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The Response of Rat Alveolar Macrophages to Chronic Inhalation of Coal Dust and/or Diesel Exhaust[1]

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The use of diesel-powered equipment in underground mines has raised questions regarding possible synergistic effects of coal dust and diesel emissions. Therefore, the effects of chronic exposure of rats to coal dust and/or diesel exhaust on various properties of alveolar macrophages were investigated. Inhalation exposure of rats was 7 hr/day, 5 days/week for 2 years. Exposure groups were: filtered air controls, 2 mg/m^3 coal dust, 2 mg/m^3 diesel particulate, and 1 mg/m^3 coal dust plus 1 mg/m^3 diesel exhaust. Exposure to coal dust and/or diesel exhaust had little effect on oxygen consumption, membrane integrity, lysosomal enzyme activity, or protein content of alveolar macrophages. However, exposure to coal dust increased macrophage yield, enhanced chemiluminescence, and increased the activity of the cell membrane (i.e., increased cellular spreading and surface ruffling). In contrast, diesel emissions depressed chemiluminescence and decreased the ruffling of the cell membrane. Therefore, the data suggest that exposure to coal dust and/or diesel exhaust does not affect the viability of alveolar macrophages. However, coal dust may activate alveolar macrophages while diesel emissions may depress the phagocytic activity of these cells. The combination of exposures to coal dust and diesel exhaust results in a phagocytic activity which is an average of the effects of separate exposures.

INTRODUCTION

In recent years there has been increasing interest in the use of diesel-powered units in coal mines. Diesel engine exhaust contains a diversity of potentially toxic materials in the form of gases, metals, chemical aggregates, polycyclic organic matter, and particulates (Santodonate *et al.*, 1978). Therefore, the biological effects of exposure to diesel exhaust are currently receiving a great deal of attention. In addition, a number of studies have been conducted concerning the health consequences of exposure to coal dust, especially with regard to the formation of lung lesions characteristic of coal workers' pneumoconiosis. To date, little information is available concerning possible synergistic effects of coal dust plus diesel exhaust. Some studies have been conducted which measure the effect of

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diesel exhaust on pulmonary function tests with coal miners (Ames *et al.*, 1982; Reger *et al.*, 1982) while another study has investigated the effects of coal dust plus diesel exhaust on lung morphology (Karagianes *et al.*, 1981). However, little is known concerning the effects of coal dust–diesel exhaust mixtures on the function of individual lung cells.

Alveolar macrophages are free lung cells located on the surface of the small airways and the alveoli (Weibel, 1973). These cells play an important role in the protection of the lung against airborne bacteria and particles. The response of alveolar macrophages to inspired bacteria and particles is complex. Upon contact with the particles, alveolar macrophages release superoxide anion (O_2^-) (Drath *et al.*, 1976, 1978; Sweeney *et al.*, 1981), which may be involved in the detoxification of these foreign substances (Sagone *et al.*, 1976). The release of superoxide anion by alveolar macrophages after exposure to foreign particles can be monitored by the measurement of chemiluminescence (Miles *et al.*, 1978; Castranova *et al.*, 1980). Exposure to foreign particles also causes large increases in the consumption of oxygen (Drath *et al.*, 1976, 1978; Castranova *et al.*, 1980; Hoidal *et al.*, 1978). Finally, alveolar macrophages engulf these foreign particles and digest them by releasing lysosomal enzymes into phagocytic vacuoles (Myrvik and Evan, 1967; Myrvik *et al.*, 1961). In this manner, these phagocytes cleanse the lung of bacteria and of foreign particles (Green, 1972).

Since alveolar macrophages act as the lung's first line of defense against the toxic effects of inhaled substances, it is important to investigate the effects of diesel exhaust–coal dust mixtures on alveolar macrophage viability and function. Therefore, the objective of the present investigation was to characterize the effects of *in vivo* exposure to coal dust and/or diesel exhaust on various properties of rat alveolar macrophages. The physiological parameters measured in this study were as follows: cellular number, cellular volume, oxygen consumption, chemiluminescence, leakage of protein and lysosomal enzymes, cellular protein, cellular lysosomal enzyme activity, and cell surface morphology. Rats were exposed by inhalation for 7 hr/day, 5 days/week, for 2 years to a total respirable dust load of 2 mg/m^3, i.e., the current standard in coal mines. Experimental animals were divided into four exposure groups: (1) controls (filtered air), (2) coal dust (2 mg/m^3), (3) diesel exhaust (2 mg/m^3), and (4) coal dust (1 mg/m^3) plus diesel exhaust (1 mg/m^3).

MATERIALS AND METHODS

Inhalation Exposure

Specific-pathogen-free Fisher 344 male rats were separated into four exposure groups: (1) control (filtered air), (2) coal dust (2 mg/m^3 respirable dust), (3) diesel exhaust (2 mg/m^3 respirable dust), and (4) diesel exhaust (1 mg/m^3 respirable dust) plus coal dust (1 mg/m^3 respirable dust). Inhalation exposure was conducted for 7 hr/day, 5 days/week, for 2 years at the Center Hill exposure facilities of the Environmental Protection Agency (EPA, Cincinnati, Ohio). During periods of exposure, the chambers were darkened in order to increase the activity of the rats, i.e., photoperiod was set for a 7-hr dark day and a 17-hr light period so that exposure was during the active period of the rats.

The rats used in this study were immunized against Sendai virus prior to the 2-year exposure. At various times rats were tested for titers of *Mycoplasma pulmonis* and lung autopsies performed. No evidence of serious pulmonary infection was noted in any of the exposure groups. Food and water were supplied *ad libitum* during periods of nonexposure. Temperature and humidity were strictly controlled at 22°C and 50% relative humidity with a tolerable range of 10%. Emissions were monitored for particulate load, particle size, gas composition, and organic and inorganic chemicals. These variables were maintained within strict limits throughout the 2-year exposure. Concentrations of various emission components are listed in Table 1.

The coal sample was eastern bituminous coal from the Blacksville No. 1 mine in West Virginia. Coal was micronized and sealed under nitrogen until it was generated into the exposure chambers by a Wright dust feeder. The mass median diameter determined by scanning microscopy at a magnification of 1000× was 10.2 μm in the bulk sample and 8.5 μm in the chambers.

Diesel emissions were generated by a four-cylinder, four-cycle Caterpillar 3304 diesel engine identical to those commonly used in underground coal mines. The engine consumed no. 2 diesel fuel containing 0.5% sulfur. The engine was equipped with a water exhaust scrubber and operated on a duty cycle which simulated mine operating conditions, i.e., a repetitive eight-mode cycle where engine load and speed were varied and approximately 55% of the cycle was in the idle mode. The diesel exhaust was diluted to achieve a particulate concentra-

TABLE 1

ATMOSPHERIC CONCENTRATIONS OF MAJOR CHAMBER COMPONENTS OVER THE 24-MONTH EXPOSURE DURATION

	Treatment			
Component	FA	CD	CD + DEP	DEP
Respirable particles (mg/m^3)	NM	2.1 ± 0.4**	2.0 ± 0.3	1.9 ± 0.3
CO_2 (%)	0.07 ± 0.02	0.08 ± 0.04	0.17 ± 0.06	0.16 ± 0.04
CO (ppm)	2.0 ± 0.9	2.1 ± 0.8	10.3 ± 2.0	10.5 ± 2.3
NO (ppm)	0.08 ± 0.13	0.08 ± 0.28	7.6 ± 2.8	7.8 ± 3.1
NO_2 (ppm)	0.06 ± 0.04	0.07 ± 0.05	1.5 ± 0.5	1.5 ± 0.5
SO_2 (ppm)	NM	0.003 ± 0.005	0.5 ± 0.3	0.6 ± 0.4
NH_3 (ppm)	0.5 ± 0.6	0.6 ± 0.7	0.4 ± 0.3	0.6 ± 0.8

Note. Airborne concentrations in the filtered air (FA) and coal dust chambers arose from the same ambient filtered/conditioned air supply and thus should be almost identical, except for the coal dust that was introduced. CD + DEP and DEP represent contributions from the same generation source with the baghouse filtration in the former instance the only variable. A continuous reading of the respirable particulate concentration was obtained within each exposure chamber by light scattering using a cyclone preseparator and a respirable aerosol monitor. Time-weighted averages of respirable dust concentrations (data shown; $\bar{x}$ ± SD) were taken three times a day using a Mine Research Establishment gravimetric sampler (Casella London Limited, Model 113A) which measures respirable particulate concentration using a horizontal elutriator in series with a filter by criteria established by the British Medical Research Council. Readings from both methods were always in close agreement. NM, not routinely monitored. **$\bar{x}$ ± SD.

tion of 2 mg/m^3 for exposure at this level. The same air stream (2 mg/m^3) was used to obtain a diesel exhaust particulate concentration of 1 mg/m^3 for the diesel exhaust–coal dust mixture by splitting the air stream in half, running one half through a bag house which acted as an absolute filter for the particulates, and then recombining the split streams. This resulted in halving the particulates but not affecting the gases or vapors. Measurement of the respirable diesel exhaust particulate concentration was made on the unsplit and recombined diesel air streams just before entering the respective exposure chambers. The mass median diameter of the diesel particulate was measured using an electrical aerosol size analyzer (Thermal-Systems Inc., Model 3030) or by scanning microscopy to be 0.23 μm and 0.36 μm, respectively.

Cell Preparation

After 2 years of exposure, rats were transferred to the Appalachian Laboratory for Occupational Safety and Health (Morgantown, W.Va.) for study. All assays were performed within 48 hr of the last exposure. Data obtained with cells from filtered-air controls were compared with those from unexposed rats housed in Morgantown. No significant differences in yield, protein, oxygen consumption, or chemiluminescence attributable to stress in transit from Cincinnati were noted. Alveolar macrophages were obtained by tracheal lavage according to the method of Myrvik *et al.* (1961). The animals were anesthetized with sodium pentobarbital (0.2 g/kg body wt) and exsanguinated by cutting the abdominal aorta. The lungs from each rat were lavaged 12 times with a total of 80 ml of an ice-cold Ca^{2+}-free solution which contained 145 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4, 9.35 mM Na_2HPO_4, and 5.5 mM glucose (pH 7.4). The cells were separated from the lavage fluid by centrifugation at 500g for 5 min. Alveolar macrophages were then washed twice by alternate centrifugation and resuspension in a Hepes-buffered medium of the following composition: 140 mM NaCl, 5 mM KCl, 10 mM Na Hepes (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid), and 5.5 mM glucose (pH 7.4). Measurement of cellular volume, oxygen consumption, and chemiluminescence were performed with cells suspended in Hepes-buffered medium.

Measurement of Macrophage Yield and Cellular Volume

The yield of alveolar macrophages obtained per rat and the cellular volume of these cells were determined as described previously (Castranova *et al.*, 1979) by using an electronic cell counter interfaced with a cell sizing attachment (Coulter Model Z_B with a Channelyzer II attachment, Coulter Instrument Co., Hialeah, Fla.). The number of alveolar macrophages in the lavage was calculated from the cell count multiplied by the percent of cells in the sample which possess a size which is characteristic of alveolar macrophages; i.e., contaminating red blood cells, lymphocytes, and polymophonuclear leukocytes were eliminated.

Measurement of Oxygen Consumption

Oxygen consumption was measured with a Gilson K-IC oxygraph which was equipped with a Clark electrode (Gilson Medical Electronics; Middleton, Wisc.) (Castranova *et al.*, 1980). The oxygraph was calibrated by measuring the level of oxygen in Hepes-buffered medium which had been bubbled with gases of known oxygen concentrations until saturation occurred. In order to measure the amount of oxygen consumed by alveolar macrophages, 4×10^6 cells were preincubated in 1.75 ml of Hepes-buffered medium at 37°C for 15 min. After preincubation this cell suspension was transferred to the chamber of the oxygraph which was also maintained at 37°C, and oxygen consumption was measured over a period of 10 min. When the effects of particle exposure were studied, zymosan (6 mg/ml) was added to the cell suspension at the time when the cells were transferred to the oxygraph (zero time). Oxygen consumption was expressed as nanomoles of oxygen consumed per 10^7 alveolar macrophages per minute.

Measurement of Chemiluminescence

Chemiluminescence was measured according to a method which we published previously (Miles *et al.*, 1978; Castranova *et al.*, 1980). Briefly, 4×10^6 alveolar macrophages in 5 ml of Hepes-buffered medium were preincubated at 37°C for 15 min. All incubations were done in dark-adapted plastic liquid scintillation vials. After the period of preincubation, zymosan (6 mg/ml) was added to some cell suspensions and particle-stimulated chemiluminescence was measured immediately (zero-time measurement). Resting chemiluminescence was enhanced by the addition of luminol, final concentration of 10^{-8} *M* (Aldrich Chemical Co., Milwaukee, Wisc.). Chemiluminescence was measured as counts per minute in the tritium channel of a liquid scintillation counter (Model LS-345; Beckman Instrument Co.; Fullerton, Calif.) operated in the out-of-coincidence mode. After the zero-time determination, the vials were returned to the water bath where they were maintained at 37°C. Measurements of chemiluminescence were then made at various later times. The background level of chemiluminescence was measured either in the presence of zymosan and buffer or luminol and buffer. In this paper chemiluminescence was expressed as counts per minute minus the background level.

Analysis of Protein and Lysosomal Enzyme Activity

Alveolar macrophages and acellular lavage fluid were collected by modification of the method described above. Aliquots (5 ml) of Ca^{2+}-free solution were used for the lavage procedure and 16 separate aliquots were collected from each rat. The first two aliquots were combined (sample A) as were the last 14 (sample B). Both samples were centrifuged at 500*g* for 10 min to sediment cells. The supernatant from sample A was saved for determination of biochemical properties in the cell-free fraction of the lavage fluid termed acellular lavage fluid. This volume was approximately 7 ml as there was not complete recovery of the fluid from the lungs on the first two lavages. The supernate from sample B was discarded rather than combined with that from sample A to avoid dilution of acellular protein and enzymes. The cell pellets from both samples were combined, washed one time with 0.9% NaCl (w/v; isotonic saline) and resuspended in a small volume of this same solution.

The following biochemical properties were measured on the alveolar macrophage fraction and the acellular lavage fluid from each rat: protein content, assayed by the method of Hartree (1972) using bovine serum albumin as the standard; acid phosphatase activity at pH 5.0, following the procedure of Turnbull and Neil (1969) with *p*-nitrophenyl phosphate as substrate; β-galactosidase at pH 4.5, as described by Lockard and Kennedy (1976) with *p*-nitrophenyl-β-D-galactopyranoside as substrate; and β-*N*-acetylglucosaminidase at pH 4.5, according to Sellinger *et al.* (1960) with *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as substrate. For the three lysosomal enzyme activities, alveolar macrophages (4×10^5 cells) were suspended in 200 μl of isotonic saline and the lysosomal enzymes released from the cells by incubation with 50 μl of 1% Triton X-100 (w/v).

Preparation for Scanning Electron Microscopy (SEM)

Macrophage monolayers were prepared using round 15-mm plastic tissue culture cover slips (Lux Scientific) placed in the bottom of the flat-bottom wells (approx. 1.7×1.6 cm) of Linbro Multi-well Tissue Culture plates. Two milliliters of cell suspension were pipetted into each of five wells for each of the four exposure groups. The cells were incubated at 37°C in 5% CO_2 for 2 hr to allow adherence of the cells to the cover slips and then rinsed two times with phosphate-buffered medium to remove nonadherent cells and free coal/diesel particulate.

The alveolar macrophage monolayers were fixed for 24 hr in 2% Karnovsky's fixative at pH 7.2 ± 0.1 and washed in 0.2 *M* cacadylate buffer. Subsequent to critical-point drying the cells were dehydrated through a graded series, 10–100%, of ethanol with 10% gradations and finally transferred through graded concentrations of amyl acetate–ethanol solutions to 100% amyl acetate. A Denton DCP-1 critical-point drying apparatus using liquid CO_2 was used for drying the cells. The plastic cover slip with the adhering cells was glued to pure carbon planchets and mounted on aluminum SEM stubs and coated with gold/palladium (200 Å) or carbon. Coating of the samples was carried out using a Polaron E5100 Series II sputter coater or a Polaron E6100 Vacuum Evaporator, respectively.

Scanning electron microscopic examination of the alveolar macrophage monolayers was performed using an ETEC Autoscan SEM operating at 20 kV using both secondary (SEI) and backscatter (BEI) imaging.

Micrographs of the cell monolayers were taken at random for each of the four exposure groups. These micrographs were then scored on a 1 thru 5 scale for three morphologic characteristics, i.e., cellular spreading, surface ruffling, and filopodia. Scores from three independent observers were averaged for statistical analysis.

Statistical Analysis

The treatment groups were compared to the controls using a one-way analysis of variance and a Tukey's test or using student's *t* test. Significance was established at $P < 0.05$.

RESULTS

The effects of chronic exposure of rats to coal dust (2 mg/m^3), diesel exhaust (2 mg/m^3), or coal dust (1 mg/m^3) plus diesel exhaust (1 mg/m^3) on alveolar macrophage protein, volume, and yield are listed in Table 2. After 2 years of inhalation

TABLE 2
PROPERTIES OF RAT ALVEOLAR MACROPHAGES AFTER EXPOSURE FOR 2 YEARS[a]

	Protein ($mg/10^7$ cells)	Mean cell volume (μm^3)	Mean cell number (cells/rat)
Control	2.7 ± 0.2	1798 ± 27	$0.87\ (\pm 0.08) \times 10^7$
Coal dust	2.8 ± 0.2	1727 ± 49	$2.47\ (\pm 0.73) \times 10^7$*
Diesel exhaust	3.6 ± 0.5	1664 ± 43*	$1.06\ (\pm 0.08) \times 10^7$
Diesel exhaust + coal dust	3.3 ± 0.2	1739 ± 36	$0.99\ (\pm 0.08) \times 10^7$

[a] Values are the means ± SEM of at least five experiments.
* Significantly different from control ($p < 0.05$).

exposure, there was no significant diffrerence in cellular protein content between cells from exposed or unexposed rats. These data suggest that exposure did not affect protein synthesis. In addition, exposure did not cause membrane leakiness; i.e., alveolar macrophages did not swell. In fact, cells from rats exposed to diesel exhaust were approximately 7% smaller than control. Exposure to diesel exhaust or to the diesel exhaust–coal dust mixture did not affect the number of alveolar macrophages recovered by pulmonary lavage. In contrast, the cell yield was increased by approximately 184% after a 2-year exposure to coal dust.

The effect of inhalation exposure on the membrane integrity of alveolar macrophages was studied by monitoring the amount of protein or lysosomal enzymes which appeared in the acellular pulmonary lavage fluid. These data are summarized in Table 3. Chronic exposure to coal dust and/or diesel exhaust did not cause membrane leakiness; i.e., cellular protein or enzymes did not leak into the extracellular fluid lining the lungs. This maintenance of membrane integrity after exposure agrees with our data demonstrating no increase in the cellular volume of alveolar macrophages obtained from rats exposed to coal dust and/or diesel exhaust (Table 2).

We also measured the activity of lysosomal enzymes in alveolar macrophages obtained from exposed rats. These data are listed in Table 4. Inhalation exposure

TABLE 3
BIOCHEMICAL PROPERTIES OF THE ACELLULAR FLUID FROM PULMONARY LAVAGE OF 2-YEAR-EXPOSED RATS[a]

	Protein (mg/ml)	AP[b]	β-Gal[c]	β-N-AG[d]
Control	0.18 ± 0.02	ND	0.32 ± 0.05	3.6 ± 0.3
Coal dust	0.26 ± 0.05	ND	0.23 ± 0.03	3.4 ± 0.2
Diesel exhaust	0.24 ± 0.02	ND	0.24 ± 0.02	4.3 ± 0.2
Diesel exhaust + coal dust	0.19 ± 0.01	ND	0.29 ± 0.02	3.7 ± 0.2

[a] Values are the means ± SEM of at least eight experiments using acellular fluid from the first two lavages (7 ml). Values were not significantly different from control ($P < 0.05$).
[b] Acid phosphatase; ND, enzyme activity not detectable.
[c] β-galactosidase (nmol substrate hydrolyzed/min/ml).
[d] β-*N*-acetylglucosaminidase (nmol substrate hydrolyzed/min/ml).

TABLE 4
BIOCHEMICAL PROPERTIES OF RAT ALVEOLAR MACROPHAGES AFTER EXPOSURE FOR 2 YEARS[a]

	AP[b]	β-Gal[c]	β-N-AG[d]
Control	154.1 ± 14.5	82.0 ± 5.4	150.9 ± 12.2
Coal dust	167.3 ± 8.7	82.9 ± 5.1	187.5 ± 13.2
Diesel exhaust	185.6 ± 14.2	91.4 ± 7.2	209.1 ± 19.8*
Diesel exhaust + coal exhaust	185.7 ± 12.2	86.6 ± 5.1	189.0 ± 11.1

[a] Values are the means ± SEM of at least eight experiments.
[b] Acid phosphatase (nmol substrate hydrolyzed/min/10^7 cells).
[c] β-galactosidase (nmol substrate hydrolyzed/min/10^7 cells).
[d] β-N-acetylglucosaminidase (nmoles substrate hydrolyzed/min/10^7 cells).
* Significantly different from control ($P < 0.05$).

to coal dust and/or diesel exhaust for the most part did not alter cell-associated lysosomal enzyme activity. The only significant change observed was a 39% increase in the activity of β-*N*-acetylglucosaminidase after exposure to diesel exhaust. However, diesel exhaust had no significant effect on the activity of the other lysosomal enzymes tested, i.e., acid phosphatase and β-galactosidase.

The effect of chronic inhalation exposure to coal dust and/or diesel exhaust on oxygen consumption by alveolar macrophages is shown in Fig. 1. Exposure did not affect the rate of oxygen consumption by resting alveolar macrophages, i.e., cells not stimulated by zymosan. These data suggest that chronic exposure to coal dust and/or diesel exhaust did not decrease the viability of alveolar macrophages. It is known that addition of zymosan increases oxygen consumption, i.e.,

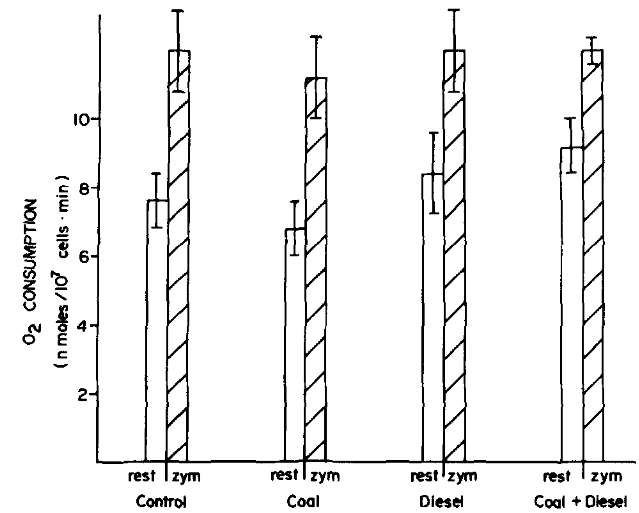

FIG. 1. Oxygen consumption by alveolar macrophages obtained from rats after 2 years of inhalation exposure to filtered air (control), coal dust (2 mg/m^3), diesel exhaust (2 mg/m^3), or coal dust (1 mg/m^3) plus diesel exhaust (1 mg/m^3). Oxygen consumption was measured with unstimulated cells (rest) and with cells stimulated with zymosan (6 mg/ml). Values are means ± standard errors of five separate experiments.

initiates a respiratory burst in alveolar macrophages (Miles *et al.*, 1978; Castranova *et al.*, 1980). Inhalation exposure had no significant effect on the magnitude of this respiratory burst.

The respiratory burst can also be monitored by measuring chemiluminescence. Chemiluminescence has been shown to result from the release of active forms of oxygen, i.e., superoxide anion, hydrogen peroxide, hydroxide ion, etc. (Miles *et al.*, 1978). The effects of inhalation exposure on the chemiluminescence generated by alveolar macrophages at rest are shown in Fig. 2. Note that resting chemiluminescence is so low that it must be amplified with luminol in order to monitor its time course. Exposure to coal dust resulted in a substantial increase in resting chemiluminescence (note points designated (a)). Therefore, these data are consistent with an activation of alveolar macrophages by coal dust. In contrast, exposure to diesel exhaust depressed resting alveolar macrophages; i.e., resting chemiluminescence was significantly lower than control as noted by points designated (a). The effects of exposure on zymosan-stimulated chemiluminescence are shown in Fig. 3. In all cases zymosan increased chemiluminescence over the resting level. However, exposure to coal dust significantly enhanced this response while diesel or diesel plus coal depressed the ability of alveolar macrophages to respond to zymosan particles (note points designated (a)).

Since data concerning chemiluminescence suggested that coal dust activated alveolar macrophages while diesel exhaust depressed these cells, we analyzed the surface characteristics of these phagocytes by using scanning electron microscopy (Fig. 4). Scanning electron microscopy indicates that alveolar macrophages obtained from control rats were a mixture of rounded and flattened cells. Most control cells appeared rounded and exhibited a ruffled surface (Fig. 4A). After exposure to coal dust, a greater proportion of alveolar macrophages appeared flattened rather than rounded. In addition, after exposure to coal dust

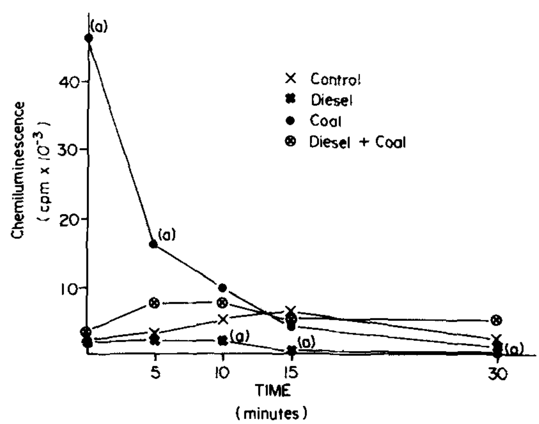

FIG. 2. Resting chemiluminescence by alveolar macrophages obtained from rats after 2 years of inhalation exposure to filtered air (control), coal dust (2 mg/m^3), diesel exhaust (2 mg/m^3), or coal dust (1 mg/m^3) plus diesel exhaust (1 mg/m^3). Resting chemiluminescence was enhanced by the addition of 10^{-8} M luminol. Values are means of five separate experiments while (a) designates values significantly different from control ($P < 0.05$).

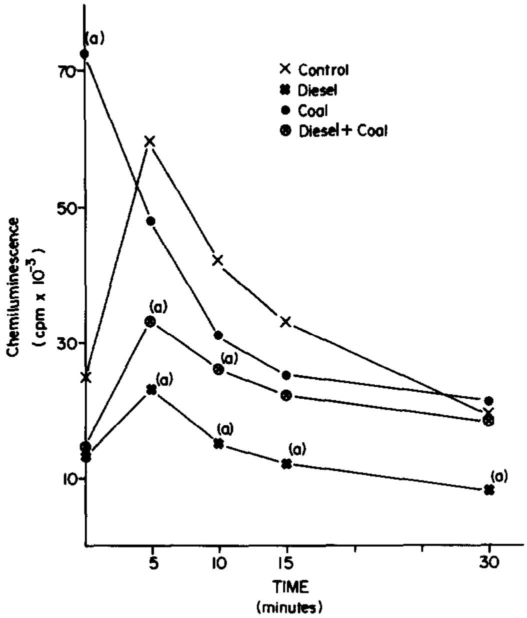

FIG. 3. Chemiluminescence generated by alveolar macrophages in response to zymosan (6 mg/ml). Values are means of five separate experiments while (a) designates values significantly different from control ($P < 0.05$).

ruffling of the membrane surface was increased (Fig. 4B). In contrast, after exposure to diesel exhaust a decreased number of flattened alveolar macrophages was observed. Diesel-exposed alveolar macrophages also exhibited decreased ruffling and a smoothing of the plasma membrane (Fig. 4C). Some diesel-exposed macrophages also exhibited surface blebbing and deterioration (Fig. 4C). These data are consistent with the hypothesis that coal dust activates alveolar macrophages and diesel exhaust depresses these phagocytes.

DISCUSSION

The data presented in this investigation indicate that inhalation exposure to coal dust and/or diesel exhaust for 2 years is not toxic to rat alveolar macrophages. However, exposure to coal dust does enhance the phagocytic activity of alveolar macrophages while exposure to diesel emissions has a depressive effect. Since few studies have investigated possible harmful interactions between coal dust and diesel emissions, our data cannot be compared with existing literature on effects of combined exposures. However, our results can be compared with data concerning exposure to either coal dust or diesel exhaust.

Our data indicate that inhalation exposure to coal dust (2 mg/m^3) for 2 years increased the number of alveolar macrophages obtained by pulmonary lavage. In contrast, other exposure regimes had no effect on cell yield. The failure of treatment with coal dust plus diesel exhaust to enhance cell yield may be due to the lower dose of coal used in the combined exposure, i.e., 1 mg/m^3 coal dust with

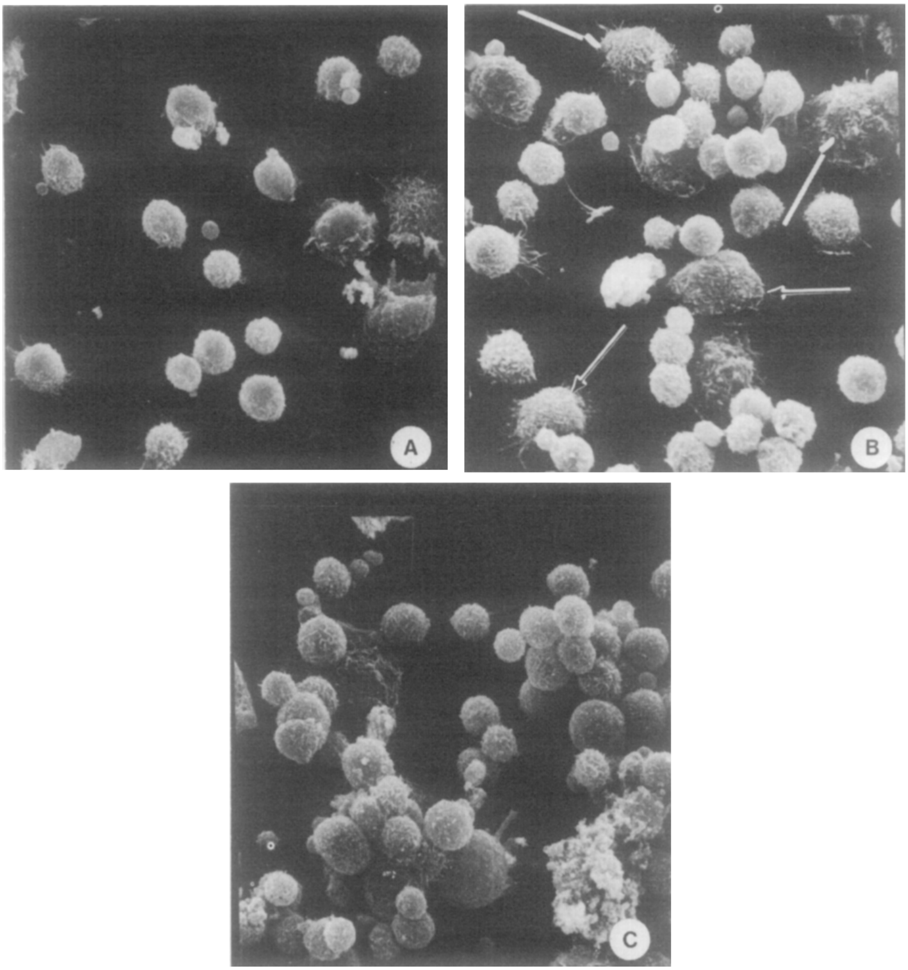

FIG. 4. Scanning electron micrographs of rat alveolar macrophages: (A) control cells at 1000×; (B) cells exposed to coal dust (2 mg/m^3 for 2 years) at 1000× (arrows indicated flattened cells); (C) cells exposed to diesel exhaust (2 mg/m^3 for 2 years) at 1000×.

the combined exposure as compared to 2 mg/m^2 for coal dust exposure alone. Other studies have also reported an increase in the number of alveolar macrophages after exposure to coal (Kissler *et al.*, 1982; Bingham *et al.*, 1977; Bowden and Adamson, 1978; Brain, 1971). One study indicates that macrophage proliferation is short lived, i.e., occurs only the first month after coal exposure (Bingham *et al.*, 1977). However, rats in our study were exposed for 2 years. Data concerning the effect of diesel emissions on cell yield are conflicting. Some reports indicate that diesel exhaust increases the number of macrophages in the lung (Vostal, 1980; Strom, 1981) while our results and other data indicate no change (Chen *et al.*, 1980 a,b). Microscopic evaluation of lungs from other diesel-exposed rats in the National Institute of Occupational Safety and Health (NIOSH) study indicates that there is an increased number of macrophages in the air spaces; however, it is likely that many of the macrophages are trapped due to hyperplasia of alveolar epithelial cells and an increase in alveolar debris associated with diesel exposure (Dr. Frank Green, personal communications). These effects were present after 2 years of exposure, but not after 1 year. Therefore, the conflicting data concerning yield after lavage of diesel-exposed lungs are probably due to differences in dose and duration of exposure.

Data from Table 2 indicate that exposure to coal dust and/or diesel exhaust had little effect on cellular volume with diesel emissions causing a slight decrease in volume. The fact that there were no changes in cellular protein is also consistent with a lack of volume change. These data conflict with other studies which suggest that diesel exposure increases macrophage diameter as measured microscopically (Strom, 1981; Chen *et al.*, 1980a). This discrepancy is probably due to the effect of diesel exhaust on the surface structure of alveolar macrophages. As shown in Fig. 4C, diesel-exposed cells do appear to be larger in diameter than control cells. However, many of the diesel-exposed cells have smoother rather than ruffled surface. This loss of surface ruffling tends to decrease volume and counter the effect of increased diameter.

Measurements of oxygen consumption and membrane integrity indicate that exposure to coal particulates and/or diesel emissions was not toxic to alveolar macrophages, i.e., cellular viability was unaffected. These data agree with reports from other laboratories concerning the lack of effect of coal dust (Adams and Timar, 1978) or diesel emission (Strom, 1981; Chen *et al.*, 1980a; Weller *et al.*, 1981) on the viability alveolar macrophages. In contrast, Mauderly *et al.* (1982) have reported a decrease in the membrane integrity of alveolar macrophages after exposure to high levels of diesel exhaust (3–6 mg/m^3). However, this discrepancy is probably due to the fact that these investigators used levels of diesel emissions which were as much as three times the levels used in our study.

In general, our data indicate that exposure to coal dust and/or diesel exhaust did not affect lysosomal enzyme activity. The only exception was a small elevation of one enzyme activity, i.e., β-*N*-acetylglucosaminidase, after diesel exposure. There is a report that diesel exhaust can enhance acid phosphatase activity (Weller *et al.*, 1980). However, another study reported a decrease in acid phosphatase in diesel-exposed alveolar macrophages (Weller *et al.*, 1981). We found no change in acid phosphatase activity in response to diesel emissions (Table 4). There is also a report that short-term exposure to coal dust increased lysosomal enzyme activity (Bingham *et al.*, 1977). This result conflicts with our data and may suggest the lysosomal enzyme activity stabilizes after long periods of inhalation exposure.

Our data indicate that the protein content of alveolar macrophages was not affected by exposure to coal dust and/or diesel exhaust. This suggests that exposure did not affect protein synthesis. Karol *et al.* (1979) reported that coal dust inhibited protein synthesis by alveolar macrophages. However, their exposure level of coal dust was very high (200 mg/m^3) compared to our level of 2 mg/m^3.

Data concerning chemiluminescence (Figs. 2 and 3) indicate that coal dust activates alveolar macrophages while diesel exhaust depresses phagocytic activity. Davis *et al.* (1979) have suggested that phagocytic activity can be determined by observation of the characteristics of the plasma membrane of alveolar macrophages with an increase in phagocytic activity being related to cell flattening and increased membrane ruffling. The scanning electron micrographs presented in Fig. 4, therefore, support the conclusions obtained from the chemiluminescence studies. Another laboratory has also suggested that diesel exhaust decreases the phagocytic activity of alveolar macrophages (Chen *et al.*, 1980a). They found that diesel exhaust decreased particle uptake by alveolar macrophages and that the surface of these cells exhibited smoothing with blebs replacing ruffling. In contrast, another report suggested that diesel exhaust had no effect on uptake (Chen *et al.*, 1980b). Our data concerning coal dust are in agreement with those of Hill *et al.* (1982) which report increased surface ruffling in response to coal fly ash.

The data concerning surface morphology and secretion of active forms of oxygen suggest that *in vivo* exposure of rats to diesel emissions results in depression of the phagocytic activity of alveolar macrophages. This suggestion is supported by results obtained by other investigators in the NIOSH coal dust–diesel exhaust study. Hahon *et al.* (1985) have shown that mice exposed *in vivo* to diesel exhaust are more susceptible to pulmonary infection by influenza virus. This result seems likely, since our data indicate that the release of antibacterial substances by alveolar macrophages is depressed after exposure to diesel exhaust. Green *et al.* (personal communications) have also shown as part of the NIOSH study that particulate clearance, as determined by particulate accumulation in the lung, is inhibited after 2 years of exposure to diesel exhaust but is not inhibited by exposure to coal dust. These data correlate with our suggestion that phagocytic activity of alveolar macrophages is depressed by diesel exposure.

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