of a change pH of the cell media. When alveolar macrophages were exposed, in vivo, to 1-7 ppm ozone for 3 hours (Coffin et al., 1968), there was a significant decrease in phagocytic activity, an increase in the number of polymorphonuclear leukocytes, a decrease in the number of macrophages, and a decrease in the number of cells that showed phagocytic activity. Gardner et al. (1969) exposed rabbits to NO₂ at concentrations ranging from ambient to 60 ppm for 3 hours. Nitrogen dioxide produced an influx of polymorphonuclear leukocytes at 8 ppm and a marked depression of phagocytic activity at 10 ppm or greater. These concentrations are higher than concentrations of ozone producing similar responses. These authors suggest that NO₂ is a less potent irritant than O₃.

**Experimental Objectives**

The purpose of the present study was to determine the effect of inhaled sulfuric acid aerosol on the phagocytic capabilities of alveolar macrophages. Phagocytic indices were determined for alveolar macrophages obtained from rabbits exposed to varied concentrations and durations of sulfuric acid aerosol. The lungs of exposed and control animals were lavaged and the following parameters examined: 1) ability of the cells to phagocytize radiolabelled microspheres in vitro, 2) percent viability of lavaged cells, 3) differential cell count of lavaged cells, 4) total protein in cell free lavage fluid, and 5) morphological changes in the bronchi by scanning electron microscopy.
METHODS AND MATERIALS

Sulfuric Acid Aerosol Generation and Characterization

A schematic drawing of the dynamic exposure system is presented in Figure 2. A stream of generating air was passed through a silica gel drying chamber and directed at the surface of a 50 ml aliquot of fuming sulfuric acid (Mallinckrodt) contained in a 500 ml single neck flask. A fresh surface was constantly exposed to the air stream by means of a magnetic stirring bar. The resultant sulfur trioxide vapors are driven through a glass column by a stream of diluting air into a 44 liter Pyrex bell jar. Once inside, the sulfur trioxide reacts with moisture in the air to form sulfuric acid aerosol. The aerosol was vented through an outlet at the bottom of the chamber door into a sodium carbonate trap.

An airtight, easy access into the chamber was provided by fastening a 1/2 inch thick lucite plate to the chamber with 16 1-3/4 inch x 1/4 inch hex bolts. The bolts were fixed in 3/4 inch x 1-1/2 inch lucite, epoxied to the outer circumference of the chamber. Two rubber tubing O-rings insured an adequate seal.

Sulfuric acid aerosol concentrations were determined by bubbling, at a rate of 1 liter/minute, an aerosol sample drawn at the nose level of the rabbit through a 100 ml sodium carbonate solution of known concentration. The time to end point, using Bromocresol green as the
Figure 2. A schematic representation of the sulfuric acid mist exposure apparatus.
indicator, was measured and the aerosol concentration calculated from the following equation:

\[
\frac{(0.100)(A) \times 9.8062 \times 10^4}{t} = \text{mg/l} \ H_2\text{SO}_4
\]

where \(A\) = the concentration of the sodium carbonate and \(t\) = minutes to reach end point = liters of aerosol.

Gran's plots (Kozarek and Fernando, 1972; Seymour, Clayton, and Fernando, 1977) were used to verify \(H_2\text{SO}_4\) aerosol concentrations. A 10 ml sample of bubbled sodium carbonate solution containing trapped \(H_2\text{SO}_4\) was titrated with 0.02 M HCl. The subsequent change in pH is recorded with the volume of acid added. After the second equivalence point, the straight line plot of \((V_{\text{acid}} + V_{\text{base}}) [H^+]\) versus \(V_{\text{acid}}\) was extrapolated to intersect the abscissa at \(V_A = V_{\text{eq2}}\). If a standard sodium carbonate solution is titrated in the same manner, the amount of \(H_2\text{SO}_4\) can be determined from:

\[
\text{moles acid}_{\text{std.}} = V_{\text{std.}} \times \text{ Conc}_{\text{HCl}}
\]

\[
\text{moles acid}_{\text{sample}} = V_{\text{sample}} \times \text{ Conc}_{\text{HCl}}
\]

therefore,

\[
(V_{\text{std.}} \times \text{ Conc}_{\text{HCl}} - V_{\text{sample}} \times \text{ Conc}_{\text{HCl}}) - \frac{\text{moles } H_2\text{SO}_4 \text{ added}}{2}
\]

\[
\times 9.8062 \times 10^4 = \text{mg } H_2\text{SO}_4 \text{ in sample}
\]
Aerosol concentrations were adjusted by manipulating the air flow rates into the chamber. An increased generating air flow and/or decreased diluting air streams produced the higher concentrations employed in this work. Conversely, a decrease in generating air flow rates and/or increase in the diluting air flow rates produced the lower concentrations. The magnetic stirring bar speed also affected aerosol concentrations with higher bar speeds producing an increase in sulfuric acid mist levels. Lower bar speeds resulted in a reduced amount of sulfur trioxide vapors and lower sulfuric acid concentrations. The bar speed was kept constant throughout the study.

The aerosol particle size (MMD) was determined using an eight-stage cascade impactor. The sulfuric acid mist was drawn through the cascade impactor at a rate of 700 ml/minute for 1 hour. The effective cut-off diameter for each stage was determined by:

\[ \text{Effective cut-off diameter} = 1265 \sqrt[3]{\frac{W^2}{F}} \]

where \( W \) = hole diameter in centimeters and \( F \) = flow rate in cubic cm/minute. \( \text{H}_2\text{SO}_4 \) accumulation on each stage was determined by Gran's plots. The percent cumulative distribution was plotted against the effective cut-off diameter. The 50% intercept is equivalent to the aerodynamic mass media diameter (AMMD). The AMMD was converted to the mass median diameter (MMD) by:

\[ \text{MMD} = \sqrt{\frac{\text{AMMD}}{P}} + 0.01 - 0.1 \quad \text{(in microns)} \]
The relative humidity of the air inside the chamber was determined using a Bendix Psychron, model 566 (Bendix Corp.). Air was drawn past wet bulb and dry bulb thermometers by a battery operated fan. Temperatures were plotted on a calibration curve and the percent relative humidity recorded.

**Determination of Phagocytic Indices by Percent Phagocytosis**

New Zealand white rabbits, 1-2 kg, were individually exposed to sulfuric acid mist aerosols for 0.5, 2, or 4 hours. Aerosol concentrations ranged from 0.043 to 0.522 mg of sulfuric acid per liter of air. Each rabbit was sacrificed by injection of a 40 ml bolus to a marginal ear vein. The trachea was exposed and clamped to prevent infiltration by red blood cells. The lungs were excised and lavaged with 4-10 ml aliquots of sterile, prewarmed (37°C) physiological saline. Alveolar macrophages were extracted from the lavage fluid by centrifugation at 1000 RPM (approximately 300 g) 2-4°C for 10 minutes. Lavage fluid was analyzed for total protein content by Coomassie Binding Technique (Bradford, 1976). The resultant pellet was resuspended in 10 ml media consisting of 88% Eagle Minimum Essential Media containing phenolphthalein indicator (Flow Laboratories Inc.), 10% heat inactivated fetal calf serum, 1% penicillin G-streptomycin sulfate, and 1% L-glutamine. The pH was adjusted to 7.2 by the addition of sterile 8% (w/v) NaHCO₃ until the color changed to cherry red. An aliquot of this cell suspension was taken for a cell count using a hemacytometer. A 1.0% (w/v) Trypan Blue solution was added to the aliquot to determine cell viability, noting
the number of stained (dead) cells per 100 total cells. The percent red blood cells and differential cell count were determined. Another aliquot was taken and brought up in sufficient media to give a concentration of $1 \times 10^5$ cells/ml. Sterile, disposable, 15 ml centrifuge tubes were filled with 0.5 ml of this dilution. A suspension of tracer microspheres (3M Company), 2-4 μm, labelled with $^{85}$Strontium, a gamma emitter, was prepared in a concentration of $1 \times 10^6$ particles/ml. These black, inert particles had a specific activity of 10 m Curie/gram. One-half ml of this particle suspension was added to the centrifuge tubes to give a particle-to-macrophage ratio of 10:1. Tubes were incubated at 37°C for 0, 1, 2, 3, and 4 hours. A "particles only" tube was also incubated for 4 hours and served as a reference. At the end of each incubation period, the tubes were placed in ice and 0.3 ml of 0.03 M EDTA was added to each to halt further phagocytic activity. After the final incubation period, the contents of each tube were carefully layered on 1 ml 30% Ficoll and centrifuged at 1000 RPM, 2-4°C for 3 hours. Tube contents were then divided into 3 fractions by taking 0.5 ml (primarily supernatant), 0.9 ml (macrophages with engulfed particles), and 0.9 ml (free particles). Fractions were placed into separate scintillation vials with 10 ml scintillation cocktail (Handiflur, Scientific Products) and 4 ml water. Activity was determined using a liquid scintillation counter (Beckman Instruments). The percent phagocytosis was determined from the following equation:

$$\frac{(M_x - T_x) - (M_o - T_o)}{M_x + T_x + B_x} \times 100 = \% \text{ phagocytosis}$$
where $M_x = \text{net counts per minute obtained from the middle fraction}$,
$T_x = \text{net counts per minute obtained from the top fraction}$,
$B_x = \text{net counts per minute obtained from the bottom fraction}$,
$M_0 = \text{net counts per minute obtained from the middle fraction of the 0 hour tube}$, and
$T_0 = \text{net counts per minute obtained from the top fraction of the 0 hour tube}$.

The first component of the numerator, $(M_x - T_x)$, represents the macrophages that have engulfed particles; the second component, $(M_0 - T_0)$, represents the solubility of the radioactive particles in the media; and the denominator, $M_x + T_x + B_x$, represents the total activity possible for each sample. Values for percent phagocytosis were calculated for each $H_2SO_4$ exposure at the 0, 1, 2, 3, and 4 hour incubation periods. Phagocytic indices were plotted against CT (the product of the concentration and exposure length) to determine if a dose-response relationship could be elucidated.

**Scanning Electron Microscopy**

Lungs were excised from two rabbits exposed to 211 or 296 mg/m$^3$ $H_2SO_4$ aerosol for four hours. Lungs were also excised from two unexposed rabbits. Twenty ml dilute Karnovsky's Fixative were perfused through the airways to remove mucus. The lungs were fixed in concentrated Karnovsky's for 1-2 days. The entire bronchial tree was extracted by carefully removing the fixed tissue. The bronchial ramis to the superior lobe, Figure 3, was divided into 3 segments: proximal,
Plan of the respiratory tubes as seen from the ventral surface.

Figure 3. Respiratory bronchioles as seen from the ventral surface. -- S represents the superior bronchus used for scanning electron microscopy.
medial, and distal, each approximately 8 mm long. Each segment was transected and dehydrated in a graded series of ethanol. After further dehydration by the critical point drying technique, the sections were coated with carbon and gold-palladium in a high vacuum evaporator. Tissue samples were observed for morphological changes using an ETEC Scanning Electron Microscope.
RESULTS

Phagocytic Indices versus CT Index

Along with exposure variables, the phagocytic indices obtained are shown in Table 2. Each is an average of three values obtained from the triplicate samples utilized at each incubation interval. The resulting standard deviations were variable, ranging from 0.44 to 14.81. The concentrations were computed, because of differences in exposure durations, as a CT function. This CT parameter, mg-hours/m$^3$, is the product of the concentration, mg/m$^3$, and the exposure duration, hours.

Values for percent phagocytosis in Table 2 are plotted versus CT in Figures 4 through 8, for each incubation interval. There is not a clear relationship between CT and percent phagocytosis at the levels used in this study, although there is a shift of the data points toward an increase in percent phagocytosis with an increase in time of incubation. This shift was also observed for control values (Table 2).

In order to evaluate effects of H$_2$SO$_4$ exposure on the increased % phagocytosis with increased incubation, which is evident from the data in Table 2, the values for % phagocytosis at 2, 3, and 4 hours were compared to the values observed at 1.0 hour incubation time. The results are plotted in Figure 9, 10, and 11. Similarly, % phagocytosis at 4.0 hours incubation time was compared to those obtained after 1, 2, and 3 hours incubation. These data are illustrated in Figures 12, 13, and 14. Both sets of plots demonstrate a linear progression of this particular
Table 2. Phagocytic indices and alveolar macrophage population characteristics in rabbits exposed to \( \text{H}_2\text{SO}_4 \).

<table>
<thead>
<tr>
<th>Exposure Length (hr)</th>
<th>Concentration (mg/m(^3))</th>
<th>CT (mg-hr/m(^3))</th>
<th>% Viability</th>
<th>% Red Blood Cells</th>
<th>% Phagocytosis</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 hr 1 hr 2 hr 3 hr 4 hr</td>
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</tr>
<tr>
<td>4.0</td>
<td>0.0</td>
<td>0.0</td>
<td>98 **</td>
<td>1.31 64.50 75.12 78.20 78.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±2.76* ±3.09* ±3.81* ±2.73* ±2.55*</td>
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</tr>
<tr>
<td>0.5</td>
<td>46.0</td>
<td>23.0</td>
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<td>94</td>
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</tr>
<tr>
<td>0.5</td>
<td>59.0</td>
<td>30.0</td>
<td>100 **</td>
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</tr>
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<td>52.0</td>
<td>97 **</td>
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<td>70.0</td>
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<tr>
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<tr>
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<td>90.0</td>
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<td>2.0</td>
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<td>98 26 **</td>
<td>0.05 17.28 38.16 47.53 74.04</td>
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Table 2, Continued.

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<th>Exposure Length (hr)</th>
<th>Concentration (mg/m³)</th>
<th>CT (mg-hr/m³)</th>
<th>% Viability</th>
<th>% Red Blood Cells</th>
<th>% Phagocytosis</th>
<th>Incubation Time</th>
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*Standard deviation.

**Less than 10%.
Figure 4. Percent phagocytosis at 0 hours incubation.
Figure 5. Percent phagocytosis at 1 hour incubation.
Figure 6. Percent phagocytosis at 2 hours incubation.
Figure 7. Percent phagocytosis at 3 hours incubation.
Figure 8. Percent phagocytosis at 4 hours incubation.
Figure 9. Phagocytic activity at 1 hour versus 2 hours.
Figure 10. Phagocytic activity at 1 hour versus 3 hours.
Figure 11. Phagocytic activity at 1 hour versus 4 hours.
Figure 12. Phagocytic activity at 4 hours versus 1 hour.
Figure 13. Phagocytic activity at 4 hours versus 2 hours.
Figure 14. Phagocytic activity at 4 hours versus 3 hours.
index of phagocytosis with the prolongation of incubation. Control values also revealed the same progression.

**Lavage Cell Population Characteristics**

Table 2 also contains values for percent viability and percent red blood cell contamination obtained from each cell population. The percent viability showed no changes attributable to the concentration of \( \text{H}_2\text{SO}_4 \) aerosol and exposure duration.

The percent red blood cells was determined during the viability assay. The addition of Trypan Blue to the cell suspension gives the red blood cells an orange tinge when observed on a hemacytometer. This coloration, together with their concave appearance and relatively small size, made them readily distinguishable from the macrophages.

The red blood cell contamination for the majority of cell populations was less than 10 percent. There was a dramatic increase in red blood cells present as the aerosol concentration increased. This was a toxic response due to direct action on pulmonary vascular tissue by the sulfuric acid aerosol resulting in infiltration of the bronchi by the red blood cells. It is apparent that some rabbits were more susceptible to this effect. One exposure at 66 mg/m\(^3\) for four hours resulted in 16 percent red blood cells while another exposure at 92 mg/m\(^3\) for the same duration produced less than 10 percent. The maximum red blood cell contamination, 58%, occurred at an aerosol concentration of 550 mg/m\(^3\). No signs of toxicity were observed during the one-half hour exposure although dark red petechia were found on the lungs at necropsy.
Each animal was observed during exposure for signs of toxicity, eye irrigation (blinking, red rimmed), and respiratory distress. Animals exposed to low concentrations, 48-63 mg/m$^3$, exhibited no signs of discomfort after a brief, 2-3 minute, period during which time there was sneezing and/or pawing at the nose. As the H$_2$SO$_4$ concentration increased, the frequency of sneezing and irrigation also increased. Aerosol concentrations above 158 mg/m$^3$ resulted in intermittent sneezing throughout the exposure. A decrease in rate of respiration was evident at concentrations of 317 mg/m$^3$ and above. In addition, blinking and half-closed eyes were observed at all concentrations, during the 2 hour and 4 hour exposures. Necropsy results revealed tissues which appeared normal.

The particle size of the H$_2$SO$_4$ aerosol ranged from 1.18 μm mass median diameter (MMD) at the lowest aerosol concentration, 46 mg/m$^3$, to 0.47 μm MMD at the highest, 522 mg/m$^3$. The decrease in particle size with an increase in H$_2$SO$_4$ concentration was the result of a reduced availability of moisture in the chamber environment due to the increase in sulfur trioxide levels.

Table 3 contains the differential cell count representing the cell populations employed in this study. The differential cell count does show that the cell populations used were predominantly macrophages. Three hundred to four hundred cells were counted after staining with May-Grunwald and Giemsa. The range for percent macrophages was 62 to 99 percent, and the mean was 90 percent. The mean percent lymphocytes and mean percent neutrophils were 4 and 6, respectively. Although it is
Table 3. Differential cell counts for lavage cell populations in rabbits exposed to sulfuric acid mist.

<table>
<thead>
<tr>
<th>Exposure Length (hr)</th>
<th>Concentration (mg/m³)</th>
<th>CT (mg-hr/m³)</th>
<th>Macrophages (%)</th>
<th>Lymphocytes (%)</th>
<th>Neutrophils (%)</th>
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</thead>
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<td>46.0</td>
<td>23.0</td>
<td>91</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>0.5</td>
<td>48.0</td>
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<td>95</td>
<td>4</td>
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apparent from Table 2 that there is a high degree of variability in the
cell types, there does not seem to be a meaningful relationship between
the various cell types and the $H_2SO_4$ acid mist concentration, the
exposure length, or the CT function.

Control versus $H_2SO_4$ Exposed Phagocytic Indices

The dynamic property of phagocytosis is illustrated in Figure 15. The mean phagocytic index from exposed cell populations, calculated for
each incubation period, was plotted against incubation time. Mean
values for cell populations from control animals are also displayed in
Figure 15. It is evident that the sulfuric acid aerosol at the levels
employed in this study caused a significant decrease in the phagocytic
capabilities of the alveolar macrophages. While the percent phagocytosis
for control populations appears to have reached a maximum at 4 hours, the
$H_2SO_4$ exposed populations continues to increase and could conceivably
attain the level reached by the control populations.

The least squares technique was used to find the best fit for
the log of the incubation time versus control and test phagocytic
indices (Figure 16). The slope of the lines serve as an indicator of
response if the phagocytic rate is affected by exposure to $H_2SO_4$ mist.
The slope of the control and $H_2SO_4$ exposed lines was 24.09 and 63.32,
respectively. This indicates that the phagocytosis was significantly
depressed by exposure to $H_2SO_4$ mist at the concentrations and exposure
times used here. Maximum depression occurred at the 1.0 hour incubation
and decreased at the longer incubation times. The phagocytic index
approached the control index at 4 hours incubation, suggesting recovery.
Figure 15. Change in phagocytosis with time.
Figure 16. Log incubation time versus percent phagocytosis. -- Slope of $\text{H}_2\text{SO}_4$ exposed line indicates an increase in phagocytic rate, suggesting some recovery.
Total protein levels in the pulmonary lavage fluid were determined using the Coomassie Binding Technique to determine whether a relationship existed between total protein concentrations in lung lavage collections in exposure to $H_2SO_4$ aerosol using the CT index. There was a great deal of variation in results as shown in Figure 17 and no meaningful conclusions could be derived from the data. However, the trend suggests a depression in total protein as CT increases.

**Statistical Analysis**

Statistical analyses are summarized in Table 4. The Scattergram subprogram contained in the Statistical Package for the Social Sciences system was used to compute linear regression for Figures 4-17.

The Pearson product-moment correlation coefficient, symbolized by R, was used to determine the goodness of fit of the regression line. When there is a perfect fit, R is equal to +1.0 or -1.0, the negative sign indicating an inverse relationship. When the linear regression line is a poor fit to the data, R will be close to zero.

The square of Pearson's R is another statistic, denoted as $R^2$. It ranges from a minimum of 0 to a maximum of 1 and therefore is a better measure of the strength of the relationship between two variables.

A widely used statistic is the standard error of estimate which is the standard deviation of actual Y values from the predicted Y values. The standard error of estimate evaluates the accuracy of the predicted equation. Thus, the smaller the standard error of estimate, the more accurate the prediction.
Figure 17. Total protein in lavage fluid. -- A slight negative correlation exists.
Table 4. Statistical analysis obtained by linear regression. -- Phg. = phagocytosis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation R</th>
<th>$R^2$</th>
<th>Y Intercept</th>
<th>Slope B</th>
<th>Standard Error of Estimate</th>
<th>Standard Error of A</th>
<th>Standard Error of B</th>
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<tr>
<td>CT vs. 0 Hr. Phg.</td>
<td>0.325</td>
<td>0.105</td>
<td>146.914</td>
<td>114.994</td>
<td>153.270</td>
<td>33.932</td>
<td>65.725</td>
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<tr>
<td>CT vs. 1 Hr. Phg.</td>
<td>-0.168</td>
<td>0.028</td>
<td>231.985</td>
<td>-1.808</td>
<td>159.745</td>
<td>69.305</td>
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<td>-0.061</td>
<td>0.004</td>
<td>224.685</td>
<td>-0.834</td>
<td>161.742</td>
<td>153.767</td>
<td>2.683</td>
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<tr>
<td>CT vs. 3 Hr. Phg.</td>
<td>-0.149</td>
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<td>-2.283</td>
<td>160.233</td>
<td>189.791</td>
<td>2.971</td>
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<td>CT vs. 4 Hr. Phg.</td>
<td>0.023</td>
<td>0.000</td>
<td>151.285</td>
<td>0.389</td>
<td>161.999</td>
<td>226.384</td>
<td>3.287</td>
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<tr>
<td>1 Hr. Phg. vs. 2 Hr. Phg.</td>
<td>0.683</td>
<td>0.466</td>
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<td>0.868</td>
<td>10.992</td>
<td>10.450</td>
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<td>1 Hr. Phg. vs. 3 Hr. Phg.</td>
<td>0.773</td>
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<td>1 Hr. Phg. vs. 4 Hr. Phg.</td>
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<td>1.017</td>
<td>11.386</td>
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Table 4, Continued.

<table>
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<th>Variables</th>
<th>Correlation</th>
<th>$R^2$</th>
<th>Y Intercept</th>
<th>Slope B</th>
<th>Standard Error of Estimate</th>
<th>Standard Error of A</th>
<th>Standard Error of B</th>
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<td>4 Hr. Phg. vs. 1 Hr. Phg.</td>
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<td>4 Hr. Phg. vs. 3 Hr. Phg.</td>
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<td>Phg. Means vs. Incubation Time</td>
<td>0.944</td>
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<td>Percent Phg. vs. Log Incubation Time</td>
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<td>CT vs. Total Protein</td>
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<td>-0.340</td>
<td>146.829</td>
<td>46.017</td>
<td>0.114</td>
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The standard error of A and the standard error of B constitute the standard deviations of the normal distributions of A and B values, respectively. The standard error aids in determining the potential degree of discrepancy between the sample mean and the population mean.

The absence of a relationship between CT and percent phagocytosis illustrated in Figures 4-8 is supported statistically in Table 4. The close approximation of Pearson's R and $R^2$ to zero for CT versus percent phagocytosis indicates that there is no linear relationship between the two variables. The relatively high values obtained for the standard error of estimate and standard error of B are in agreement with this.

A weak relationship appears when phagocytic indices for the various incubation times are compared to one another.

A strong correlation exists between an increase in percent phagocytosis and an increase in incubation time. This conclusion is based on the values obtained for Pearson's R and $R^2$, 0.944 and 0.890, respectively, which approach the maximum correlation of 1.00. The strength of the relationship increases when percent phagocytosis is plotted against the log of the incubation time. The resultant Pearson's R of 0.981 and $R^2$ of 0.963 indicates an almost ideal linear relationship.

Statistical analysis performed on Figure 17 reveals a weak negative relationship between total protein in the lung wash and the CT index. This, together with the negative slope, implies that, as CT increases, the total protein decreases. The small $R^2$, 0.241, and large standard error of estimate, 146.829, are indications of the variations
that occurred which contribute to the weak correlation between these variables.

**Morphological Observations by Scanning Electron Microscopy**

Bronchiolar sections of exposed and control lungs were examined at 800X and 2000X by scanning electron microscopy. Photomicrographs, Figures 18-22, were taken at 2000X to facilitate comparisons. Observations of control lung preparations revealed cilia of uniform density and length in the terminal and medial sections of the bronchi, whereas cilia in the proximal segment appeared matted. Mucus was not present in control bronchi. Nonciliated cells were randomly distributed throughout the bronchi of the three control sections. Nonciliated cells were also observed in the bronchi obtained from rabbits exposed to H₂SO₄ aerosols. Cilia were matted in the medial and terminal portions and appeared shorter in the medial segment when compared to control photomicrographs. Random patches of mucus were found throughout the terminal piece of the exposed bronchi.
Figure 18. Scanning electron micrograph of the proximal section of a superior bronchus. -- a) control segment; b) H₂SO₄ exposed segment.
Figure 19. Scanning electron micrograph of the medial segment of a superior bronchus. -- a) cilia appear matted and shortened; b) control section.
Figure 20. Scanning electron micrograph of the distal segment of a superior bronchus. -- a) control section; b) H$_2$SO$_4$ exposed section, cilia appear matted, covered with a thin mucus layer.
Figure 21. Scanning electron micrograph of the distal segment of a superior bronchus from a control rabbit.
Figure 22. Scanning electron micrograph of the distal segment of a superior bronchus from an H$_2$SO$_4$ exposed rabbit. -- a) non-ciliated cells appear poorly delineated; b) cilia appear less dense.
DISCUSSION AND CONCLUSIONS

The salient finding in the present study of the phagocytic index of lung macrophages from rabbits inhaling several concentrations of sulfuric acid and exposed from 0.5 to 4.0 hours was the absence of a concentration (CT) dependent effect. This implicates an all-or-none type of effect which may result when normal body deacidification processes are overcome. Larson et al. (1977) hypothesized that inhaled H₂SO₄ concentrations may be reduced in two ways: 1) by dilution resulting from hydration of the aerosol following inhalation, and 2) by neutralization with ammonia. Ammonia is the more probable way in which acidity may be changed. They found that the mouth, which is less efficient than the nose in scrubbing inhaled aerosols, might have the greater effect on the chemistry of acid aerosols. They found the range of oral concentrations of NH₃ to be 29 to 250 μg/m³, which they claim has the potential for converting 84 to 1500 μg H₂SO₄ per cubic meter to a nearly neutral salt, (NH₄)₂SO₄. The lower nasal concentrations of ammonia, 13 to 46 μg/m³, could convert 37 to 137 μg H₂SO₄ per cubic meter to (NH₄)₂SO₄. Thus, the so-called all-or-none effect could result from the overwhelming of the natural deacidification processes followed by a marked effect without the gradual ascent of the dose-response curve which is the usual curvilinear pattern observed.

Further evidence in support of this is found in the report of Sim and Pattle (1957) who used ammonia in their studies with sulfuric
acid mist. They found that when ammonia was used as the agent for nullifying the effects of $\text{H}_2\text{SO}_4$ mist there was almost immediate relief of symptoms when the ammonia was in sufficient concentrations. These studies suggest that the ammonia concentration might be the rate-limiting step in the neutralization of inhaled acid aerosols. Once neutralization is overwhelmed by an abundance of $\text{H}_2\text{SO}_4$, a response could be elicited. This indicates that a threshold concept may exist for $\text{H}_2\text{SO}_4$.

The primary function of pulmonary alveolar macrophages is the maintenance of a clean alveolar surface. Foreign material which reaches the alveoli is ingested and removed. Thus, alveolar macrophages should, conceivably, be quite resistant to inhaled toxic or irritant aerosols. For example, Weissbecker et al. (1969) investigated the effects of various gases on in vitro alveolar macrophage viability. Hanging drop suspensions of alveolar macrophages were exposed to several gases for 1 hour, then stained with Trypan Blue to assess all viability. Hydrogen cyanide and carbon monoxide had no apparent effects on macrophage viability at high concentrations above 607 ppm. Ozone, on the other hand, was more toxic than HCN and CO, reducing macrophage survival to 7% at 25 ppm. Nitrogen dioxide decreased survival to 38% at 3548 ppm. At a concentration range of 98-6789 ppm, nitric oxide lowered the viability from 81% to 30%. Exposure to sulfur dioxide reduced cell viability to 55% at 317 ppm and to 35% at 19,100 ppm.

No significant effects on macrophage viability resulted from the sulfuric acid mist concentrations, 46 mg/m$^3$ (9.48 ppm) to 522 mg/m$^3$
(130.23 ppm), used in the present study. Although sulfuric acid mist is considered to be a more potent irritant than sulfur dioxide, the concentrations used were well below those employed by Weissbecker et al. (1969). Thus, the absence of an effect of viability in the present study was not surprising.

Richmond (1974) found the viability of pulmonary alveolar macrophages after 2 hours of phagocytosis to be 100%. He then changed the pH of the medium to correspond to that obtained with exposure to contaminant gases, 6.5 for 5% CO$_2$ and 7.4 for 0.8 ppm ozone, and discovered no change in viability after 2 hours. Murphey et al. (1975), in studies on the mechanism of alteration of pulmonary antibacterial activity by O$_2$, found no significant differences in viability of PAMs exposed to 100% O$_2$ for 24-72 hours from control animals. These results attest to the resistive properties of lung macrophages and indicate that the decrease in phagocytosis observed in this study at all levels of exposure cannot be attributed to changes in viability of the cell population.

Other changes in population characteristics after exposure to various toxicants have been noted by several investigators (Coffin et al., 1968; Gardner et al., 1969; Murphey et al., 1975). Coffin et al. (1968) reported an increase in polymorphonuclear leukocytes and a decrease in the number of PAMs after 3 hour exposures to 1-7 ppm ozone. Ozone, at these levels, also produced a significant decrease in phagocytosis and a decrease in the number of macrophages that were phagocytic, i.e., exposed cells had ingested fewer bacteria cells than controls. They suggested that the influx of polymorphonuclear leukocytes was a
reflection of a chemotactic effect, while the decreased macrophage population was a result of cell destruction in situ by ozone or sensitization of the cell membrane to lysis during lavage. Rabbits exposed to NO\textsubscript{2} at concentrations ranging from ambient to 60 ppm showed a significant rise in the number of polymorphonuclear leukocytes at 8 ppm NO\textsubscript{2} (Gardner et al., 1969). At 25 ppm NO\textsubscript{2}, there was an increase in polymorphonuclear leukocytes equal to 20% of the population. Ozone produced an equivalent response at 2 ppm, indicating that NO\textsubscript{2} is less potent than ozone. A 3 hour exposure to NO\textsubscript{2} at 10 ppm or greater also produced a marked decrease in phagocytic activity, reducing the median number of bacteria/cell from 4.1 to 2.1. In addition, only 66% of exposed macrophages showed phagocytic activity versus 83% of control macrophages.

One possible mechanism postulated by Gardner et al. (1969) for the action on lung macrophages by ozone and/or nitrogen dioxide, which could also apply to sulfuric acid aerosol is that the air pollutant inactivates an opsonogenic factor in the extracellular environment. This could account for the decrease in phagocytic activity. This mechanism may be particularly applicable to the action of H\textsubscript{2}SO\textsubscript{4} aerosol for the following reason: although variations were observed in the macrophage recoveries in this study, they could not be attributed to exposure to H\textsubscript{2}SO\textsubscript{4} aerosol concentrations employed nor the exposure duration. The mean percent macrophages was 90%, indicating that some other factor was responsible for the decline in phagocytic activity that was observed here (Figure 15).
No attempt was made to purify or isolate the macrophages from the other cells found in the lung lavage. Phagocytic indices were not corrected for populations containing less than 100% macrophages in keeping with the objective of evaluating phagocytic activity representative of a natural cell population. Thus, large variations were encountered in the measurements. These were attributable to the natural biological variations inherent in the heterogeneous cell populations harvested.

A moderate range of variability in the absolute values for phagocytosis was also noted in normal rabbit populations by Murphey et al. (1975). They demonstrated that the variability was due to both humoral factors and cellular factors, in addition to the expected individual variations.

The ability of the macrophage to seek out, ingest, and inactivate invading material results from the integration of a number of complex reactions. Macrophages are attracted to foreign particulates by chemotactic factors that may be released by the particles themselves, or formed from the interaction of the particle and the host tissue. Simultaneously, serum opsonins could attach to the particle's surface, making the particle susceptible to ingestion by the macrophage. Once ingested, the particle may be inactivated and broken down or remain unaltered if completely undigestible.

This complex series of events provides numerous potential sites for the interference of an environmental contaminant with phagocytosis. The toxin could impair chemotaxis by destroying chemotactic substances
or by reducing the phagocyte's mobility. The toxin could inhibit the ingestive process by damaging the phagocyte's membrane. Furthermore, the toxin could damage the cytoplasm or the intracellular enzyme systems involved in bacterial inactivation and degradation (Goldstein et al., 1976).

This study was not designed to investigate these factors but to determine the effect of inhaled H\textsubscript{2}SO\textsubscript{4} aerosol on phagocytic activity. Thus, the mechanism for the inhibition of phagocytosis observed here can only be surmised. The probable mechanism is most likely a combination of the ensuring hypothesized events. Direct attack upon the cell by H\textsubscript{2}SO\textsubscript{4} may result in a change in charge, surface tension, or adhesiveness of the cell membrane. This attack and the sequelae would affect the rate of engulfment, because recognition of material as foreign occurs when the particle first adheres to the cell surface. If it sticks, ingestion will result. Therefore, discrimination between foreign or endogenous materials may depend on the presence of a suitable layer of opsonizing protein. If, as suggested by Gardner et al. (1969), this opsonogenic factor is destroyed by an air pollutant (e.g., O\textsubscript{3}, NO\textsubscript{2}, H\textsubscript{2}SO\textsubscript{4}), engulfment would not occur.

Inhibition of intracellular enzymes which are necessary for optimum cell function and therefore phagocytosis is also a possible mechanism of the depression of phagocytosis (Elvick, 1978). Gardner et al. (1969) observed decreased \textit{in vitro} phagocytosis of BCG activated macrophages and inactivation of the hexose monophosphate shunt, which normally accompanies phagocytosis following NO\textsubscript{2} exposure.
Total protein in the lavage fluid was found to decrease with increasing $H_2SO_4$ concentrations and/or exposure duration in this study. This could be due to an increase in lung proteolytic activity resulting from a low pH, the probable consequence of $H_2SO_4$ exposure. Rynbrandt and Kleinerman (1976) have shown that extracts from lungs exposed to 30 ppm NO$_2$ for 2 days demonstrated greater proteolysis in the acid pH range. This increase in pulmonary proteolytic activity at pH values below the physiological range may predispose lung tissue to greater destruction when the tissue's pH is lowered, possibly accounting for some of the histopathological damage observed with $H_2SO_4$.

The frequency of occurrence of morphological changes observed in this preliminary work by Scanning Electron Microscopy (SEM) in the bronchi of $H_2SO_4$ exposed animals was too low to be meaningful. However, similar responses have been observed by other investigators. Schwartz et al. (1977) found variability in density and length of cilia in 6 of 9 guinea pigs exposed continuously to 30 to 38 mg/m$^3$ $H_2SO_4$ for 7 days. A mucous coating and matted cilia were present in the bronchus of mice exposed repeatedly to $H_2SO_4$-carbon particle mixtures ranging from 200 mg/m$^3$ to 50 mg/m$^3$ $H_2SO_4$ in combination with 5 mg/m$^3$ carbon particles (Ketels et al., 1977). Holes in the cell surface of nonciliated cells and dying ciliated cells were also observed by Ketels et al. (1977) in the mice bronchi. Damage to the surface of the respiratory tract by irritant aerosols could influence the ability to clear inhaled bacteria from the lungs. This could enhance the susceptibility of the host to airborne respiratory infections.
In summary, the various concentrations and exposure durations of H$_2$SO$_4$ aerosols employed in this study produced:

1. No relationship between percent phagocytosis and a CT index (concentration x exposure length). A steady increase in phagocytosis with an increase in incubation time was seen in test and control rabbits. The phagocytic index was depressed in all exposed rabbits at all incubation times used. The maximum depression occurred at the 1.0 hour incubation and decreased at the longer incubation times. The phagocytic index approached the control index at 4 hours incubation, suggesting recovery.

2. Viability of recovered macrophage populations remained unaltered, regardless of H$_2$SO$_4$ concentration or exposure length.

3. The percentage of macrophages in lavage fluids was not changed by any of the exposures to H$_2$SO$_4$ aerosols.

4. The total protein in all-free lavage fluids appeared to decrease with an increase in the CT index. This may be a result of an increase in pulmonary proteolytic activity produced by a decrease in pH.

5. Preliminary morphological changes, observed by scanning electron microscopy, revealed shortened and matted cilia in the deeper segments of the superior rami.

6. Signs of toxicity exhibited by the rabbit, i.e., sneezing and pawing at the nose, increased in frequency and duration with an increase in the H$_2$SO$_4$ aerosol concentration.
Future studies with sulfuric acid mist should include provisions for examination of the lung macrophage ultrastructure for membrane effects attributable to $\text{H}_2\text{SO}_4$. The effects of inhaled $\text{H}_2\text{SO}_4$ on mucociliary transport rates, bactericidal activity, and metabolic requirements of macrophages should also be investigated. Data from these evaluations should significantly enhance the assessment of the hazard from exposure to airborne sulfuric acid aerosol.
SELECTED BIBLIOGRAPHY


_________. "Long Term Exposure to \( \text{SO}_2 \), \( \text{H}_2\text{SO}_4 \) Mist, Fly Ash and Their Mixtures: Results of Studies in Monkeys and Guinea Pigs," Arch. of Environ. Health, 30:254-262, May 1975.


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"Influence of Particle Size on Phagocytosis by Rabbit Alveolar 
Macrophages In Vitro," Inhalation Toxicology Research Institute 

Higgins, Ian T. T.  "Effects of Sulfur Oxides and Particulates on 


Junge, C. E. and T. G. Ryan.  "Study of the SO\textsubscript{2} Oxidation in Solution 
and Its Role in Atmospheric Chemistry," Quart. J. Royal Met. 

Karnovsky, Manfred L.  "Metabolic Basis of Phagocytic Activity," 

Katz, G. V. and S. Laskin.  "Pulmonary Macrophage Response to Irritant 
Gases," in Air Pollution and the Lung, E. F. Aharonson, A. Ben- 
David, and M. A. Klingberg (eds.), New York, John Wiley and 
Sons, 1976.

Ketels, Kathleen V., Jeannie N. Bradof, James D. Fenters, and Richard 
tory Tract of Mice Exposed to H\textsubscript{2}SO\textsubscript{4} Mist Carbon Particle 

Klika, E., V. Rychterova, I. Tesik, D. Jarkovska, and A. Borecka. 
ACTA Universitatis Carolinae, Monographia LXIX, 1975.

Kozarek, W. J. and Q. Fernando.  "Location of the Equivalence Point in 
Potentiometric Titrations," J. Chemical Education, 49:202-204, 
March 1972.

Larson, Timothy V., David S. Covert, Robert Frank, and Robert J. 
Charlson.  "Ammonia in the Human Airways: Neutralization of 
Inspired Acid Sulfate Aerosols," Science, 197:161-163, 8 July 
1977.

Campbell.  "Toxicity of Long Term Exposure to Oxides of Sulfur," 

Lockard, Virginia G. and Ronald E. Kennedy.  "Alterations in Rabbit 
Alveolar Macrophases as a Result of Traumatic Shock," Lab. 

Mackaness, G. B.  "The Phagocytosis and Inactivation of Staphylococci by 


