ALTERATIONS IN PULMONARY XENOBIOTIC METABOLIZING ENZYME SYSTEMS IN ASBESTOTIC ANIMALS

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INTRODUCTION

The synergistic interaction of asbestos and tobacco smoke in the genesis of bronchogenic carcinoma has been reported extensively by both epidemiological and experimental studies.[1,30,42,18,26,31,49] Inspite of several efforts in this direction, the precise biochemical mechanisms involved in the potentiating effects of asbestos in the development of bronchogenic carcinoma remains obscure. It is conceivable that the fibres may adsorb the known carcinogens of cigarette smoke on their surface and thus facilitate their entry and retention in the system.[27,28] Experimental studies have shown the adsorption of benzo(a)pyrene, a major component of cigarette smoke on the surface of asbestos and their poor excretion from experimental animals.[41,44] Alternatively, asbestos fibre may directly modify carcinogen metabolizing enzyme system, viz. activation and conjugation reactions. It has been reported that asbestos fibres partially inactivate microsomal mixed function oxidase (MFO) system.[25,40] The present paper is concerned with the alterations in the xenobiotic metabolizing enzyme system, lipid peroxidation, antioxidant levels in the lung of rats at progressive stages of dust exposure.

MATERIALS AND METHODS

Dust

Chrysotile UICC standard reference sample, particle size <30 μ, was obtained as a gift from Dr. J.B. Leinweber, John-Manville Mills, U.S.A.

Chemicals

Benzo(a)pyrene, 3-hydroxy benzo(a)pyrene, styrene epoxide and bovine serum albumin were procured from Sigma Chemical Co., U.S.A. All the other chemicals and reagents were either purchased from V.P. Chest Institute, New Delhi or Sisco Research Laboratories (SRL), Bombay, India, and were of analytical grade.

Treatment of Animals

Male albino rats from the ITRC colony, weighing 150–180 gm were used. The dry dust and 0.15 M NaCl were separately autoclaved at 15 lbs pressure for 15 min, suspended and mixed thoroughly just before inoculation. The animals were intratracheally instilled with 5 mg of dust suspended in 0.5 ml of normal saline, according to the procedure, described by Zaidi.[54] Corresponding controls received 0.5 ml of normal saline only. The animals were maintained on a commercial pellet diet, supplied by Hindustan Lever Limited, Bombay, India and tap water *ad libitum*. Six animals from each group were sacrificed at 1, 4, 8, 16, 90 and 290 days after inoculation.

Isolation of Microsomes

The rat lung microsomal fraction was isolated by the procedure of Johannesen et al.[22]

Enzyme Assays

Benzo(a)pyrene hydroxylase was assayed by the fluorimetric techniques as described by Dehnen et al.[8] The quantitation of phenolic metabolites was based on comparison of fluorescence to a standard solution of 3-hydroxy benzo(a)pyrene. Epoxide hydratase activity was measured by the fluorimetric technique according to the method of Dansette et al.[7] by using styrene epoxide as substrate.

Glutathione-S-transferase activity was determined by the procedure described by Habig et al.,[15] using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate.

Chemical Estimation

Microsomal cytochrome P-450 was quantitated from carbon monoxide plus dithionite-reduced difference spectra as described by Omura and Sato.[38] An extinction coefficient of 91,000 cm^{-1}, M^{-1} was used for absorbance change between 450 and 490 nm. Glutathione content was measured in rat lung cytosolic fraction, according to the method of Ellmann.[11]

Ascorbic acid content was estimated in lung cytosol according to the procedure of Schaffert and Kingsley.[43]

Enzymatic and non-enzymatic lipid peroxidation was determined by the procedure of Ottolenghi[39] as modified by Hunter et al.[17]

Protein was estimated by the method of Lowry et al.,[29] with crystalline bovine serum albumin as standard.

Statistics

The values presented mean ± standard error of six animals, statistical significance was determined by Students' 't' test.

RESULTS

Effect of Chrysotile on Lung Weight

There was significant increase in lung weight of experimental animals at 90 and 290 days after treatment (Figure 1). At 290 days of exposure, the increase was 95% in fresh lung weight over the untreated group.

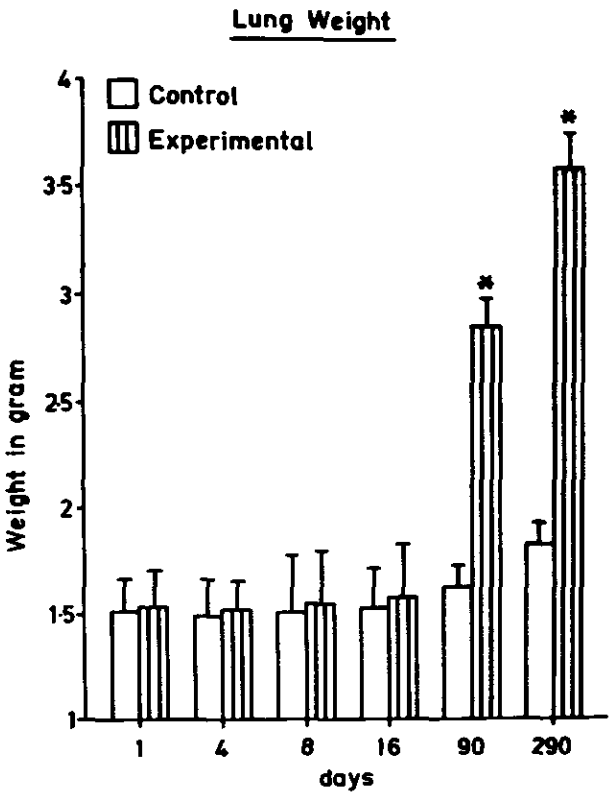

Figure 1. Fresh lung weight of control and chrysotile treated rats. The values are expressed as mean ± SEM of six animals. *p>0.001.

Effect of Chrysotile on Rat Lung Microsomal and Cytosolic Fractions

The biochemical changes related to drug metabolizing enzyme system induced by chrysotile at different time intervals are given in Figures 2, 3, 4, 5. As Figure 2 shows there was decrease in lung microsomal cytochrome P-450 content from 1 to 16 days but at 90 and 290 days there was significant increase in the content of P-450. At 290 days, the increase was recorded (45%). Same pattern was obtained with the activity of benzo(a)pyrene hydroxylase as shown in Figure 3. At 90 and 290 days, 49% and 48% increase were obtained over their controls, respectively. In case of epoxide hydratase as shown in Figure 4, till 16 days of exposure there was a decrease in the activity but at 90 and 290 days after treatment 90% and 96% increase were recorded respectively, over their controls. However, in cytosolic fraction there was a continuous decrease in the activity of glutathione-S-transferase in experimental animals as shown in Figure 5.

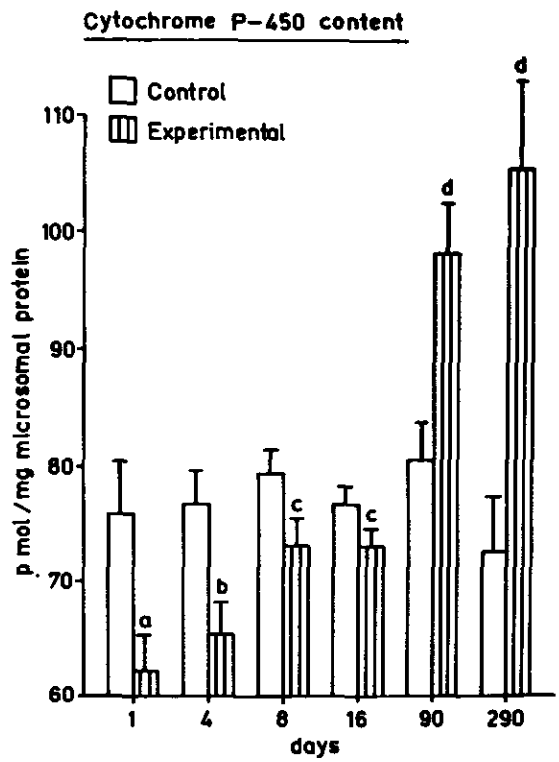

Figure 2. Lung cytochrome P-450 content of control and chrysotile treated rats. The values are expressed as mean ± SEM of six animals. $^{a}p<0.02$; $^{b}p<0.01$; $^{c}p<0.05$; $^{d}p<0.001$.

At 90 and 290 days, the decrease in the activity was 21% and 39% which is statistically quite significant.

Effect of Chrysotile on Water Soluble Antioxidants

As recorded in Figure 6, at 90 and 290 days after exposure, a significant decrease in the content of ascorbic acid was observed in the lung cytosolic fraction of the experimental animals. In case of reduced glutathione content as given in Figure 7, there was continuous decrease in experimental animals at all the stages of exposure but at 90 and 290 days after treatment the decrease is very significant.

Effect of Chrysotile on Lipid Peroxidation

As shown in Figure 8, at all the stages of exposure there was significant induction in microsomal lipid peroxidation in asbestotic animals.

DISCUSSION

It is revealed from the study that asbestos fibre alters mixed function oxidase system of rat lung at all the stages of the exposure. At the initial stages of exposure, the content of cytochrome P-450 and the activity of benzo(a)pyrene hydrox-

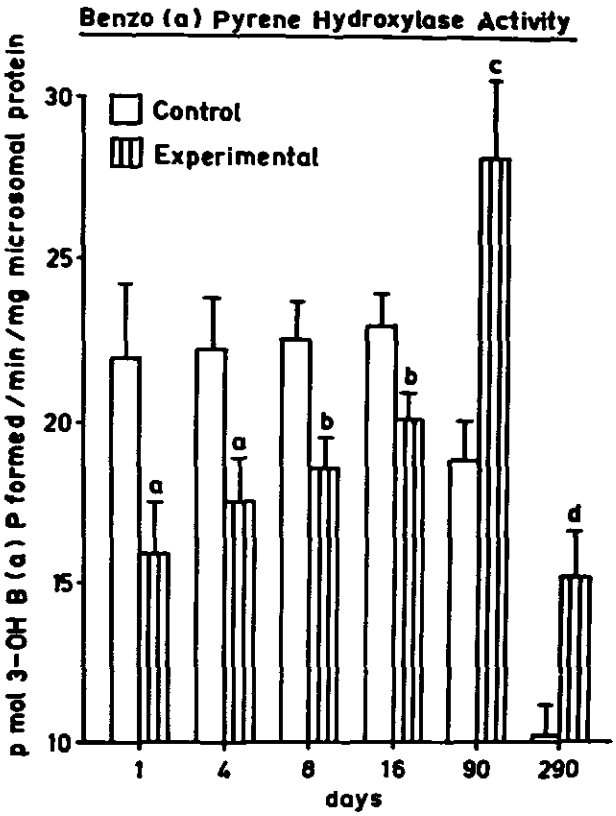

Figure 3. Benzo(a)pyrene hydroxylase activity in lung microsomes isolated from control and chrysotile treated rats. The values are expressed as mean ±SEM of six animals. $^{a}p<0.05$; $^{b}p<0.02$; $^{c}p<0.001$; $^{d}p<0.01$.

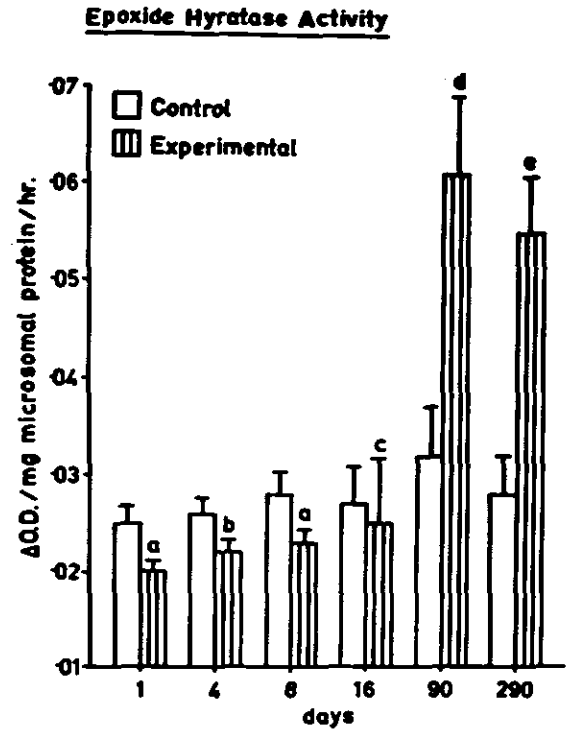

Figure 4. Epoxide hydratase activity in lung microsomes, isolated from control and chrysotile treated rats. The isolated from control and chrysotile treated rats. The values are expressed as mean ± SEM of six animals. $^{a}p<0.02$; $^{b}p<0.05$; ^{c}p-NS; $^{d}p<0.01$; $^{e}p<0.001$.

ylase and epoxide hydratase were reduced as compared to their respective controls, but at later stages a reversed pattern with a progressive increase was observed. The decrease in the content of cytochrome P-450 at initial stages of exposure may be due to destabilization of heme proteins.[10,34,52]

Partial inactivation of microsomal mixed function oxidase system *in vivo* and *in vitro* at the initial stages of the disease have been reported.[25,40,24,36] The inhibition in the activity of phase I reaction suggests that at the early stages chrysotile prolongs the tissue retention of carcinogens. However, the content of cytochrome P-450 and the activity of benzo(a)pyrene hydroxylase and epoxide hydratase increased at 90 days and thereafter, thus indicating that chrysotile fibre participates actively in the activation of phase I reaction at the advanced stages of the disease when fibrosis developed. At this stage, our results are in agreement with Naseem et al.[35] and Dzugaj et al.,[19] who have reported high activity of benzo(a)pyrene hydroxylase in lymphocytes isolated from asbestos workers and liver of asbestos exposed mice. The increase in the activity of aryl hydrocarbon hydroxylase system is very important because they play the major role in the regulation of the microsomal biotransformation of polycyclic aromatic hydrocarbons (PAHs), the major carcinogen of cigarette smoke.[13] It is associated with the activation of PAHs in chemical carcinogenesis and also the epoxide hydratase catalyzed formation of dihydrodiols.[37] Therefore, the higher activities of microsomal benzo(a)pyrene hydroxylase and epoxide hydratase on prolonged period of asbestos exposure may produce more reactive metabolites from the known carcinogens present in the cigarette smoke in the target tissue thereby increasing the possibility of higher DNA adduct formation.[40] A linear decrease in the activity of glutathione-S-transferase in the case of chrysotile treated animals was observed. The maximum inhibition for the activity was observed at 290 days of treatment, registering a 39% inhibition. This is in agreement with the findings of Brown et al.[3] Glutathione-S-transferase is involved in the detoxification of metabolically modified carcinogens by conjugation with reduced glutathione. The decrease in activity of this enzyme as observed in this study may, in turn, result in the accumulation of unscavenged reactive metabolites which may find access to other sites and exert deleterious effects like the adduct formation with DNA. The hydrolytic enzymes which released from lysosomes have been reported in asbestotic

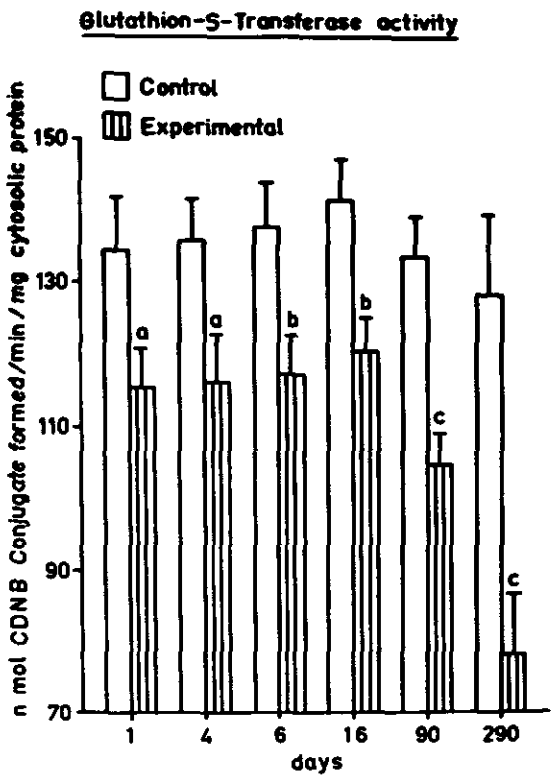

Figure 5. Glutathione-S-transferase activity in lung cytosol, fractionated from control and chrysotile treated rats. The values are expressed as mean ± SEM of six animals. $^{a}p<0.05$; $^{b}p<0.02$; $^{c}p<0.001$.

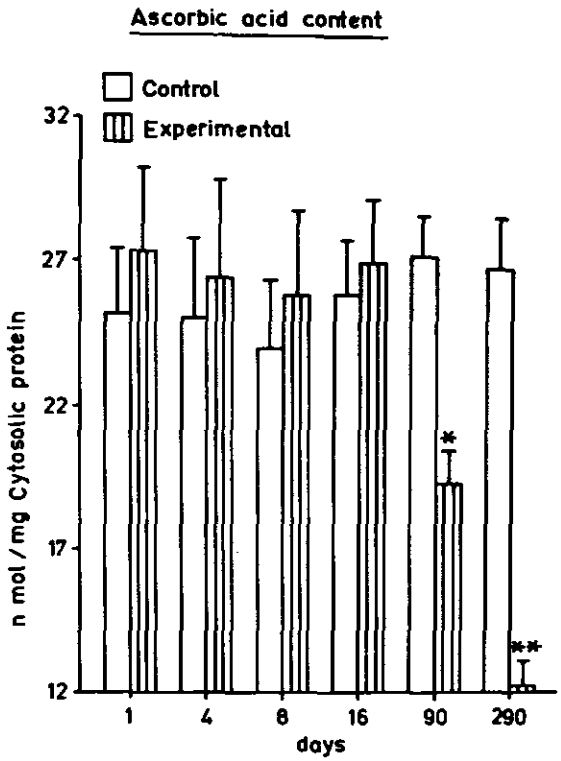

Figure 6. Ascorbic acid content in the lung of control and chrysotile treated rats. The values are expressed as mean ± SEM of six animals. *$p<0.001$; **$p<0.001$.

animals at the advanced stages of the disease[50] may further negate the clearance by the hydrolysis of preformed conjugates releasing reactive metabolites in the cells.

A higher rate of both enzymatic and non-enzymatic lipid peroxidation have been recorded in pulmonary microsomal fractions isolated from chrysotile treated rats after 1, 4, 8, 16, 90 and 290 days of exposure. Recently, other investigators have also reported similar findings.[12,21] The lipid peroxides generated due to the peroxidative damage of polyunsaturated fatty acids of the biological membrane have tremendous toxic potential in the biological systems.[9,14,33,53] These include alterations in membrane fluidity, initiation of free radical chain reactions, and effects on intermediatory metabolism. The lipid peroxide also stimulates the metabolism of benzo(a)pyrene.[14] There are several evidences to prove that hydroxyl and superoxide radicals are involved in asbestos induced lipid peroxidation[5,16,20,32,51] Therefore, the enhanced lipid peroxidation of the lung microsomes in asbestotic rat may contribute to the delayed toxic and carcinogenic effect of these mineral fibres.

A remarkable decrease in the contents of water soluble antioxidants like ascorbic acid and reduced glutathione have been recorded in chrysotile treated rats after 90 and 290 days of exposure. However, insignificant depletion in the levels of these antioxidants was observed at the initial stages of exposure. Several antioxidants are known to inhibit tumors induced by a variety of carcinogens, including PAHs.[2,4,6,23,45-48] The low contents of antioxidants in the lung after chrysotile inhalation may hamper the defense of the tissue against other environmental and occupational contaminants. It may be concluded from the above study that the events like quick generation of active carcinogens, their poor elimination from the tissue, hydrolysis of preformed conjugates, generation of free radicals and low antioxidants level in the lung following exposure to asbestos dust may be initiating, favouring and stimulating the process of bronchogenic carcinoma.

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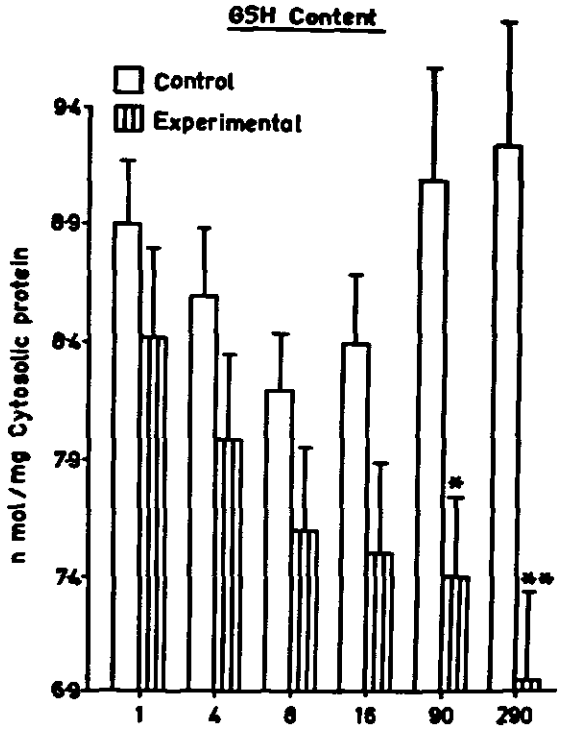

Figure 7. GSH content in the lung of control and chrysotile treated rats. The values are expressed as mean ± SEM of six animals. *p<0.01; **p<0.001.

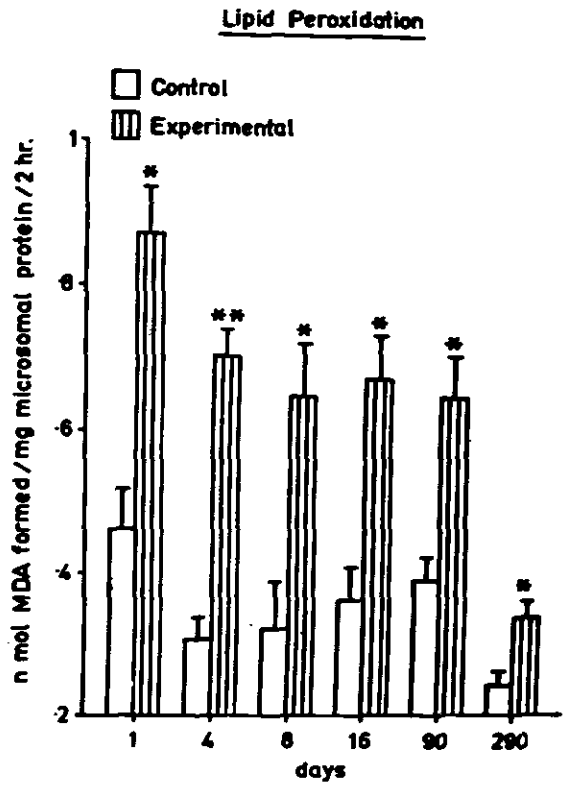

Figure 8. Lipid peroxidation in lung microsomes isolated from control and chrysotile treated rats. The values are expressed as mean ± SEM of six animals. *p<0.001; **p>0.001.

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THE ASSOCIATION OF SMALL IRREGULAR OPACITIES ON CHEST RADIOGRAPH WITH AGING IN A NONSMOKING POPULATION WITHOUT OCCUPATIONAL DUST EXPOSURE

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ABSTRACT

Small opacities on chest radiograph have been found to increase with age in several studies which have been confounded by dust exposure and/or cigarette smoking. To analyze the association of small opacities with age, we used the ILO 1980 Classification to categorize 159 radiographs of asymptomatic, lifetime nonsmokers without occupational exposure to dusts. The study population included 84 males and 75 females. Age ranged from 15 to 85 years with a mean of 51.2 years and a standard deviation of 19.9 years. Chest radiographs with ages concealed were classified independently by two B readers, Reader 1 found 133 (83.6%) to have profusion category 0/0 and 26 (16.4%) to have category 0/1. Reader 2 found 125 (78.6%) to have category 0/0 and 34 (21.4%) to have category 0/1. No subject had a profusion category greater than 0/1. Significant point biserial correlation coefficients (r_{pb}) were found between profusion category and age (r_{pb}=.1659 and .1611 for readers 1 and 2 respectively; both $p<.05$). Analysis by gender demonstrated an association of small opacities with age only in females (r_{pb}=.2761 and .3091 for readers 1 and 2 respectively; both $p\leq.01$). Changes of the breasts which take place with aging may account for this association.

The International Labor Office (ILO) International Classification of Radiographs of Pneumoconioses is used for epidemiologic research and surveillance of workers in dusty occupations.[1] It may also contribute to the evaluation of a worker for compensation. A variety of normal and abnormal structures produce radiographic patterns similar to those of the pneumoconioses complicating interpretation of the ILO 1980 Classification.[2,3] Studies have described an increase in small opacities on chest radiograph associated with age.[4-9] These investigations have been confounded by dust exposure and/or cigarette smoking which also increase small opacities on chest radiographs.[10-15]

To test the hypothesis that small opacities increase with age independent of dust exposure and cigarette smoking, we used the ILO 1980 Classification to categorize chest radiographs of asymptomatic, lifetime nonsmokers without occupational exposure to dusts.

METHOD

Subjects were volunteers, predominantly from the Church of Jesus Christ Latter-Day Saints (Mormons). A modified version of the Medical Research Council questionnaire for respiratory symptoms was administered to each individual.[16] A detailed occupational history was also obtained. Height (in meters) and weight (in kilograms) were measured with the subject wearing light outdoor clothing without shoes. A pulmonary physician examined all subjects. A 14" × 17" posteroanterior (PA) chest radiograph was taken at six feet on full inspiration with the patient in the standing position. PA radiographs with ages concealed were classified independently by the National Institute for Occupational Safety and Heatlh (NIOSH) certified B readers (readers 1 and 2). ILO 1980 Classification standard films were used. Results were reported with OMB Form No. 68-5 1322 provided by NIOSH for the complete classification.

Subjects were included in the study population if they met the following criteria: 1) a lifetime nonsmoker (total smoking of less than 0.5 pack-year and no smoking within six months of the study); 2) no symptoms of chest wall, lung, or heart disease; 3) no history of work in a mine, quarry, foundry, or pottery; 4) no occupational exposure to asbestos, irritating gases, or chemical fumes; 5) a normal physical examination of the chest wall, lungs, and heart; and 6) a PA radiograph of technical quality acceptable to both readers.

The Chi-square goodness of fit test was used to examine the relationship of profusion category with gender.[17] To analyze associations of small opacities with age and an obesity index (weight/height^2), the point biserial correlation coefficient (r_{pb}) was applied.[18] The r_{pb} allows correlation of a continuous variable (age and obesity index) with a categorical variable which has two values (all chest radiographs were classified into two profusion categories).

RESULTS

Eight volunteers were excluded from the study as a result

of work in mines or exposure to asbestos. Technical quality prevented classification of six radiographs. The study population included the remaining 159 subjects. There were 84 males and 75 females. Ages of males and females were comparable and were uniformly distributed from 15 to 85 years (Table I). Males were, as expected, taller and heavier.

Table I

Ages and Anthropometric Measures

	Males	Females
n	84	75
Age in years		
Range	15-85	17-84
Mean	52.1	50.2
Stand. Dev.	19.7	20.0
Height in meters		
Range	1.490-1.940	1.460-1.780
Mean	1.733	1.611
Stand. Dev.	0.073	0.068
Weight in kilograms		
Range	59.6-110.9	43.8-104.7
Mean	78.9	67.7
Stand. Dev.	11.4	12.2

Reader 1 categorized 133 (83.6%) radiographs as profusion category 0/0 and 26 (16.4%) as category 0/1. Reader 2 categorized 125 (78.6%) radiographs as category 0/0 and 34 (21.4%) as category 0/1 (Table II). No chest radiograph was found to have a profusion category greater than 0/1. Agreement between the two readers was 80.5%.

In subjects with radiographs categorized 0/1, small opacities were found only in the lower zones. The predominant shapes and sizes were *s* and *t* varieties. There were no large opacities.

Males had a higher prevalence of radiographs categorized as 0/1 but this difference between genders reached statistical significance with reader 2 only (Chi square = 4.08, p=.04). Correlation of profusion category with age, with males and females included, provided r_{pb} values of 0.1659 and 0.1611 (readers 1 and 2 respectively). Both were statistically significant (Table III). This association was then analyzed separately for each gender. Males were found to have no statistically significant correlation of profusion category with age while females had significant r_{pb} values of 0.2761 and 0.3091 (Table III). There was an association of profusion category with the obesity index in females with reader 1 only (Table III). However when age and obesity index were simultaneously regressed against profusion category, the association of the obesity index with the profusion category was not found to be significant.

DISCUSSION

No subject in our group of asymptomatic, lifetime nonsmokers without occupational exposure to dusts had a profusion category greater than 0/1 and only 16 to 21 percent were classified as category 0/1. Small opacities were predominantly *s* and *t* in shape and size and were located in the lower zones. Males were found to have category 0/1 radiographs more frequently than females. We found small opacities on chest radiograph to increase with age. However, when analyzed by gender, this association was statistically significant for females only.

A possible explanation of the association of profusion category and age in females is that the small opacities result

Table II

ILO 1980 Classification of Profusion Category

	Reader 1		Reader 2	
	Male	Female	Male	Female
0/0	67	66	59	66
0/1	17	9	25	9

Table III

Correlation of Profusion Category with Age and Obesity Index

	Reader 1		Reader 2	
	r_{pb}*	P Value	r_{pb}*	P Value
AGE				
All Subjects	0.1650	.03	0.1611	.04
Males	0.0732	.51	0.0562	.61
Females	0.2761	.01	0.3091	.00
OBESITY INDEX (WEIGHT/HEIGHT^2)				
All Subjects	0.0225	.77	0.1478	.06
Males	0.0904	.41	0.0978	.38
Females	0.1334	.24	0.2246	.04

*r_{pb} is the point biserial correlation coefficient between profusion category and either age or obesity index

from changes in breast tissue. The lobules of glandular parenchyma and its stroma are hormonally dependent. With age, involution of these tissues occurs with replacement by adipose tissue.[19,20] Fat absorbs relatively few X-rays and is therefore less radiopaque than the other tissues of the breast. As a result of this differential absorption, fat would provide sharp contrast on a radiograph to other tissues including persistent strands of fibrous connective tissue, veins, and calcified arteries. These structures may be seen as small opacities in older females and would explain the location of the small opacities and their association with aging in females.

Age in females explained less than 10% (coefficient of determination) of the variance of profusion category in this population without occupational exposure to dusts. Ten percent is an underestimate since the maximal coefficient of determination obtained using the point biserial correlation coefficient is approximately 0.80.[18] In addition, as the proportion of the study population in each of the two categories varies from 0.50, both r_{pb} and the coefficient of determination will be underestimated.[18] In our study, the inequality of subjects in categories 0/0 and 0/1 leads to an error in our determination of the true variance of profusion category explained by age. Although the exact value cannot be determined, it can be concluded that the majority of the variance of profusion category with both genders is unexplained.

Soft tissues overlying the chest wall are thought to account for small opacities[8] but could not be demonstrated to explain any variance of profusion category in our group. Radiographs of females should be categorized 0/1 more frequently than males as a result of overlying breasts if soft tissues explained a significant portion of the variance of profusion category. There was an effect of gender in our study but both readers categorized more radiographs of males as 0/1. Our study also showed that an obesity index (weight/height^2) had no association with small opacities when a multiple regression was done with age as another independent variable. Subject characteristics not investigated in our study, technical quality of the radiograph, or error in classification may explain the majority of variance of profusion category in nonsmoking, unexposed populations.

Two other investigations have categorized unexposed populations using the ILO Classification. Castellan et al. studied 1422 blue collar workers without exposure to known occupational respiratory hazards.[8] Only ten workers had profusion category 0/1 and three had categories 1/0 and 1/1. Small opacities were irregular in shape. A statistically significant difference in ages of workers with profusion category $\geq$ 0/1 was detected when compared to those with category 0/0. Almost all workers with small opacities were smokers. Epstein et al. found 35 of 200 radiographs of hospitalized patients had a profusion category of 0/1 and 22 had category $\geq$1/0.[21] Small opacities were predominantly irregular in shape and located in either the lower zones or all zones of the lungs. The higher prevalence (11%) of radiographs with categories $\geq$0/1 may have been the result of classifying a hospitalized population. An association of profusion category with age was not described.

Some studies in exposed populations have also shown profusion category to increase with age.[5-7,9] These findings may have resulted from incomplete accounting for the effect of dust exposure, decreased clearance of the dust by the respiratory tract with aging, confounding factors (e.g., other occupational exposures, environmental exposures, cigarette smoking), or an age associated increase in small opacities independent of dust exposure and confounding factors.

We conclude there is an association of small opacities on chest radiograph with age independent of dust exposure and cigarette smoking in females only. Changes in breast tissue occurring with age may account for this finding.

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PULMONARY EFFECTS OF ACUTE EXPOSURE TO SULFUR TETRAFLUORIDE DURING ELECTRICAL CABLE REPAIR WORK

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ABSTRACT

Six electrical workers were accidentally exposed to sulfur tetrafluoride (SF4) while repairing an electrical cable in an underground confined space. Repairs began 4 days after a burnout at a nearby substation. Symptoms noted approximately 1 hour after beginning work were shortness of breath, chest tightness, productive cough, nose and eye irritation, and headache. Some workers also experienced fatigue, nausea, and vomiting. Partial resolution of symptoms occurred when exposure was interrupted while attempts to identify the cause of the problem were made. Although exposure ended after several hours, 4 workers remained symptomatic for over one week. Chest radiographic abnormalities included, several discrete areas of transitory platelike atelectasis in 1 worker, and soft hazy infiltrates in another. Pulmonary function changes included reversible decrements in FVC and FEV_1.

Sulfur hexafluoride (SF6), an inert gas, used in circuit breakers as an electrical arc-interrupting medium, decomposes to SF4 and other compounds when subjected to intense heat. SF4, an irritant gas, with toxic effects similar to phosgene, was eventually identified by mass spectrometry of worksite air samples, and is the likely cause of the illness developed by these workers. Effects of SF4 exposure on humans have not been reported in the medical literature, although available information indicates that it is a highly irritant compound. Occupational health personnel should be aware that exposure to SF4 is an important health hazard for workers repairing damaged electrical systems containing SF6.

INTRODUCTION

Unpredicted exposures to industrial chemicals place workers at serious risk, as they are both unprepared for the event and may not know the compound's toxic effects. We wish to report the consequences of an unexpected, unpredicted, and unrecognized exposure to the irritant gas, sulfur tetrafluoride (SF4). The exposure occurred after initiation of repairs following a burnout (small explosion) in an electrical substation. During the burnout circuit breakers using sulfur hexafluoride (SF6) as an insulating gas were damaged. When SF6 is subjected to intense heat it decomposes to SF4 and other compounds.[1] Although SF4 has been reported to have toxicity similar to that of phosgene,[2] we could find no published documentation of illnesses in humans caused by this compound.

CIRCUMSTANCES OF EXPOSURE AND ACUTE SYMPTOMS.

On January 12th 1988 approximately 15,000 gallons of polybutene insulating oil was lost from an electrical transmission cable. Concurrently, a burnout occurred in circuit breakers at a substation several miles further down the line. Four days later a team of six gas operators (workers #1-6) began repair work approximately 100 yards from the substation by cutting the surrounding pipe to gain access to the enclosed electrical cable. The worksite was an underground space, 10'×4'×8', with two three foot diameter openings. Four members of the crew (#1-4) worked underground, while the safety officer, (#5), remained on the surface. The foreman, (#6), worked above or below ground as needed. All workers were previously healthy, except for (#5) who had a history of emphysema.

Prior to entering the worksite, routine measurements for natural gas and oxygen concentrations were found to be satisfactory. At approximately 9:00 AM the team began using compressed air powered smith cutters to open the pipe. About one hour later, five underground workers began experiencing burning eyes, tearing, dry and burning throat, and chest tightness. An odor similar to a "burning car battery" was noticed. The crew stopped working and went above ground; symptoms decreased fifteen minutes later.

A fan was obtained to improve air circulation at the worksite. The previously mentioned symptoms, however, recurred shortly after work was resumed. In addition, some workers started experiencing headache, fatigue and cough productive of clear sputum. Concern was raised that the air compressor was the cause of the problems. Work was again halted and a different compressor ordered. Symptoms subsided when the men stopped working underground and went into

the fresh air. Nevertheless, the same symptoms recurred one hour after work was resumed with the new compressor.

Due to the persistence and worsening of symptoms a worksite investigation was performed by the company chemist at approximately 3:00 PM. No abnormalities were detected on routine air monitoring and samples from the partially opened pipe were taken for analysis. Although no problems were identified, a second fan was brought to improve the air circulation. Two hours later workers again reported chest tightness and/or shortness of breath. Two complained of headache, fatigue and nose bleeds; two felt nauseous and one worker vomited. Work was again halted and the entire crew waited above ground for the chemist's final report. At 10:00 PM workers were sent home as the report had not yet arrived.

At approximately 1:00 AM all six workers were notified by telephone that SF4, a potentially hazardous material, was identified in the air samples taken from the partially opened pipe at the worksite. In addition they were instructed to immediately go to the nearest hospital emergency room. Five of the workers went to one hospital and the sixth to another. Oxygen was administered by mask in the emergency room of the first hospital. Chest radiographs were not taken until approximately 11:00 AM, approximately 26 hours after the onset of exposure. Five of the workers were discharged a few hours later.

Worker #1, complained of headache, cough productive of blood streaked sputum, and wheezing. Three discrete areas of atelectasis were observed on chest radiograph. He was admitted to hospital and treated with bronchodilators and antibiotics. Pulmonary function testing (PFTs) performed January 19th were normal. While in hospital he became febrile. The headache and productive cough persisted for over one week.

All six workers elected to come to the Occupational Medicine Center at the Mount Sinai Hospital for evaluation between January 26 and February 1, 1988. Initial and persistent symptoms are summarized in Table I. Three workers, (#2-4), complained of fatigue at the time of evaluation. Physical examination did not reveal any pertinent abnormalities.

On reviewing the initial radiographs, worker #3 had hazy infiltrates in the lower lung fields, while worker #6 had a slight infiltrate in his left lower lung field. All follow-up radiographs taken between 10–21 days after the accident were normal.

Pulmonary function testing was not performed during the initial emergency room evaluation. PFTs were ordered by the company physician between three and ten days after the event for five of the six workers, the sixth a few days later. Three of these were normal. Workers #5 and 6 had slight

Table I
Symptoms of Workers Exposed to Sulfur Tetrafluoride

	Worker					
Symptom	#1	#2	#3	#4	#5	#6
Burning/ Tearing eyes		*	*	*	*	*
Nasal irritation/ Epistaxis	°		°	°		
Throat irritation	*			*		°
Chest tightness/ Wheezing/ S. O. B.	*	*	°	°		*
Cough	°	*	*			
Nausea/Vomitting	*	*				
Fatigue		°	°	°		
Headache	*		°			

* Symptoms following exposure
° Symptoms lasting longer than one week

decreases in FVC, 75% and 77% percent of predicted, which normalized to 89 and 98% on follow-up testing a few days later. PFT results are summarized in Table II. Interpretation of these findings is limited by the fact that different equipment was used at each location.

Worker #3 had three sets of PFTs, the first set performed on January 19, 1988 was normal. PFTs taken prior to resuming work one week after the event, revealed an obstructive pattern, FVC 109% and FEV_1 67% of predicted. He did not have a history of asthma, but did complain of chest tightness and shortness of breath on exposure to cold air for approximately one week following the exposure. Repeat testing when he was asymptomatic was normal. DLCOs were normal in all workers except for #5 who had a history of asbestos exposure and emphysema.

DISCUSSION

SF6 was first synthesized by Moissen and Lebeau in 1902 by burning sulfur in a fluorine atmosphere.[3] SF6 has been used in electrical equipment in the United States since 1953.[4] It is a heavy, colorless, odorless gas of high chemical stability. By being an effective electron scavenger SF6 can efficiently retard electrical conduction. These properties have led to its use as an electrical insulating material in circuit breakers, cables, capacitors, and transformers.[5] SF6 containing equipment has allowed the creation of compact electrical substations requiring one twentieth the land of previous designs.[6]

The use of SF6 has increased markedly in recent years. The National Occupational Hazard survey initiated in 1971, ap-

Table II
Pulmonary Function Results

Worker ^				
#3	Date	Jan. 19*	Jan. 26 '	Feb. 1"
	FVC	4.15	4.40 (109%)	4.87 (116%)
	FEV1	3.7	2.08 (67%)	3.92 (120%)
	FVC/FEV1	89%	47%	80%
	FEF25-75		2.00 (62%)	2.69 (81%)
	DLCO			38.1 (141%)
#5	Date	Jan. 19 '	Jan. 28 "	
	FVC	3.58 (77%)	4.52 (98%)	
	FEV1	2.17 (60%)	2.71 (78%)	
	FVC/FEV1	60%	64%	
	FEF25-75	1.08 (34%)	1.50 (33%)	
	DLCO		16.2 (60%)	
#6	Date	Jan. 21 '	Jan. 26 "	
	FVC	3.64 (75%)	4.23 (89%)	
	FEV1	3.28 (87%)	3.97 (107%)	
	FVC/FEV1	90%	94%	
	FEF25-75	5.96 (160%)	7.66 (153%)	
	DLCO		24.3 (82%)	

* Private Physician's office
' Company medical facility
" Mount Sinai Medical Center
^ Workers #1,2,4 all had unchanged results on repeat testing
Percentages in parenthesis are % perdicted

proximated that 177 American workers were potentially exposed to this compound.[7] Preliminary information from the early 1980's estimates over 9,000 potentially exposed workers, over half repairers of electrical and electronic equipment.[8]

SF6 is an inert gas; in experimental studies no ill effects were found in mice breathing a mixture of 80% SF6 and 20% O_2 for 12–16 hours.[2] SF6 will break down to toxic sulfur oxyfluorides during electrical arcing in the presence of oxygen.[1,9] Worker exposure to these gases can be significantly reduced by the presence of properly maintained absorptive filters. In experiments specifically designed to identify the decomposition products of SF6, SF4 was only generated by higher energy arcs after the consumption of available oxygen.[1] Temperatures above 150°C have been reported to lead to the decomposition of SF6 to SF4 and other compounds.[10]

SF4, a highly reactive, colorless gas which fumes in moist air, has an irritating odor similar to sulfur dioxide.[11] No comprehensive studies of this compound's toxicity could be found in the medical literature. The material safety data sheet on this compound reports it to be extremely irritating and corrosive to the upper and lower respiratory tracts, skin, and eyes.[9] SF4 hydrolyses in air to form hydrofluoric acid. Thus skin and mucous membranes lesions similar to those caused by this acid can be expected in workers exposed to SF4. SF4 may cause chemical pneumonitis and pulmonary edema.[4] Animals exposed to 10 ppm SF4 for one hour developed rapid labored breathing, weakness, and cyanosis.[12] The manufacturer has reported that animals exposed to 50 ppm for 4 hours died from pulmonary edema.[13] Ten repeated exposures of 4 ppm for 4 hours produced signs of respiratory effects in rats. Pulmonary damage was observed in rats sacrificed immediately after the tenth exposure. Those subsequently unexposed for 14 days recovered clinically and showed no anatomical lesions.[14] In 1959 investigators for E.I. Du Pont de Nemours & Company recommended that SF4 should be treated with extreme caution as it has an inhalation toxicity comparable to phosgene.[2] Consistent with this high level of toxicity the ACGIH has set a ceiling exposure limit of 0.01 ppm for this compound.[15]

Electrical substations contain switches, circuit breakers, conductors, and transformers to switch power circuits and transform power from one voltage to another or from one system to another. At the station in question three circuit breakers were connected to the damaged cable. Each of these were approximately the size of a 55 gallon drum and filled with SF6.

Although the exact sequence of events leading to the SF4 exposure has not been determined, a likely sequence is as follows. Due to damage at a distant site insulating oil was lost from the cable. This, or the following burnout led to a disruption of the valve separating the circuit breakers from the cable. Due to the intense heat of the burnout SF6 decomposed and all oxygen in the system was consumed. Further breakdown of SF6 occurred leading to the production of SF4 and possibly other compounds. As the circuit breakers were not externally damaged the SF4 was forced into the pipe containing the cable and was released when the pipe was cut at the worksite. Although other breakdown products may have been present, SF4 was the only one qualitatively identified. The level of exposure was not quantified. Repeat testing the following day revealed barely detectable levels.

Workers were exposed to SF4 for about 6 hours over a 12 hour period while repairing the cable. Chest radiographs were not taken until 26 hours after the start of exposure. In addition any early PFT changes may have been missed as testing was not performed until a few days after the event when the majority of acute symptoms had already subsided.

Radiographic evidence of multilobar atelectasis was present in one worker. In addition a second worker, who did not have a previous history of asthma, complained of chest tightness on exposure to cold air and developed a transitory obstructive pattern on pulmonary function testing. His chest radiograph revealed hazy infiltrates in his lower lung fields. These findings are consistent with known toxic effects of irritant gas exposure.

All five underground workers had respiratory tract symptoms, the sixth worker who remained above ground, experienced only eye irritation. The intermittent nature of the exposure most probably prevented the development of more severe effects such as chemical pneumonitis or toxic pulmonary edema.

These workers were unaware that their job could lead to exposure to SF4. Although they had worked for many, some for over twenty years in this field, none had heard of SF4 before or were aware that their job may lead to exposure to irritating chemicals in general and to SF4 in particular. Had they, or the company management, physician, chemist, or industrial hygienists been aware of the potential for this exposure, it is likely that the exposure would have been of much shorter duration. The potential for toxic exposures, however, is documented in the material safety data sheet describing SF6.[9] Proper education may have prevented the adverse health effects suffered by these workers.

Although the presence or absence of odor should not in general be relied upon to identify toxic exposures, odors present in areas containing heated SF6 must be considered to be coming from decomposition products and be a signal for the use of proper safety procedures.[1,4] An odor similar to a "burning car battery" was identified by workers, but no one involved in the initial investigation recognized this to be a warning signal. Fortunately, no worker developed severe complications and all have been able to return to work.

In order to limit the potential adverse effects of a similar event in the future, the following recommendations were given to both workers and management:

1. Comprehensive air tests be conducted before work is begun after accidents.
2. Knowledgeable individuals should be available for immediate on sit consultation if needed.
3. If a problem is presumed to exist work should not be resumed until the evaluation has been completed.
4. Proper respiratory protective equipment should be available at the worksite.

Due to the expanding role of SF6 in the electrical transmission industry[16] it is likely that exposures to its decomposition products will occur in the future. Occupational health personnel should thus be aware that exposure to SF4 is an important health hazard for workers repairing damaged electrical systems containing SF6.

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EXPERIMENTAL STUDIES ON THE EFFECT ON THE IMMUNE SYSTEM OF EXPOSURE TO COALMINE DUST AND QUARTZ.

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INTRODUCTION

Immune effects of mineral dusts may influence the development and progression of pneumoconiosis. In coalworkers' pneumoconiosis, progressive massive fibrosis and Kaplan's Syndrome are said to be related to immunologic abnormalities.[1] In addition, experimental studies have revealed that fibrogenic mineral dust alter immune responses.[2,3]

As part of a programme to examine the effect of silica and coalmine dust on the immune system, two approaches were taken: firstly immunocompetent cells from the rat spleen were exposed to dust *in vitro* and their mitogenic responses were assessed; secondly, dusts were intratracheally administered to rats and the effects of elicited bronchoalveolar leukocytes on splenocyte mitogenesis were studied.

MATERIALS AND METHODS

Animals

Twelve to fifteen-week old, female, SPF-maintained, inbred PVG rats were supplied by the Institute of Occupational Medicine breeding unit.

Dusts

Four kinds of dusts were used in the experiments: two were coalmine dusts collected from the air of British collieries mining 1) anthracite coalmine dust (A) and 2) low rank coalmine dust (L), 3) titanium dioxide (TiO_2; rutile, Tioxide Ltd. Stockton on Tees), a dust of low biological activity, 4) quartz dust (DQ12 standard).

Splenocyte Mitogenesis

Rats were killed by intraperitoneal injection with Nembutal and spleens were aseptically removed and disaggregated with a glass homogenizer. After lysing the erythrocytes, splenocytes were suspended in Hepes-buffered RPMI 1640 medium supplemented with 50mM 2-Mercaptoethanol, 2mM glutamine, 100mg/l kanamycin and 10% fetal calf serum (cRPM1). Finally 2×10^5 splenocytes, in medium were delivered to each well of 96-well microtiter plates.

The splenocytes, with or without dust suspensions, supernatant or bronchoalveolar cells, were cultured in the presence or absence of a suboptimal dose of phytohemagglutinin (PHA, 10 µg/ml) for three days at 37°C in 5% CO_2. The cultures were then pulsed with 0.25uCi tritiated thymidine, incubated overnight, and the uptake of ^3H Thymidine was determined by liquid scintillation counting.

Effect of Dusts on Splenocyte Mitogenesis

The four kinds of dusts were autoclaved and suspended in cRPMI. Each aliquot was added to splenocytes to obtain a final concentration in the well of 10, 50 or 100 µg/ml. They were then co-cultured at 37°C in 5% CO_2 for 24 hours and stimulated with suboptimal PHA for a further three days in culture. A preliminary study showed that 24 hours of co-culture of the splenocytes with dusts led to the optimal response to PHA. The cultures were assessed for mitogenesis as described above.

Effect of Supernatants on Mitogenesis

Splenocytes were adjusted to 1×10^6 cells/ml and aliquots of 5ml were delivered to plastic flasks. The splenocytes were allowed to adhere for six hours (adherence efficiency 27 ± 10%, $\bar{x} \pm$ sd) and non-adherent cells were removed by washing. The adherent splenocytes were cultured with dusts at a final concentration of 100 µg/ml for 24 hours and supernatants were collected which were spun, filtered and frozen until use. The supernatants, at various dilutions were delivered to wells containing 2×10^5 splenocytes and these were cultured and harvested as described above.

Interleukin-1 Activity in Spleen Cell Supernatants

Three-fold dilutions of supernatants from cultures of adherent spleen cells exposed to dust at 100 µg/ml, were incubated with C3H mouse thymocytes at 6×10^5 per well in microtiter plates. PHA was added at a final concentration of 4 µg/ml and the plates cultured for 72 hours. Thymocyte proliferation was determined by the incorporation of tritiated thymidine added during the final 16 hours of culture. Supernatant from unfractionated spleen cells cultured with 10 µg/ml Concanavalin A (Con A) served as a positive control. Con A activity was neutralized with methylmannoside before use in the thymocyte assay.

Effect of Bronchoalveolar Leukocytes from Dust-Exposed Rats on Splenocyte Mitogenesis

Rats were intratracheally instilled with 1mg of the four different kinds of dusts suspended in 0.5ml Phosphate Buffered Saline (PBS). PBS alone was injected into rats as a control. Bronchoalveolar cells (BAC) were obtained by lavage seven days later. BAC were washed with RPMI164O and sus-

pended in cRPMI. BACs from quartz-treated rat were separated into a macrophage and neutrophil-enriched populations by density gradient centrifugation through Sepra-cell medium. Total and differential counting was done on Diff-quick stained cytospin preparations and viability was assessed by trypan blue exclusion. Total or separated BACs were added to splenocytes at final ratios of from 1:4 to 1:128. The cultures were incubated and assessed for mitogenesis as above.

Statistical Analysis

Since variation between experiments was large, the data were expressed as mitogenic indices for each condition: the mitogenic indices were obtained by dividing the suboptimal PHA-driven splenocyte mitogenesis with dust, supernatant or bronchoalveolar leukocytes, by the mitogenesis without these treatments. The differences in mean values of mitogenic indices between treated and untreated were tested by paired t-test. The differences were considered as significant if values were less than 0.05. In the IL-1 assay, the ^3H uptake by the cultures with various supernatants were compared to those with control supernatant (no dust treatment).

RESULTS

Effect of Dusts on Splenocyte Mitogenesis *In Vitro*

Both quartz at 10, 50 or 100 μg/ml and low rank coalmine dust L at 50 and 100 μg/ml significantly enhanced mitogenesis. Quartz especially augmented splenocyte proliferation even without mitogen (data not shown). On the contrary, both TiO_2 and coalmine dust A suppressed splenocyte proliferation in a dose-dependent manner (Figure 1).

Effect of Supernatant from Dust-Exposed Adherent Splenocytes on Mitogenesis

The supernatant, tested at various dilutions did not cause enhancement of mitogenesis and, in fact, supernatant from splenocytes treated with quartz at a high dose were inhibitory to mitogenesis (typical results of 1:16 dilution shown in Figure 2).

Interleukin-1 Activity in Supernatants

Despite the lack of enhancement in the spleen cell mitogenesis assay, the thymocyte assay did show interleukin 1-like activity in the quartz supernatant diluted at 1:7.5 (Figure 3).

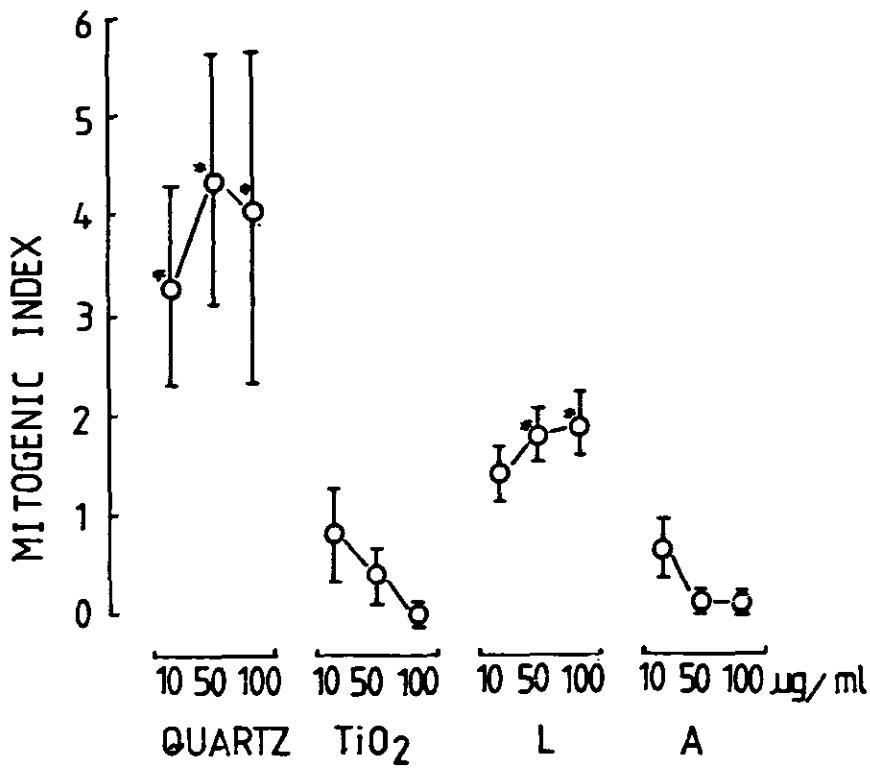

Figure 1. Mitogenic indices (means with standard errors) of splenocytes cultured with four kinds of dusts. Mitogenic index derived as the ratio of mitogenesis with dust:mitogenesis without dust. An asterisk denotes a significant ($p<0.05$) difference from the control.

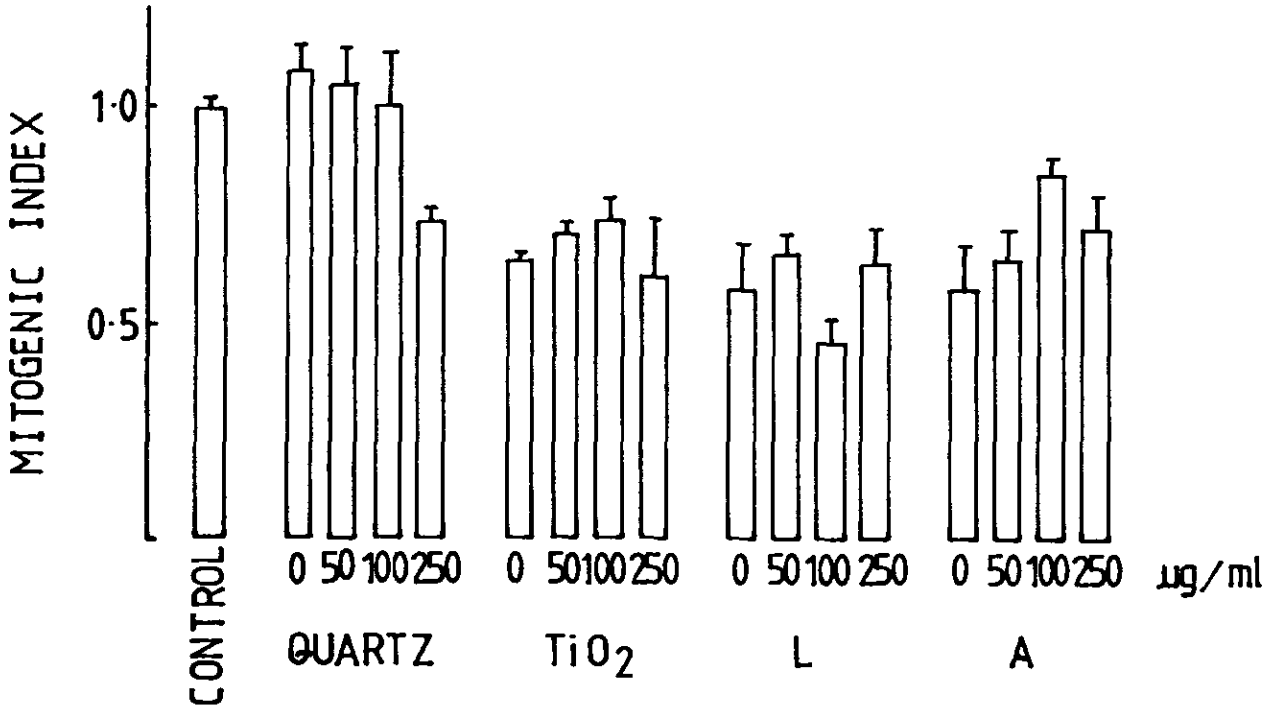

Figure 2. Effects of supernatants from dust-exposed adherent splenocytes on mitogenesis. Data are shown as mitogenic indices (means with standard deviations). Mitogenic index derived as the ratio of mitogenesis with supernatant from dusted or not-dusted adherent splenocytes:mitogenesis without supernatant.

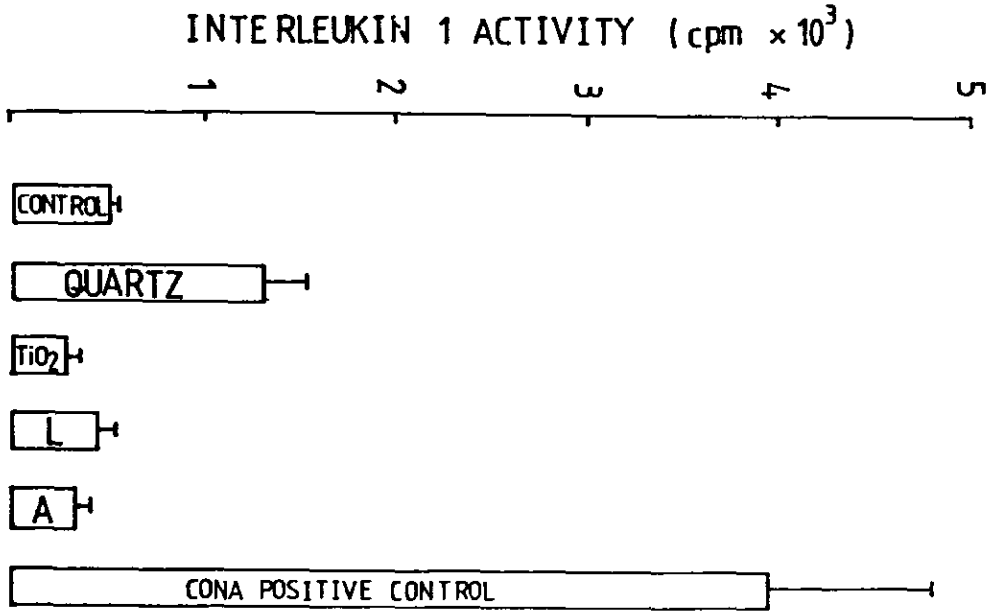

Figure 3. Interleukin 1 activity in supernatants from dust-exposed adherent splenocytes. An asterisk denotes a significant ($p<0.05$) difference from the control supernatant.

Higher concentrations of supernatant were inhibitory and supernatant from spleen adherent cells treated with other dust had no detectable Il-1 activity.

Effect of Bronchoalveolar Leukocytes from Dust-Exposed Lungs on Mitogenesis

Control, PBS-elicited bronchoalveolar leukocytes (total 4.16 $\pm$ 0.33 $\times$ 10^6 ($\bar{x}$ $\pm$ sd) cells per rat, macrophages >98%, viability >95%) showed an inhibitory effect on splenocyte mitogenesis which was effector:indicator cell ratio-dependent. The bronchoalveolar leukocytes from coal dust L (total 3.08 $\pm$ 0.76 $\times$ 10^6 cells per rat, macrophages 96 $\pm$ 2%, neutrophils 3 $\pm$ 2%, viability >91%), coalmine dust (A) (total 2.58 $\pm$ 0.33 $\times$ 10^6 cells per rat, macrophages >99%, viability >92%) or TiO_2 (total 4.61 $\pm$ 0.33 $\times$ 10^6 macrophages >99%, viability >93%) did not affect mitogenesis. Figure 4 shows the results for BAC from coalmine dust A.

The BAC from quartz-treated rats (total 16.83 $\pm$ 4.64 $\times$ 10^6 cells per rat, macrophages 42 $\pm$ 4%, neutrophils 57 $\pm$ 4%) was significantly less inhibitory to splenocyte proliferation, at ratios of 1:64, 1:32 and 1:16, than the control. After the separation into macrophage- and neutrophil-enriched fractions (recovery rate 60 $\pm$ 1%), the macrophage-enriched population (macrophages 89 $\pm$ 5%) also showed less inhibition at ratios of 1:64 and 1:32. In contrast to the inhibitory effect of the total leukocytes or separated macrophages, the neutrophil-enriched population (neutrophils 82 $\pm$ 2%) markedly enhanced mitogenesis compared to control BAC (Figure 5).

DISCUSSION

In our rat model system, we have examined the effects of exposure to mineral dusts on the immune system. The splenic lymphocytes were taken as indicator cells for the direct effect of dust on the immunomodulatory role of leukocytes within the lung.

Both quartz and coalmine dust with a high (>5%) quartz component, enhanced splenocyte mitogenesis *in vitro*. Supernatant from adherent splenocytes, presumed to be mostly macrophages, treated with quartz showed increased IL-1 activity, whilst supernatant from coalmine dust or TiO_2-treated macrophages had no such activity. None of these supernatants caused enhanced mitogenesis. These apparently conflicting findings may be explained as follows. Adherent macrophages secrete, in addition to IL-1, a variety of substances including prostaglandins and hydrogen peroxide, which are inhibitory to lymphocyte proliferation.[4,5] Subsequently the ability of any supernatant to influence mitogenesis is likely to be the product of both the inhibitory and enhancing activity present in it. Evidence that inhibitory factors were present, and could be diluted out was

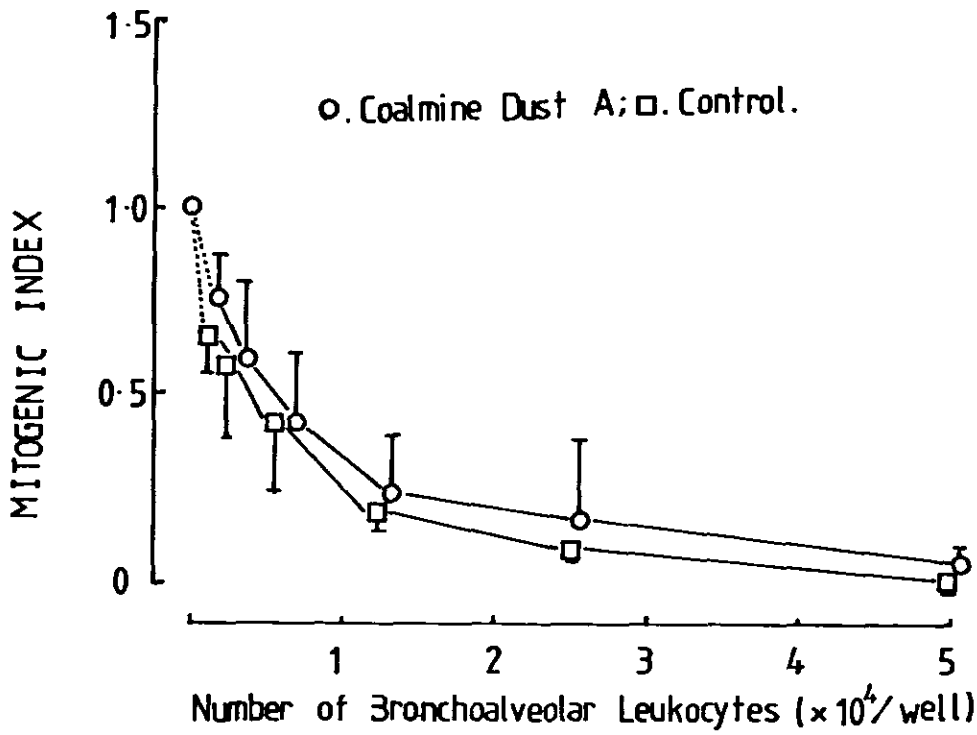

Figure 4. Bronchoalveolar leukocytes from coalmine dust L—instilled rats inhibited splenocyte mitogenesis in a dose-dependent manner. No significant differences from control leukocytes were present. Mitogenic index derived as the ratio of mitogenesis with bronchoalveolar leukocytes:without leukocytes.

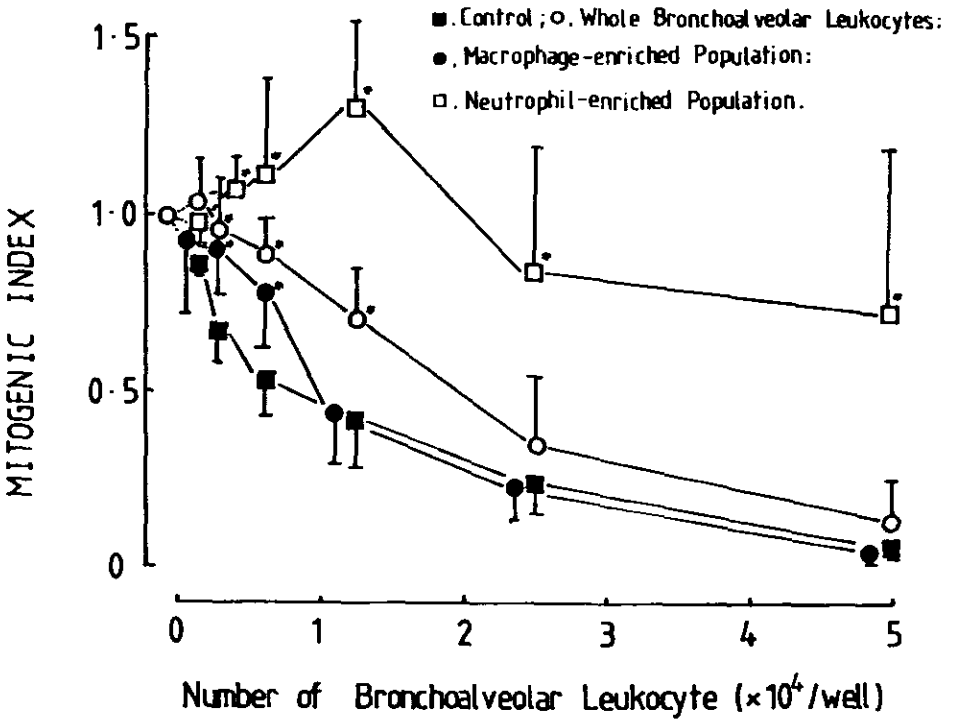

Figure 5. Effects of bronchoalveolar leukocytes from quartz-injected rats on splenocyte mitogenesis. Data are shown as mitogenic indices (means with standard deviations). An asterisk denotes a significant difference ($p<0.05$) from controls treated with PBS.

shown by the fact that IL-1 activity in the supernatant from quartz-exposed splenocytes was expressed only at higher dilutions. Further studies are needed to elucidate the mechanism of enhanced mitogenesis by quartz and low rank coalmine dust *in vitro* including further characterization of the secreted product present in supernatant form dust-treated macrophages.

Alveolar macrophages are situated at the air-tissue interface, strategically located for initial contact with inhaled particulates. They also play a crucial role in pulmonary immune responses. Alveolar macrophages in some species including rats are said to be poor accessory cells for mitogen or antigen-derived lymphocyte proliferation.[6] In our study normal bronchoalveolar macrophages inhibited splenocyte mitogenesis in a dose-dependent manner. TiO_2 and two kinds of coalmine dusts did not affect this down-regulatory function of alveolar macrophages. However the whole BAC and the alveolar macrophage-enriched population from quartz-treated rats inhibited lymphocyte response to a lesser extent than control BAC although this may be due to contaminating neutrophils as described below. Further studies are needed to confirm whether alveolar macrophages elicited by exposure to quartz have altered immunomodulatory properties, as suggested by this study.

The neutrophils separated from quartz BAC strikingly enhanced mitogenesis and this could be mediated through protease[7] or an Il-1 analogue which has been described in secretions from peritoneal neutrophils.[8]

Inhalation exposure to asbestos fiber, another type of fibrogenic dust, causes recruitment of Ia-positive alveolar macrophages and secretion of Il-1 by alveolar macrophages.[9,10] Additionally, alveolar macrohpages from asbestos-exposed rat enhanced T lymphocyte proliferation *in vivo*.[11] *In vitro* fibrogenic dust such as asbestos and silica stimulated alveolar macrophages to secrete IL-1.[12] Inhalation exposure to silica also causes secretion of IL-1 by alveolar macrophages when stimulated with endotoxin.[13] These studies suggest that fibrogenic dusts have immunostimulatory effects on alveolar macrophages and our results partially support these findings. However the complex effect of recruitment of newly exudated, monocyte-derived populations with altered cytokine production and the role of neutrophils, which are also found in dust exposed lung,[14] remain to be resolved.

This study suggests that, in the lungs of individuals inhaling quartz or quartz-containing coalmine dusts, the alveolar macrophages may be affected by phagocytosed dust to release a range of mediators which could modulate lymphocyte responses in the local environment of the lung. Additionally, neutrophils which are recruited into dust-exposed lung could also enhance immune responses leading to localized immunodulation. Any "adjuvant-type" effect on the immune system could contribute directly to heightened responses within the lung both to dust itself and to infectious agents, both of which could contribute to the tissue injury and fibrosis found in pneumoconiosis.

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BRONCHOALVEOLAR LAVAGE IN SUBJECTS EXPOSED TO OCCUPATIONAL DUSTS

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INTRODUCTION

Alveolar macrophages are free lung cells located on the surface of small airways and alveoli. These phagocytes play an important role in the protection of the lung against airborne bacteria and particles.[1] However, hyperactivation of pulmonary phagocytes can lead to excessive secretion of enzymes and reactive oxygen species which could result in lung injury, emphysema, or fibrosis.[2,3]

Analysis of bronchoalveolar lavage effluents for cell types and cellular activity has yielded information concerning the etiology of various pneumoconioses. For example, emphysema associated with inhalation of coal dust or cigarette smoke has been related to enhanced secretion of reactive oxygen species from alveolar macrophages.[4-7] In contrast, hypoactivation of alveolar macrophages has been associated with inhalation of diesel particulates[4] and has been related to increased susceptibility to pulmonary infection.[8] Inhalation of cotton dust has been associated with dramatic increases in lavagable polymorphonuclear leukocytes[9] while pulmonary sarcoidosis and silicosis have been related with high numbers of lymphocytes in lavage effluents[10,11]

The objective of the present study was to obtain lavage effluents from 8 control subjects, 8 healthy power plant workers exposed to fly ash, 1 healthy coal miner, and 1 rock driller with acute silicosis. These effluents were analyzed for total numbers of alveolar macrophages, lymphocytes and neutrophils and for secretory activity of alveolar macrophages. Data were then analyzed to determine what response patterns were characteristic for given dust exposures.

METHODS

Selection of Subjects

The 8 control subjects were all adult males from the area of Morgantown, WV. Two subjects among the controls had smoked cigarettes for approximately three pack-years and had stopped more than ten years ago. The 8 power plant workers were all healthy adult male employees of a power facility in Hatfield, PA, approximately thirty miles from Morgantown. One of the power plant personnel was a comparable ex-smoker. The remaining subjects were all lifelong non-smokers. The coal miner was a healthy non-smoking male from Morgantown, WV. The rock driller was a patient under treatment with corticosteroids for acute silicosis. He had shown considerable clinical improvement with this treatment but still manifested significant radiologic and pulmonary functional abnormalities at the time of lavage. The mean age of the 8 control subjects was 36 years (range: 31 to 49 years); the mean age of the remaining subjects studied was 38 years (range: 29 to 53 years).

Each volunteer was interviewed prior to participation in the study. Each completed the British Medical Research Council standardized questionnaire concerning respiratory and occupational history. One individual among the controls occasionally used a metered-dose inhaler for the management of mild asthma; otherwise there was no history of significant concurrent respiratory illness among the subjects except for the one with acute silicosis. Other than these two individuals, none of our volunteers received any medications regularly.

None of the control subjects had a history of significant exposure to occupational dusts. The coal miner had worked in underground mines for approximately 20 years and rarely wore a respirator. The power plant employees had variable histories of exposure to dusts at their plant, ranging from 4 to 15 years of employment. Although some of their work brought them into contact with both asbestos and coal dust, the primary dust exposure was to fly ash. None of them wore respirators consistently. The rock driller was exposed to significant levels of sandstone, coal and rock dust over a 12 year period during which time he never wore a respirator.

Experimental Procedures

Protocols were approved by HSRB at West Virginia University. In each subject, pulmonary function tests were obtained (spirometry and diffusion capacity) as well as a 12-lead electrocardiogram, PA and lateral chest x-ray films, and screening blood tests. All of these studies were normal except for the presence of mild obstruction in our one asthmatic control and moderate restriction and reduced diffusion capacity in the patient under treatment for acute silicosis.

Flexible bronchoscopy and bronchoalveolar lavage was performed in a consistent fashion in all subjects.[5-7] After the tip of the bronchoscope had been wedged into a distal subsegment in the right middle or right lower lobe, this area was

lavaged with 10 aliquots of 20 cc of 0.9 saline. The pooled return from the lavage was passed through nylon mesh (150 mesh) to remove mucus from the specimen. The specimen was then centrifuged at 500 g for 5 min. at 2°C, the supernate decanted, and the pellet of cells resuspended in HEPES buffered medium (145 mM NaCl, 5mM KCl, 10 mM HEPES, 1 mM $CaCl_2$, 5mM glucose; pH:7.4). These cells were then washed twice by alternate centrifugation and resuspension in HEPES-buffered medium.

Total cell counts in the lavage effluent were determined using an electronic cell counter. Lymphocytes, neutrophils, and alveolar macrophages were identified by their distinctive cellular volumes using an electronic cell sizing attachment as described previously.[12,13]

Chemiluminescence was measured in the presence of 1.7 mg luminol and 8 serum using a Berthold 9505 Luminometer. Chemiluminescence was monitored at rest (Rest CL) and after stimulation with either 3×10^{-6}M phorbol-12-myristate acetate (PMA-CL) or 2 mg/ml zymosan (Zym-CL). Chemiluminescence was expressed as total counts per second/10 min/1.63 $\times$ 10^6 alveolar macrophages.

RESULTS

The data are shown in Table I. Fly ash exposure resulted in significant increases in lavagable alveolar macrophages, lymphocytes, and neutrophils compared to controls. In addition, zymosan-stimulated chemiluminescence was significantly elevated while resting CL and PMA-CL exhibited a trend toward elevation. Coal dust exposure resulted in only a slight increase in resting chemiluminescence. In contrast, neither PMA-CL or Zym-CL was enhanced. The most striking changes were observed in acute silicosis where very large increases in lymphocytes, neutrophils, resting CL, PMA-CL, and Zym-CL were noted.

DISCUSSION

Our data indicate significant differences in alveolar cell populations and phagocytotic activity when asymptomatic occupationally-exposed individuals are compared with controls. Subjects exposed to fly ash exhibited significant pulmonary inflammation, i.e., their bronchoalveolar lavage contained approximately twice the number of macrophages, lymphocytes, and neutrophils as controls. In addition, phagocytotic activities as measured by chemiluminescence were all increased, with the activity after zymosan stimulation being significantly enhanced.

No significant activation of alveolar macrophages was noted after coal dust exposure. This contrasts with coal-induced activation noted in animal studies.[4] Clearly more coal miners are needed before definitive conclusions can be drawn.

The acute silicotic exhibited the most striking changes. Lavagable cells were elevated by 32% for alveolar macrophages, 14.6 fold for lymphocytes, and 10.5 fold for neutrophils. Similar increases in lymphocytes and neutrophils due to silica exposure have been reported previously in both rats and humans.[14,11] These observations are more dramatic when one considers that this subject had been on corticosteroid therapy prior to lavage. Indeed, such treatment would tend to decrease the yield of lavagable cells.[15] The high levels of chemiluminescence seen in the silicotic patient (i.e., Rest CL increased 5.3 fold, PMA-CL increased 3.7 fold, and Zym-CL increased 12.4 fold) suggest that a substantial oxidant burden exists in the lungs of this subject. Oxidant injury could explain the restrictive lung disease and diminished diffusion capacity observed in this patient.

CONCLUSION

We present data on bronchoalveolar lavage in normal control subjects and individuals occupationally exposed to in-

Table I

Characterization of Bronchoalveolar Lavage

Parameter[1]	Controls (mean ± SEM)	Fly Ash (mean ± SEM)	Coal Dust	Acute Silicosis
# Alveolar macrophages	7.4 ± 1.2	14.1 ± 2.1*	4.0	9.8
# Lymphocytes	4.5 ± 0.8	8.4 ± 1.1*	4.6	65.6*
# Neutrophils	2.9 ± 0.7	7.3 ± 2.5*	3.1	30.3*
Rest CL	27.0 ± 6.0	38.9 ± 6.0	65.8	144.6*
PMA CL	68.2 ± 22.6	83.0 ± 10.7	64.5	250.2*
ZYM CL	41.4 ± 12.4	84.0 ± 8.4*	47.4	509.0*

[1]Cell counts are given as millions/lavage.
CL values are millions of counts per second/10 minutes/1.63 X 10^6 alveolar macrophages.
*Significantly greater than control at $p < 0.05$ using a Student's T test.

dustrial dusts. Asymptomatic exposed subjects who were normal by clinical, radiologic and pulmonary function criteria nevertheless showed significant changes in cell populations and phagocytotic activity when compared with unexposed individuals. Much more extreme changes were observed in one subject suffering acute silicosis.

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EXPOSURES OF PRODUCTION EMPLOYEES TO AIRBORNE CONCENTRATIONS OF FIBROUS GLASS DURING THE MANUFACTURING PROCESS

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INTRODUCTION

Airborne concentrations of fibrous glass can be evaluated either gravimetrically or by optical fiber counting methodologies. However, numerous studies[1,2] have demonstrated that there is little correlation between gravimetric results and concentrations of fibrous glass present. For this reason, optical fiber counting methodologies rather than gravimetric analysis have become the methods of choice for fibrous glass analysis.

Prior to the publication of the Occupational Safety and Health Administration's Revised Asbestos Standard (29CFR 1910.1001) in June of 1986,[3] the generally accepted procedure for the determination of airborne concentrations of asbestos, fibrous glass, and other man-made mineral fibers was the NIOSH P&CAM 239 method.[4] However, with the promulgation of this Standard, a new methodology for the evaluation of airborne concentrations of fibrous materials was introduced, the NIOSH 7400 method.[5]

This method introduced a new sampling train for fiber collection (i.e. 25 mm cassette with 5Omm extension cowl) as well as alternative methods for fiber counting (Rules "A" and "B"). Though similar in other respects to the NIOSH P&CAM 239 method, the new NIOSH 7400 method quickly began to receive increased attention from the industrial hygiene community as it was utilized to evaluate individuals' exposures to not only asbestos, but other man-made mineral fibers as well. Of particular concern was the notable adherence of fibers to the sampling cowl and the differing results obtained when fibers were counted via the "A" versus the "B" rules.[6]

With a considerable body of data on employees' exposures to fibrous glass obtained through use of the NIOSH P&CAM 239 method, it became imperative for Owens-Corning Fiberglas to evaluate the correlation between the two methods in terms of the sample results produced and to determine if the NIOSH 7400 method should be adopted for future exposure evaluations. Furthermore, since most of the information concerning use of the NIOSH 7400 method had been generated as a result of asbestos monitoring, it was felt that additional information could be gleaned through the use of the method to evaluate airborne concentrations of a man-made mineral fiber such as fibrous glass. Thus, the following study was designed and implemented.

MATERIALS AND METHODS

Seventy-five paired personal and area samples were collected in parallel on 0.8 micron pore size mixed cellulose ester filters mounted in 37 mm diameter polystyrene plastic cassettes with 16 mm non-electrically conductive extension cowls (i.e. NIOSH P&CAM 239 sampling method) or in 25 mm diameter polystyrene plastic cassettes with 50 mm electrically conductive extension cowls (i.e. NIOSH 7400 sampling method). During the initial phase of the study, additional samples were collected using 0.45 polycarbonate filters mounted in 37 mm diameter cassettes with 16mm extensions cowls for analysis by scanning electron microscopy. However, this approach was quickly discontinued due to the poor fiber retention (i.e. fibers were collected but were easily dislodged during transportation).

All samples were collected at a flow rate of two liters per minute (i.e. 2.0 l/m) using constant flow sampling pumps. The pumps were calibrated, with the filter and sampling train in line, before and after sampling using a precision rotameter calibrated against a primary standard (i.e. soap bubble meter for volumetric rate of air flow). Samples were collected at specific sites along plant manufacturing lines during the production of a variety of fibrous glass insulating products (e.g. batts, blankets, and loose fill). Samples were collected over significant portions of the work shift and are believed to be representative of full shift exposures.

All sample filters were mounted using the acetone/triacetin clearing method and analyzed via phase contrast optical microscopy (PCOM) at a magnification of 400X. Fiber counts for all sample filters were derived utilizing the procedures specified in both the NIOSH P&CAM 239 method as well as the NIOSH 7400 "A" method (i.e. all fibers >5 microns in length with aspect ratios egual to or greater than 3:1 were counted). Glass fibers were differentiated from other fibers by shape recognition using polarized light microscopy. Additionally, fiber length and diameter measurements were determined for a fraction of the samples.

To address fiber adherence to the sampling cowls, after filter removal, all cowls were rinsed with 25% isopropanol in distilled water. Rinse solutions were then filtered through 0.4 micron polycarbonate filters, and analyzed using the counting procedures described above.

After all sample results had been obtained, matched pair results were analyzed statistically to determine differences between the 37 and 25 mm diameter filters and corresponding cowls. Natural log transformed data were used to determine statistical difference at the 0.05 significance level.

RESULTS AND DISCUSSION

The sample results obtained from this study are indicated in Table I. The mean total fiber exposure and the lower and upper 95% confidence limits are shown for the forehearth, line, packer, bagger, rollup, repack cubed, and repack milled operators. The overall mean total fiber (both glass fiber and all other fiber) exposures of employees in OCF production facilities involved in the manufacture of fibrous glass insulation products were 0.024 f/cc for filters only and 0.03 f/cc for filters and cowls combined (NIOSH P&CAM 239 and 7400 methods combined). Additional analyses revealed that 70 to 75% were glass fibers and that 60% of the glass fibers were of a respirable size (i.e. diameters <3.5 microns, lengths of 5 to 250 microns, and length to diameter ratios of 3:1 or greater). Furthermore, these sample results were consistent irrespective of the type of product produced (i.e. faced vs. unfaced insulation) or the physical parameters of the product produced (i.e. R 30 vs. R19 or 24" width vs. 18" width).

Because a significant concentration of fibers were found adhering to the sidewalls of the cassettes (i.e. NIOSH P&CAM 239 Procedure) and to the sampling cowls (NIOSH 7400 Procedure), these fibers were also counted. Results are reported on Table I and Figure I as filter only and as filter and cowl combined. Figure I also includes results of samples collected in end-user applications. The data indicate that there was no statistically significant difference in sample results obtained from the NIOSH P&CAM 239 and 7400 methods when the "A" counting rules were used (see Figure 1). Furthermore, this result was consistent irrespective of the fiber type or size analyzed (i.e. total fiber, total glass fiber, or respirable glass fiber).

Statistical analysis also indicated that there was no difference between the total fiber results obtained from the NIOSH P&CAM 239 and 7400 methods using the "A" counting rules when the fibers on the filters and cowls were combined, Table II. Table II also includes results of samples collected in end-user applications. As indicated in Table II, the ratio, R, of (fibers deposited on cowls + fibers deposited on filters)/ fibers deposited on filters, was 1.7 for the NIOSH P&CAM 239 method (i.e. 16 mm sampling cowl) and 1.5 for the NIOSH 7400 method. There was no statistical difference between these ratios.

Table I

Total Airborne Fiber Concentrations Obtained by Using the NIOSH P&CAM 239 and 7400 "A" Methods (Combined) Fibers per Cubic Centimeter

ITEM	ALL FIBERS							
	Filters				Filters and Cowls			
	# Samples	Exp. Value	95% LL	95% UL	# Samples	Exp. Value	95% LL	95% UL
FOREHEARTH	20	0.017	0.006	0.028	19	0.025	0.003	0.048
LINE	2	0.003	0.000	0.032	2	0.006	0.000	0.076
PACKER	30	0.028	0.017	0.040	29	0.036	0.017	0.055
BAGGER	8	0.023	0.012	0.033	7	0.030	0.002	0.057
ROLLUP	2	0.021	0.001	0.041	2	0.028	0.000	0.082
REPACK-CUBED	9	0.024	0.008	0.040	9	0.033	0.002	0.065
REPACK-MILLED	4	0.040	0.013	0.068	3	0.045	0.000	0.110

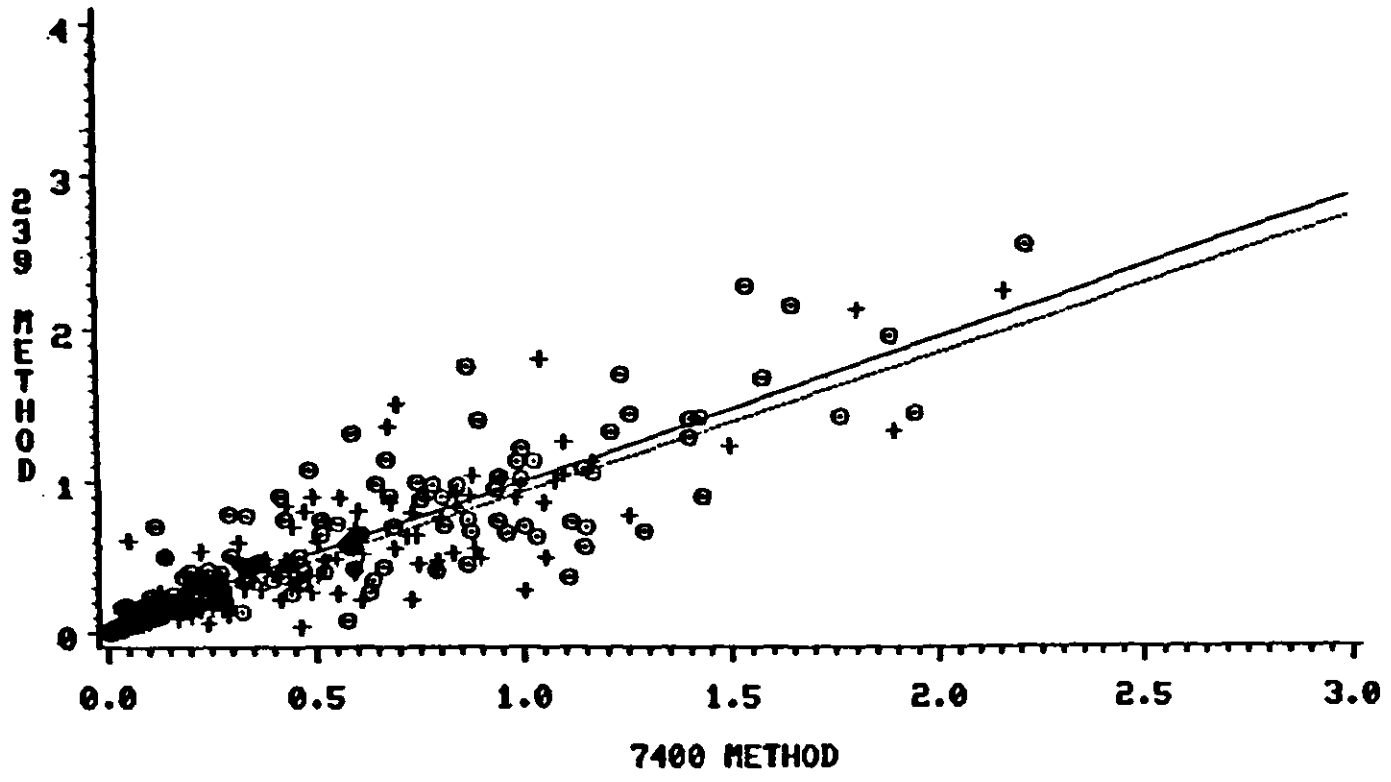

Figure 1. Total fibers per cc—random field counts.

Table II

Ratio of (Fibers Deposited on Cowls + Fibers Deposited on Filters)
Fibers Deposited on Filters for NIOSH P&CAM 239 and NIOSH 7400 Methods*

METHOD	# OF SAMPLES	AVERAGE	MEDIAN	STANDARD DEVIATION
239	162	1.7	1.5	0.90
7400A	160	1.5	1.4	0.52

NOTE: STATISTICALLY THE RATIOS FOR METHODS 239 AND 7400A ARE NOT DIFFERENT.

Significant fiber deposition on samgling cowls has been reported previously by Seixas et. al.[6] Also, in commenting on this phenomenon, some investigators have suggested that a high ratio of fibers detected on sampling cowls versus fibers found on filters is merely an artifact produced by undercounting of fibers deposited on filters.[7] This was not found to be the case, however, since the ratio, R, was consistently high for low as well as medium and high fiber counts.

CONCLUSIONS

The results obtained from our studies indicated that mean employee exposures to total fibers in Owens-Corning Fiberglas manufacturing facilities was 0.024 fibers/cc for filters only and 0.03 f/cc for filters and cowls combined. There was no statistically significant difference in the results obtained when the NIOSH P&CAM 239 and 7400 "A" methods were utilized. However, the study also demonstrated that there was a significant concentration of fibers deposited on the sampling cowls used for both methods which should conceivably be considered in determining the total level of exposures to fibrous glass.

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