ALUMINUM INHALATION REDUCES SILICOSIS IN A SHEEP MODEL

R. BÉGIN, M.D. • F. Dubois, M.D. • A. Cantin, M.D. • S. Massé, M.D. • A. Dufresne, Ph.D. • G. Perrault, Ph.D. • P. Sébastien, Ph.D.

Université de Sherbrooke, Sherbrooke (QC), Institut de Recherche en Santé et Sécurité au Travail, Montréal (QC) and Dust Disease Research Unit, McGill University, Montréal (QC)

INTRODUCTION

Although effective methods of prevention of silicosis have been known for years and implemented in the workplace through legislation, the disease remains of public health interest with some 200 new cases per year from an estimated workforce of 150,000 exposed workers in Canada.[8]

The recent availability of a soluble and inert compound, aluminum lactate (Al) has contributed to the renewed interest of aluminum therapy in silicosis. We have recently documented that Al suppresses the biological activity of quartz up to 10 months after exposure with faster clearance of the Al coated quartz particles.[4] In this study, we evaluate the efficacy of soluble Al aerosol inhalation to alter the biological activity and disease process associated with silica exposure in the sheep tracheal lobe model.

MATERIALS AND METHODS

Experimental Design

The flock of 40 sheep was randomly divided in 4 groups of 10 sheep. The first group was exposed to 100 ml phosphate buffered saline (PBS) infusion in the tracheal lobe followed by monthly inhalation of 10 ml PBS (group PBS-PBS). The second group was exposed to 100 ml PBS followed by monthly inhalation of 100 mg Al in 10 ml PBS (group PBS-Al). The third group was exposed to 100 mg Minusil-5® (Pennsylvania Glass Y Sand Co., Pittsburg, PA) in 100 ml PBS followed by monthly inhalation of 10 ml PBS (groups Si-PBS). The fourth group was exposed to 100 mg Minusil-5® in 100 ml PBS followed by monthly inhalation of 100 mg Al in 10 ml PBS (group Si-Al).

Minusil-5® particles have been well characterized,[3] 99.9% of diameter <5 μm and 95% <1 μm.

Exposures were carried out via bronchoscopic catheterization of the tracheal lobe bronchus and slow infusion of the suspension in the lobe. Inhalations were carried out 24 hr after bronchoalveolar lavage (BAL) with the animal intubated and breathing a mist of nebulized 0.01 to 4 μ-sized liquid particles with a Bird Mark 8 pressure ventilator (Bird Corp. Richmond, Ca) set at a maximal pressure of 25 cm H_2O for 20 minutes. Exhaled gases were vented outside the room. BAL were carried out after wedging the distal tip of the bronchoscope in the tracheal lobe bronchus by slow infusion of four 50 ml 39°C aliquots of PBS through a 50-ml syringe attached to the work channel of the bronchoscope and by gentle aspiration of the effluent. BAL were performed prior to exposures and at monthly intervals after. Animals were sacrificed and autopsied at month 6.

Bronchoalveolar Lavage

The BAL cell differential populations were determined on cytocentrifuge smears stained with Wright-Giemsa. In the supernatant, albumin, IgG and IgM were determined by the immunochemical method (Cappel Lab. Inc., Downington, PA). The activity of lactate dehydrogenase (LDH) was measured by spectrophometric method. BAL phospholipids were measured by the technique of Bartlett[1,2] and contribution of lecithin and phosphatidylglycerol determined on the basis of their PO_4 content.

To assess interstitial lung matrix changes we looked at the glycoaminoglycan and fibronectin accumulation in BAL fluid. Oxidant production by alveolar macrophages was evaluated according to methods previously developed.[6]

Histopathology

The tracheal lobe was identified and 9 samples of the lobe of each sheep were obtained and each evaluated histologically for intensity and profusion of lesions to yield our average pathologic index of disease.

Determination of Quartz Concentration in Lung Tissue and Lavage

For each sheep in the study, a large fragment of the tracheal lobe was analyzed for quartz concentration using X-ray diffractometry.[7]

RESULTS

Lung Lavage Cellularity

The total and differential cell counts per lavage were similar in the group PBS-PBS and the group PBS-Al throughout the study. All silica-exposed sheep demonstrated at month 1 a 3 to 10-fold increase in cellularity which was sustained in the group Si-PBS but significantly attenuated to control levels in the group Si-Al ($p < 0.01$). In the Si exposed sheep, macrophages, lymphocytes and neutrophils were increased but there was no significant change in the eosinophil counts which were less than 4% at all times.

Lung Lavage Biochemistry

Albumin averaged 70 ± 8 μg/ml in the group PBS-PBS and did not vary significantly over time. In the group PBS-Al, albumin levels were comparable ($p > 0.05$). In the Si-PBS group, there was a transient increase to 200% control level at month 1 with gradual return to control levels by month 5. In the Si-Al group, albumin remained at control level after Al inhalation. Lactate dehydrogenase levels in the PBS-PBS group averaged 6 ± 1 ml U/ml and did not vary significantly; in the PBS-Al group, levels were comparable ($p > 0.05$). In the Si-PBS group, there was a significant sustained 6 to 8-fold increase but in the group Si-Al, after initial increase, the levels of lactate dehydrogenase returned to the PBS-PBS group levels. Surfactant showed patterns of response similar to that of lactate dehydrogenase in the 4 groups.

Fibronectin in Macrophage Supernatant

The production of fibronectin by alveolar macrophages in culture at month 6 was undetectable in groups PBS-PBS, PBS-Al and Si-Al but significantly increased at 2.1 ± 1 ng/10^6 cells per 24 hours in the SI-PBS group ($p < 0.01$).

Oxidant Production and Glutathione

Lung cells of the PBS-PBS group at time 0 spontaneously released low amounts of superoxide (1.77 ± 0.55 nmol cytochrome-C reduced/10^6 cells-hr) and hydrogen peroxide (0.67 ± 0.34 μM/10^6 cells-hr), and the release of oxidants did not change during the study period in any of the groups. Glutathione in the bronchoalveolar lavage fluid of the PBS-PBS group at time 0 was 0.23 ± 0.05 μM and did not differ between groups throughout the study period.

Lung Silica Content

The concentration of quartz in the lung parenchyma of the tracheal lobe of the sheep 6 months after initial exposure was as follows: in the group PBS-PBS and in the group PBS-Al, it was undetectable. In the group Si-PBS, it was 2.83 ± 0.98 μg/mg and in the group Si-Al it was 1.01 ± 0.74 ($p < 0.05$).

Pathological Scores of Disease

The lung morphology of the sheep in the group PBS-PBS and PBS-Al remained normal. In the group Si-PBS, we found early nodular silicotic lesions composed largely of macrophages and lymphocytes with no evidence of collagen deposition comparable to those reported earlier,[3-5] with a pathological score of disease of 2.9 ± 1.0. In marked contrast, the group Si-Al had milder histological changes and a significantly lower score of 1.0± 0.3 ($p < 0.05$). In the Si-Al group, there was significant reduction of both the profusion and the severity scores ($p < 0.05$). Whereas well-defined silicotic nodules were seen in 8/10 sheep in the Si-PBS group, they were seen in only 1/10 of Si-Al sheep.

DISCUSSION

This study documents that soluble aluminum lactate aerosol inhalation does not alter the normal biological processes in the bronchoalveolar milieu and does not produce significant pathological lung damage. In this study, we have observed that Al inhalation at monthly intervals significantly suppresses the alveolitis of silcosis, reduces the intensity and profusion of the disease process, and accelerates the clearance of quartz particles from the lung tissue.

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PULMONARY TOXICITY OF ILLITE AND KAOLIN DUSTS

H. DANIEL • L. Le Bouffant • A. Wastiaux • P. Sëbastien
CERCHAR, BP No. 2, 60550 Verneuil en Halatte, France

INTRODUCTION

Clay minerals kaolin and illite are present in some mixed dusts of industrial origin. Kaolin is an industrial mineral with many applications. It is used as a filler in the paper industry, as a filler and extending agent in rubber, paints, inks, plastics and insecticides, in the manufacture of China, refractory bricks, crucibles, saggars and glass, as a mild abrasive in soaps and toothpastes and as stiffener of textile. Dust possibly produced in these industries, but also smoking,[2] can be sources of exposure to fine kaolin particles. It is now accepted that the long term inhalation of high quantities of kaolin dust can lead to the development of a specific type of pneumoconiosis.[11] From the pathological viewpoint, the fibrosis is mainly nodular or massive,[9] with important dust retention.[14]

Sources of exposure to illite and possible related-health effects are much less documented. The interest for illite came essentially from its presence in coal mine dust.[3] It has been suggested that the in-vivo leaching of aluminum from illite particles could reduce the activity of the accompanying quartz particles.[7] To our knowledge, the toxicity of illite particles has been tested in only few in-vitro or in-vivo experiments.[1,5,8]

We have some evidence that both minerals can exhibit acute pulmonary toxicity after a single intratracheal injection.[4] In a previous experiment, two groups of 50 female Wistar rats were injected with 50 mg of fine particles of either illite from Le Puy, or kaolin from Cornwall. Respectively 12% (illite group) and 45% (kaolin group) of the animals died of pulmonary oedema in the first week following the injection.

In this context, we found it useful to conduct long-term experiments to comparatively assess the fibrogenicity of illite and kaolin dust, alone or in combination with quartz.

METHODS

In a first series of experiments, illite (Le Puy), kaolin (Cornwall), quartz (Madagascar) and coal (Courrières low rank) were tested in the rat exposed by inhalation. Wistar female rats were exposed for 3 months (5 h/d, 5 d/w) to 300 mg/m^3 of respirable dust. Aerosol generators and inhalation facilities are described in detail elsewhere.[6]

In a second series, animals received a single intratracheal injection of either quartz (12.5 mg), quartz + illite (12.5 mg + 37.5 mg) and quartz + kaolin (12.5 mg + 37.5 mg). Injected particles were prepared by cyclone separation and were of respirable size.

In both series, the pulmonary response was assessed at month 6, 12, 18 and 24. Animals (10 per subgroup) were killed and the lungs removed. The weight of fresh lung was recorded for each animal.

Left lobes were used for histopathological examination. They were perfused under 25 cm H_2O pressure and fixed in 10% neutral buffered formalin. Sections stained by hematoxylin eosine and Picrosirius were examined at three different locations under crossed polaroid filters. In each group, remaining fragments of lung tissue were pooled, dried and analyzed for collagen, lipids and dust. Collagen was measured by the method of Stegeman.[12] Coal in the lung was measured gravimetrically after extraction by the formamide technique.[13] For quartz and clay, lung dust was extracted by low temperature ashing, ash suspension, and filtration through a polycarbonate membrane filter. Quantity of quartz on the membrane was determined by X-ray diffractometry. Quantity of clay was deduced from aluminum concentration measured by X-ray fluorescence.

RESULTS

Main results of the inhalation experiments are reported in Figure 1. Similar conditions of exposure yielded to different dust retentions in the lung. The highest retention was observed with coal and the lowest with quartz, clay retention being situated in between. For clays, there was no evidence of pulmonary clearance after month 12. At month 6, the mean weight of fresh lung was 5 times above control value in the quartz group; it was only slightly elevated in the other groups. In the following periods, the lung weight increased much more in the quartz group than in the other groups. A similar pattern was observed with pulmonary collagen.

Main results of the intratracheal injection experiments with the quartz and quartz/clay mixtures are reported in Figure 2. At month 6 and 24, respectively 48% and 37% of the injected dose was still present in the lungs of animals exposed to quartz alone. Clay admixture had no clear effect on the clearance of quartz. It seemed, however, that overall quartz retention was somewhat higher in the quartz/illite group and somewhat lower in the quartz/kaolin group. Mean weight of fresh lung was 4–5 times above control value in the quartz group and in the quartz/kaolin group. Interestingly enough, the lung weight was only slightly elevated in the quartz/illite group. In all groups, the lung weight increased in the period 6–24 months. Results of collagen measurement clearly discriminated the three groups. The admixture of illite or kaolin to the injected quartz, respectively reduced or greatly enhanced the production of pulmonary collagen.

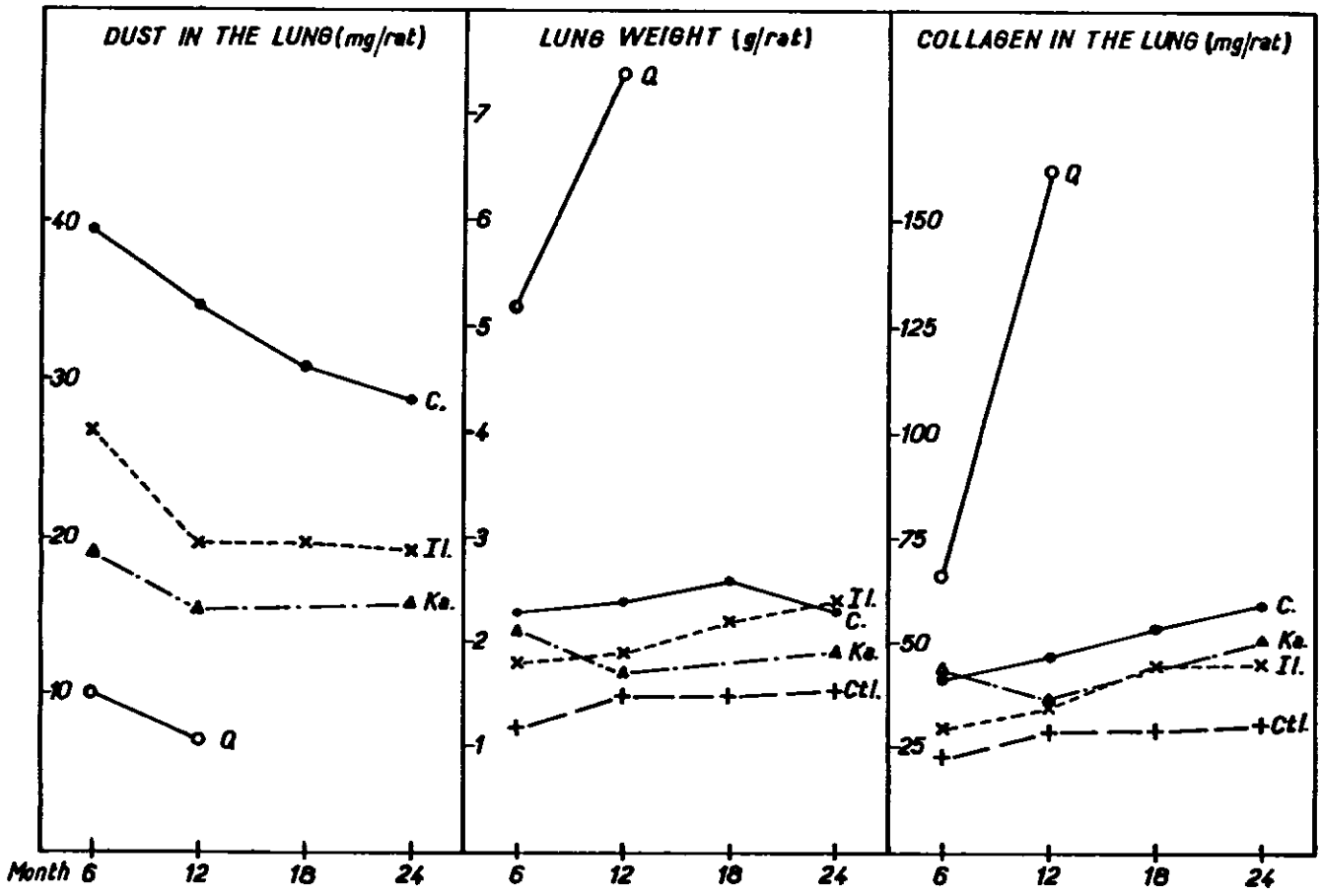

Figure 1. Wistar female rats exposed by inhalation (300 mg/m^3, 3 months) to quartz (Q), kaolin (K), illite (I) and coal (C). Measurement of lung dust, weight of fresh lung and pulmonary collagen at month 6, 12, 18 and 24.

DISCUSSION

In our inhalation experiments kaolin and illite exhibited similar activities. During a two year period they produced very little collagenous fibrosis. These results agree with previous experimental observations.[9] They are also similar to those obtained after inhalation of coal dust by experimental animals. It should not be concluded however, that kaolin, illite and coal dust have similar biological activities. First it must be remembered that results of these experimental tests are poor predictors of the pneumoconiotic risk in humans. Inhalation of coal mine dusts for example, can lead to disabling pneumoconiosis in miners, but these dusts exhibit very moderate activity in most of experimental tests. Secondly, there is some evidence from our experiments by intratracheal injection that clay and coal dust behave differently in the lung.

An interesting observation was the pulmonary response to intratracheal injection of quartz/clay mixtures. Combination of quartz and kaolin gave rise to pronounced collagenous fibrosis, as already noticed.[10] By contrast animals exposed to quartz/illite produced mixtures of less pulmonary collagen than animals exposed to the same dose of quartz alone. This clearly indicates that kaolin and illite behave differently in the lung. But apparently, this difference in behaviour had no detectable effect when the two clay minerals were tested individually. These findings illustrate once more how complex are the mechanisms of action of inhaled coal mine dust, which generally contains quartz, kaolin and illite.

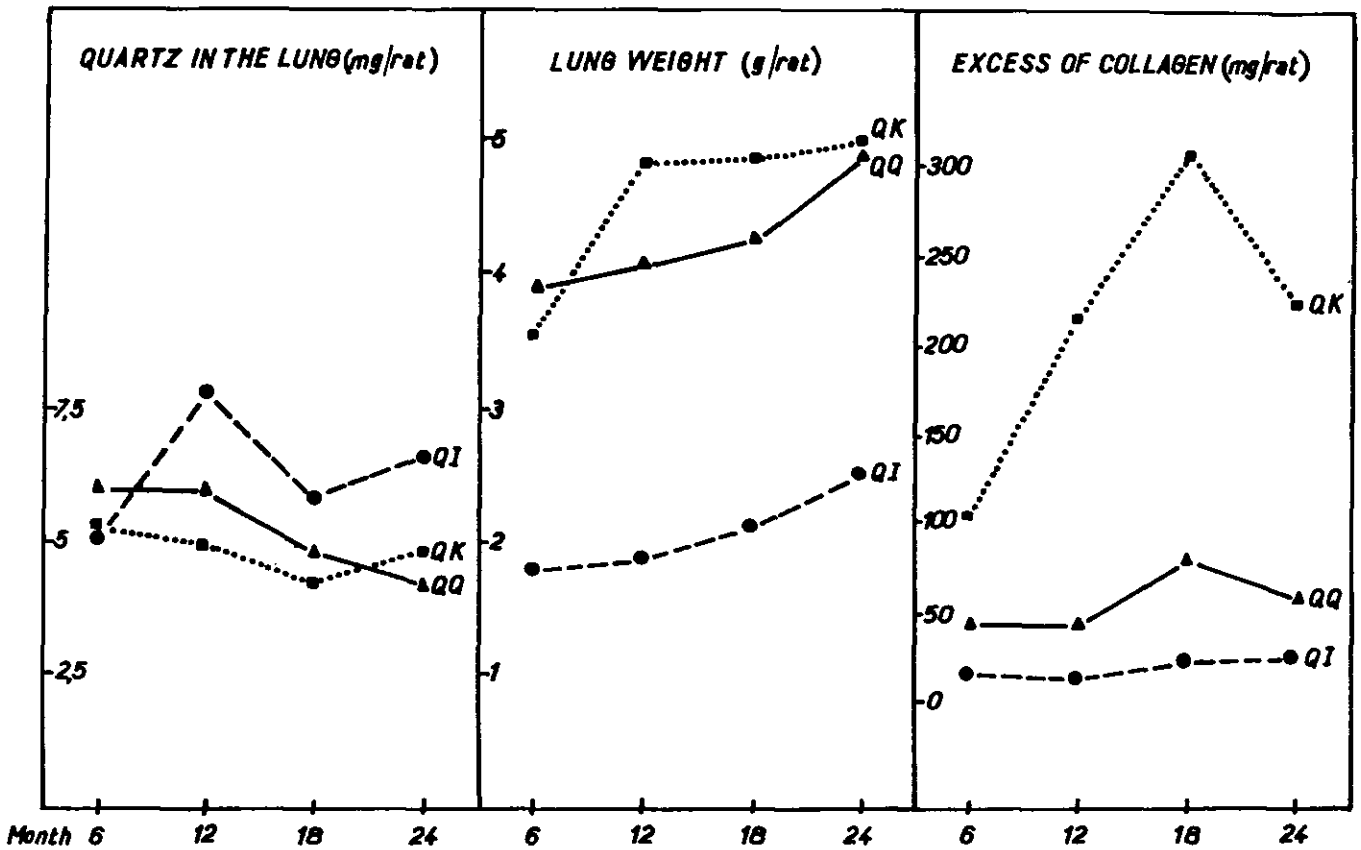

Figure 2. Pulmonary response to quartz/clay mixtures injected intratracheally in Wistar female rats. Measurement of lung quartz, weight of fresh lung and pulmonary collagen at month 6, 12, 18 and 24. Three dusts were injected: QQ 12.5 mg of quartz, QK 12.5 mg of quartz + 37.5 mg of kaolin, QI 12.5 mg of quartz + 37.5 mg of illite.

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TOXICOLOGICAL EVALUATION OF ASBESTOS SUBSTITUTE

O. RAHMAN, Ph.D. • S.G. Khan, M. Phil. • M. Aslam, M. Phil. • P. K. Agarwal, M.D. • S. Ali, Ph.D.

Industrial Toxicology Research Centre, Post Box No. 80, Lucknow—226 001, India
Dept. of Pathology, K.G's Medical College, Lucknow—226 003, India

INTRODUCTION

Epidemiological and experimental studies have proved that asbestos induces certain pathological changes in the lung like fibrosis knows as asbestosis and two forms of malignancies, i.e. mesothelioma and bronchogenic carcinoma.[30,35] In order to safeguard the workers from the hazardous effects of these fibres, scientists all over the world are trying to replace them with other natural and man-made mineral fibres. Among them, wollastonite is a promising asbestos substitute under trial in India. They are acicular or fibrous calcium silicates ($CaSiO_3$), and have attained additional significance due to their high thermal resistance properties. The use of wollastonite in ceramic tiles, etc., has given this mineral considerable attention as a substitute for asbestos fibres.[1,25] A few reports are available on their biological effects. Skaug and Gylseth[38] have reported, on the basis of hemolytic studies of two natural and three synthetic calcium silicates, that synthetic silicates are more toxic than the natural. Bolton et al[2] have reported no major pulmonary damage in rats exposed to three different varieties of calcium silicate insulation materials. Moreover, a few cases of lung fibrosis, pleural thickening, chronic bronchitis and impairment of lung ventilatory capacity in wollastonite exposed workers have also been reported.[11,20] However, no report is available on the biological activity of Indian varieties of wollastonite dusts. Therefore, in the present study, three varieties of Indian wollastonite, namely, kemolit A-60, kemolit-N and kemolit ASB-3 have been evaluated for their toxicity. Besides cytotoxic studies *in vitro* and fibrogenic responses *in vivo*, the effect of these fibres on the pulmonary xenobiotic metabolizing enzyme system was also evaluated, to monitor their influence in the presence of other carcinogens, if present in the system simultaneously either by smoking or from the other environmental sources. The results obtained from these studies were compared with chrysotile, the most toxic variety of asbestos and also a carcinogenic enhancer in the presence of tobacco smoke.[3]

MATERIALS AND METHODS

Dust

Wollastonite dust samples, kemolit A-60, kemolit-N and kemolit ASB-3 were obtained from Mr. Salil Singhal, Director, Wolkem Private Ltd. Udaipur (India). Particle size below 30 u were prepared as described by Zaidi.[43] Chrysotile UICC standard reference sample particle size $<30\ \mu$ was obtained as a gift from Dr. J.B. Leinweber, Johns-Manville, U.S.A.

Chemicals

Benzo(a)pyrene, 3-hydroxy benzo(a)pyrene, styrene epoxide, 1-chloro, 2,4,-dinitrobenzene and bovine serum albumin were procured from Sigma Chemical Company, USA. All the other chemicals and reagents were either purchased from V.P. Chest Institute, New Delhi, India or Sisco Research Laboratory (SRL) Bombay, India, and were of analytical grade.

Hemolytic Studies

The lysis of 0.2% suspension of human erythrocyte in 0.01M Tris-HCl buffer pH 7.35 in 0.15M NaCl caused by 2 mg/ml each of different dusts was measured at 37°C after two hours except chrysotile where it was 10 minutes to avoid adsorption.[33]

Treatment of Animals

Female albino rats from ITRC Colony, weighing 150-180 gm, were used. The dried dusts and 0.15 M NaCl were separately autoclaved at 15 lbs pressure for 15 min. The dusts were separately suspended in 0.15 M NaCl just before inoculation. The animals were divided into five groups. Intratracheal treatment of animals with dust were done according to the procedure as described by Zaidi.[43]

Each animal of the experimental groups was instilled intratracheally with 5 mg of different dust samples separately, suspended in 0.5 ml of normal saline. Control groups received 0.5 ml of normal saline solution only. The animals were maintained on commercial pellet diet, supplied by Hindustan Lever Limited, Bombay, India, and tap water *ad libitum*. The animals were sacrificed at 90 days after the instillation of dusts. Lungs were taken out, weighed and a portion was fixed in 10% formal saline for histopathological studies, while the other portion was cut into small pieces and dried at 110°C for chemical estimation. Another set was taken for microsomal and cytosolic fractionations.

Histopathological Studies

Representative 5 μ paraffin sections were cut and stained with hematoxylin-eosin and VanGieson.

Isolation of Microsomes

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

Enzyme Assays

Benzo(a)pyrene hydroxylase was assayed by the fluorimetric technique as described by Dehnen et al.[8] The quantitation of phenolic metabolite was based on comparison of fluorescence to a standard solution of 3-hydroxy benzo(a)pyrene.

Epoxide hydratase activity was assayed by the fluorimetric technique, according to the method of Dansette et al.[6] by using styrene epoxide as substrate.

Glutathione-S-transferase activity was determined by the procedure, described by Habig et al.,[16] by 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.[31] 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.[12]

Ascorbic acid content was estimated in lung cytosol according to the procedure of Schaffert and Kingsley.[34]

Enzymatic and non-enzymatic lipid peroxidation was determined by the procedure of Ottolenghi[32] as modified by Hunter et al.[19] estimating the malonaldehyde formed with 2-thiobarbituric acid.

Hexosamine and sialic acid were estimated in the fresh tissue by the methods of Dische and Broenfreund[10] and Warren respectively. Uronic acid and collagen were estimated by the method of Dische,[9] and Stegmann et al.[41] respectively in dry tissue. Phospholipids were extracted from dry tissue in chloroform: methanol (2:1) and were estimated by their phosphorous content by the method of Fiske and Subba Row.[13]

Protein content in trichloroacetic acid precipitate was estimated by the method of Lowry et al.[27] by using crystalline bovine serum albumin as standard.

RESULTS

In vitro Studies

Table I shows that all the wollastonite dust samples induced hemolysis and the order of hemolysis was 43.4%, 41.2% and 35.3% by kemolit A-60, kemolit-N and kemolit ASB-3 respectively. In comparison to chrysotile all the samples were less hemolytic.

In vivo Studies

Fibrogenic Response. The changes in the chemical composition and dry weight of the lung by different wollastonite samples are recorded in Table II. When compared with chrysotile,[7] the increase in the collagen content and phospholipids by kemolit A-60 was significantly very high. There was no significant increase in the mucopolysaccharides by these dusts. Kemolit A-60 increased only the content of sialic acid.

The histopathological studies revealed mild to moderate amount of fibrosis of the lung alveoli (Figure 1). The animals exposed to Kemolit A-60 showed peribronchiolar areas of fibrosis (Figure 2) which was not found with any other dust. In some cases collection of chronic inflammatory cells and few abscesses were also seen on scanning the tissue (Figures 3 and 4).

Table I

Comparative Hemolysis of Chrysotile and Wollastonite Using Rat Erythrocytes

Dust sample	% Hemolysis
Chrysotile	72.16±3.29
Kemolit A-60	43.37±2.50
Kemolit - N	41.19±3.03
Kemolit ASB-3	35.28±1.65

The values represent mean of six separate experiments ± S.D.

Lung Weight

A significant increase in lung weight of all the experimental animals was observed, kemolit A-60 showed a higher increase in the lung weight as compared to kemolit-N and kemolit ASB-3 (Figure 5).

Effects of Different Dusts on Lung Microsomal and Cytosolic Fractions

Figure 6 shows the increase in the cytochrome-P-450 content by different dusts. Chrysotile showed the maximum increase followed by kemolit A-60. Activities of benzo(a)pyrene hydroxylase and epoxide hydratase is recorded in Figure 7 and 8. Among all the dust samples kemolit A-60 induced maximum increase but in comparison to chrysotile the increase was of lower magnitude. The alteration in the activity of glutathione-S-transferase is recorded in Figure 9. Chrysotile decreased the activity of this enzyme significantly, while kemolit A-60 decreased the activity 9% which was three times less than observed by chrysotile. Kemolit-N and kemolit ASB-3 increased the activity of glutathione-S-transferase.

Effect on Water Soluble Antioxidants

Statistically, chrysotile and kemolit A-60 induced significant decrease in the content of ascorbic acid as reported in Figure 10, while glutathione content was significantly decreased only by chrysotile (Figure 11).

Effect on Microsomal Lipid Peroxidation

Chrysotile and kemolit A-60 induced significant lipid peroxidation both enzymatically and non-enzymatically, followed by kemolit-N. There was no change by kemolit ASB-3 (Table III).

Table II

Changes in the Lung Weight and Composition of Control and Wollastonite Treated Rats

Parameters	Control	Kemolit A-60	Kemolit N	Kemolit ASB-3
Dry Weight (mg/g fresh tissue)	204±23	237±21[d]	218±19	205±19
Lung protein (mg/g fresh weight)	100±8.2	105±5.8[d]	103±2.5	101.4±1.9
Hexosamine (mg/100 mg fresh tissue)	2.00±0.14	2.16±0.054	2.09±0.08	2.04±0.08
Sialic acid (mg/100 mg fresh tissue)	3.34±0.14	3.70±0.23	3.47±0.48	3.44±0.56
Uronic acid (mg/g dry weight)	23.38±3.98	28.10±4.84	26.65±3.57	25.96±2.71
Collagen (mg/g dry weight)	39.36±3.87	61.45±11.87[c]	48.93±7.32	45.76±5.56
Phospholipids (mg/g dry weight)	7.96±0.46	13.31±0.33[a]	9.31±0.09[b]	8.57±0.13[d]

The values are expressed as mean ± S.D. of six animals.

[a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.02$; [d] $p < 0.05$.

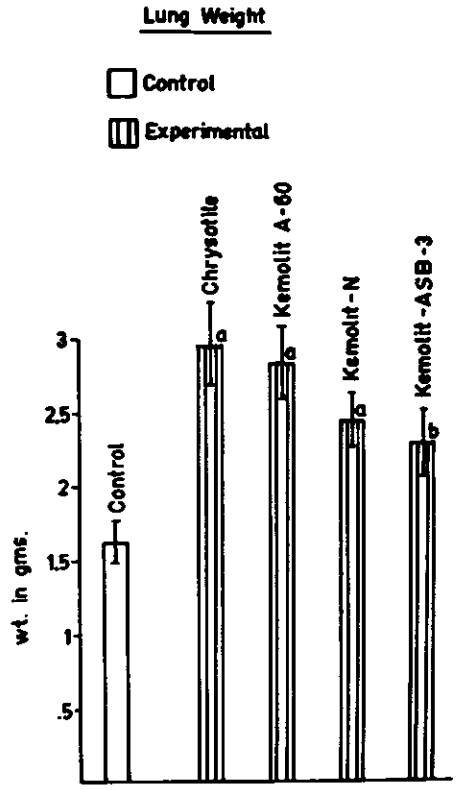

Figure 1. Section of rat lung tissue taken 90 days after the intratracheal inoculation of wollastonite showing interstitial fibrosis. 400×

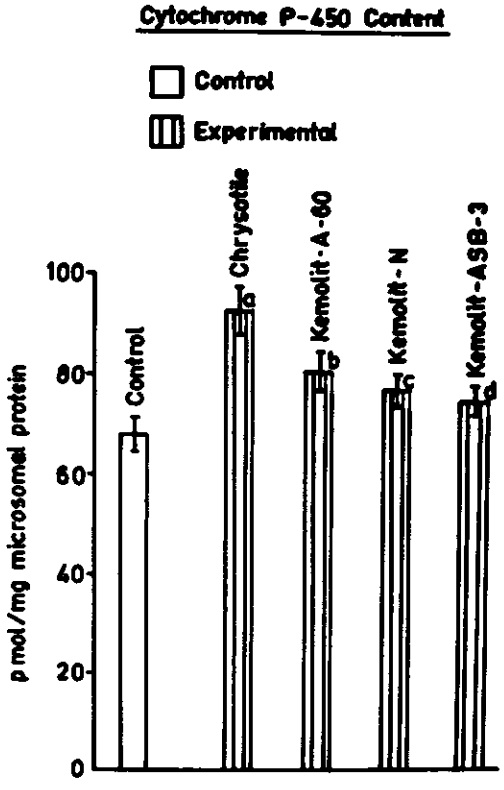

Figure 2. Section of rat lung tissue taken 90 days after the intratracheal inoculation of wollastonite showing peribronchiolar area of fibrosis. 400×

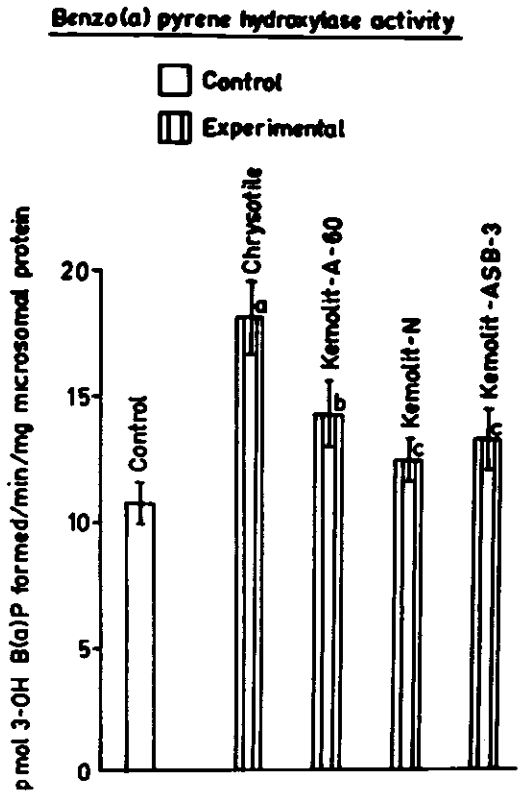

Figure 3. Section of rat lung tissue taken 90 days after the intratracheal inoculation of wollastonite showing inflammatory cells. 400×

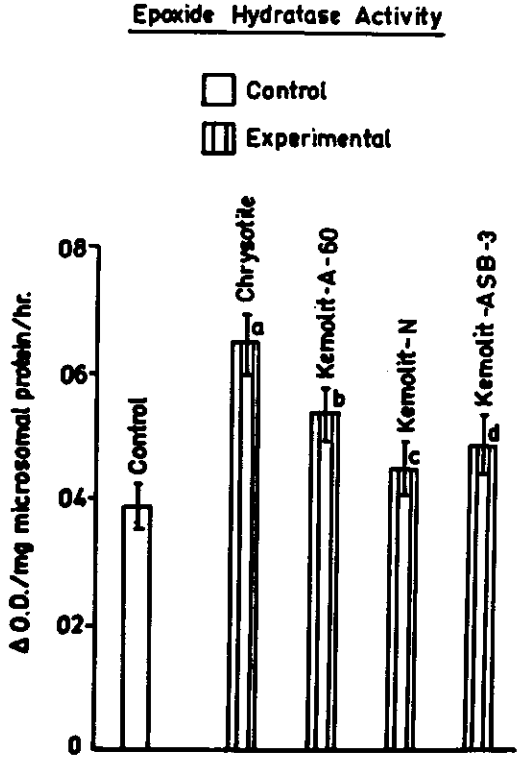

Figure 4. Section of rat lung tissue taken 90 days after the intratracheal inoculation of wollastonite showing abscess. 400×

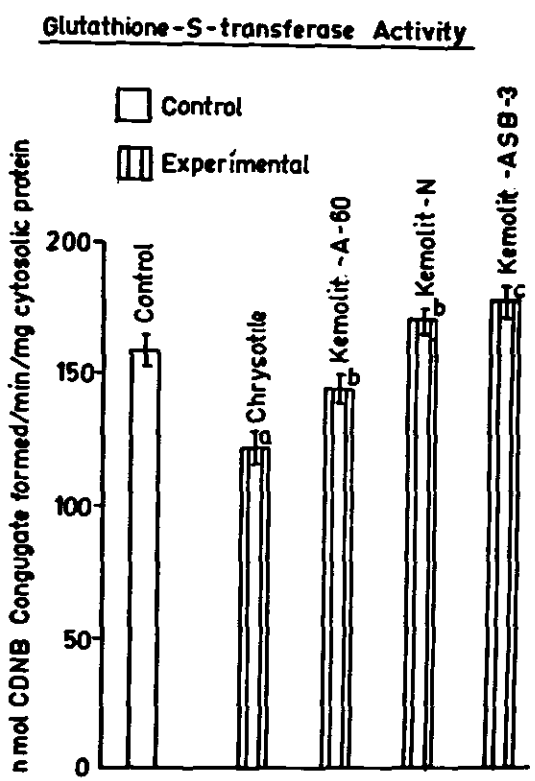

Figure 5. Fresh lung weight of control and dust treated animals. The values are expressed as mean ± SEM of six animals $^{a}p < 0.001$; $^{b}p < 0.02$.

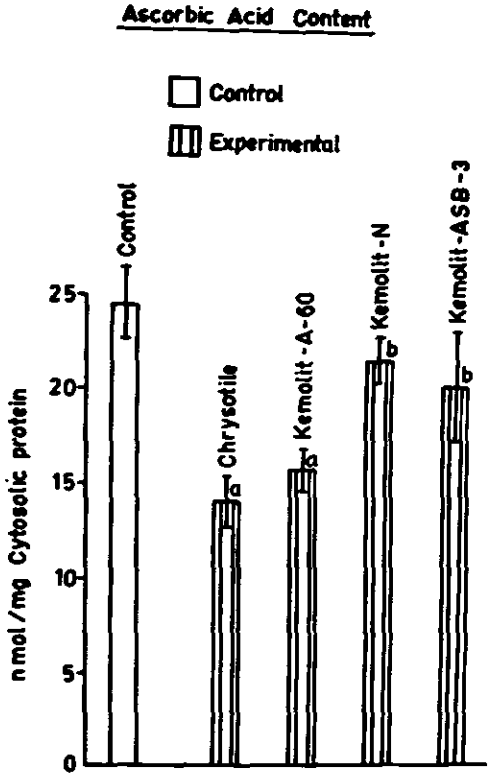

Figure 6. Lung cytochrome P-450 content of control and dust treated rats. Results represent mean ± SEM of six animals, $^{a}p < 0.001$, $^{b}p < 0.02$, $^{c}p < 0.05$, ^{d}p - N.S.

DISCUSSION

In the present study among the three varieties of wollastonite, kemolit A-60 was found most toxic. The cytotoxic index of these samples were much less than chrysotile[18] using erythrocytes as *in vitro* model system[37] Kemolit A-60 was found to be the most fibrogenic dust. The fibrogenic pattern of these dusts tallied with those of Bolton et al.[2]

It appeared from the results that only kemolit A-60 could influence the activity of benzo(a)pyrene hydroxylase and epoxide hydratase in the diseased animals where fibrosis had already developed and collagen content was very high. However, when compared with chrysotile exposed animals where fibrosis had just begun and collagen content was lower than kemolit A-60 exposed animals, the activities of these enzymes were much less. Benzo(a) pyrene hydroxylase and epoxide hydratase play a crucial role in the formation of ultimate carcinogen derived from polynuclear aromatic hydrocarbons.[14,24] Further activation of these enzymes induced by chrysotile may aggravate the situation in the presence of other carcinogens, if present in the system. The effect of these dusts on glutathione-S-transferase activity was very interesting. This enzyme catalysed the conjugation of the ultimate carcinogens with glutathione in the lungs, which are eventually eliminated.[5] The inhibition of these enzymes and the reduction of glutathione by chrysotile decelerate the above reaction, hence providing the accumulated metabolites with the opportunity of interacting with DNA.[42] Similar results were also reported by Brown et al.[3] On the other hand kemolit ASB-3 increased the activity of glutathione-S-transferase significantly which could facilitate the elimination of reactive metabolites of the PAHs from the system. The other two dusts, i.e., kemolit A-60 and kemolit-N did not have any significant effect on the activity of this enzyme. Chrysotile inhibited the content of water soluble antioxidants, like glutathione and ascorbic acid significantly. It is important to note this, since antioxidants are known to inhibit tumors induced by PAHs.[23,36,39,40] Among the asbestos substitutes, kemolit A-60 decreased the content of ascorbic acid only and had no significant effect on glutathione but when compared with chrysotile the magnitude of decrease was much less. The decrease in ascorbic acid content by kemolit A-60 could be associated with its high fibrogenic response at this stage. Ascorbic acid is one of the important components of mammalian lungs defense against environmental pollutants.[26,28] It is closely associated with environmental stress in man and animals.[4] Therefore, its low level in lungs may hamper the defence of tissue against environmental pollutants. A higher rate of enzymatic and nonenzymatic lipid peroxidation was observed by chrysotile and kemolit A-60 while the induction

of LPO with kemolit-N was of lower magnitude and that with kemolit ASB-3 was insignificant. It is well documented that the induction of free radicals may be responsible for the pathogenicity produced by asbestos.[15,17,21,29]

From these studies it is evident that in comparison to chrysotile asbestos, wollastonites were less cytotoxic and did not bring significant alterations in the drug metabolizing enzyme system. Concluding this paper we would like to emphasize that kemolit-N lies at one end of the spectrum being closely followed by kemolit ASB-3 with kemolit A-60 as the most toxic form of wollastonite.

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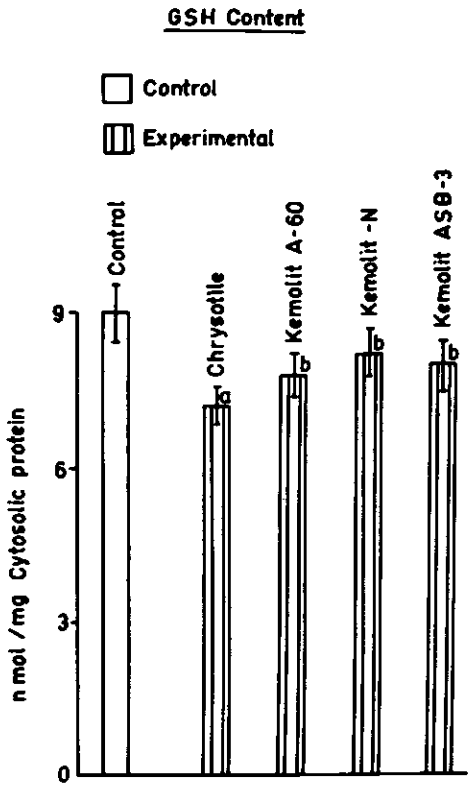

Figure 7. Benzo(a)pyrene hydroxylase activity in lung microsomes, isolated from control and dust treated rats. The values represent mean ± SEM of six animals $^{a}p < 0.001$, $^{b}p < 0.02$, ^{c}p – N.S.

Table III

Lipid Peroxidation in Control and Dust Treated Animals

Treatments	Control	Chrysotile	Kemolit A-60	Kemolit N	Kemolit ASB-3
Microsomes	0.28±0.022	0.374±-.018[a]	0.361±0.024[a]	0.346±0.032[b]	0.295±0.038[d]
Microsomes + NADPH	1.28±0.12	2.154±0.16[a]	2.054±0.13[a]	1.73±0.18[c]	1.55±0.14[d]
Microsomes + Fe^{++}	4.68±0.23	7.041±0.52[a]	6.753±0.45[a]	5.85±0.38[b]	5.12=0.43[d]

The values are expressed as mean ± SEM of six animals.

$^{a}p < 0.001$; $^{b}p < 0.02$; $^{c}p < 0.05$; ^{d}p-N.S.

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Figures 8-11 not provided

STUDY OF EFFECT OF DIFFERENT KIND OF SHORT ASBESTOS ON LUNG OF RATS

YANG MEIYU • Zhao Jinduo • Ku Guihau • Zhao Xiuqing • Fu Bo • Zhang Jifang
Shenyang Research Institute of Industrial Hygiene and Occupational Disease, Shenyang, P.R. China

Asbestos has many properties which include acid and alkali resistance, fire resistance, and ability of electric resistance which make it indispensable in modern industry. It is well known that inhalation of asbestos dust can lead to pulmonary fibrosis and carcinoma of the lung or to the development of diffuse mesothelioma of the pleura and peritoneum. But the mechanism of asbestos carcinogenesis is not clear. Some authors believe that asbestos carcinogenesis depends on chemical action. Other authors think that the physical nature of asbestos fibre is the main factor. Especially long fibre asbestos is the main reason for either fibrosis or tumors of lung. In order to elucidate hazards of short asbestos fibre, an experiment study of effects of four kinds of short asbestos on lungs of rats was observed. (Figures 1-4)

MATERIALS AND METHODS

Dust

Chrysotile and crocidolite were obtained from a Shenyang asbestos processing factory and Qingdao No. 2 asbestos processing factory respectively. Tremolite and actinolite were obtained from mining department of a college. These fibres were prepared by grinding in a ceramic ball mill. The length and diameter distribution of these fibres were obtained by phase-contrast microscopy as in Table I. Morphology of fibres was observed by electron micrograph.

Animals

Wistar rats were used. Weights of rats were 180-220g with nearly equal number of male and female rats. They were divided into 5 groups: chrysotile, crocidolite, tremolite, actinolite and saline control group. There were seventy rats in each group. All rats were injected intratracheally with 20mg dusts suspended in 1 ml saline; one month later they were injected repeatly with suspended dust of same dose. Total dose was 40 mg in each rat. Some rats were killed at the end of 2, 4, 6, 12 and 18 months respectively after the initial injection of dust. The one third rats were allowed to live out their full lifetime. The living condition of rats was observed. The body weight of rats was measured every other month. The wet and dry weight and collagen content of the lungs were determined. The lungs and hilar nodes were examined. The native death and the development of pulmonary neoplasms of the one third rats were observed.

RESULTS

The body weight of all the animals were increasing with time. There was no difference significantly among the experimental and control groups. The dry weights of lungs of rats in all groups are shown in Figure 5.

The increasing of wet weight and collagen of lungs was similar to that of dry weight of lungs, during the sixth to twelfth months after onset of injection dust. There was significant increasing of collagen in the lung. There was significant difference among experimental and control groups. The increasing of collagen in chrysotile group was the highest among the experimental groups as in Table II.

PATHOLOGY

Gross

There were a lot of small grey-white or brown-tan spots at the surface of lungs in every group, after 2-4 months of injection dust. In the crocidolite group, the spots often were grey-blue in color. There were obvious spots at the cut surface and slight pulmonary emphysema after 6-12 months. Lymph nodes in the experimental groups were larger and harder than those of control group, especially in the chrysotile group.

Microscopic Appearance

Chrysotile group: after 2-4 months, a lot of macrophages, dust cell, asbestos fibres and debris were seen in alveoli adjacent to respiratory bronchioles and there was increasing of reticulate fibre, thickness of bronchioles and arteriolas wall (Figures 6, 7). During 6-12 months, there was slight pulmonary emphysema, a few collagen fibres in the interstice (Figure 8). At the end of 18 months, these changes were similar to former. No asbestos bodies were found in the lungs. There were a few reticular fibres and dust in the lymph nodes (Figure 9).

In the tremolite and actinolite groups, pathologic changes were nearly the same and both slighter than chrysotile group (Figure 10).

Crocidilite group: Reaction of lung tissue was initially slighter than other experimental groups. Later there was also reticular fibres hyperplasia in pulmonary interstice.

At 18th months, the epithelium hyperplasia of bronchioles and alveolus in some rats was present in the crocidolite and chrysotile groups (Figure 11).

The incidence of pulmonary malignant tumor: The asbestos fibres produced pulmonary malignant tumor (the exclusion of spontaneous lymphoblastoma) in all experimental groups. No pulmonary malignant tumor happened in the control group. The first tumor was found in the chrysotile group; rat died after 15 months of injection dust. Later, two rats with cancer were found at 22 months after injection dust, two cases in the crocidolite group and one case in both tremolite and actinolite group separately, as in Table III and Figures 12, 13.

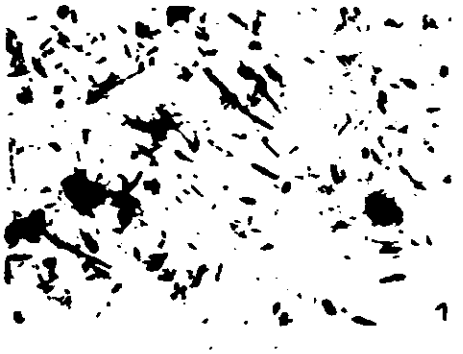

Figure 1. Electromicrograph of chrysotile (× 2500).

Figure 2. Electromicrograph of crocidolite (× 2500).

Figure 3. Electromicrograph of tremolite (× 2500).

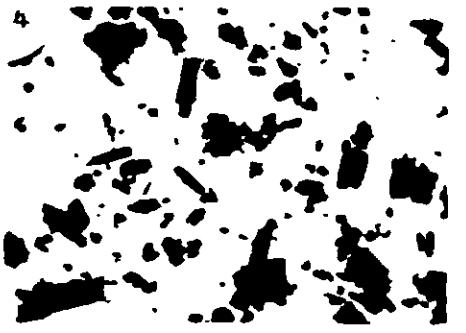

Figure 4. Electromicrograph of actinolite (× 2500).

Table I

Length and Diameter Distribution of Different Asbestos and SiO_2

Asbestos	Length of fibre(%)					Fibre in diameter (%)					SiO%
Fibre	<3	-5	-10	-20	>20	<1.2	-1.6	-2.5	-5	>5	
Chrysotile	76	15	6	2	1	100					0.31
Crocidoite	88	8	3	1	0		100				4.7
Tremolite	88	6	3	3	0	19	55	20	4	2	0.13
Actinolite	76	14	7	2	1	26	55	11	6	2	0.34

Table II

Result of Collagen Content of Lungs in Every Group

Group	2			4			6			12			18		
	No. (T P)	$\bar{X}$	S.E.	No. (T P)	$\bar{X}$	S.E.	No. (T P)	$\bar{X}$	S.E.	No. (T P)	$\bar{X}$	S.E.	No. (T P)	$\bar{X}$	S.E.
Cont	5	45.79	5.04	7	48.15	3.22	6	54.98	2.94	8	57.17	2.31	7	71.94	4.80
Chr	4	53.66	3.74	5 (2.49, <0.05)	68.40	6.40	4 (6.59, <0.001)	81.78	2.20	8 (3.08, <0.01)	80.10	1.40	7	85.86	5.10
Cro	4	35.59	2.70	6	48.64	2.33	6	55.89	3.37	8 (2.25, <0.05)	72.26	6.29	7	78.05	4.14
Tre	4	49.20	3.88	5	53.30	6.46	6	69.30	3.93	8 (2.93, <0.01)	71.20	4.21	8	77.23	5.0
Act	5	40.37	2.03	6 (2.32, <0.05)	59.78	3.64	5 (2.761, <0.05)	71.71	6.70	5 (2.396, <0.05)	78.07	10.67	6	79.10	5.18

Table III

Pathologic Type and Time of Inducing Tumor

Group	No. of Animals	No. of Pulmonary Tumors	15	17	19	21	23	25	27(Months)
Chrysotile	38	3	sq			Fibro			Adeno
Crocidolite	39	2			Adeno		Fibro		
Tremolite	40	1				Adeno			
Actinolite	35	1				sq			
Control	38	0							

Sq, Squamous cell carcinoma;
Fibro, Fibrosarcoma;
Adeno, Adenocacinoma;

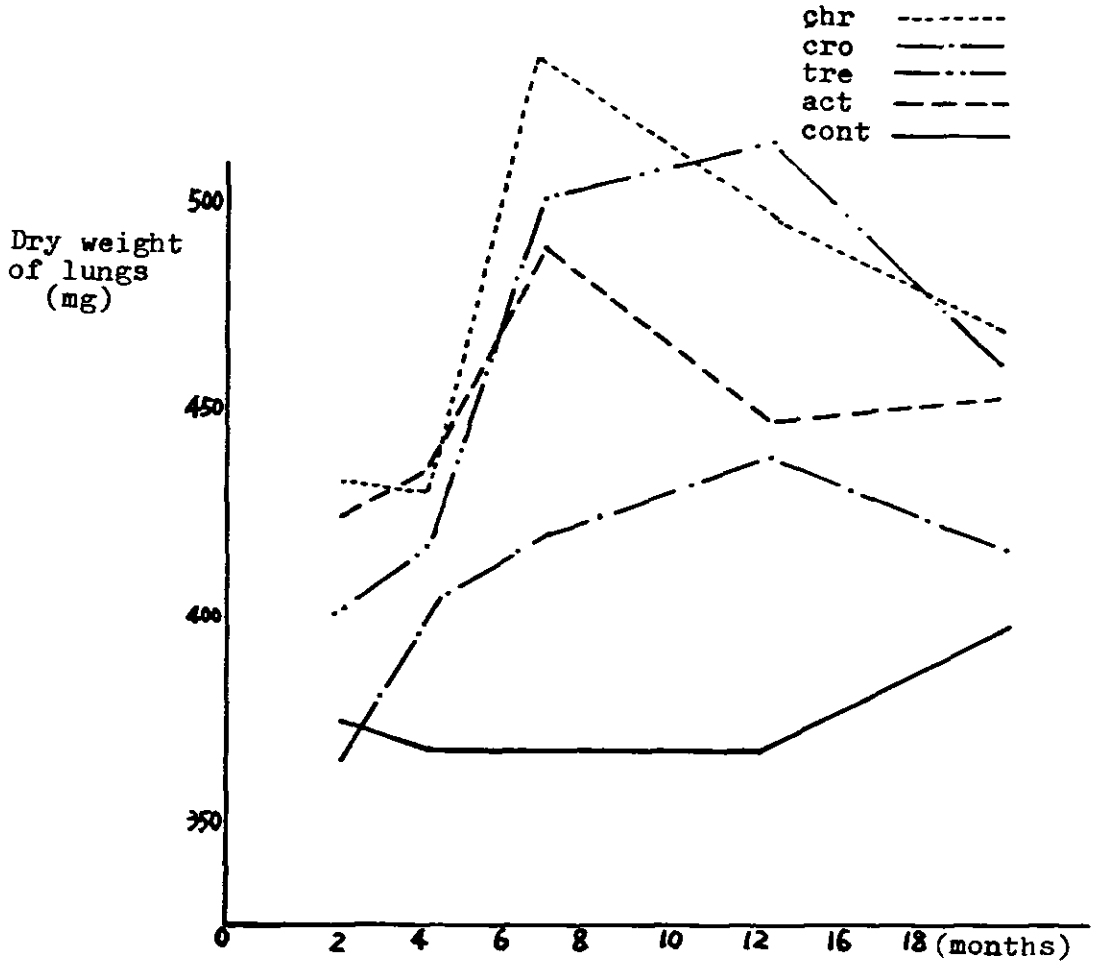

Figure 5. Changes of dry weight of lung in rats of every group.

DISCUSSION

For the different types of asbestos or the same asbestos type in different area, inhalation or injection asbestos can lead to various reaction of lung in the animal.[1,2,3] In addition inhalation of same asbestos in different kinds of animals produced also various results. All four short asbestos in the experiment produced increasing of wet and dry weight of lung in rats. A lot of reticular fibres and a few collagen were found in the pulmonary interstice. These results correspond to Fu Shao Chang's report.[4,5,6]

Inhalation of injection of asbestos fibres may produce pulmonary tumors.[7,8,9] Seven rats with lung tumors in this experiment, the first lung tumor was found in a rat that had died at the 15th month after initial injection of chrysotile fibres. Most tumors were found during 21-25 months. No cancer happened in the control group. This experiment indicated that short asbestos fibres produced not only pulmonary fibrosis but also pulmonary cancer. This result corresponds with Gross's report. Besides, epithelium hyperplasia of bronchioles and alveoli was sometimes present. There changes were not seen in the control group. It seems possible that pulmonary malignant tumors resulted from these changes.

Therefore, we hold that the effect of short fibre asbestos must be considered as recommended hygienic standard.

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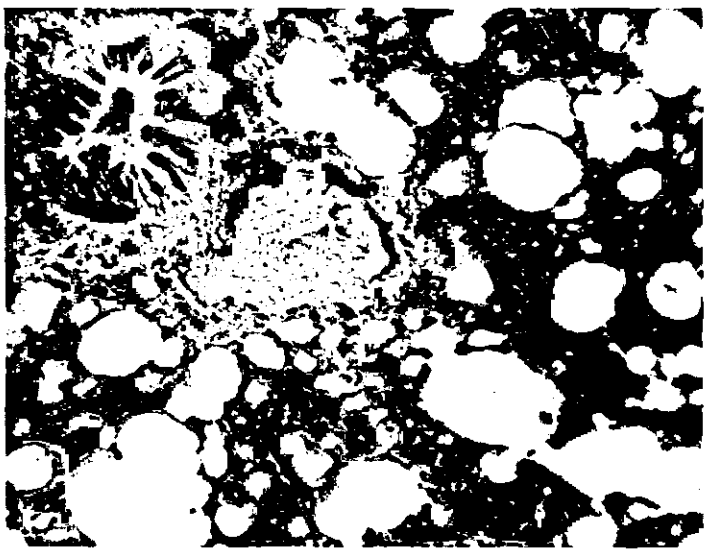

Figure 6. Chrysotile: Extensive areas of reticular fibre abutting on the terminal bronchioles and involving respiratory bronchioles. At the 2 months after injection of dust (H.E. × 78).

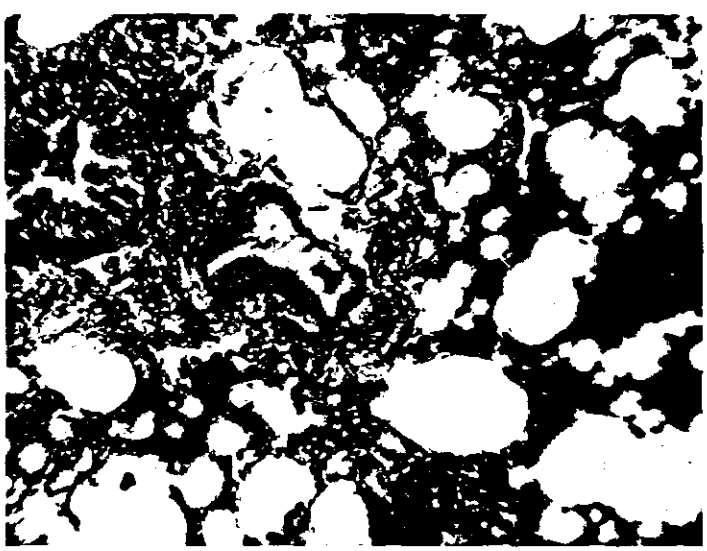

Figure 7. Bid (G.S. × 78).

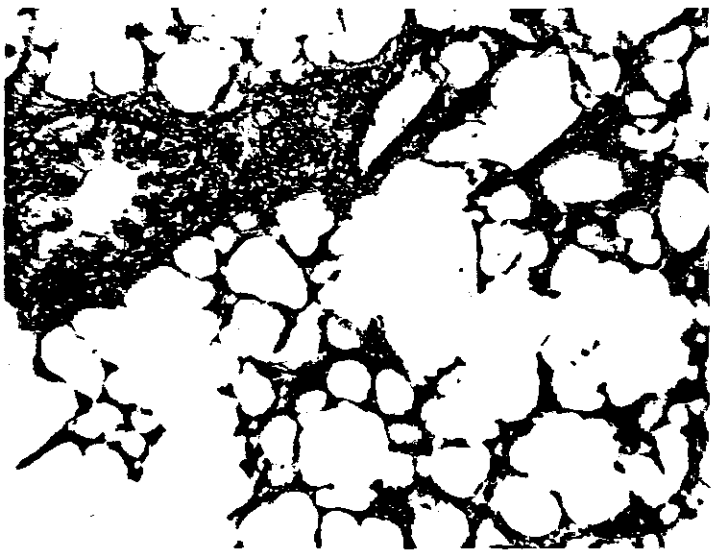

Figure 8. Chrysotile: Slight centrilobular emphysema and slight thickening of alveolar wall in different areas of the lungs. 6 months (G.S. × 78).

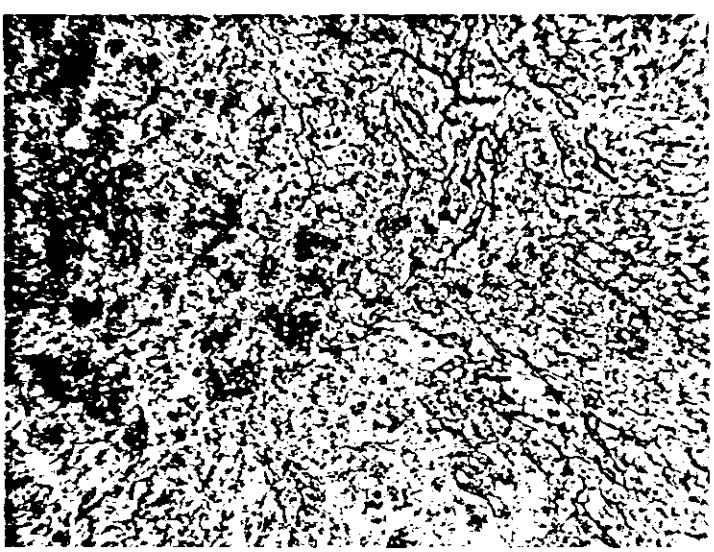

Figure 9. Chrysotile lymph node: Many more reticular fibres. 18 months (G.S. × 78).

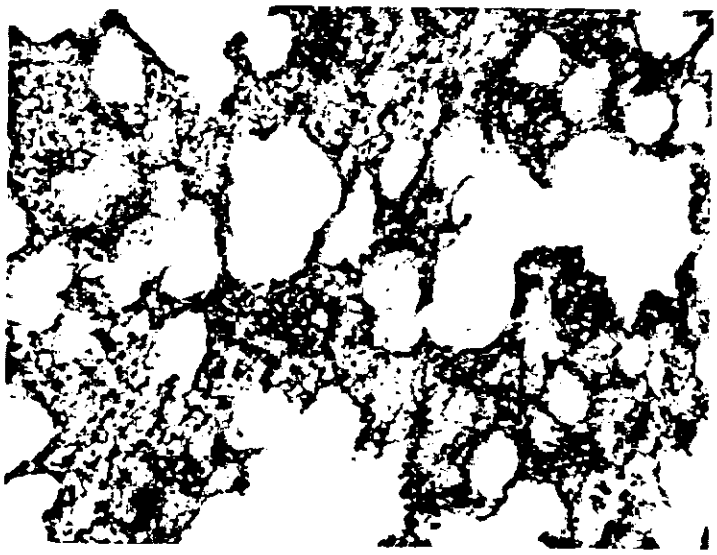

Figure 10. Tremolite: Extensive reticular fibres in pulmonary interstice. 12 months. (G.S. × 78).

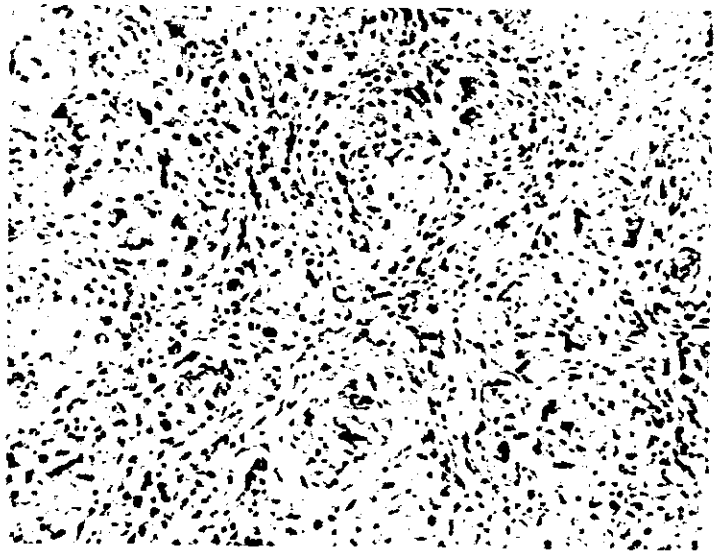

Figure 12. Chrysotile: Squamous cell carcinoma. 15 months (H.E. × 78).

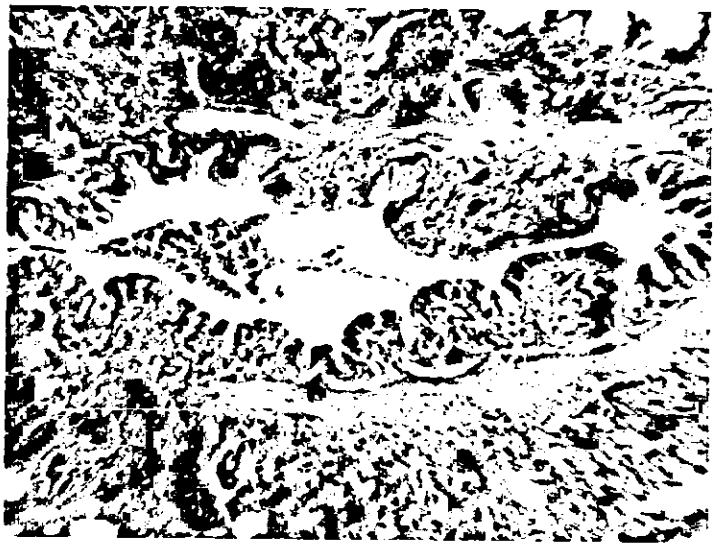

Figure 11. Chrysotile: Epithelium hyperplasia of bronchioles. 18 months (H.E. × 120).

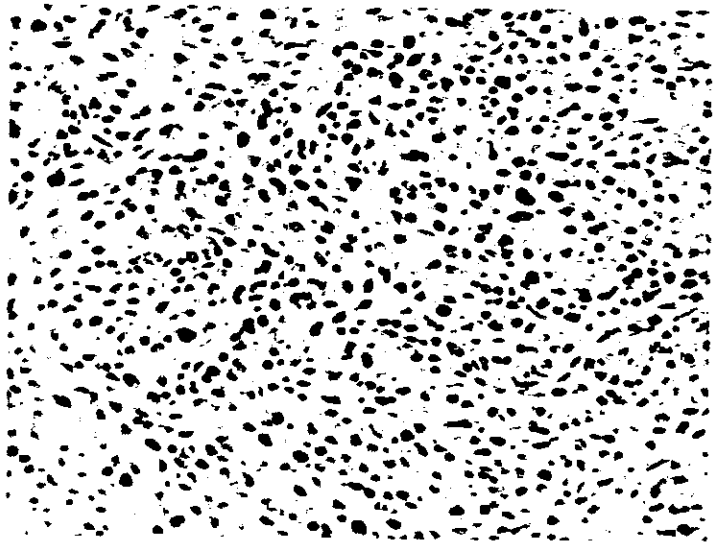

Figure 13. Chrysotile: Fibrosarcoma. 22 months (H.E. × 78).

ACUTE TOXICITY OF FLY ASH COLLECTED FROM A MUNICIPAL INCINERATOR BURNING TRASH

Y. ALARIE • M. Iwasaki • M. Stock • R.C. Pearson • D.J. Lisk

Graduate School of Public Health, University of Pittsburgh Pittsburgh, PA, and Toxic Chemicals Laboratory, Cornell University, Ithaca, NY, USA

INTRODUCTION

There are about 70 operating municipal refuse incinerators in the United States and about 250 more are planned. The ash produced typically contains high concentrations of heavy metals[1] and a wide range of toxic organics including polychlorinated dibenzodioxins and dibenzofurans.[2] Therefore, there is a concern about possible health effects of the ash among those residing downwind from such incinerators as well as on workers within the plants. We have undertaken some preliminary studies with exposures of guinea pigs to very high concentrations of fly ash collected from one municipal refuse incinerator.

Experimental

Twenty kilograms of fly ash was collected from a municipal incinerator. After drying and mixing the ash was analyzed for cadmium, lead and zinc by wet ashing with nitric and perchloric acids followed by anodic stripping voltametry.[3] Mercury was determined by flameless atomic absorption analysis.[4]

The ash was placed into a Pitt No. 3 aerosol generator[5] for resuspension in air of fine particles which were delivered to an exposure system for guinea pigs.[6,7] This system consisted of a central glass chamber to which four glass chambers were attached, each holding one guinea pig. Each of these animal chambers functioned as a flow-through whole body plethysmograph.[8] Therefore they permitted indirect measurement of tidal volume (VT), from the pressure changes ($\triangle P$) created by each breath as monitored by a sensitive pressure transducer attached to each chamber.[8] Four male Hartley guinea pigs (300-350g) were obtained from Hazleton Research Products, Inc. and were exposed to the ash 6 hours/day for 5 consecutive days. The exposure concentration was 314 mg/m^3 and the particle size was 3.2 μm mass aerodynamic diameter. Prior to and immediately following each exposure each animal was challenged with 10% CO_2 in 20% O_2 and 70% N_2. $\triangle P$ and respiratory frequency (f) were measured during air breathing and CO_2 challenge.[8,9] Similar CO_2 challenges were also conducted on days 6-9, 14, 16, 21, 26-30, 35 and 50 following exposure. Euthanasia was performed on day 50 using pentobarbital. Kidneys, livers and lungs were removed. Lungs were fixed using intratracheal infusion of 10% buffered formaldehyde held at 25 cm H_2O for two hours prior to continued fixing in the same solution. Before and after fixation lung weights were taken and lung volumes were measured by water displacement. Four guinea pigs were used as controls and treated as the exposed animals except that no dust was delivered to the exposure system.

RESULTS

Table I lists the heavy metals and carbon content of the ash and Table II lists the heavy metals found in tissues of guinea pigs 45 days after termination of exposure. Significant elevation was found in the lungs of the exposed animals as compared to the controls.

Following the first exposure and during the five exposure days there was no change from preexposure for VT measured during air breathing. However f was lower. During CO_2 challenge both VT and f were lower. This effect persisted for all exposure days. Measurements made after the 5 exposure days and until sacrifice at day 50 indicated recovery towards control values. However, there was histopathological findings in all animals ranging from moderate to severe pneumoconiosis. This consisted of interstitial macrophage reaction with a number of dense, black granule-laden macrophages. Airways were moderately constricted and a moderate degree of smooth muscle hypertrophy of the airways and vessels was present. Thickening of alveolar septa by macrophages and foci of granule-laden macrophages was observed. There was no increase in lung weights in comparison to the controls. Lung volumes after fixation were reduced by 50% in two animals, probably because of the constricted airways preventing the entry of fixative as in controls.

DISCUSSION

Fly ash from refuse incinerators will vary greatly because of the nature of the operation. Nevertheless, the results indicate that a very high concentration was needed to induce an abnormal ventilatory response to CO_2 on an acute basis. The reduction in VT during CO_2 was just below 50% of control. This level of effect can be induced by 13 mg/m^3 of cotton dust,[10] 1.5 mg/m^3 of paraquat,[11] or 50 mg/m^3 hexamethylene diisocyanate trimer.[12] Therefore the dust tested was not very potent in inducing an acute pulmonary effect. The delayed effects, as indicated from microscopic examination of the lungs were important and it would therefore be appropriate to investigate such dusts with repeated exposures at low concentrations to investigate the possible chronic effects. Airways constriction with smooth muscles hypertrophy suggest the possible development of chronic obstructive lung disease. This could be followed functionally by flow-volume measurements which can be made in guinea pigs.[9]

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Table I

Heavy Metals and Carbon Content of Collected Fly Ash for Exposure of Guinea Pigs. Concentration Given on the Basis of Dry Weight.

Item (units)		
Cadmium	(ppm)	477
Lead	(ppm)	2134
Mercury	(ppm)	25
Zinc	(ppm)	14301
Carbon	(%)	7.34

Table II

Concentrations of Heavy Metals in Tissues of Guinea Pigs Exposed to Refuse Incinerator Fly Ash. Measurements Made 45 Days After 5 Daily Exposures of 6 Hours Each at an Exposure Concentration of 314 mg/m^3

	(Parts per million (dry weight of metal in tissue)	
	Controls	Exposed
	[a]	
Cadmium in lung	0.20 ± 0.02	1.45 ± 0.17
Cadmium in kidney	1.16 ± 0.22	1.91 ± 0.23
Cadmium in liver	0.41 ± 0.07	0.97 ± 0.11
Lead in lung	1.25 ± 0.22	6.58 ± 0.46
Lead in kidney	0.40 ± 0.06	0.62 ± 0.14
Lead in liver	0.23 ± 0.03	0.34 ± 0.03
Zinc in lung	68.50 ± 7.06	110.48 ± 5.63
Zinc in kidney	89.45 ± 4.18	94.39 ± 7.31
Zinc in liver	93.63 ± 6.12	115.48 ± 12.74
Mercury in lung	0.16 ± 0.04	0.32 ± 0.03

[a] Means ± standard error.

ASBESTOS RELATED DIFFUSE PLEURAL FIBROSIS

A.R. GIBBS,* MB, ChB, MRC Path. • D.M. Griffiths† • M. Stephens,‡ MB, BCh • F.D. Pooley,§ Ph.D., MIMM., MAIME., C Eng.

*Department of Pathology, Llandough Hospital, Penarth
†Medical Research Council External Staff on Occupational Lung Disease, Llandough Hospital, Penarth
‡Department of Pathology, University Hospital, Nottingham
§Institute of Materials, University College, Cardiff

Diffuse pleural fibrosis (DPF), particularly when severe may cause reduction in vital capacity and contribute to respiratory disability.[1] Recently DPF has been accepted as a consequence of asbestos exposure.[2,3] We previously studied 7 cases of asbestos related DPF and reported on the pathological and mineralogical findings.[4] In this study we have extended these observations to 13 cases and also performed mineralogical analysis on tissues sampled from the central, subpleural and pleural regions of the lung. The aims of the study were to investigate the type of occupational exposure, the total mineral fibre burdens and the type and distribution of asbestos mineral fibres within the lungs associated with this form of asbestos related disease.

METHODS

The 13 cases were selected on the basis that there was a history of asbestos exposure and at autopsy the DPF was bilateral, covered more than 25% of the lung surface and exceeded 5 mm in thickness at some points. Clinical and occupational histories were obtained from the hospital notes or Medical Boarding Centres. At least one of the lungs was inflated and tissue blocks were taken for histology as follows:

1. Subpleural region of the apex of upper lobe.
2. Subpleural region of the apex of lower lobe.
3. Subpleural region of the base of lower lobe.
4. Central region of the upper lobe.
5. Central region of the lower lobe.
6. Several blocks from areas of pleural fibrosis.

The degree of parenchymal fibrosis was graded from 0 to 4 for each histological slide by an established system and an average value obtained for each case.[5,6]

For mineralogical examination samples were taken from the same areas. The mineralogical analysis was performed for the subpleural region by pooling samples adjacent to blocks 1-3, for the central region by pooling the samples adjacent to blocks 4 and 5 and the pleura by pooling the samples adjacent to the blocks from 6. The samples were divided into two, and one from each of the areas was dried to a constant weight so that the wet/dry ratio could be calculated. The remaining portions were digested in a wet state by 40% potassium hydroxide. For light microscopy: after centrifugation, the sediment was examined in a Fuchs Rosenthal chamber by phase contrast and the number of fibres and bodies counted, from which could be calculated the number per gramme of dried lung.[7] At least 100 asbestos forms were counted. For electron microscopical examination: following digestion, the final deposits were ashed at 300C for 4 hours. The suspensions were then passed through 0.2 pore sized nucleopore filters.

They were then carbon coated and examined by transmission electron microscopy. The fibres were identified by type and the number per gramme of dried lung calculated. At least 200 fibres were counted and identified for each sample. Statistical analysis was performed using the Wilcoxon ranking test for paired data.

RESULTS

The occupational details for each case are given in Table I. All were males and the age at death ranged from 47 to 80 years. Duration of exposure to asbestos varied from 1 to 35 years. In all cases macroscopical examination revealed extensive diffuse visceral pleural fibrosis which was at least greater than 5 mm but which went up to 4 cm in thickness and mimicked pleural mesothelioma in some cases. In several cases there were extensive adhesions between the visceral and parietal pleura. In some cases recognisable parietal pleural plaques were also present. Case 10 also showed severe diffuse pericardial fibrosis similar to that of the pleura. Microscopically the pleura showed mature collagen arranged in a basket weave pattern. The degree of pulmonary fibrosis for each case is given in Table I.

Total mineral fibre counts obtained by light and electron microscopy at the central, subpleural and pleural sites are given for each subject in Table II. Table III shows the mean total and specific fibre counts for each of the anatomical sites.

The total asbestos fibre count was significantly greater in the central and subpleural region than in the pleura ($p<0.01$). There were no significant differences in the distribution of chrysotile in the central, subpleural or pleural regions but there was a statistically significant difference in the distribution of amosite and crocidolite ($p<0.01$). The amosite and crocidolite levels were much lower in the pleura than in the other regions.

Table I

Ages, Occupational Histories and Histological Fibrosis Grades of 13 Cases of DPF with a History of Asbestos Exposure

SUBJECT	AGE/YRS	OCCUPATIONAL HISTORY	GRADE OF PULMONARY FIBROSIS
1	80	Pipe fitter 25 yrs	2
2	67	Engineer, 2 yrs cutting asbestos sheets	2/3
3	64	Boiler maker	1/2
4	75	Electrical welder	2
5	71	Carpenter, cutting roofing sheets	1/2
6	64	Asbestos sprayer	2/3
7	62	Boiler lagger for 33 years	1
8	64	Unloaded asbestos from sacks (4 yrs) and production of refractory slab (7 yrs)	2
9	47	Mixing and moulding blue asbestos for battery moulds for 1 yr	0/1
10	69	Marine engine fitter 35 yrs	ND
11	54	Gas fitter and plumber	0/1
12	75	Refitting ships 25 yrs	1
13	74	Ship yard joiner	2/3

DISCUSSION

We consider that the DPF in 11 of these cases is likely to have been caused by asbestos but in two it is debatable. Subject 13 had been treated for pulmonary tuberculosis nine months prior to death; the lungs showed quite severe fibrosis and very low asbestos fibre counts which were well within the range of our normal controls. Tuberculosis therefore seems the most likely cause of the pleural fibrosis in this case. Subject 11 had a very vague history of asbestos exposure and the lung asbestos counts were well within the normal control levels. The cause in this case is unknown but unlikely to be due to asbestos.

The light and electron microscopical mineral fibre counts showed a good correlation with each other but the light microscopical counts were in general two orders of magnitude lower than the electron microscopical counts. Although light visible counts obtained by phase contrast cannot give an accurate value for total lung asbestos burden, they are a useful indicator of amphibole exposure.[9,10] Small thin fibres are not visible by this method. Nevertheless, the method is inexpensive, quick and more widely available than EM analysis and in this group of cases the LM counts appeared to be a useful indicator of whether the DPF was likely to have been asbestos induced.

The total counts were generally raised above normal and in

Table II

Total Fibre Counts Obtained by LM and EM Analysis in the Central (C), Subpleural (S), and Pleural (P) Regions of the Lung

SUBJECT	LM (X 10^3)			EM (X 10^6)		
	C	S	P	C	S	P
1	102.8	76.5	5.7	10.7	25.5	2.0
2	232.7	105.5	26.7	8.4	19.0	9.2
3	79.4	131.0	7.0	18.0	19.1	7.5
4	55.6	88.5	0.65	ND	ND	ND
5	68.4	66.5	0.83	18.9	12.2	2.2
6	95000.0	5700.0	306.0	24769.5	32722.3	123.5
7	5330.0	4200.0	0	143.3	124.7	10.1
8	1210.0	1800.0	3.7	162.8	225.0	11.0
9	48.5	100.0	0	15.4	40.6	2.5
10	ND	744.0	11.5	18.6	80.3	17.0
11	12.2	7.7	3.1	21.9	13.1	3.3
12	7000.0	2500.0	0	93.9	164.5	2.1
13	0	25.0	0	26.1	32.4	13.2

Table III

Geometric (Arithmetic) Mean Asbestos Fibre Counts by Type in the Central (C), Subpleural (S) and Pleural (P) Regions of the Lung (10^6)

	Total Asbestos fibres	Chrysotile	Crocidolite	Amosite
C	36.8 (2089.3)	5.24 (9.5)	5.2 (1907.9)	6.6 (182.9)
S	50.4 (2776.5)	13.3 (56.3)	4.9 (2559.0)	7.4 (160.9)
P	6.7 (16.2)	5.5 (7.7)	0.15 (7.4)	0.05 (1.1)

the range we have seen with pleural plaques and minimal asbestosis. However, three subjects had counts which were well above this range (subjects 6, 7 and 12). Subject 6 had an extremely high count, which we have usually encountered in severe asbestosis; he was an asbestos sprayer, an occupation which may be associated with very high asbestos exposures.

The counts obtained at the various sites within the lung confirm the nonuniform distribution of asbestos as shown by others.[11] Chrysotile parenchymal levels were similar to normal controls except for subject 6. Amphibole parenchymal levels were raised above normal in all but two (subjects 11 and 13) which we consider unlikely to be caused by asbestos. The pleura contained relatively little fibre and by far the majority of this was chrysotile. Sebastien et al[12] in a previous study of asbestos fibres from the lung parenchyma and pleura of cases suffering from a variety of asbestos related diseases, found no relationship between parenchymal and pleura concentrations and the pleura contained almost exclusively chrysotile. Although amphibole fibres were found in the pleura of all but one of our cases, they were extremely sparse in number.

In conclusion the findings of this study confirm previous observations that the distribution of fibres within the lung is not uniform. It also shows that in these cases of DPF the majority of fibre within the pleura is chrysotile but small numbers of amphibole fibres are also present. Also the amount of amphibole fibres within the pleura is much less than that in the parenchyma. As in other asbestos related diseases the parenchymal levels of amphibole asbestos but not chrysotile appear raised above controls.

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HYALINE PLEURAL PLAQUES AND ASBESTOS EXPOSURE

C. BIANCHI • A. Brollo • L. Ramani

Laboratory of Pathological Anatomy, Hospital of Monfalcone, Monfalcone, Italy

INTRODUCTION

The importance of hyaline pleural plaques as possible markers of previous exposure to asbestos and to other mineral fibres, is now recognized.[1,2,11,12] In the present study the characteristics of pleural plaques have been analyzed in a series of necropsies, carried out in Monfalcone, Italy. The first results of this investigation have been the object of previous papers.[3-7]

MATERIALS AND METHODS

The Territory of Monfalcone is a small industrial district, in northeastern Italy, at the border with Yugoslavia. It covers an area of 152 square kilometers and has a population of 59,599 (1981 census). The Monfalcone Territory includes eight towns, the major of which are Monfalcone (30,259 inhabitants), and Ronchi dei Legionari (10,052 inhabitants).

The Monfalcone shipyard, opened at the beginning of this century (1908), is the most important industry in this area. At present about 3,000 persons are employed in the Monfalcone shipyard; but the workforce was greater in the past, having reached nearly 10,000 workers in the late 1930's.

During the period October 1979-December 1987 a total of 1,620 necropsies were performed at the Monfalcone Hospital. The necropsies were carried out by three pathologists (Claudio Bianchi, Lucia Bittesini, and Alessandro Brollo). In all the cases a careful inspection of the thoracic cavities was performed, in order to detect the presence of hyaline pleural plaques. In a large majority of the necropsies, the costal and the diaphragmatic pleuras were detached from the thoracic cavity, placed on a table, and then examined. All white, fibrous patches, calcified or not, involving parietal pleura were defined as pleural plaques. Unilateral and very small patches were also considered as hyaline plaques. The cases with pleural plaques were classified in 3 classes: (1) mild, (2) moderate, and (3) severe, on the basis of the size of plaques. Small plaques (few centimeters in major diameter) were classified as mild, while very large unilateral and bilateral plaques involving the major part of a hemithorax were defined as severe. The term moderate was applied to the intermediate conditions. In expressing the results, a simplified classification ("small" and "large") is sometimes adopted, "small" corresponding to class 1, and "large" including the remaining two classes.

In a number of cases the macroscopic features of pleural plaques were photographically documented. Moreover samples were generally obtained from the pleura for histological examination.

In all the cases a sample, measuring about 3x3x2 centimeters, was taken from the lung base and formalin fixed. The piece was taken from the right lung, or from the left base when the right was largely involved by tumor. In 464 cases the sample was completely digested in a commercial 5% sodium hypochlorite solution. Asbestos bodies were then isolated and quantitated according to the method of Smith and Naylor.[16] The results were expressed as the number of asbestos bodies per gram of dried lung tissue.

In 745 cases the patients' relatives were interviewed to obtain detailed occupational data. Generally the interviews were carried out at our laboratory by one of us (C.B.). In some cases the interviewer was another doctor of our staff, and in a few cases telephone interviews were performed. The questions concerned the various occupations of the patient, the place and the time of the activities, and the occupations of his/her relatives. When sufficient information was not obtained, further members of the family were interviewed. In many cases the "work-book" (a personal document in which all the occupations and the names of employers are listed) was consulted.

The chi-square test was used to compare the prevalence of pleural plaques among the various groups of patients. The relation between pleural plaques and asbestos body content was examined by determination of the Spearman's correlation coefficient.

RESULTS

The series consisted of 1,040 men and 580 women. Tables I and II show the sex and age distribution of pleural plaques. Between the two sexes there were significant differences in the prevalences of total plaques ($p<0.001$), small plaques ($p<0.01$), and the large plaques ($p<0.001$).

In Table III the patients are subdivided according to sex, and place of residence at death. The men resident in Monfalcone Territory more frequently showed pleural plaques ($p<0.001$) and large plaques ($p<0.01$), compared with men resident in other areas. Among women higher prevalences of total plaques ($p<0.001$) and of small plaques ($p<0.01$) were observed in Monfalcone Territory residents.

The amounts of lung asbestos bodies showed marked differences between the two sexes (Table IV). A good correlation was visible between the amount of asbestos bodies and extent of pleural plaques (Table V) ($r=0.61$, $p<0.001$).

Table I

Hyaline Pleural Plaques and Age (Men)

Age (years)	No. of cases	Hyaline Pleural Plaques (%) Absent	Class 1	Class 2	Class 3
0 - 24	13	100.0	0.0	0.0	0.0
25 - 34	4	75.0	0.0	25.0	0.0
35 - 44	24	29.2	33.3	12.5	25.0
45 - 54	99	34.3	20.2	21.2	24.2
55 - 64	209	31.6	26.8	19.1	22.5
65 - 74	327	27.5	24.8	24.8	22.9
75 - 84	305	26.2	23.3	28.2	22.3
85 - 94	59	37.3	22.0	22.0	18.6
Total	1040	30.3	23.9	23.6	22.2

Table II

Hyaline Pleural Plaques and Age (Women)

Age (years)	No. of cases	Hyaline Pleural Plaques (%) Absent	Class 1	Class 2	Class 3
0 - 24	5	100.0	0.0	0.0	0.0
25 - 34	5	100.0	0.0	0.0	0.0
35 - 44	13	84.6	15.4	0.0	0.0
45 - 54	26	80.8	15.4	3.8	0.0
55 - 64	69	71.0	26.1	1.4	1.4
65 - 74	125	72.8	18.4	7.2	1.6
75 - 84	236	77.1	19.5	3.4	0.0
85 - 94	96	82.3	12.5	5.2	0.0
95 - 99	5	100.0	0.0	0.0	0.0
Total	580	77.2	18.1	4.1	0.5

Table III

Hyaline Pleural Plaques and Residence

	No. of cases	Hyaline Pleural Plaques (%) Absent	Class 1	Class 2	Class 3
MEN					
MT Residents	872	25.8	23.6	25.5	25.1
Others	168	53.6	25.6	13.7	7.1
WOMEN					
MT Residents	468	73.9	20.5	4.9	0.6
Others	112	91.1	8.0	0.9	0.0

MT = Monfalcone Territory

Table VI shows the prevalence of pleural plaques in men classified on the basis of work history data. There were marked differences from one occupational category to another, with shipyard workers and clerks being at the two extremes. The subjects employed in industries (shipbuilding, chemical, construction, and various), showed a significantly higher prevalence of pleural plaques, compared with persons employed in agriculture ($p<0.001$), or with persons included in the remaining groups ($p<0.001$).

Among the chemical industry workers there were 18 patients, who had been employed in the sodium carbonate factory of Monfalcone; a very high prevalence of pleural plaques was observed in this subgroup, large plaques having been found in 14 cases, and small plaques in 3.

Among women a large number of patients had histories of household exposure to asbestos having cleaned the work clothes of their family members employed in shipbuilding or in the chemical industry. A double classification of the cases had therefore been adopted, according to the presence (Table VII) or the absence (Table VIII) of data indicating domestic exposure. Eight women with incomplete histories have not been included in the tables. The women exposed to asbestos at home significantly differed from the others in the prevalence of pleural plaques ($p<0.001$).

In a large majority of the cases the patients with pleural plaques and with histories of employment in the shipyard or in other industries, had begun their work before 1950. However, a small number of subjects, who had their first employment in the shipyard in the 1970's, were observed; these workers showed small plaques and widely variable amounts of lung asbestos bodies (between 100 and 200,000/g dried lung tissue).

Table IV

Asbestos Bodies Amounts in 464 Cases

AB *	MEN Cases	%	WOMEN Cases	%
0 - 1	6	1.6	4	4.4
1 - 2	16	4.3	14	15.6
2 - 3	80	21.4	37	41.1
3 - 4	108	28.9	31	34.4
4 - 5	118	31.6	3	3.3
5 - 6	40	10.7	1	1.1
6 - 7	6	1.6	0	0.0
Total	374	100.0	90	100.0

* Asbestos bodies, Log10 /g dried tissue

Table V

Hyaline Pleural Plaques and Lung Asbestos Bodies

AB *	No. of cases	Hyaline Pleural Plaques (%) Absent	Class 1	Class 2	Class 3
0 - 1	10	60.0	40.0	0.0	0.0
1 - 2	30	60.0	26.7	13.3	0.0
2 - 3	117	41.9	37.6	15.4	5.1
3 - 4	139	28.8	18.7	25.2	27.3
4 - 5	121	7.4	11.6	33.1	47.9
5 - 6	41	0.0	9.8	26.8	63.4
6 - 7	6	16.7	0.0	0.0	83.3
Total	464	26.5	21.6	23.3	28.7

* Asbestos bodies, Log10 /g dried tissue

Table VI

Hyaline Pleural Plaques and Occupations (Men)

	No. of cases	Hyaline Pleural Plaques (%) Absent	Class 1	Class 2	Class 3
Shipbuilding industry	141	7.1	22.0	33.3	37.6
Shipbuilding and others	217	18.4	18.9	25.8	36.9
Chemical industry	26	19.2	23.1	26.9	30.8
Sailors and dock workers	19	31.6	42.1	10.5	15.8
Various industries	46	50.0	21.7	19.6	8.7
Construction industry	33	54.5	27.3	9.1	9.1
Agriculture	26	61.5	30.8	7.7	0.0
Artisans and traders	24	66.7	25.0	8.3	0.0
Clerks	12	91.7	0.0	8.3	0.0
Other	11	63.6	27.3	0.0	9.1
Insufficient data	4	25.0	25.0	50.0	0.0
Total	559	27.4	22.0	23.4	27.2

Table VII

Hyaline Pleural Plaques and Occupations in Women with Histories of Domestic Asbestos Exposure

	No. of cases	Hyaline Pleural Plaques (%) Absent	Class 1	Class 2	Class 3
Shipbuilding industry	9	22.2	77.8	0.0	0.0
Various industries	19	26.3	52.6	15.8	5.3
Housewives	24	33.3	54.2	12.5	0.0
Textile industry	14	50.0	35.7	14.3	0.0
Agriculture	20	55.0	30.0	15.0	0.0
Artisans and traders	18	61.1	38.9	0.0	0.0
Maids	11	63.6	27.3	9.1	0.0
Other	6	50.0	50.0	0.0	0.0
Total	121	44.6	44.6	9.9	0.8

Table VIII

Hyaline Pleural Plaques and Occupations in Women without History of Domestic Asbestos Exposure

	No. of cases	Hyaline Pleural Plaques (%) Absent	Class 1	Class 2	Class 3
Various industries	2	50.0	0.0	0.0	50.0
Clerks	6	66.7	16.7	16.7	0.0
Shipbuilding industry	10	70.0	30.0	0.0	0.0
Textile industry	4	75.0	25.0	0.0	0.0
Maids	5	80.0	20.0	0.0	0.0
Artisans and traders	8	87.5	12.5	0.0	0.0
Housewives	18	94.4	5.6	0.0	0.0
Agriculture	4	100.0	0.0	0.0	0.0
Total	57	82.5	14.0	1.8	1.8

DISCUSSION

Data on the prevalence of hyaline pleural plaques, in the general population or in specific occupational groups, are available for various regions.[1,2,6-8,11-13,15] Several studies are based on chest X-ray findings, and others on necropsy material. Since the sensitivity of X-ray examination in detected pleural plaques is low,[12] the comparisons between the two types of investigation are of limited value.

The prevalence we observed in the present series appears to be very high when compared to those found in other necropsy series.[1] The residents in the Monfalcone area seem to be particularly involved.

The marked difference in the prevalence of pleural plaques between the two sexes is a first indication that an occupational source might represent the most important cause of the plaques in our cases. This idea is strongly corroborated by occupational histories.

Some researchers[12] believe that at retirement probably nearly all the shipyard workers have pleural plaques. The present findings confirm such an opinion. In our material over 90% of the subjects, who had worked only in the shipyard, showed hyaline pleural plaques. Moreover other industries have been identified as causes of plaques. In particular, working in a sodium carbonate factory, a plant active in Monfalcone until 20 years ago, was almost constantly associated with the presence of plaques.

In clarifying the etiology of pleural plaques in the Monfalcone series, occupational data as well as the findings concerning lung asbestos content have to be considered.

A history of employment in the shipyard represents a strong indication of asbestos exposure so that pleural plaques, we found in shipyard workers, may be attributed to asbestos. As far as the majority of the other workplaces appearing in the occupational histories were concerned, we were able to ascertain that they implied an asbestos exposure. However, the meaning of some data remains uncertain. For instance pleural plaques (mostly small) have been observed in some subjects with histories of employment in agriculture. We could not ascertain the etiology of the plaques in these cases; an environmental exposure to asbestos is a possible cause, but incompleteness of the history data or the role of factors other than asbestos cannot be excluded.

Information furnished by quantitation of lung asbestos bodies supports the idea that asbestos is by far the most important cause of pleural plaques in the present series. In fact a good correlation was observed between the amount of asbestos bodies and pleural plaques. Nevertheless in several cases with histories indicative of important occupational asbestos exposure and with large pleural plaques, low numbers of asbestos bodies were found. Conversely some heavily exposed subjects showed large amounts of asbestos bodies, not associated with the presence of pleural plaques.

The low number of asbestos bodies in exposed subject may be explained by different factors, such as the clearance of asbestos fibers,[14] or individual differences in the production of asbestos bodies.[9] Moreover it should be remembered that the sensitivity of the Smith-Naylor method, the techniques used in the present investigation, has recently been questioned.[10] Concerning the absence of pleural plaques in heavily exposed persons, in our material this situation was usually associated with the presence of firm, diffuse adhesions between the visceral and parietal pleura.

CONCLUSIONS

In the Monfalcone area a consistent portion of the male population has spent some part of their life in the shipyard. Consequently it is not surprising that a very high prevalence of hyaline pleural plaques has been observed in this territory. However, the present investigation furnishes data on the intensity of asbestos exposure in the Monfalcone shipyard. Moreover other sources of asbestos exposure, before unsuspected, have been identified and the magnitude of the phenomenon "domestic asbestos exposure" in this territory has been defined. In our experience detection of pleural plaques represents a valid way of monitoring asbestos exposure.

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MORPHOLOGY, CHARACTER AND FEATURES OF ANTOPHYLLITE-INDUCED MESOTHELIOMAS

K. KOLEV

Institute of Hygiene and Occupational Health, Medical Academy, Sofia, Bulgaria

ABSTRACT

A histological, cytological, histochemical and electron-microscopic observation was carried out on 109 mesotheliomas, induced by /UICC/, Bulgarian and Soviet antophyllites. Intraperitoneal and intrapleural method of application were used. Most characteristic of the antophyllite-induced mesotheliomas is the fact that in 55 to 72% in terms of their infrastructure they conform to the sarcomatous variant. This feature is in complete contradiction with science in the experimental and human mesotheliomas caused by crocidolite, amosite and chrysotile, where the prevailing variant is the carcinomatous one—about 60% while the sarcomatous account for about 10-15%. The prevailing position of the sarcomatous variant in the first case is not influenced by the kind of antophyllite, the morphology of the dust applied, nor by the way of application.

The author proposes a new histogenetic classification of antophyllite-induced mesotheliomas.

No Paper provided.

CELL TYPES OF ASBESTOS LUNG CANCER

COLEMAN B. RABIN, M.D. • Irving J. Selikoff, M.D. • Yasunosuke Suzuki, M.D. • Ruth Lilis, M.D.

Div. of Environmental & Occupational Medicine, Mt. Sinai School of Medicine of the City University of New York, USA

ABSTRACT

We have been following a cohort of asbestos insulation workers from January 1, 1967. By January 1, 1985, there were 526 lung cancer deaths in which we had opportunity to review initial diagnostic X-rays, histological material, and clinical findings. We will present the distribution of cell types, including small cell carcinoma, squamous cell, adenocarcinoma, large cell carcinoma, bronchoalveolar carcinoma, and less common cell types, in relation to topographical findings and other parameters.

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